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Pathophysiology of the Enterotoxic and Viral Diarrheas

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INTRODUCTION

As amply documented in other papers in this volume, there is no doubt that diarrheal diseases are the world's biggest health problem. They constitute the commonest cause of death among young children in the most populous developing countries (Tables I–III).[1,2] Although more difficult to quantify, even greater is the impact of 3 to 12 severe, dehydrating illnesses per year on the physical and mental development of those children who survive. Also alarming is the recent "modernization" trend toward reduced breast-feeding in areas where very poor socioeconomic conditions result in a significant lag in the development of adequate water supply and sanitation facilities. The findings in northeastern Brazil of Nations-Shields suggest that the striking mortality of 15 to 25% in the first 5 years of life will likely increase even further as this trend proceeds (M. Nations-Shields, personal communication, 1981).

The nutritional impact of what should be an acute, self-limited derangement of water and electrolyte balance is further accentuated by the transient malabsorption state and by reduced oral intake, while catabolic demand may be increased during the acute phase of diarrheal illness. The leading etiologies are enterotoxigenic *Escherichia coli*, rotaviruses, *Shigella*, and *Campylobacter jejuni*. The frequency and severity of *Giardia* and amoebic infections vary with the setting.

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Table I. Mortality in First 5 Years of Life in Northeastern Brazil[a]

Total	14.7%
Diarrhea	
as 1° cause, or 35% of all deaths	5.1%
as associated cause	2.6%
Respiratory disease	
as 1° cause	1.8%
as associated cause	5.8%
Measles	1.6%

[a]Source: reference 1.

Table II. Causes of Death in Fortaleza, Brazil (1976–1977)[a]

Diarrheal disease	2129	(22.4%)
Malignancy	1394	(14.6%)
Perinatal mortality	1185	(12.4%)
Cerebrovascular disease	1119	(11.8%)
Cardiac disease	825	(8.7%)
Pneumonia	677	(7.1%)
Motor vehicle accidents	655	(6.9%)
Ischemic heart disease	549	(5.8%)
Other	989	(10.4%)

[a]Source: Dr. Ana Rosa dos Santos, Division of Epidemiology and Biostatistics, Secretary of Health, Ceará, Brazil.

Table III. Age-Specific Mortality, Fortaleza, Brazil (1976–1977)[a]

	<1	1–4	5–14	15–24	25–44	45–64	>65
Diarrhea	1828	196	30	3	9	16	47
Malignancy	22	16	39	47	236	545	489
Perinatal	1185	—	—	—	—	—	—
Cerebrovascular	5	1	9	28	98	290	688
Cardiac	10	17	14	19	94	240	431
Pneumonia	331	155	31	12	24	33	91
Motor vehicle	—	29	104	117	216	122	67
Ischemic heart disease	—	—	—	4	47	165	333

[a]Source: Dr. Ana Rosa dos Santos, Division of Epidemiology and Biostatistics, Secretary of Health, Ceará, Brazil.

It is useful, both conceptually and in practical field diagnosis, to separate acute diarrheal illnesses into two groups according to pathogenesis and site of disease in the intestinal tract. The first, arising from the action of enterotoxins or viral agents that impair absorption and elicit net isotonic fluid secretion in the upper small bowel, results in a noninflammatory, often watery, diarrhea. The second type arises from destruction or invasion of the distal small bowel or colonic mucosa by organisms such as *Shigella* or *Campylobacter,* or by cytotoxins, that produce an inflammatory dysentery in which the stool may contain blood or pus. Keusch (chapter 3, this volume) focuses on the latter inflammatory colitides. After brief mention of enteric host defenses and microbial virulence factors, I will confine my comments to the former, more common, noninflammatory diarrheas caused primarily by bacterial enterotoxins, or by viral infections of the upper small bowel.

Types of Acute Diarrhea

	1. Noninflammatory	2. Inflammatory
Mechanism: ⟷	Enterotoxin or reduced absorptive surface	Mucosal invasion
Site:	Small bowel	Colon
Type:	Watery	Dysenteric
Diagnosis:	No fecal leukocytes	Polymorphonuclear leukocytes in feces

Enteric Host Defenses

Several host factors influence the outcome of enteric infections, among which poor personal hygiene and unavailability of sanitary facilities are contributing factors. Space permits only a cursory mention of the normal host gastrointestinal defense mechanisms, including gastric acidity, normal microflora, motility, mucus, and humoral and cellular immunity.

