The Pathogenesis of Infectious Diarrhea

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1. INTRODUCTION

During the past decade much has been learned about the events that convert a peaceful state of coexistence between microbes and the gut into a diarrheal illness. Research into the pathogenesis of infectious diarrhea has been concerned with several aspects of the problem—the events leading to colonization of the intestine by pathogens and their attachment to or invasion of the intestinal wall, the mechanisms by which intestinal water and solute transport is distorted, leading to diarrhea, and finally, the factors that regulate shedding of offending organisms and repair of the damaged intestine. The effective application of new basic pathophysiology concepts to the care of patients with diarrhea has been widely publicized. Fortunately, infants and children in the developing world, for whom diarrheal diseases are so devastating, have been the major beneficiaries. Promoted by The Programme for Control of Diarrhoeal Diseases of the World Health Organization (WHO), treatment programs based on modern concepts of diarrhea pathogenesis have led to a dramatic reduction in mortality and morbidity in many Third-World countries.¹ Perhaps because of a lesser level of concern over the problem, physicians in developed countries have been relatively slow to take up these rational approaches to treatment.

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Another less immediate fallout from research into the pathogenesis of diarrhea is the insight it brings to our understanding of normal gastrointestinal function, and, indirectly, the response of the gut to a variety of diseases. The global cost of diarrheal diseases, although less than it was 10 years ago, remains colossal. An essential basis for future improvements in care and consequent reductions in cost will continue to be contributed by basic and applied research. The following brief account of some current research activities in this field is intended to indicate that, indeed, the future is promising. This chapter emphasizes the gastroenterological, rather than microbiological and immunological, aspects of the subject. Before abnormal interactions between bowel and bugs are discussed, certain recently developed concepts of normal intestinal function will be reviewed.

2. THE NORMAL INTESTINE

The small intestine is the site of many enteric infections and the region where most of the intestinal absorption of salt and water occurs. Although deranged smooth muscle motility patterns,^{2,3} altered neural function,⁴ and abnormal vascular⁵ and lymphatic flow could influence water and salt transportation in the gut, little is known about the response of these factors to enteric infection. What is clear is that the small intestinal epithelium and underlying lymphoid tissue are of central importance to the pathogenesis of infectious diarrhea.

2.1. Small Intestinal Epithelium

In the mammalian gut, epithelial proliferation is confined to the crypts. From proliferating undifferentiated crypt cells several cell types develop (Table 1). Some points relevant to the potential role of these cells in infectious diarrhea are the following.

Table 1. Small Intestinal Epithelial Cells

Membranous ("M") cells
Goblet cells
Paneth cells
Endocrine cells
Columnar absorptive cells

2.1.1. Membraneous (M) Cells

These cells are found in the dome epithelium overlying Peyer patches and may be confined to those sites. Owen's microscopic studies showed uptake of intact protein by M cells,⁶ which, he suggested, may serve an antigen-sampling role. Certainly, their configuration is appropriate for this purpose. Studies in our laboratory used the Ussing chamber technique to quantitate macromolecular transport in piglet jejunal mucosa *in vitro* and demonstrated enhancement of horseradish peroxidase uptake across patch-containing segments compared with patch-free segments.⁷ This process was partially inhibited by sodium fluoride, suggesting that it was an active cellular phenomenon. Possibly the sparsity or absence of surface mucus or glycocalyx, the special properties of the overlying unstirred water layer, or increased porosity of the underlying basement membrane in the patches may play some part in this enhanced macromolecular absorption. Nevertheless, available evidence favors an antigen-sampling role for the M cell. Theoretically, these cells could influence interactions between intestinal microorganisms, dietary antigens, and the host immune system.

2.1.2. Goblet Cells

These polarized, mucin-secreting cells appear to arise from undifferentiated precursors in the crypts.⁸ The quality and quantity of intestinal mucus probably exert an important influence on interactions between the gut and microorganisms too.⁹ Quality varies depending on the rate of production, on the cosecretion of certain enzymes, and on the chemical structure of constituent glycoproteins, or mucins. As a viscous gel, the mucus barrier may trap microorganisms and dietary proteins in the lumen or enzymes and proteins released from the wall; as a fluid sol, mucus could exert a cleansing action in the lumen. Control of the amount and nature of secreting mucus is poorly understood.

