Prolonged Small Intestinal Mucosal Injury as a Primary Cause of Intractable Diarrhea of Infancy

Emanuel Lebenthal

Department of Pediatrics, State University of New York at Buffalo, and Division of Gastroenterology and Nutrition, Children's Hospital of Buffalo, Buffalo, New York 14222

Prolonged or intermittent diarrhea early in life has been shown to be one of the main causes of infant death in the developing countries. Many factors are involved in the persistence of the diarrhea that are unique to early infancy; these in turn may lead to serious complications such as severe malnutrition, intercurrent infections, and even death of the child. Emphasis has been put on issues related to the vantage point of certain research groups such as secretion or malabsorption of certain nutrients in the small intestine or intolerance of protein or carbohydrates secondary to the chronic diarrhea. The main thrust of this chapter is the presentation of an overview of the determinants that lead to the prolongation and eventually the intractability of diarrheal episodes. Specifically, changes in gastrointestinal function and morphology during acute diarrhea that affect the infant with compromised nutritional status are reviewed. Based on our experiences at the Buffalo Children's Hospital from 1977 to 1983 in the care of infants with intractable diarrhea, we believe that the main underlying mechanism is prolonged mucosal injury of the small intestine.

In the normal small intestine, absorption of nutrients is very efficient per unit time or length and the flow of contents is relatively rapid. In contrast, in the colon, absorption of nutrients is much less efficient than in the small bowel but the flow is comparatively slow, making possible the salvage of materials that are not absorbed in the small intestine. Any deviation from the normal situation will upset the delicate balance of absorption and secretion. From both mechanistic and empiric points of view, prolonged small intestinal mucosal injury can serve as a central issue for further understanding of multiple variables involved in prolongation of the diarrheal state. At the same time, it will also provide a basis for designing therapeutic and nutritional regimens.

The possible underlying mechanisms operant in prolonged mucosal injury can be treated as either contributing factors or as consequences of the injury itself. Diarrhea can be a result of small intestinal secretion of electrolytes and fluids, insufficient colonic salvage either because of overloading of a normal
colon or because a diseased colon cannot handle the normal small intestinal volume and electrolyte load (1,2). Different groups of investigators have emphasized disturbances of secretion, motility, and transit time in diarrhea. Substantial advancement has been made in the understanding of these disturbances of normal function in relation to secretory diarrhea not only from the standpoint of the etiologic agents, such as cholera and E. coli enterotoxins, but also with respect to the molecular basis of their action. These mechanistic descriptions of the pathogenesis of diarrhea have provided new insight into the changes occurring on the cellular level, including cyclic AMP concentration, calcium flux, and intracellular levels of calmodulin. But these have not been adequately investigated to be directly applicable to the clinical management of acute and chronic diarrheal syndromes.

Central to our understanding of the current topic are the pathophysiological mechanisms of injury to the small intestinal epithelium and the unique features of mucosal injury in early infancy. The relationship of the small intestinal mucosal injury to other secondary effects, such as malabsorption, diminished exocrine pancreatic secretion, deconjugation of bile acids, loss of gut hormones synthesized in the epithelium, and increases in macromolecular absorption and their corresponding consequences, are further detailed in graphic form. The central concept is also shown to have special importance in infancy, where delayed mucosal healing can be linked to inefficient villus repair, primarily on the basis of slower cellular turnover in the infant epithelium.

We believe that the emphasis on the prolonged small intestinal mucosal injury that we have shown to be present in intractable diarrhea in industrialized countries can serve as a model to evaluate the relative importance of the determinants that promote the deterioration of acute diarrhea into chronic diarrhea in the underdeveloped countries. We concentrate in this chapter on the many intricate variables related to this specific issue and suggest new avenues for possible research to be conducted in developing countries.

**INTRACTABLE DIARRHEA OF INFANCY**

The syndrome of intractable diarrhea of infancy (IDI) was originally defined by Avery in 1968 (3). The definition originated from his observation of a 45% mortality rate in infants who satisfied the following criteria: age less than 3 months, duration of diarrhea greater than 2 weeks, and no evidence of enteric infection. The criteria are arbitrary and serve to emphasize that the young infant is more susceptible than older infants, children, or adults to the devastating effects of protracted diarrhea. The last criterion is particularly controversial; although it is true that in the vast majority of infants no etiology for the diarrhea is identified, in many cases a specific entity is identified and treated appropriately, but a protracted diarrheal state ensues nonetheless. The explanation for this effect, we believe, is the fact that most cases are associated with persistent injury
to the small intestinal epithelium. This injury may exist for prolonged periods even after the disappearance of the factor that elicited the injury. Therefore, we have modified the criteria to include all cases of prolonged diarrhea in infancy including those in which a specific etiology is identified (4).

Intractable diarrhea of infancy is a syndrome and not a disease. Many entities are capable of initiating the syndrome, which may ultimately lead to malnutrition and death (Table 1). The mechanisms by which these disorders operate vary; we believe injury to the small intestinal epithelium is the central pathophysiological event that acts as a final common pathway for the perpetuation of the diarrheal state. This is graphically illustrated in Fig. 1, where a number of pathogenic mechanisms can be seen to converge on the central theme. In this scheme, it can easily be seen that the prolonged mucosal injury may, in itself, be responsible for sustaining the initial mechanism, thereby establishing a vicious cycle that acts to prolong the mucosal injury.