Appropriate hygienic measures and sanitary facilities should limit the ingestion of the large inocula of 10^5 to 10^8 bacteria usually required for an infectious dose of bacteria (with the exception of *Shigella*). Second, normal gastric acidity provides an important barrier to bacterial and parasitic infection. Its neutralization by antacids or, perhaps, food results in increased attack rates or increased severity of infections ranging from cholera and salmonellosis to giardiasis.[3] The importance of normal

microflora in preventing infections is often overlooked. That antibiotics predispose to increased risk of infection in experimental animals has been known for some time, and this relationship is increasingly being recognized as significant for humans.[4–6]

Likewise, inhibition of normal gastrointestinal motility enhances susceptibility to infection and impedes rather than helps normal absorptive processes.[7,8] Mucus throughout the gastrointestinal tract probably plays a far greater role than currently appreciated, whether by binding organisms or toxins or by protecting the mucosa from toxins or microbial invasion. The major roles of humoral, secretory, and cell-mediated immunity in protection from enteric infection are beyond the scope of this paper.

Microbial "Virulence" Factors

As our understanding of the etiology and pathogenesis of enteric infections develops, it becomes increasingly apparent that the capacity of many microbes to cause disease may be determined by variable gene codes, frequently transmissible among organisms, as well as by the species itself. For example, *E. coli* may be enterotoxigenic like *Vibrio cholerae,* invasive like *Shigella,* or harmless normal flora, depending on the gene code they happen to carry.

Among the virulence traits felt to be important in pathogenesis of diarrhea are colonization factors, enterotoxin production, cytotoxin production, and invasiveness. While several traits may be present in the same organism, the focus of this paper is on the extent of colonization required for enterotoxigenic organisms to act in the upper small bowel, the mechanism of action of the enterotoxins, and specific attack on certain intestinal epithelial cells by viral agents.

Bacterial Adherence to Upper Small Bowel

Numerous surface fimbriate and fibrillar adhesins have been described for *E. coli, Salmonella, Shigella, Klebsiella,* and *Proteus,* as well as for *Bordetella, Corynebacteria,* and *Mycoplasma.*[9,10] Among the best understood fibrillar adhesins are those required for colonization of enterotoxigenic *E. coli* in porcine, bovine, and human small bowel. These plasmid-encoded adhesins appear to be species-specific, such as K88, K99, and

Table IV. Effect of K88 and ENT Plasmids on *E. coli* Capacity to Cause Porcine Diarrhea[a]

Plasmids	Diarrheal attack rate (No. Ill/Total No. Fed)
$K88^{-}ENT^{-}$	0/8
$K88^{-}ENT^{+}$	0/11
$K88^{+}ENT^{-}$	6/20
$K88^{+}ENT^{+}$	20/25

[a]Source: reference 11.

CFA/I for piglets, calves, and humans, respectively, and are probably necessary for colonization and thus production of disease.

Even colonization alone (without enterotoxin) may occasionally cause mild or chronic diarrhea, as described in piglets studied by Smith and co-workers (Table IV).[11] It has been suggested that these adhesins may adhere by lectinlike interactions with specific carbohydrates. They are detected by immunoassay, specific hemagglutination patterns, or bioassay *in vitro*.[10,12,13] The concepts of developing specific immunity to these adhesins, or of exploiting carbohydrate or lectin competition for their adherence in the upper small bowel, hold great promise. However, it is becoming increasingly apparent that human enterotoxigenic *E. coli* exhibit multiple antigenic and biologic types of adhesins, such as colonization factor antigen/I, colonization factor antigen/II, type I fimbriae, and probably several others.[13,14]

There have been recent descriptions of a close, disruptive adherence of classical enteropathogenic *E. coli* to villous brush border with associated reduction in disaccharidase activities.[15] Whether the less tightly associated colonizing, fimbriate coliform organisms, enterotoxigenic or not, or their metabolities or products are responsible for the well-recognized morphological, enzyme deficiency, and clinical manifestations of malabsorption associated with acute diarrheal illnesses of bacterial, or even viral or parasitic, etiology is unclear.[16–21]

Normal Small Bowel Physiology

In order to understand the small bowel secretory derangement caused by enterotoxins or by rotaviruses, or possibly by both simulta-

neously, one must first examine the normal physiology of upper small bowel electrolyte absorption and secretion.