2.1.3. Paneth Cells

Little is known of the function of these distinctive granulated cells, confined to the crypts and derived also from undifferentiated crypt cells. The presence of degenerating protozoa and bacteria in their lysozyme fractions suggests a possible involvement in regulating enteric flora.⁸

2.1.4. Endocrine Cells

At least 15 endocrine paracrine cells have been identified in the mammalian gut.¹⁰ Most of them produce active peptides, many of which

can alter intestinal transport function, but a role for these cells in infectious diarrhea has not been elucidated.

2.1.5. Columnar Absorptive Cells

The dominant cell type in the small intestine epithelium, the columnar absorptive cell, differentiates as it migrates up from the crypt to be shed from the tip of the villus.^{11,12} This process of cell renewal is extremely relevant to the evolution of at least some, if not all, types of infectious diarrhea. Earlier studies demonstrated a range of enzymatic markers of enterocyte differentiation. Activities of the brush border membrane (BBM) disaccharidases and alkaline phosphatase and basolateral membrane Na-K-ATPase increase as enterocytes differentiate.¹³ Based on studies of winter flounder small intestine, which lacks a crypt compartment, Field proposed that absorptive function might reside primarily in villus cells whereas secretory events reside in the crypts.¹⁴ Using a reproducible experimental model, described in more detail in Section 3.2.2., we have characterized some transport properties of the crypt and villus compartments of the piglet small-intestinal epithelium. For these studies we have been using jejunal epithelium taken from piglets 40 hr after experimental infection with transmissible gastroenteritis (TGE) virus; at that stage of the disease the virus, after invading the mucosa, has been shed, leaving an epithelium composed of undifferentiated crypt-type epithelial cells.¹⁵ Studies attempting to define pathogenetic mechanisms of a disease, and a veterinary disease at that, are teaching us about the function of the normal intestine!

Recent experiments in our laboratory have examined ion, glucose, and amino acid transport in piglet small intestine, comparing acute TGE-infected mucosa with normal controls. To assess ion transport, we exposed jejunal epithelium mounted in Ussing chambers to theophylline, which inhibits phosphodiesterase in normal intestine causing intracellular accumulation of cAMP,¹⁶ inhibition of mucosa to serosal fluxes of Na and Cl, and a brisk secretion of Cl. TGE-infected tissue responds to theophylline with a normal accumulation of cAMP but no inhibition of NaCl absorption. This cryptlike epithelium shows a brisk Cl secretory response to theophylline, suggesting that secretory function does reside in the crypts. The failure of furosemide to inhibit NaCl absorption in TGE jejunum further supports the conclusion that this cryptlike epithelium lacks the capacity for electroneutral NaCl absorption at its luminal membrane.

Earlier marker perfusion experiments *in vitro* and Ussing chamber studies *in vitro* demonstrated impaired capacity of the TGE intestine to absorb glucose. Recent experiments used BBM vehicles to delineate distinctive features of D-glucose transport in this experimental preparation of crypt cells.¹⁷ Initial rates for Na-gradient-dependent D-glucose uptake into vesicles prepared from TGE mucosa were reduced, and overshoot was blunted. To determine whether this blunted response in crypt-type cells was due to an actual defect in the BBM glucose transporter, or whether it was secondary to other factors such as an altered Na gradient indirectly affecting glucose transport, we undertook equilibrium kinetic studies using vesicles incubated in gramicidin to clamp transmembrane potential. Glucose uptake was linear for 5 sec under these clamped conditions, allowing us to measure stereospecific sodium-dependent D-glucose uptake over a range of D-glucose concentrations and analyze the results in an Eadie-Hofstee plot. In control tissue the plotted points were curvilinear, a least-squares analysis deriving a "best fit" for two distinct straight lines, one representing high-affinity sites, the second low-affinity sites. For the TGE vesicles, the same calculations derived a single straight line which did not differ from the low-affinity line of the control vesicles but did differ significantly from the high-affinity line. We concluded that crypt cell luminal membranes lack an effective high-affinity D-glucose-Na cotransporter and assume that this high-affinity carrier appears as the cell differentiates during its migration along the crypt-villus axis.

We had expected that neutral amino acid-Na cotransport across intestinal luminal membranes would conform to a pattern similar to that described earlier for glucose transport. We were wrong. First, in Ussing chambers, we observed that 3-O-methyl glucose and L-alanine together had a greater stimulatory effect on net Na absorption than did either substrate alone, suggesting separate transporters for glucose and alanine in normal and TGE intestine.¹⁸ Measuring initial Na-gradient-dependent L-alanine uptake rates into BBM vesicles, we found normal accumulation ratios in vesicles from TGE jejunum.¹⁹ These *in vitro* data strongly suggest that neural amino acid-Na cotransport is intact in the luminal membrane of crypt cells, a pattern quite distinct from that for glucose.