TABLE 1. Disorders identified with intractable diarrhea of infancy listed by groups according to pathophysiology

<table>
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<tr>
<th>Disorders associated with villous atrophy</th>
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<tr>
<td>Viral gastroenteritis</td>
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<tr>
<td>Secondary disaccharidase deficiency</td>
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<td>Secondary monosaccharide malabsorption</td>
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<td>Cow’s milk protein enteropathy</td>
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<td>Soy protein enteropathy</td>
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<td>Egg protein enteropathy</td>
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<td>Eosinophilic gastroenteropathy</td>
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<td>Congenital crypt hypoplasia</td>
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<td>Sprue</td>
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<td>Celiac disease</td>
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<td>Dermatitis herpetiformis</td>
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<td>Immunodeficiency syndromes</td>
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<td>Bacterial overgrowth</td>
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<td>Pathogenic E. coli</td>
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<td>Giardia lamblia</td>
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<td>Hirschsprung’s disease</td>
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<td>Malnutrition</td>
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<th>Disorders associated with secretory diarrhea</th>
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<td>Familial chloride diarrhea</td>
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<td>Bacterial toxins:</td>
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<td><em>Escherichia coli</em></td>
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<td><em>Vibrio cholera</em></td>
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<td>Hormones elaborated by tumors</td>
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<td>VIP-oma</td>
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<td>Zollinger–Ellison syndrome</td>
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<td>Medullary carcinoma of the thyroid</td>
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<td>Basophilic leukemia</td>
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<td>Systemic mastocytosis</td>
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<td>Inflammatory lesions of the colon</td>
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<td>Bile acid induced secretory diarrhea</td>
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<th>Anatomical problems associated with intractable diarrhea</th>
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<tr>
<td>Short bowel syndrome</td>
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<td>Hirschsprung’s disease</td>
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<td>Gastrochisis</td>
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<td>Malrotation</td>
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<td>Ileal atresia</td>
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<td>Intestinal lymphangiectasia</td>
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<td>Congenital crypt hypoplasia</td>
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<th>Metabolic entities associated with intractable diarrhea</th>
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<tr>
<td>Acrodermatitis enteropathica</td>
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<td>Abetalipoproteinemia</td>
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<td>Wolman’s disease</td>
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<td>Hypoparathyroidism</td>
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<td>Hyperthyroidism</td>
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<td>Adrenal insufficiency</td>
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<th>Congenital lack of an enzyme or protein carrier in intestine</th>
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<td>Glucose-galactose malabsorption</td>
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<td>Sucrase-isomaltase deficiency</td>
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<td>Primary lactase deficiency</td>
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<td>(a) developmental</td>
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<td>(b) congenital</td>
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<td>(c) adult type</td>
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<td>Enterokinase deficiency</td>
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<th>Pancreatic insufficiency</th>
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<td>Cystic fibrosis</td>
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<td>Shwachman-Diamond syndrome</td>
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<td>Congenital lipase deficiency</td>
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<td>Congenital trypsin deficiency</td>
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Some studies have investigated the role of small intestinal mucosal injury and secondary disaccharidase deficiency in the pathogenesis of IDI (Fig. 3). In 1965, Burke et al. (5) demonstrated villous blunting, increased cellular infiltrate, and cuboidal epithelial cells in the biopsies of 9 of 10 infants with refractory diarrhea. In 1973, Shwachman and colleagues (6,7) documented disaccharidase deficiency and small intestinal mucosal injury in 9 of 11 infants with intractable diarrhea of infancy. Similarly, we found mucosal injury in each of 30 infants with this syndrome; in our series, 28 of 30 (93%) had significant (grades II–IV) mucosal injury (8). Our experience also indicates that the cellularity in the lamina propria of patients with IDI is actually decreased, rather than increased as reported by Burke et al. (5).

A congenital familial variant of the syndrome of IDI has recently been described by Davidson et al. (9). In this syndrome, protracted severe diarrhea exists from birth. Steatorrhea, carbohydrate malabsorption, dehydration, and acidosis occur. Excess stool water and electrolyte losses persist even in the fasting state. The intestinal mucosal biopsies of the reported infants exhibited the characteristic features of villous atrophy and, in sharp contrast to celiac disease, crypt hypoplasia without a compensatory increase in mitoses or an inflammatory infiltrate in the lamina propria. Villous epithelial cells exhibited the absence of a brush border and an increase in lysosome-like inclusions. It was postulated that a failure in the normal maturation of crypt cells was operative, since the surface epithelial cells of the villus resembled the immature undifferentiated crypt cells.

Our experience has been similar to that of Davidson et al. in that we have observed infants who exhibit hypoplastic “short crypts”. These infants have
required prolonged treatment with total parenteral nutrition. Evaluation of serial biopsies has revealed that the histologic abnormality persists for many months before nutrients by mouth can be absorbed.

LENGTH OF TIME FOR RECOVERY OF SMALL INTESTINAL INJURY

The length of time necessary for recovery of mucosal injury is uncertain. Barnes and Townley (10) demonstrated recovery of histologic injury by 8 days and 3 to 7 weeks after acute infectious enteritis. Greene et al. (11) indicated that total histologic and biochemical recovery may occur as early as 6 to 9 weeks. We have shown, however, that mucosal injury may persist for prolonged periods. Sixteen of 23 (70%) infants with IDI had significant (grades II-IV) atrophy 6 months after presentation (8).

In examining the reasons why mucosal injury may be prolonged in the small intestine, one must examine certain aspects of normal turnover of cells and the postulated mechanisms of repair. In the epithelium of the small intestine, cells are continuously lost from the villus tip and replaced by cells found in crypts. The crypts are manufacturing areas for villus cells. They contain the stem cells, which are located in the lower portion of the crypts and which give rise to each of the major cell types found in the intestine, i.e., mature enterocyte, goblet cell, enteroendocrine cell, and Paneth cell.

The crypts may be divided into several different zones. The proliferative zone is restricted to the 18 lowest cell positions (lower half of the crypt). It is here that cell division occurs, and, through cell isolation techniques, the enzymes involved in DNA synthesis have been shown to occur exclusively in this area.

FIG. 2. Effect of impaired villus regeneration in perpetuation of malabsorption.
Between positions 18 and 22, the cells differentiate. As enterocytes migrate from
the proliferative compartment through the differentiation compartment, there
is a gradual increase in the enzymes for RNA and protein synthesis, intracellular
organelles, and finally enzymes and proteins for digestion, absorption, and trans-
port (12,13). Migration time, from crypt–villus junction to villus tip, has been
estimated to be between 38 and 46 hr (12-14).