The small bowel is a remarkable and complex organ, the function of which determines our nutritional and hydration status, and, to a significant degree, our quality of life. It is in the upper small bowel that most macro- and micronutrients are absorbed. These include calcium, magnesium, iron, glucose, and other carbohydrates, often after the action of small bowel disaccharidase enzymes. Water-soluble vitamins and, depending on normal hepatic and pancreatic function, fat-soluble vitamins and essential long chain fatty acids are also absorbed. Furthermore, excessive bacterial overgrowth in the small bowel can alter the intact absorption of substances such as bile salts or vitamin B_{12} farther distally in the colon.

Integrally linked to the micronutrient absorptive function of the small bowel are constant, large, bidirectional fluxes of electrolytes and water. It is a relatively slight shift in this delicate balance toward secretion that results in diarrhea. Shown in Figure 1 are the striking bidirectional fluxes measured in ligated canine jejunum with separate sodium isotopes simultaneously placed intraluminally (^{22}Na) and intravenously (^{24}Na) (R. L. Guerrant and J. E. Rohde, unpublished observations, Cholera Research Laboratory, Dacca, Bangladesh, 1970). In the normal (control) state, the absorptive flux slightly exceeds the secretory flux, resulting in net absorption. From these types of data one might expect the equivalent of 40 to 50 liters per day of isotonic fluid to be exchanged in each direction across the normal human small bowel.

Although specific studies are difficult because of the dynamic status of rapid cell turnover, the small bowel is a complex organ that clearly has regional and subcellular specialization for secretion and absorption. These specialized regions have particular relevance for current hypotheses about the effects of cyclic AMP and cyclic GMP nucleotides

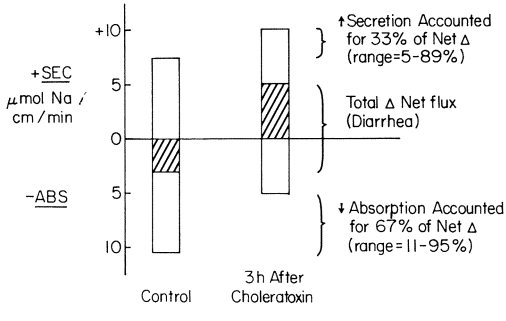

Figure 1. Effect of cholera toxin on unidirectional sodium fluxes in ligated canine jejunal segments (N = 22). (Source: R. L. Guerrant and J. E. Rohde, unpublished observations from the Cholera Research Laboratory, Dacca, Bangladesh, 1970.)

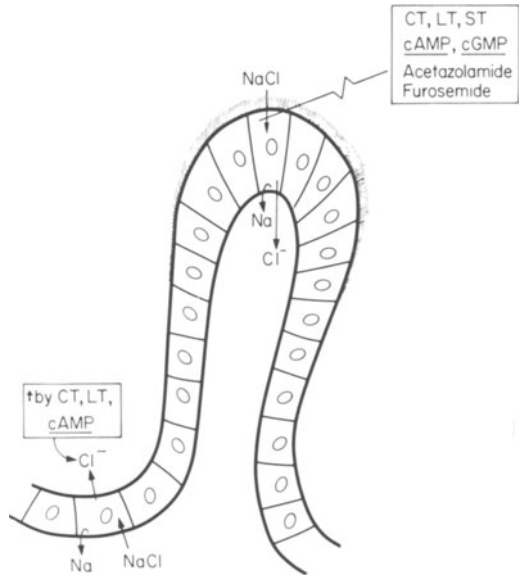

Figure 2. Specialization of villus tip and crypt cells in the small intestinal mucosa and postulated sites of enterotoxin action.

and possible interactions with viral infections of specific cells such as those in villus tips (Figure 2).

The cuboidal crypt cells multiply and provide a continuous supply of differentiating cells that migrate toward the villus tip, where they specialize for absorption by developing microvillous brush borders and producing enzymes such as disaccharidases and alkaline phosphatase. Based largely on studies with enterotoxin probes of ion transport in short-circuit current chambers, current data suggest regional differences in absorptive and secretory function, with villus tip cells being primarily absorptive and intervillus crypts being primarily secretory.