From these studies of disease a picture of transport function in the normal poorly differentiated crypt cell and, by inference, in the normal mature villus cell emerges. Alterations in the balance between these types of cell in the mucosa and the regulation of cell renewal in the small intestine can be expected to be important determinants of the capacity of the intestine to conserve water and salts.

2.2. Gut-Associated Lymphoid Tissue (GALT)

Since the intestinal epithelium is not completely impermeable to potential antigens, this lymphoid tissue is probably an important component of the intestine's defenses. The GALT consists of lymphocytes and plasma cells in the lamina propira, intraepithelial lymphocytes, lymphoid nodule (Peyer patches), and mesenteric lymph nodes.

Two lines of antigen-sensitive lymphoid cells, T and B lymphocytes, have been characterized. The B lymphocyte can differentiate into plasma cells that synthesize and secrete immunoglobulins, the major portion of which is IgA; little is known of the role of IgE, IgM, or IgG in mucosal defense, but IgA is assumed to be important. Lymphoid populations in the gut are interrelated. Peyer patches appear to contain a precursor population of lymphocytes that can disseminate to the lamina propria and epithelium to participate in locul mucosal immunity. IgA produced in the lamina propria is transported through epithelial cells into intestinal secretions as a dimer linked by a J chain to which secretory component is attached. Intestinal T cells are less well characterized. Forty to seventy percent of Peyer's patch cells are B cells whereas most intraepithelial lymphocytes are T cells. Lymphoid cells of gut origin also migrate to extraintestinal sites, the mammary gland, genital tract, and bronchial tree, for example.

3. INTESTINAL RESPONSE TO ENTERIC PATHOGENS

3.1. Colonization by Pathogens

3.1.1. Attachment and Invasion

A number of nonspecific host factors, listed in Table 2, maintain a sparse flora in the stomach and small intestine of healthy people.^{20,21} To

Table 2. Intestinal Defenses against Infection

Gastric acid
Lactoferrin
Enteric flora
Intestinal motility
Mucus
Immune factors
Passive—colostrum, breast milk
Active—local immune response

colonize the small intestine, organisms must first survive passage through a region of low intraluminal pH in the stomach. Additional nonspecific intraluminal protective factors include lactoferrins contained in human milk, other flora, and their metabolic products (e.g., short-chain fatty acids). Administration of antibiotics and dietary adjustments, particularly changes in fiber content, are two clinical situations in which a change in the balance of the normal flora can allow certain species to proliferate. Also, intestinal motility and perhaps villus motility are significant mechanisms for clearing enteric organisms. Small-bowel bacterial overgrowth is a well-known consequence of defective motility, and experimentally, inhibition of motility can be a requisite for creating models of enteric infection.²² A possible role for the mucus gel layer has been discussed already. Several factors that may be associated with enteric pathogens, *Escherichia coli* and *Vibrio cholerae* enterotoxins, immune complexes, PGE₂, and perhaps bile salts may stimulate mucus release *in vitro*.

The M cells and a lack of surface mucus may render the dome epithelium relatively permeable to pathogens, which would then come quickly into contact with lymphoid tissue. Our preliminary collaborative studies of lapine rotavirus with Dr. Martin Petric indicate that although the virus does infect villus epithelium it is the patch epithelium that is particularly vulnerable to invasion by this virus.²³ Also, in the patches, virus antigen could be seen by immune fluorescence not only in the epithelium but also in adjacent lymphoid tissue, suggesting a process of invasion followed by an immunological response.

Certain properties of enteric organisms themselves can be important factors in determining their pathogenicity.²⁴ For example, the motility of *V. cholerae* probably enhances its penetration of the mucus layer, such patterns of propulsion being further enhanced by chemotactic properties of the bacterium. The importance to pathogenicity of various bacterial enzymes has been noted too; mucinase produced by *V. cholerae* and *Shigella flexneri*, glycosidase by a strain of *S. flexneri*, and *V. cholerae* protease are considered important determinants of pathogenicity.