The number of cells on the villus tip depends on two ongoing factors: (a)
cell production from the crypts and (b) cell loss secondary to extrusion of villus
tip cells. The mechanisms that regulate this dynamic equilibrium and maintain
homeostasis between cell loss and cell production are not well understood. Two
mechanisms of control of cell production in the small intestinal crypts have,
however, been postulated: (a) feedback control and (b) adaptive control (12).

The feedback control mechanism has evolved from investigation of the in-
testine under two experimental conditions: transient ischemia (15) and irradiation
(16). Under these circumstances, there is loss of villus cells followed by a com-
pensatory response in the crypts. The proliferative zone has been found to
increase in size, thus expanding the growth fraction of the crypts. To explain
this phenomenon an “antimitotic substance,” which is believed to be secreted
by the villus cells, has been proposed (17). When mature villus cells are absent,
as in cases of severe mucosal injury, a lack of the antimitotic substance would
allow crypt cells to increase their mitotic rate, thus expanding the crypt com-
partments. Others have proposed the existence of “intestinal chalones,” which
are secreted by stem cells and found in high concentrations in the stem cell
area (18). As cells move from the proliferative compartment and are exposed
to lesser concentrations, they are allowed to mature.

Another hypothesis for the control of the villus/crypt ratio has stemmed from
examination of the intestinal response during lactation and following resection
of a segment of the intestine (19,20). Under these experimental conditions, an
increase in the height of the villi is noted without a corresponding increase in
mitoses in the crypts. This has been linked to an adaptative response.

FACTORS INFLUENCING EPITHELIAL CELL KINETICS

A large number of factors have been identified that are thought to influence
epithelial cell kinetics. These may either be intrinsic or extrinsic to the intestine
and can be categorized into dietary, hormonal, neural, pharmacologic, and
physical classes (12). One important determinant that may adversely affect ki-
netics in the intestinal epithelium of infants with intractable diarrhea is mal-
nutrition. This has been examined by Guiraldes and Hamilton (21), who have
demonstrated a decrease in labeling and mitotic indices as well as migration
rates in malnourished weanling animals relative to controls. This adverse effect
may be one factor that delays mucosal repair in infants with IDI who are
malnourished and may partially explain the prolonged mucosal injury and
ineffective villus repair (13) (Fig. 2).
POSSIBLE INTERRELATED CHANGES IN PROLONGED SMALL INTESTINAL MUCOSAL INJURY

The individual mechanisms may also be interrelated in other ways, as can be demonstrated in the figures that follow. According to the severity of the mucosal injury, changes occur in the absorptive, secretory, and reabsorptive capacities for such substances as minerals, carbohydrates, proteins, and fats, resulting in a generalized malabsorptive state (Fig. 3).

Decreased Enteric Hormones

The malabsorption of nutrients (Fig. 3) may also be aggravated by an impaired release of the enteric hormones synthesized in the proximal small intestine, i.e., secretin and pancreozymin–CCK (lower part, Fig. 3). A deficiency in secretagogues then may lead to a secondary pancreatic insufficiency and further loss of carbohydrates, proteins, and fats, as depicted in the upper part of Figs. 3 and 4.

A decrease in the enteric hormone gastrin (Fig. 4) may have additional complications. A deficiency in gastrin would result in a reduction in hydrochloric acid and pepsin secretion. Defects in acid and proteolytic activity may then alter processing of antigens, allowing for increased absorption of foreign proteins as well as for bacterial colonization (Fig. 3).

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**FIG. 3.** Pathogenesis of malabsorption following injury to the small intestinal epithelium.
Corroborating data for the above hypotheses exist in a study by Creutzfeldt evaluating enteric hormone responses and insulin and glucose homeostasis in patients with malabsorption resulting from celiac disease and other causes of villus atrophy (22). The hormones that have been evaluated include gastrin, pancreatic polypeptide, and gastric inhibitory polypeptide (GIP). The reason for selection of gastrin for study is that gastrin is synthesized and released from the gastric mucosa, which is not affected in the malabsorptive syndromes. Gastric inhibitory polypeptide, on the other hand, is exclusively situated in those regions of the small intestine that are affected by mucosal injury; GIP has also been assigned to be a major incretin, an insulin-releasing substance. Impairment of GIP release, therefore, could be followed by an impairment of insulin secretion and glucose utilization. Pancreatic polypeptide, on the other hand, arises from the exocrine and endocrine pancreas and is released by indirect hormonal and neural stimuli, which mainly originate in the proximal intestine.

Patients with severe mucosal injury exhibited elevated basal serum gastrin, GIP, and pancreatic polypeptide levels. The postprandial responses of all three hormones, however, were markedly reduced, as were the glucose and insulin responses. The magnitude of these findings correlated with the extent of mucosal injury. A similar diminished response of secretin and pancreozymin (CCK) following intraluminal duodenal acid instillation has also been found in patients with celiac disease when compared to healthy controls (23).

A somewhat confusing finding, however, has been the demonstration of an increased enteroendocrine (secretin and GIP) cell number as demonstrated by
immunofluorescence in biopsies exhibiting severe mucosal injury (24-26). A discrepancy therefore exists in view of the finding of diminished postprandial response of hormone secretion although increased cell numbers exist in the duodenum. Since impaired glucose absorption was documented, there may as well exist inadequate absorption of a "stimulus" that is responsible for hormone release. As a result, diminished exocrine pancreatic secretion and gallbladder emptying occur, which in turn contribute to the malabsorption of nutrients.

Role of Bile Acids in Protracted Diarrhea

The low levels of pancreozymin-CCK following intraluminal duodenal acid instillation in celiac disease patients might suggest a lower enterohepatic circulation of bile acids. Bile acids have been shown to have another role in the development of the protracted diarrheal state. The deconjugated bile acids, deoxycholic and chenodeoxycholic acids, have been detected in the duodenal fluids of infants with intractable diarrhea of infancy (27). The unconjugated bile acids have been shown to have cytopathic effects (28) on the small intestinal mucosa and also to inhibit the absorption of water and electrolytes by the colon (29).