The driving force both for the electrically neutral sodium chloride absorption in villus tip cells and for the electrogenic chloride secretion in crypts may well be the same contralumenal sodium-potassium-activated ATPase-linked sodium pump that extrudes sodium from the cell.[22] The different effects of the same sodium pump that cause neutral sodium chloride absorption or electrogenic chloride secretion can be explained by relative differences in the location of the neutral sodium chloride coupled transport. For electrically neutral absorption of sodium in villus tips, the pump may extrude sodium to the lumenal membrane, while for electrogenic chloride secretion in the villus crypts, the pump may extrude sodium through the contralumenal membrane.

Mechanism of Action of Cholera Enterotoxin and Heat-Labile Enterotoxin of *E. coli*

As predicted by John Snow over a century ago, the entire syndrome of clinical cholera appears to result from the action of an enterotoxin that shifts the delicate balance toward secretion of isotonic fluid in the upper small bowel. As shown in Figure 3, the effect of cholera toxin on net water (and sodium) fluxes following even a brief experimental exposure results in a net secretory response that occurs after a 30- to 60-minute lag period and becomes maximal at 2 to 3 hours. On the basis of similar effects of dibutyryl cyclic AMP, theophylline, and cholera toxin on ion fluxes across isolated rabbit ileal mucosa in short-circuited chambers,[23] several other investigators and we have explored the effects of cholera toxin on intestinal mucosal adenylate cyclase activity. This secretory

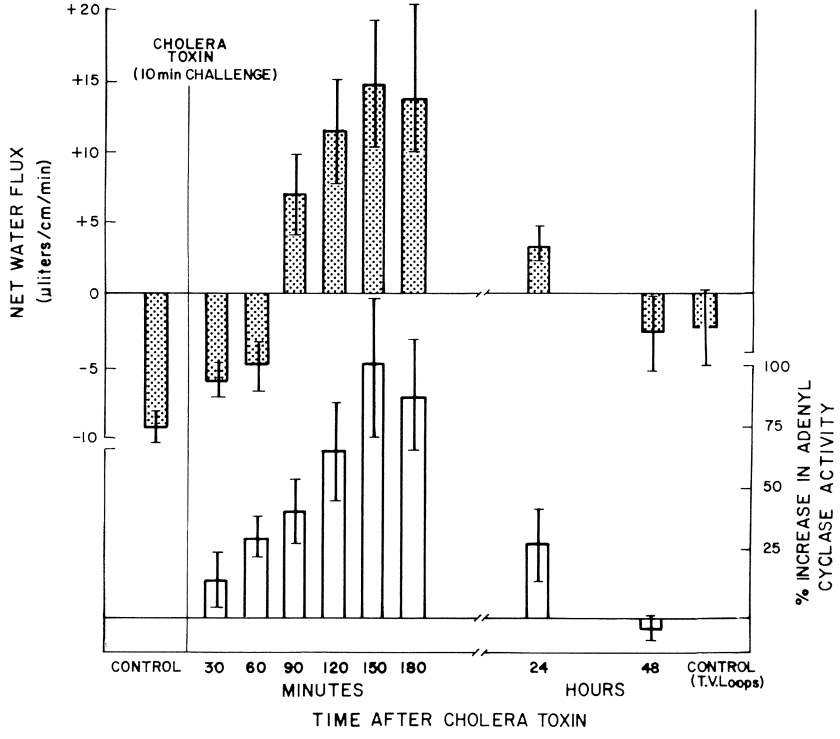

Figure 3. Time course of cholera toxin effects on net fluid transport and on mucosal adenylate cyclase in canine jejunum. (Source: reference 24.)

response parallels precisely the activation of intestinal mucosal adenylate cyclase (Figure 3).[24] Cholera toxin is thus a unique pharmacologic agent that activates intestinal mucosal adenylate cyclase and, after a lag period, causes fluid secretion for longer than 24 hours after exposure, despite removal of the toxin. Only after 48 hours, a time sufficient for renewal of most mucosal epithelial cells, do absorptive function and adenylate cyclase activity return to normal levels.