Of great current research interest are the factors that determine a bacterium's capacity to attach to the intestinal surface.^{25,26} If organisms succeed in overcoming the defensive strategies described earlier, they must attach to or invade the mucosa itself or its surface coat in order to colonize. *V. cholerae* and enterotoxigenic strains of *E. coli* cause no structural damage to the mucosa, but they colonize either the glycocalyceal coat or the brush border surface itself. Genetic studies have identified piglets with intestinal brush borders differing in their capacity to bind enterotoxigenic *E. coli*, and it has been suggested that these differences depend on glycolipids in the surface membranes. Other strains of diarrhea-produc-

ing bacteria, for example, the rabbit diarrhea *E. coli* (RDEC-1), attach to the mucosal surface, effacing microvilli; they may produce cytotoxins but they have not been shown to produce enterotoxins. Plasmid-associated fimbriae have been demonstrated on this latter organism. The initial site of their attachment, the M cells overlying lymphoid follicles rather than absorptive columnar cells, is fimbria mediated.

Some pathogens invade the epithelium and deeper structures of the bowel wall. Salmonellae are engulfed by an invagination of the apical membrane. The biochemical processes that regulate this uptake, like the factors involved in invasion of the small intestinal epithelium by viruses such as the rotaviruses and the TGE virus, are unknown. The fact that these pathogens preferentially invade villus cells suggests a role for brush border receptors in the differentiated columnar epithelium.^{26,27} The existence of receptors is further supported by the relative consistency of the region of the bowel involved in a range of invasive enteric infections. The determinants of invasion of the deeper layers of bowel observed in several bacterial enteritides, while the above viruses are mainly confined to the epithelium, are not known.

3.1.2. Immune Response

The dome epithelium is not the only site, but it may be a preferred site for attachment or invasion by enteric pathogens, providing direct access to lymphoid tissue in Peyer's patches. Enteric immunization can prime or suppress both intestinal and extraintestinal immune responses.²⁸ A variety of enteric antigens are known to stimulate production of specific IgA antibody in the gut. The local nature and protective effect of this response has been clearly demonstrated in studies using attenuated polio virus given by mouth. To generate a significant IgA response, it appears that at least one enteric priming dose is needed. The status of the antigen also is an important determinant of the local immune response; for example, immune responses of the intestinal lymphoid tissue to cholera toxin and cholera toxoid vary greatly. Colostrum and milk also contain IgA antibodies²⁹; like those released into the gut lumen, they are linked to a secretory component and therefore are relatively resistant to proteolytic degradation by digestive enzymes. Furthermore, the mucus gel on the epithelial surface may trap quantities of IgA, thereby concentrating it at the epithelial surface and enhancing its protective effect.³⁰

3.2. Mechanisms of Diarrhea

Diarrhea is the state of excessive loss of fluid and electrolyte in stools. Many factors could contribute to its pathogenesis, but to date,

most research has focused on epithelial transport.³¹ Some organisms remain in the gut lumen or on the outer surface of the mucosa and alter transport via enterotoxins they produce. Others invade the mucosal wall directly to cause changes in epithelial function. Some invasive organisms also produce enterotoxins, but in many cases mechanisms are simply not understood. The pathogenesis of the diarrhea resulting from enteroadherent *E. coli* infections is still a mystery, for example.

There are two models of infectious diarrhea for which considerable pathophysiological data have been accumulated: toxigenic diarrheas as exemplified by cholera and certain *E. coli* infections, and invasive enteritides caused by human rotavirus (HRV) and piglet TGE virus.

3.2.1. Toxigenic Diarrhea

Studies elucidating the actions of cholera toxin, the toxin of *V. cholerae*, have provided an extremely important stimulus to research into mechanisms of all types of diarrhea.^{32,33} Cholera toxin binds by its B subunits to specific GM₁ ganglioside receptors in the brush border; its A₁ subunit activates adenylate cyclase in the basolateral membrane leading to intracellular cAMP accumulation. The heat-labile toxin of *E. coli* (LT) closely resembles cholera toxin in its structure, its binding to the brush border, and its capacity to activate adenylate cyclase leading to cAMP accumulation. The heat-stable *E. coli* toxin differs from LT in its structure; it activates guanylate cyclase leading to cyclic GMP accumulation.³⁴ The end results are similar, although cAMP appears to exert a more potent effect than cGMP on ion transport.