Recently, a deficiency in intraluminal concentrations of bile acids as a result of increased fecal excretion was demonstrated in patients with IDI (30). Even when the terminal ileum is involved to only a limited extent, or when a short segment is removed, bile acid malabsorption may occur. As a result of increased fecal loss of bile acids, a compensatory increase in hepatic synthesis may be demonstrable. If the increase is sufficient to maintain normal intraluminal bile concentrations, micelle formation will not be impaired, and lipid malabsorption will not occur. The increase in fecal bile acids may, however, lead to diarrhea unaccompanied by steatorrhea. If loss of bile acids is so great that hepatic synthesis cannot compensate and maintain bile acids above the critical micellar concentration, steatorrhea will also occur (31).

In the evaluation of persistent diarrhea in infancy, the relatively inefficient fat absorption of prematures and full-term infants must be considered. The decreased fat absorption is caused by several factors including low mean concentrations of bile acids in the duodenal fluid. Watkins et al. (32) found that premature infants had a concentration of 1.8 mM. This contrasts with mean levels of 5.3 mM in full-term, 7.0 mM in older infants, and 8.5 mM in adults (33). The bile acid pool is similarly reduced in the newborn infant to approximately one-half that of the adult values corrected for surface area (34). Premature infants, between 32 and 36 weeks, have inadequate concentrations such that steatorrhea is common. In addition, the pancreatic lipase content is low in the neonatal period (approximately 5 to 7% of specific activity of children 2 years and older) (35). Complicating the problem of low lipase activity is the finding that there is no increase in the enzyme concentration in the duodenum after i.v. stimulation with pancreozymin and secretin (36).

Ileal transport mechanisms for the active reabsorption of bile acid in the
newborn are immature but improve with age. Fetal and neonatal animals exhibit only passive transport in the jejunum and ileum. Within the first few days of life, the kinetics of absorption indicate that an active carrier-mediated transport process is developing. By 1 month of age, the ileal absorption has been shown to be comparable to that of adults. In addition, the jejunum of the newborn appears to be able to absorb conjugated bile acids by passive diffusion; this might result in premature absorption, further reducing the intraluminal concentration (37–39).

Increased Absorption of Native Foreign Protein

This scheme has been most clearly described for cow’s milk protein intolerance (Fig. 5). In this situation, the milk protein intolerance may in fact be a result of minimal digestion of proteins in the stomach and small intestinal mucosal injury. Mucosal injury may permit excessive antigen to be absorbed or have access to the mucosal surface (40). Indeed, an excess of antigen absorption (egg albumin) has been documented following gastroenteritis (41); this presumably occurs on the basis of mucosal injury. In vitro animal experiments using horseradish peroxidase (HRP) as the probe have corroborated these findings (42). In addition, recent work utilizing mucosal explants maintained in organ culture indicate extensive absorption of HRP into both the enterocytes and the lamina propria of histologically injured mucosa as compared to biopsies with normal morphology (43). The mechanism of uptake is believed to be by both passive

![Diagram](Image)
diffusion and pinocytosis. The significant increase in the number of enterocytes diffusely penetrated with HRP in injured biopsies suggests that passive diffusion of macromolecules into the mucosa may be important in children with mucosal injury. These findings may be of significance for the initiation of cow’s milk protein intolerance following gastroenteritis.

A defect of the local immune system (IgA in particular) has also been postulated to play a permissive role (40). The relationship of serum IgA deficiency to cow’s milk protein intolerance has been recently supported by the finding of decreased number of IgA-staining cells in mucosal biopsies of children with the postenteritis syndrome (43,44).

The exact mechanism by which the immunological events associated with cow’s milk protein intolerance lead to a persistence of mucosal injury and disaccharidase deficiency is not known. One hypothesis would be via interference with trophic factors that are essential for villus repair. The factors influencing cell division in the small intestine with which the immune system might adversely interact are outlined below.

PROTEIN ENERGY MALNUTRITION AND PROTRACTED DIARRHEA

The interaction of protein energy malnutrition, depressed immune function, infection, and the GI tract appears to be another important factor in the perpetuation of the syndrome of intractable diarrhea of infancy (Fig. 6). Infection appears to adversely affect nutrition in many ways (45). Minor systemic and local infections increase losses of nitrogen in the urine (45). These losses may be coupled with metabolic losses of K\(^{+}\), Mg\(^{2+}\), Zn\(^{2+}\), phosphorus, sulfur, and vitamins A, C, and B in more severe infections (45,46). The excessive nitrogen

![Diagram of Protein Energy Malnutrition](attachment:protein-energy-malnutrition.png)
wasting occurs as a result of the shunting of amino acids from peripheral muscle to compensatory metabolic pathways, i.e., those involved with gluconeogenesis and acute-phase reactants such as haptoglobins, C reactive protein, α1 antitrypsin, and α-II macroglobulins. The negative nitrogen balance is reflected by the observation of decreased serum amino acid concentrations (47).

When infection occurs in the gastrointestinal tract, synergy between the metabolic effects of infection and the specific effects on the intestinal mucosa occurs. The rate and severity of the nutritional disturbance may result in increased metabolic derangements. The infectious diarrheas are associated with decreased absorption of nitrogen, carbohydrates, and vitamins A, B₁₂, and folate. Caloric losses may exceed 500 to 600 calories per day, and a further addition to the nutritional disturbances is induced by the metabolic effects previously discussed (45,46).