Unidirectional sodium flux measurements in ligated canine jejunal segments with ^{22}Na given intraluminally and, in some instances, simultaneously with intravenous ^{24}Na, have shown that, in each of 22 consecutive studies, the net secretory response to cholera toxin was due, in part, to an increase in unidirectional sodium secretion (accounting for a mean of 33% of the net change) *and* reduced unidirectional sodium absorption (accounting for an average of 67% of the net change) (Figure 1) (R. L. Guerrant and J. E. Rohde, unpublished observations from the Cholera Research Laboratory, Dacca, Bangladesh, 1970). The increased short-circuit current and secretory response to cholera toxin and cyclic AMP appear to result from two effects: There is reduced neutral NaC1 absorption, primarily in villus tips,[25] *and* an increase in the electrogenic secretory flux of chloride and possibly bicarbonate, presumably from crypt cells.[26–28] As noted above, the driving force for both active chloride absorption with electrically neutral sodium-coupled transport in "leaky" epithelia in many species and even for the electrogenic chloride secretion, may be the same Na-K-dependent, ATPase-linked sodium pump in the lateral and basal membranes.

Three lines of indirect evidence suggest that the secretory effect of cholera toxin and cyclic AMP probably arises from crypt cells. First, selective damage of villus tip tissue with hypertonic saline does not impede the cholera toxin-induced secretory response.[29] Second, studies of marine teleost intestine that, like the gall bladder, has no crypt tissue, show that cyclic AMP and theophylline cause only reduced absorption and no net secretion.[26] Third, DeJonge and co-workers showed that a 5-minute exposure of rat intestine to cholera toxin resulted in activation of only villus tip adenylate cyclase and reduced absorption. In contrast, a 30-minute exposure to cholera toxin resulted in crypt as well as villus tip adenylate cyclase activation and a secretory response.[30]

Cholera toxin is a polypeptide with binding (B) and active (A) subunits. The binding portion contains five identical units and appears to be responsible for the avid association of cholera toxin with the monosialoganglioside (G_{M1}) receptor on epithelial cell surfaces ($Ka = 10^{-9}M$).[31] As shown by Gill, the A_1 subunit (after being split from A_2 by reduction of a disulfide bond), in the presence of NAD, cell cytosol, and ATP, is

capable of activating adenylate cyclase directly in broken cell preparations.[32,33] Prior work had shown that cholera toxin, in addition to activating adenylate cyclase, actually enhances the responsiveness to other hormones such as epinephrine (Table V).[34] From the outstanding work of Cassel and Selinger,[35,36] it now appears that the active subunit of cholera toxin, by ADP-ribosylating the GTP-binding component of adenylate cyclase (? a GTPase), blocks the normal turnoff reaction for activated adenylate cyclase (Figure 4). This explains the enhanced responsiveness to other hormones and the prolonged activation of adenylate cyclase by cholera toxin, possibly for the life of the intestinal epithelial cell.

This secretory effect of cholera toxin leaves the glucose and amino acid-coupled sodium transport mechanism intact, thus providing the basis for oral glucose-electrolyte therapy.

Table V. Effect of Cholera Toxin on the Response of Rat Fat Cell Membranes to Epinephrine[a]

	Adenylate cyclase activity (mol/min per mg protein)		
	Basal	+ epinephrine	∇ in response to epinephrine
Control	32.2	124.5	+ 92.3
Cholera toxin	100.8	361.0	+260.2

[a]Source: reference 34.

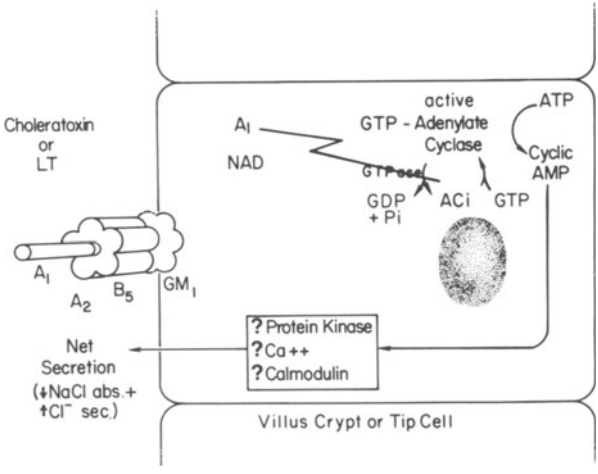

Figure 4. Proposed mechanism of cholera toxin and LT action.