In the differentiated columnar cells that populate villi described earlier, cyclic AMP or cyclic GMP inhibit NaCl absorption at the brush border membrane. The disaccharidases, glucose-Na cotransport, and alanine-Na cotransport all are intact, as is the basolateral membrane Na pump, the Na-K-ATPase system. In the crypts, where proliferating, relatively immature cells are located, the response to cyclic AMP and presumably to cholera toxin is brisk Cl secretion.¹⁶ Our preliminary data suggest that this secretion, which results from increased permeability of the luminal membrane to chloride, depends too on an intact Na⁺-K⁺-ATPase system and intact NaCl uptake across the basolateral membrane. The dramatic result is a secretory diarrhea that can be massive and life threatening. The stools rarely contain significant amounts of organic solute, and fecal Na and Cl concentrations are approximately those of extracellular fluid.

3.2.2. *Viral Diarrhea*

Our current understanding of viral diarrhea is based on studies of TGE, a reproducible, experimentally inducible piglet corona-virus enteritis.¹⁵ The TGE virus differs from HRV, but the pathophysiological response to TGE is the same as that seen when piglets are infected with HRV.³⁵ At the height of diarrhea, 40 hr after oral inoculation of piglets with TGE virus, several defects in epithelial transport have been found.¹⁷⁻¹⁹ Glucose-Na cotransport is blunted, and electroneutral NaCl absorption at the luminal membrane is not detectable in acute TGE jejunum. Although L-alanine-stimulated Na absorption is blunted, Na-gradient-stimulated alanine uptake is normal in TGE brush border membrane vesicles, suggesting that the suppression of alanine-stimulated Na absorption is not a brush border phenomenon. In Ussing chambers, neither dipeptides, susceptible to BBM hydrolysis nor those resistant to hydrolysis stimulated Na absorption more than individual amino acid.³⁶

Impaired BBM Na-gradient-dependent glucose transport in TGE appears to result from the absence of a functional high-affinity BBM carrier. The basolateral membrane pump, Na-K-ATPase, is reduced in activity as are the brush border membrane disaccharidases. We suspect that reduced Na-K-ATPase activity is not an important factor limiting the response to glucose since the TGE epithelium does possess a normal secretory response to cyclic AMP. Disaccharidase activities are low, but clinical trials have suggested that reduced levels of these enzymes are unlikely to be important determinants of acute viral diarrhea. Perhaps in some cases, late in the course of their illness, dietary disaccharide can contribute to persisting diarrhea because of intestinal disaccharidase deficiencies.

The functional profile of epithelial function in TGE diarrhea is similar, if not identical, to that described earlier for normal immature crypt cells. The virus invades the epithelium within 12 hr of inoculation, after which epithelial proliferation is stimulated in the crypts, cells migrate up along the crypt-villus axis at an accelerated rate, and the infected cells are shed rapidly into the lumen. Diarrhea becomes severe when the epithelium is composed of poorly differentiated crypt cell-type cells, and apparently when most virus has been shed. In babies, HRV probably remains in the mucosa during the early stages of diarrhea since virus is detectable in diarrheal stools in the third and fourth days of the illness.²⁷ Similarly, our preliminary studies in rabbits suggests that lapine rotavirus persists in the mucosa during the early stages of infection.²³

Rotavirus and TGE diarrheal stools contain concentrations of Na and Cl (45-55 meq/liter), considerably less than those seen in toxigenic diarrheas like cholera.³⁷

Another consequence of viral invasion and distorted epithelial dynamics may be a structural lesion. Severe flattening of villi and hyperplasia of crypts are reported in babies with HRV diarrhea and in piglets with TGE. Reduced surface area with reduced epithelial mass in theory could contribute to reduced absorptive function in viral enteritis, but from piglet studies we know that severe impairment of function can occur without a measurable structural lesion.³⁸ We believe that surface area is a relatively insignificant factor in the pathogenesis of viral diarrhea.

3.3. *Repair*

Obviously, clinical recovery depends on reversal of the pathophysiological abnormalities that led to the diarrheal illness in the first place. Cessation of nausea, vomiting, cramps, and anorexia undoubtedly contributes to the patient's recovery, but reversal of the pathophysiological determinants of diarrhea is of central importance.