Protein energy malnutrition (PEM) has additional adverse effects on the intestine and the other digestive organs (48,49). At autopsy, malnourished children exhibit thin intestinal walls and atrophic mucosa. On peroral biopsy, variable degrees of abnormalities are seen, e.g., severe villus atrophy and decreased mitotic index. These changes are more common in marasmus than in kwashiorkor (49–51). A reduction in the height of surface enterocytes and increased cellularity of the lamina propria have also been described. Ultrastructural changes include disarray of the brush border, the presence of large autophagosomes and residual bodies, depletion of collagen filaments, and a dense granular material below the basal lamellae. These histological features, however, are nonspecific and similar to the findings in celiac disease (51,52).

The pancreas of clinically malnourished children has also been affected by PEM. Pathological alterations have included (a) acinar cell atrophy, (b) disorganization, (c) decreased content of zymogen granules, (d) vacuolization and epithelial metaplasia, and (e) dilatation of pancreatic ducts. In a limited number of patients, irreversible fibrosis has also been described. It has been suggested that the earlier the age at which a child develops malnutrition, the more likely that fibrotic lesions that ensue will be irreversible (53,54). Aspirates of duodenal fluid after stimulation with pancreozymin and secretin have exhibited decreased protein and enzyme outputs. Total fluid and bicarbonate concentrations are often preserved, suggesting that the ductular and centroacinar cells are unaffected. The activities of the pancreatic enzymes are depressed to varying degrees. Chymotrypsin was found to be severely affected, whereas trypsin was least affected. The activities of amylase and lipase were only moderately depressed (55,56).

Altered immunity in PEM extends and exaggerates the increased risk of infectious complications in the previously compromised infant. Figure 6 emphasizes the effects that PEM has been shown to have on the immune system. These have been summarized by Suskind (57). Suppression of total antibody production has not been demonstrated. Concentrations of serum immunoglobulins may be normal or even elevated (57,58). However, antibody production in response to certain antigens is diminished when compared to the response
of healthy children. Antibody response to heterologous red blood cells, Salmonella typhi, yellow fever, and polio vaccine has been diminished (59–61).

In addition, local antibody production has been found to be diminished. Secretory IgA in nasopharyngeal secretions, tears, and saliva is decreased in patients with kwashiorkor and marasmus. Secretory IgA does not rise in response to an infection (62,63). These findings suggest a reduced synthesis of IgA secondary to a decreased number of IgA-producing plasma cells, which are found predominantly in the small intestine. Another possibility would include ineffective secretory component production by atrophic epithelial cells. The impairment of secretory antibody response may play an important role in permitting excessive antigen absorption, as outlined above.

Defects in cellular immunity have also been documented in PEM. This is not surprising since for many years profound atrophy of all lymphoid organs, including peripheral lymph nodes, tonsils, and spleen, has been observed in PEM. The most severely affected organ appears to be the thymus (57). Thus, T-cell number and responsiveness to PHA stimulation have been shown to be decreased in these patients. An impairment of delayed hypersensitivity both to PPD and DNCB has been found in malnourished patients (64,65).

The complement system also seems to be adversely affected by PEM. This system augments the specific immune response to infection by promoting chemotaxis, adherence, opsonization, and microbial lysis. Reduced concentrations of complement have been demonstrated in patients with kwashiorkor and marasmus. In addition, the hemolytic component, CH50, is reduced as well, indicating a functional impairment of this system (66). The above findings serve to emphasize the increased risk to infection in those patients who suffer from malnutrition.
BACTERIAL OVERGROWTH AND INFECTION

Recent investigations have shed new light on the mechanisms by which a wide variety of microorganisms overcome host defenses and disrupt intestinal morphology and function (Fig. 7). Many host protective factors are important in maintaining a barrier to invading microorganisms. These factors are also crucial in maintaining the normal intestinal microflora, which in turn deters the overgrowth of enteric pathogens (67).

The establishment of the normal bacterial and viral inhabitants of the intestine is thus dependent on the development of host defenses as well as environmental factors (67). Prevention of bacterial overgrowth in the small intestine depends on a normally functioning gastrointestinal tract: gastric acidity, intestinal motility, and intact local and systemic immune mechanisms.

The secretion of hydrochloric acid by the gastric mucosa is one of the important rate-limiting factors of bacterial colonization. In the fasting state, the stomach is virtually sterile, and the proximal small intestine is sparsely populated with bacteria. After feeding, there is a transient wave of microorganisms that pass rapidly through the gastrointestinal tract. In this light, the relative hypochlorhydria that exists both in the first weeks of life (68) and in infants with malnutrition (69) suggests that young infants may be at risk for small intestinal bacterial overgrowth.

Intestinal motility is also believed to be a regulator of the intestinal microflora by preventing excess colonization. Stasis syndromes are associated with bacterial overgrowth and have subsequent adverse effects on nutrition (70).

The local immune defenses operative in the small intestine are dominated by secretory IgA, although all classes of immunoglobulins are present. The immunoglobulins are important in recognition, complement fixation, and exclusion of antigens at the mucosal surface (71). These mechanisms are operative against toxins as well as microorganisms, and may prevent adhesion by coating the bacterial cell wall and binding toxins.

Other nonspecific mechanisms may also be operating to limit invasion of the gastrointestinal tract by microorganisms, e.g., mucin production by goblet cells.

Bacterial Pathogens in Infantile Diarrhea

The bacterial pathogens that commonly overcome protective host factors include Shigella, Salmonella, Escherichia coli (E. coli), Campylobacter, and Yersinia enterocolitica.

Escherichia coli is found throughout the distal small intestine and colon. However, only certain strains of E. coli seem to be capable of producing diarrheal disease in humans. Several pathophysiological mechanisms have been described, including enterotoxigenic, enteroinvasive, enteropathogenic, and enteroadherent E. coli.
The enteroinvasive organisms tend to fall within certain serotypes. They produce disease by invading epithelial cells of the terminal ileum and colon, thus producing symptoms of diarrhea with blood, mucus, and fever. The syndrome is indistinguishable from shigellosis. Sheets of leukocytes may be identified by Wright stain of fecal mucus.