The heat-labile enterotoxin (LT) of *Escherichia coli,* and probably of other Enterobacteriaceae (including *Klebsiella, Citrobacter,* and *Salmonella*), is remarkably similar to cholera toxin in its genetic code, antigenicity, and mechanism of action. Although there appear to be subtle differences in the nature of the binding subunits, both cholera toxin and heat-labile toxin activate adenylate cyclase in a similar fashion in many mammalian cell types. It is this promiscuous activation of adenylate cyclase in multiple cell types that has been exploited in the development of tissue culture bioassay methods, such as the Chinese hamster ovary (CHO) cell[37,38] and Y-1 adrenal cell assays[39,40] for LT and cholera toxin. The precise intracellular mechanism by which both cholera toxin and LT cause fluid secretion after intracellular cyclic AMP is formed remains unclear at present. Whether this is related to the phosphorylation of protein in crypt cell basolateral plasma membranes, as shown by DeJonge,[41] resulting in increased lumenal chloride conductance, remains conjectural. A calcium/calmodulin-dependent step appears to be involved, inferred from indirect studies with lanthanum chloride and chlorpromazine inhibition of cholera toxin-induced secretion (Figure 4).[42,43] Furthermore, calcium ionophore[44] and hormones such as serotonin[45] cause a calcium-dependent secretory response independently of changes in cyclic nucleotides.

Mechanism of Action of *E. coli* Heat-Stable Enterotoxin (ST)

Initally known to cause diarrhea in piglets and calves, ST-producing *E. coli* are now recognized as common causes of human diarrhea in adults and children.[46–49] Although its genetic code may coexist on the same plasmid with LT, ST is quite different from LT both antigenically and biologically. ST is a much smaller molecule (molecular weight 1,500 to 4,400).[50,51] In contrast to cholera toxin and LT,[38,52] ST effects are not inhibited by ganglioside G_{M1},[53] ST is inactive in nonintestinal tissue culture assay systems,[37,39] and it requires the suckling mouse assay or calf or piglet ligated segments for its detection. It now appears that there is a family of heat-stable enterotoxins, some of which, such as STb, may be inactive in mice but active in piglets.[54] The role of STb in human disease and its mechanism of action are unknown.

Initial data suggested that early effects of ST- plus LT-containing culture filtrates were associated with an immediate, measurable effect on fluid secretion and on apparent increased intestinal adenylate cyclase activity, in addition to the more prolonged activation of adenylate

cyclase by whole enterotoxigenic *E. coli* cultures.[52,55] However, in retrospect, this early effect was most likely caused by ST and the now-recognized ability of activated guanylate cyclase to form some cyclic AMP *in vitro*.[56] Indeed, after ST exposure, intestinal tissue concentrations of cyclic GMP are increased without significant changes in cyclic AMP concentrations, and the 8-bromo analogue of cyclic GMP induces a magnitude and time course of secretory responses in the suckling mouse identical to those of ST.[56]

Thus, the effect of cholera toxin (CT) and LT on cyclic AMP, and the effects of ST on cyclic GMP cause the same (net secretory) rather than the opposite direction of response (Figure 5). ST has been shown to activate guanylate cyclase rapidly, to increase short-circuit current, and to reduce sodium and chloride absorption in rabbit ileal mucosa.[57]

Other studies have shown that ST specifically activates only the particulate, *intestinal* guanylate cyclase,[53,58] an effect that appears primarily to occur in jejunum and ileum.[59] This is in striking contrast to the widespread effect of cholera toxin and LT in many different tissues, and it has sharply limited the development of new bioassay methods for ST.

Particulate guanylate cyclase activity and cyclic GMP in intestinal mucosa are located primarily in the mucosal brush border at the apical regions of villous tips where they are associated with disaccharidase and alkaline phosphatase enzymes.[60–62] This localization is consistent with the principal effect on ion fluxes of decreased absorption with ST and cyclic GMP, rather than the increased chloride secretion seen with cholera toxin, LT, or cyclic AMP. Thus, the effect of ST appears to be reduced villus tip electrolyte absorption, an effect that could be additive with crypt adenylate cyclase-activating toxins.