The relative importance of different mechanisms available for clearing pathogenic organisms from the gut is unknown, but peristalsis and the constant rapid turnover of the epithelium must be very important. Peristaltic activity may be increased in certain enteric infections,² and theoretically, secondary metabolic disturbances could also interfere with intestinal motility in the course of severe diarrhea. When organisms invade and injure the villus epithelium, at least in the case of a viral pathogen, crypt cell proliferation is stimulated, and cell shedding from the villi and migration of cells up from the crypts along the crypt-villus axis are accelerated.¹⁵ Presumably, inhibitory factors emanating from normal villus cells are lost when the virus damages these cells. Goblet cell mucin discharge is increased also, but whether quantitative or qualitative changes in intestinal mucus actually contribute to clearing of pathogens remains to be determined. A local immune response occurs, but the relative importance of the various populations of lymphoid tissue is not known. Furthermore, the actual processes involved in clearance of organisms from the lamina propria are unknown although probably macrophages are involved.

Recovery of epithelial function involves more than shedding of organisms and replacement of shed cells with newly divided ones. Repair also involves the generation of an epithelium with adequate absorptive function; it is mature differentiated cells that must replace the villus enterocytes lost in the course of an infection. A consideration of mucosal repair, therefore, becomes a consideration of the determinants of epithelial proliferation and differentiation.

Several factors listed in Table 3 may regulate cell proliferation in the gut although their effect on differentiation is unclear.³⁹ Many of these fac-

Table 3. Factors Regulating Small Intestinal Proliferation and/or Differentiation

Nutritional factors
Chronic protein-calorie malnutrition
Specific substrate deprivation (e.g., CHO)
Trace element deprivation (e.g., Zn, vitamin B ₁₂)
Intraluminal food
Normal flora
Hormones
Adrenal corticoids
Thyroxin
Growth hormone/somatomedin
Enteroglucagon
Epidermal growth factor
Neural pharmacological factors
α-Adrenergic
β-Adrenergic

tors are of little relevance to repair after enteric infection. At present no major role for hormones or neural factors in repair after infection is apparent, but the known impact of chronic protein-calorie malnutrition on cell renewal is undoubtedly relevant. Chronic starvation has been shown, consistently, to reduce cell proliferation in crypts and to reduce cell migration along the crypt-villus axis.⁴⁰ Whether it is the deprivation of energy and protein that is the key determinant of this response or whether micronutrients like iron and zinc play a pivotal role is not known. Animal experiments suggest that oral nutrients may be particularly important in promoting intestinal epithelial repair.^{41,42} Our animal studies indicate that chronic malnutrition has a powerful impact early in life when the normal postnatal development of enterocyte function is disturbed.⁴⁰ With these considerations in mind, we postulated that repair of the gut after injury might be inhibited in the malnourished host. In the piglet, experimentally infected with TGE virus, this postulate has proven to be true.⁴³ Structural and functional recovery of the mucosa after TGE infection was delayed in nutritionally deprived animals, an observation that could not be attributed to a particularly severe initial injury in the malnourished animal. However, it seems that it is only after a very extensive intestinal injury, such as that encountered in viral enteritis, that repair is significantly delayed by malnutrition. When we produced ischemic injury to a short intestinal segment in the malnourished rabbit, structural and functional repair was scarcely affected although epithelial turnover was delayed.^{42,44} We conclude that separate mechanisms exist for the regulation of cell proliferation and cell differentiation as reflected

by the independent response of these two variables to undernutrition. However, a potential "vicious cycle" contributing to persisting intestinal dysfunction and malnutrition becomes apparent. Enteric infection and attendant diarrhea and anorexia cause undernutrition, which in turn delays repair of the mucosal lesion.

To date experimental models of malnutrition have dealt mainly with generalized deprivation of both macronutrients and micronutrients; the impact on intestinal cell proliferation or differentiation of specific micronutrient deficiencies has not been assessed.

The cellular mediators that control proliferation and differentiation are not completely understood.³⁹ Because villus cell damage leads to stimulation of crypt proliferation, mature villus cells are thought to contain "chalones" that inhibit cell proliferation. Under experimental conditions cAMP stimulates cell proliferation, and it has been suggested that mesenchymal and even lymphoid tissue in the villus core exerts some influence on epithelial renewal. Polyamines that may serve some role in mediating cell proliferation also are of considerable current research interest.⁴⁵ An important limiting role in polyamine metabolism is served by ornithine decarboxylase; this cytoplasmic enzyme is increased in activity when proliferative activity is increased in the gut after massive resection.⁴⁶ Our preliminary studies show marked increases in activity after viral invasion and after ischemic injury, again associated with heightened proliferative activity. The possibility that polyamines may play some regulatory role in gut epithelial renewal is appealing, but the observation of enriched activity along the villus rather than in the proliferative zone of the crypts does not support the hypothesis⁴⁷; identification of isoenzymes in the mucosal epithelium should allow a more detailed study of this issue.⁴⁸