More controversy surrounds the enteropathogenic organisms. The fact that enteropathogenic *E. coli* have their effect only in infancy is unique and might be related to developmental changes of gut morphology and function. The pathogenic organisms have been reported in the past as causes for newborn nursery outbreaks of diarrheal disease that were associated with substantial morbidity and mortality (72). The serotypes associated with these infections, however, failed to exhibit toxin production or invasiveness by the assay techniques available at that time (73). However, subsequent investigations with material stored for several years has indicated that an atypical enterotoxin may be responsible for the pathogenicity of the organisms (74). Thus, at least some of the enteropathogenic varieties of *E. coli* may induce diarrhea by toxin production, but this hypothesis has yet to be confirmed.

In addition to the invasive and pathogenic varieties, certain strains of *E. coli* have been demonstrated to cause intestinal secretion by the elaboration of enterotoxins (75). Two types of toxins have been described: heat labile (LT) and heat stable (ST). The LT of *E. coli* is quite similar to that of cholera in immunological and physiological properties. Both are of high molecular weight, are immunogenic, and are capable of stimulating adenylate cyclase. This process begins after attachment of the toxin to a GM₁ ganglioside receptor on the cell surface. The result is an increase in isotonic fluid production in the small intestine. Enterotoxin does not cause fluid secretion in the colon (76). In contrast, the stable toxin of *E. coli* is of low molecular weight and is minimally immunogenic. Its mechanism of action is believed to be via stimulation of cyclic GMP (76).

The genes regulating synthesis of both the stable and labile toxins of *E. coli* are found on transferrable plasmids, whereas the labile toxin of cholera is chromosomally determined. Many enterotoxigenic *E. coli*, particularly those that elaborate toxins, also possess hairlike appendages (pili), which serve as colonization factors. These allow the enterotoxigenic *E. coli* to remain adherent to proximal intestinal mucosal cells, thereby overcoming the potent peristalsis of the upper small intestine that acts as a nonspecific defense mechanism. The toxin-producing organisms cause copious watery diarrhea. However, except for an occasional outbreak, they are an uncommon cause of diarrhea in most industrialized nations.

Several unusual strains of *E. coli* have recently been incriminated in the syndrome of IDI. Rothbaum et al. (77) reported 15 infants with protracted diarrhea, poor weight gain, anemia, and hypoproteinemia. In many infants, multiple attempts at enteral feedings with standard and elemental diets were unsuccessful, necessitating the use of total parenteral nutrition (TPN). Stool and duodenal fluid cultures yielded *E. coli* serotype 0119-B14 in many infants.
Those with organisms found only in the stool had less severe diarrheal disease than those with organisms in both stool and duodenal fluid.

The jejunal mucosa of infected infants exhibited several interesting findings. Light microscopy revealed moderate to severe villous atrophy, disordered arrangement of enterocytes, subnuclear vacuolization of crypt cells, and a moderately increased number of plasma cells, lymphocytes, eosinophils, and Paneth cells.

With the use of immunoperoxidase staining of the epithelium, adherent clusters of *E. coli* 0119-B14 were demonstrated as dark caps on the enterocyte surface. Interestingly, the organisms were not adherent to the goblet or crypt cells. The affected enterocytes were further found on examination by electron microscopy to have a decreased number of microvilli, which were rather “stubby” in appearance. In areas of bacterial adherence there was dissolution of the glycocalyx and flattening or loss of microvilli. The actin filaments and part of the terminal web of the microvilli had disappeared. In addition, damaged enterocytes had disordered cytoplasm with numerous large lysosomes, and disorganized endoplasmic reticulum and mitochondria. The prolonged mucosal injury in the patients reported was attributed to the adherent properties of *E. coli*, permitting them to resist peristaltic clearance mechanisms.

The bacterial attachment factors (adhesins) that have been described seem to reside in their hairlike pili or fimbrae. An adherence function may also reside in the bacterial capsule. They may only be expressed under certain conditions (78). These and other bacterial properties, including chemotaxis and motility, may determine whether or not a particular pathogen is swept away by the secretions, colonizes the mucus layer, or penetrates the mucus layer and glycocalyx to attach to the cell membrane.

Other bacterial pathogens may also cause illness in infancy. *Campylobacter* may affect infants, children, and adults and thus develop the associated features of fever, abdominal cramps, and presence of blood and mucus in the stools. *Yersinia enterocolitica* may be invasive or enterotoxigenic. In addition to diarrheal disease, it has also been associated with mesenteric adenitis with abdominal pain mimicking acute appendicitis. *Salmonella* and *Shigella* invade intestinal epithelial cells as a prerequisite to initiating diarrheal disease. Enterotoxins produced by some strains of *Shigella* and *Salmonella* may be involved in pathogenesis following invasion (72).

Other bacterial enteric pathogens, including *Bacillus cereus*, noncholera *Vibrio*, and *Aeromonas hydrophilia*, have been reported to cause diarrhea in adults but have yet to be described in infancy or childhood.

**Viral Gastroenteritis**

Until 1972, viral enteritis was only a hypothesis. Support for the suspicion was lent by transmission experiments in which bacteria-free stool filtrates from patients with diarrhea induced diarrhea in human volunteers or experimental animals (79,80).
Prolonged Small Intestine Mucosal Injury

With the development of electron microscopy and other new technologies (81,82), the identification of two groups of viral agents capable of causing acute nonbacterial gastroenteritis was accomplished. Rotavirus was found to be the major cause of episodic diarrhea in infants and young children, whereas the Norwalk agent (parvovirus) appeared to be the responsible agent in outbreaks of diarrhea in adults. Many other viruses have been identified in stools, but at present, none have met the criteria of Koch for acceptance as true agents of diarrheal disease (83).