The activation of guanylate cyclase by ST is quite different from the activation of adenylate cyclase by LT or CT. Free radicals can activate guanylate cyclase,[60] and the free radical scavenger, butylated hydroxyanisole (BHA), significantly reduces the activation of guanylate cyclase as well as the fluid secretory response to ST.[53] Furthermore, both indo-

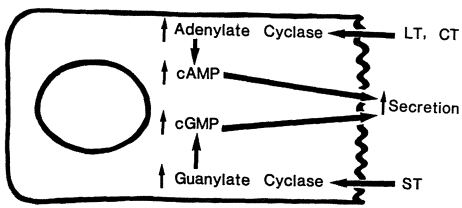

Figure 5. Effect of both adenylate and guanylate cyclase activating enterotoxins (cholera toxin or LT and ST, respectively) on net small intestinal secretion.

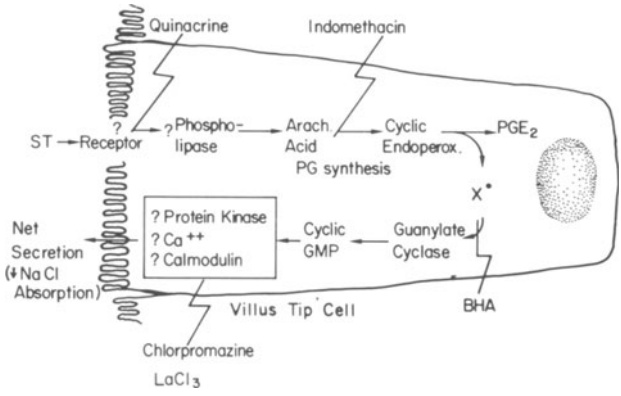

Figure 6. Possible pathways of ST effects on particulate small intestinal villous tip guanylate cyclase and reduced absorption (to cause net secretion).

methacin and antimalarial compounds such as quinacrine and amodiaquine significantly inhibit ST responses before activation of a guanylate cyclase.[63]

As shown in Figure 6, one possible interpretation of these data would be that ST involves initial membrane phospholipase activation (that is altered by quinacrine), followed by the prostaglandin synthesis pathway (that is inhibitable by indomethacin),[58,64] both before formation of a free radical that would activate guanylate cyclase. Recent work by R. N. Greenberg in our laboratory has shown that chlorpromazine and lanthanum synergistically inhibit ST- or 8 Br-cyclic GMP-induced secretion.[64,65] These findings suggest that calmodulin- and lanthanum-sensitive calcium pools may be involved in cyclic GMP-induced net secretion. Thus, cholera toxin and LT may share with ST some of the final, calcium-dependent secretory mechanisms in the small bowel (see Figure 4 and 6).

Pathogenesis of Viral Enteritis

Although several enteroviral and other viral illnesses may be associated with diarrhea, the principal viral agents now recognized as common causes of diarrhea are the small, 27-nm Norwalk-like parvoviral agents and the 70-nm rotaviruses. Norwalk-like agents typically cause winter vomiting disease[66] and are associated with delayed gastric emp-

tying,[67] reduced small bowel brush border disaccharidases,[21] and transient fat and xylose malabsorption,[68] without changes in jejunal adenylate cyclase activity.[69]

Rotavirus are much more easily detected than Norwalk-like viruses by electron microscopy or by enzyme-linked immunosorbent assay (ELISA), and are associated with diarrheal illnesses in 11 to 50% of cases in children less than 2 years of age throughout the world, with peaks in cooler, drier months.[70,71] With fecal shedding often exceeding 10^{11} per gram, rotaviruses are commonly acquired and occasionally produce symptoms in adult contacts as well.[72] Rotavirus diarrhea may be severe, often with vomiting and low-grade fever at the onset. The diarrhea is usually noninflammatory and watery, with slightly increased fecal sodium excretion and reduced brush border disaccharidase and Na-K ATPase activities.[73] Increased fecal reducing substances have also been described during rotavirus diarrhea (Table VI)[74] that might, theoretically, when challenged with a carbohydrate load during severe rotavirus infection, result in an acidic stool or clinically significant malabsorption, or worsened acidosis.[75]