4. TREATMENT

Preventive measures must be the first priority in dealing with a health problem that kills millions of the world's babies each year and a significant number of children and adults. For the young infant, a degree of protection is available in the form of breast feeding. Not only is mother's milk the most appropriate food for the baby, but also it contains elements known to confer some passive protection against enteric pathogens. Rotavirus IgA antibodies identified in human milk⁴⁹ can be expected to resist intraluminal digestion because of their attachment to secretory component. Fortunately, earlier trends away from the breast are

being reversed in many developing countries, where high attack rates are a consequence of poor sanitary and hygienic conditions.

As more enteric pathogens are identified and more is learned about their interaction with the gut, efforts increase to develop effective vaccines. The goal is to develop attenuated live organisms capable of generating an effective local immune protection against pathogenic organisms. Ironically, the success of a vaccine also depends on the organism's capacity to bypass or overcome, at least in part, existing host defense mechanisms like gastric acid and breast milk IgA. Clinical trials will be required to determine whether bicarbonate administration or withholding of breast feeding is essential to the efficacy of various oral vaccines. The most promising contenders for human diseases, to date, are two rotavirus vaccines, one an attenuated calf virus, found to be safe and protective in limited trials.^{50,51} An attenuated simian rotavirus also has been shown to protect, but to date it has been associated with an incidence of febrile reactions.⁵¹ Progress has also been made in the development of vaccines against animal bacterial pathogens.⁵² Programs are being directed at the development of vaccines for several human bacterial pathogens but none is ready for clinical use.

4.1. *Fluid and Electrolyte Balance*

The foremost consideration, particularly for the young patient, must be rehydration of the dehydrated baby or preservation of hydration for the child who is not dehydrated. The application of sound pathophysiology principles to oral fluid therapy of diarrhea is one of the important medical advances of the century. Formulation of a balanced, buffered isotonic mixture of glucose and electrolytes, an oral rehydration solution (ORS) (Table 4), promoted by a highly successful WHO Diarrheal Disease Control Program, has had a dramatic impact on mortality and morbidity from diarrheal disease in the developing world.⁵³ This formulation, usually dispensed in a cachet or tablet for dissolution in a standard vol-

Table 4. Oral Rehydration Solution (ORS)—
World Health Organization

	mM
Glucose	111
Na	90
K	20
Cl	80
Citrate	10

ume of water, provides a balance of solute and water to make the best possible use of any absorptive function that remains in the gut after infection.⁵³ Designed initially for patients with cholera, it has been found effective for all but the most severely affected babies with viral enteritis.⁵⁴ Certainly, ORS is a vast improvement over many traditional beverages used for infant diarrhea, such as fruit juices and soft drinks, all of which are highly hyperosmolar sugar solutions.⁵⁵ Pediatricians have worried about the 90-mM Na concentration in ORS since it exceeds stool concentrations in viral enteritis, but actually the solution has been used effectively to treat hypernatremic infants with viral enteritis.⁵⁶ It is strongly recommended that WHO ORS not be force-fed and that, for very young infants, it be given with 1 volume of water per 2 volumes ORS; for worldwide use WHO must advocate the use of a single oral rehydration formulation.

Regrettably, medical practices in developed countries, particularly in North America, have lagged behind the developing world in instituting this very effective treatment technique. Although some extremely ill infants will require intravenous fluids and electrolytes, rational oral therapy is simple, effective, and a preferable approach for most.⁵⁶

Several proposed modifications of oral fluid therapy based on pathophysiological considerations described previously appear promising. Many have speculated that additional organic solutes, amino acids, peptides, and even more complex carbohydrates might further enhance water absorption if incorporated into oral treatment solutions. Since even in viral enteritis our studies showed both an additive response of Na absorption to alanine and glucose and intact BBM alanine absorption, there is a theoretical basis for incorporating an amino acid like L-alanine into oral hydration solutions. Furthermore, *in vivo* studies suggest that amino acids are absorbed more efficiently when presented to the mucosa as dipeptides than when provided as amino acids alone.⁵⁷ Current clinical studies are evaluating dipeptides in ORS mixtures, but our *in vitro* data do not indicate that they will have a particular advantage.³⁵ It has been suggested too that polysaccharides might be provided as a source of glucose in ORS to provide extra carbohydrates at lower osmotic cost,⁵⁸ assuming that some digestive function is preserved in patients with enteric infections. These concepts tend to support the suggestion that some foods might serve as an appropriate source of organic solute for ORS solutions. These latter solutions, most of which have utilized cooked rice, might be suitable as a prepared product, but they might also lend themselves to preparation in the home. Field trials have shown that rice-based cereal ORS is effective in reducing diarrheal stool output.⁵⁹