The characteristic clinical features of rotavirus gastroenteritis have been well described. The incubation period ranges between 48 and 72 hr. Fever and vomiting usually precede the diarrhea and last 1 to 3 days; the diarrhea lasts for 4 to 7 days. The illness can be severe and can lead to death from dehydration in all age groups. The lower prevalence of symptomatic infection under 6 months of age may be related to the presence of maternal antibodies (83). In addition, breast feeding has been found to offer protection. Rotavirus antibody has been found in colostrum and may be present in breast milk for up to 2 years (84). The protective effect of breast feeding has been demonstrated in experimental animals (85). Breast-fed babies have a lower incidence of rotavirus infection than do bottle-fed infants and excrete fewer virus particles per gram of feces (86).

Rotavirus infection is accompanied by a seroconversion with persistence of high antibody titer for at least 2 years. Complement-fixing antibodies to rotavirus have been found in approximately 70 to 80% of newborns studied. This antibody most probably is of maternal origin, declines to 10 to 15% of early concentrations in infants by 6 months, and then is followed by a rise to adult concentrations in 50 to 90% of patients studied by 3 years of age (87). The decline in rotavirus infection in older children may therefore be related to the acquisition of the antibody. Reinfection has been recorded only rarely, but it has been well documented that rotavirus infection can occur despite the presence of circulating rotavirus antibodies. This implies that other factors (e.g., intestinal secretory IgA in the host or strain differences in the virus) are important in protection or infection (83).

Mucosal biopsy specimens obtained from infected infants have exhibited rotavirus particles, detectable on electron microscopy, within the cisternae of the endoplasmic reticulum. Only the absorptive cells appear to be infected (88). Studies of jejunal mucosa in piglets infected with human rotavirus reveal that the diarrhea occurs at a time when the intestinal villi are populated with immature cells that have failed to differentiate into mature absorptive cells during an accelerated transit from the crypts. These enterocytes exhibit defective glucose Na+ cotransport and decreased activity of Na+–K+ ATPase. There was no evidence of adenylate cyclase stimulation or accumulation of intracellular cyclic AMP (89).

The progression of an acute viral illness to one of chronic and protracted diarrhea may be related to the above pathogenic features of rotavirus. This
concept is expanded on in the section on dealing with delayed healing of small intestinal mucosal injury.

Detection of the virus is now possible by a number of methods. Direct examination by electron microscopy was the earliest and simplest method and is still widely used. Multiple variations have been made on this direct method, but all methods requiring electron microscopy require a substantial amount of time and sophisticated equipment, if large numbers of samples are to be analyzed on a cost-effective basis. Other techniques have emerged that reportedly offer sensitivity equal to electron microscopy. Counter immunoelectro-osmophoresis, complement fixation, and indirect immunofluorescence of duodenal biopsies or tissue culture monolayers have been employed by some investigators (83).

Recently, two techniques were developed for the rapid diagnosis of rotavirus: a microtiter solid-phase radioimmunoassay and an enzyme-linked immunosorbent assay (ELISA) (90,91). The ELISA has a number of advantages over the RIA technique including elimination of radioactive agents and the sophisticated equipment required for the detection of radioactivity. In addition, the ELISA is probably more sensitive than electron microscopy, which requires the presence of greater than $10^7$ particles per gram of stool.

Electron microscopy of feces during acute infectious diarrhea has revealed a number of additional possible viral agents. The role of these agents in acute and chronic diarrhea remains to be clearly established. The agents suspected include adenovirus, astrovirus, coronavirus, calcivirus, and "mini reo" virus.

Parasitic Infestation in the Small Intestine

The association between acute and chronic diarrhea and presence of intestinal parasites has been well established. *Giardia lamblia* is considered to be the most common parasite causing intestinal disease in the United States. This organism may affect infants, children, and adults and may be associated with clinical and/or laboratory features suggestive of malabsorption, the extent of which is variable (92).

*Giardia lamblia* (duodenalis) is a binucleate flagellate. Infestation follows after ingestion of cysts contained in contaminated water or food. Excystation then occurs in the acidic environment of the stomach. Subsequently, the trophozoites colonize the proximal small intestine, where conditions for survival are optimal (pH 6.4–7.0). The mechanisms related to the resultant pathogenesis of malabsorption in giardiasis are still evolving and have been recently summarized by Poley (92). The variety of mechanisms postulated include mucosal injury, mechanical destruction, bacterial overgrowth, mucosal invasion, increased cell turnover, and increased secretion of mucus.

Small intestinal mucosal injury, in this condition, appears to be variable. Often, no pathologic changes are identifiable by either light or electron microscopy. The more severe pathologic changes, i.e., flat mucosa, appear to occur only in the immunocompromised host. A transient lactase deficiency is thought
to be related to an increased cell turnover, allowing immature cells to occupy the highest cell positions.

The organisms have been found to be present in such great numbers on the villus tip that some have suggested they actually physically block villus function. An additional postulate, that the organisms may compete with the host for nutrients and thus compromise the host's nutrition, has been brought forth.

In those cases where mucosal injury exists, bacterial or fungal overgrowth has been felt to contribute to the mucosal lesion. The degree of colonization of the small intestine with bacteria has been related to the severity of the mucosal injury, but the presence of bacteria is not essential for the production of histological lesion, as bacteria are not invariably isolated.

*Giardia* have also been demonstrated to invade the epithelium; they have been demonstrated in villus and crypt cells and in the lamina propria. Invasion, however, is usually insignificant, and other than rare isolated cases there is no evidence that invasion of the mucosa by *Giardia* is a major contributor to malabsorption.

Changes in epithelial cell kinetics have been reported in man and in animals infected with *Giardia*. The surface epithelial cells that are damaged are presumed to be sloughed at a greater rate and therefore must be compensated for by increased cell synthesis in crypts. The cells that migrate up the villus at an increased rate are not fully mature, and as a consequence the digestion and transport of nutrients from the surface into the cell is impaired.

With electron microscopy, the presence of increased mucoid coat on epithelial cells and increased secretion of mucus by crypt cells have been recognized. The increased secretion of mucus by crypt and villus cells is probably stimulated by the presence of parasites and/or microorganisms and may serve as an important defense mechanism (Fig. 7). By virtue of its physical properties and immunoglobulins, it may result in entrapment and immobilization of trophozoites. It is conceivable, however, that increased mucus production may create a physical barrier resulting in a limitation of diffusion.