A potential explanation for the watery diarrhea and carbohydrate malabsorption is suggested by the original studies of rotavirus diarrhea in which duodenal biopsies were taken from infected children.[20] Patchy, irregular mucosal changes were noted, with short and blunted villi and intracytoplasmic rotaviral particles in the villous epithelial cells. Normal columnar epithelium at villus tips was replaced by irregular, cuboidal cryptlike epithelial cells. As would be expected from this histopathology, brush border disaccharidase enzymes were abnormally low in seven of eight children studied. These transient abnormalities have been confirmed by several other investigators, who have shown the rotaviral infection to be localized to the duodenal and upper jejunal villous epithelial cells.[76,77] The degree of microvillous and mucosal damage appears to parallel the severity of clinical diarrhea and dehydration.[78]

Table VI. Stool-Reducing Substances on Hospital Admission for Diarrhea[a]

	Rotavirus (N = 52 patients)	Tox+ *E. coli* or *Shigella* (N = 10)
Prehydrolysis	189 mg/dl	64 mg/dl
Posthydrolysis	3063 mg/dl	1139 mg/dl

[a]Source: reference 74.

Similar findings have been noted in infections with the closely related agent of epizootic diarrhea in infant mice (EDIM), and in experimental infection of gnotobiotic, colostrum-deprived calves with human rotaviruses.[79] In the calf model, denuded villus tips are replaced with cuboidal epithelial cells. It has been postulated that the brush border enzyme, lactase, plays a role as a receptor and as an uncoating enzyme, because beta-galactosidase removes the outer capsid layer of rotaviruses *in vitro*.[80] However, lactase-deficient populations remain clearly susceptible to rotavirus infections.[81]

Hamilton and his colleagues have suggested that the pathogenesis of human rotaviral diarrhea may be similar to that of transmissible gastroenteritis (TGE), a coronavirus infection of piglets. In a number of studies of this process, these investigators have shown that TGE viral infection occurs first in the villus tip epithelial cells, and that this is followed by shedding of infected cells and blunting of villi that are replaced by cuboidal, cryptlike epithelial cells with increased thymidine kinase and decreased sucrase activities.[82–85] These morphological abnormalities progress over the first 40 hours of experimental infection and are associated with reduced Na-K ATPase activity, reduced sodium efflux from epithelial cells, and impaired glucose-coupled sodium transport, without changes in adenylate cyclase activity. In these studies, abnormalities disappeared within 6 days.

Similar findings have been described in 8- to 10-day-old weaned piglets infected with human rotaviruses.[86] Eleven animals developed diarrhea or intestinal fluid accumulation within 72 hours after infection, at which point the small intestinal mucosa showed shortened villi, reduced sucrase activity, increased thymidine kinase activity, and no change in cyclic AMP concentration. Although net sodium and chloride fluxes measured in short-circuited chambers were not significantly altered from those in controls, the absorptive response to glucose was blunted in jejunum from infected animals.

When understood in light of the proposed normal small bowel physiology mentioned earlier, the hypothesis that villus tip cell destruction results in a predominantly secretory epithelium could explain the diarrhea caused by rotavirus infection. Such a hypothesis might also pertain to other infections with bacterial or parasitic agents that primarily damage villus tip cell morphology or function. One might also expect accentuated secretory responses to cholera toxin or *E. coli* LT, or impaired glucose or sucrose-coupled absorption in patients with rotavirus infections. The impact of rotaviral and combined infections on nutritional status and on the efficacy of oral glucose, sucrose, or amino acid and elec-

trolyte therapy remains to be determined. The roles of protection against rotaviruses by type-specific local intestinal or breast-milk antibodies are being actively explored now with the recent successful propagation of a human rotavirus in cell culture.[87]

In summary, whether through specific alteration of normal ion fluxes by enterotoxins or through nonspecific but selective damage to villous tip epithelium by viral, parasitic, or bacterial agents (or their toxic products), acute and chronic diarrheal illnesses present major problems for the maintenance of proper nutrition and childhood development throughout tropical developing areas. In some infections, such as rotaviruses, specific nutrient absorptive defects may accompany the electrolyte absorptive defect. In others, the dehydration and tendency to withdraw food from children with diarrhea may be the major factors contributing to malnutrition. Further studies of the nutritional impact of controlling dehydration with early glucose-electrolyte oral therapy, or of controlling certain infections with specific pharmacologic therapy or vaccine prophylaxis, will further elucidate the complex interaction of infectious diarrheas with malabsorption and undernutrition.

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