The appeal of all the various improved ORS formulations is their possible capacity to reduce diarrhea. Apart from the obvious beneficial impact on fluid balance, such solutions can be expected to improve acceptance of oral fluid therapy; failure to decrease stool output is probably a major factor blocking more widespread acceptance of the current ORS solution. Unless cost or palatability problems are significant deterrents, it is likely that an improved ORS formulation capable of actually decreasing diarrhea will become available in the near future.⁶⁰

4.2. *Drugs*

In general, drugs have no place in the treatment of acute infectious diarrhea. Clearance of pathogens is swift, and in most cases antibiotics are not helpful; they may even harm. Of course, there are special situations in children with infectious diarrhea where antibiotics are needed, but these are rare. Even if viricidal agents were available, they are unlikely to benefit patients with viral diarrhea because by the time diarrhea is significant, little time for the virus remains in the mucosa because of accelerated natural shedding.

As the pathogenesis of infective diarrhea was elucidated, great hope was held for a pharmacological approach to correction of the transport defects identified. Many drugs have been shown to enhance absorption or decrease secretion *in vitro*,⁶¹ but *in vivo* they have been either ineffective or toxic. Unfortunately, antidiarrheal drugs continue to be promoted for the treatment of acute diarrhea; in children at least, they should not be used.

4.3. *Nutritional Treatment*

Since recovery from viral enteritis, and presumably other invasive infections, depends on an epithelial repair process that in turn is influenced by nutritional status, it makes theoretical sense to provide nutrients to patients with diarrhea.⁶² The digestive tract is the preferred supply route for these nutrients. If the patient is young with meager nutritional reserves, particularly if he is malnourished initially, food is particularly important. Unfortunately, it has been traditional in many societies to withhold food or limit intake for extended periods during diarrhea. Limited feeding studies indicate that at the worst early feeding does no harm,⁶³⁻⁶⁶ and a recently published epidemiological study showed that diarrheal illness was relatively prolonged among patients who were mal-

nourished.⁶⁶ The latter clinical observation might have been predicted from current concepts of pathogenesis.

The breast-fed baby should continue to receive mother's milk, although extra hydration fluid may be needed as well. The question then arises as to whether some foods may aggravate diarrhea. The answer is yes, but rarely is the aggravation significant in relation to the benefits of food. In the face of a viral enteritis, at least two possible mechanisms by which dietary constituents might increase diarrhea are worth consideration, sugar intolerance and dietary protein intolerance.

Disaccharidase activities are reduced, particularly lactase activity in viral enteritis.⁶⁷ Large dietary loads of disaccharides, therefore, may cause watery, sugar-filled stools in cases of severe, prolonged mucosal disease, but in most populations, disaccharide absorption is not a limiting problem contributing to infectious diarrhea. Lactose intolerance is particularly prevalent among older children and adults belonging to native groups in North and South America. In the latter populations it is reasonable to defer cow's milk feeds for a few days early in the course of an enteritis.

Another theoretical problem that might be induced by diet during enteric infection is milk protein intolerance. It has been postulated that the infected bowel is excessively permeable to intact protein antigens.⁶⁸ Our *in vivo* studies of piglet TGE suggest that this is the case,⁶⁹ although *in vitro* experiments showed an increased uptake only during the very early phase of infection and only in non-patch-containing regions of the small intestine.⁷⁰ There are no findings yet to suggest that such an enhanced antigen absorption is of real immunological significance, and there are no data to support a general policy of withholding milk protein from patients recovering from infectious diarrhea.

5. SUMMARY AND CONCLUSIONS

A range of pathogenic organisms interact with the gut causing diarrhea by several distinct mechanisms. Until effective preventive measures are available, these patients should be treated aggressively with oral fluid based on an understanding of the pathophysiological disorders underlying their illness. Furthermore, early provision of adequate nutrients is highly desirable in order to promote quick recovery. Ongoing research, while providing important information about the normal intestine, should provide data on which to base new approaches to preventive and active therapy.

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