**MALABSORPTION SECONDARY TO BACTERIAL INFECTION**

Many studies have confirmed the association among chronic diarrhea, carbohydrate malabsorption, and bacterial overgrowth (93). The associations are most clearly related to disorders in which intestinal motility has been altered by surgical procedures (94). Patients with acute and chronic diarrhea of other etiologies, including protein energy malnutrition, have also been documented to have bacterial overgrowth and carbohydrate malabsorption. For example, Coello-Ramirez and colleagues studied 50 infants with chronic diarrhea and bacterial proliferation in the small intestine and found carbohydrate malabsorption in 68% (95). Isolated lactose malabsorption occurred in 46%, whereas only 16% had both lactose and sucrose malabsorption. Monosaccharide malabsorption was seen in only 6%.
The mechanism of secondary disaccharide and monosaccharide malabsorption in bacterial overgrowth has been extensively studied (67,96,97). In vivo animal studies have demonstrated depression of the brush border disaccharidase activities (96,97) and disturbances in the transport of glucose and fructose. Both processes have been correlated with the degree of bacterial contamination. The disaccharidase depression has been shown to be caused by two separate effects in blind-loop experiments. Ultrastructural studies have demonstrated a focal injury to the microvilli. In addition, Jonas et al. (98) found a greater decrease in lactase, sucrase, and maltase per unit of brush border membrane than alkaline phosphatase. This indicates that the disaccharidase deficiency that is found in bacterial overgrowth may be the result of degradation of the disaccharidases by bacteria and not solely to damage of the microvilli. The disaccharidases actually project off the surface of microvilli and are thus susceptible to degradation by bacterial enzymes. This hypothesis is consistent with that of Prizont (99) that bacteria produce villous damage through the action of glycosidases that hydrolyze the disaccharidases, which are themselves glycoproteins. The action of the glycosidases leads to the release of carbohydrate moieties from the glycoproteins, yielding an energy source for the proliferation of bacteria. With the fermentation of carbohydrates by bacteria, H₂ gas is produced. That endogenous carbohydrate fermentation can occur in the gastrointestinal tract of humans is indicated by the finding of an elevated fasting H₂ concentration in the breath of patients with bacterial overgrowth compared to controls (93,100).

Impairment in the transport of the monosaccharides glucose, galactose, and fructose has also been demonstrated in intestinal stasis syndromes (101,102). This may be related to the degree of mucosal injury (96,101). Considerable evidence, however, has implicated the deconjugation of bile salts, which are commonly found in contaminated intestines, as the cause (Fig. 7, left side). Comparison of the effect of deoxycholate versus the conjugated acid taurocholate on monosaccharide uptake by the small intestine has indicated a reversible type of inhibition by the deconjugated bile acid. The conjugated bile acid, in contrast, did not appear to interfere with absorption. The exact mechanism to explain these observations has yet to be elucidated (102,103).

**DISEASES OF THE COLON ASSOCIATED WITH DECREASED REABSORPTION OF FLUID AND ELECTROLYTES**

As postulated for the mechanism of bile-acid-induced diarrhea, evidence exists to suggest the diarrhea of inflammatory diseases of the colon may be accompanied by defects of intestinal salt and water absorption. In vivo and in vitro studies have demonstrated reduced absorption or secretion of salt and water in cases of ulcerative colitis (104) and Crohn's disease (105). Similar mechanisms may be involved in antibiotic-induced pseudomembranous colitis. Most recently, the defect was shown to be impaired active sodium absorption (106). The losses occur in addition to the loss of plasma and blood that occur through an inflamed,
ulcerated mucosa. Although rare, inflammatory lesions of the colon have been reported in infancy. A 20-year review at the Hospital for Sick Children (Toronto) revealed eight cases of this condition (107). Bile-acid-induced diarrhea and familial chloride diarrhea operate by a similar mechanism.

CONCLUSION

Central to our working hypothesis of the pathophysiologic mechanisms involved in the genesis of IDI is prolonged small intestinal mucosal injury. We assume that ineffective small intestinal villous repair is the underlying initiator and perpetuator of persistent blunting of villi and disarray of the brush border membranes of mature epithelial cells. Brush border enzyme activities are diminished, active transport of nutrients is disturbed, and malabsorption of nutrients follows as a consequence. As a result of the prolonged small intestinal mucosal injury, the synthesis and release of hormones such as gastrin, secretin, pancreozymin–CCK, and pancreatic polypeptide are decreased as well. The resultant decrease in pepsin, hydrochloric acid, and pancreatic exo- and endopeptidases may allow for the exposure of antigens, as intact proteins, to the injured small intestinal mucosa, which may in turn result in increased absorption of native foreign proteins and sensitization. The end result can be secondary protein intolerance.

Similarly, greater susceptibility to bacterial overgrowth and infection because of decreased pepsin and hydrochloric acid concentrations, altered motility, and protein energy malnutrition resulting from malabsorption of nutrients can result. Bacteria have the capability to deconjugate bile salts and thereby induce fluid and electrolyte losses via osmotic diarrhea. This in turn can aggravate the protein energy malnutrition caused by malabsorption of nutrients, fermentation of carbohydrates, and formation of organic acids. The final result will be prolongation of the small intestinal mucosal injury. It is conceivable that resolution of the vicious cycle by the utilization of therapeutic modalities based on the pathophysiologic changes in digestion and absorption will have an impact on resolving the devastating effects of IDI.

REFERENCES

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20. Hanson WR, Osborne JW. Epithelial cell kinetics in the small intestine of the rat 60 days after resection of 70 percent of the ileum and jejunum. Gastroenterology 1971; 60:1087.
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100. Perman JA, Modler S. Hydrogen (H₂) and methane (H₄) are products of glycoprotein catabolism by colonic flora. Gastroenterology 1981;80:1251.