Mechanisms by Which Fluid Homeostasis is Disturbed in Disease: Fluid Loss From the Small Intestine

Michael J.G. Farthing

Faculty of Medicine, University of Glasgow, Glasgow, Scotland

Dehydration is a feature of many illnesses resulting either from increased fluid losses from the gastrointestinal tract, urinary tract, skin, and respiratory tract; or from inadequate fluid intake. The latter may occur as a feature of severe illness, particularly when the conscious level is impaired, or when there is obstruction at any point in the gastrointestinal tract resulting in a failure of fluid absorption or vomiting. Febrile illnesses can markedly increase fluid losses through increased sweating, which for infants and children, include the common viral exanthemata, pulmonary infections, invasive gastrointestinal infections; and in the tropics, malaria and other common tropical infections. Metabolic disorders such as diabetes mellitus may cause increased fluid losses through the renal tract as a result of osmotic diuresis. However, of all the varied causes of dehydration, fluid losses through the gastrointestinal tract are the most profound and clinically devastating. Infants and young children are particularly susceptible to a range of intestinal infections particularly those that are responsible for high volume watery diarrhea.

FLUID HOMEOSTASIS AND THE GASTROINTESTINAL TRACT

The gastrointestinal tract plays an essential role in fluid homeostasis. Maintenance of body water relies on achieving a balance between fluid intake and losses. Water contributes 75% of the body weight of a neonate but this decreases during the first 3 to 7 days of life with a 5% to 10% reduction of body weight due to loss of extracellular fluid. Total body water decreases gradually to about 60% of total body weight by 1 year of age. Fluid and electrolyte homeostasis is compromised in the neonate by the limited ability of the neonatal kidney to excrete and reabsorb water and an impaired ability to excrete sodium, although the term neonate is able to conserve sodium by tubular reabsorption. Fluid requirements change dramatically during the early neonatal period and through childhood into adult life (Fig. 1). Fluid requirements related to body weight are greatest during infancy and thus it is this stage of life that is most susceptible to fluid losses.
FLUID LOSS FROM THE SMALL INTESTINE

GASTROINTESTINAL FLUID BALANCE

Approximately 9 l of fluid enter the gastrointestinal tract each day. Two liters is taken in the diet and the remaining 7 l is provided endogenously from saliva and from gastric, pancreatic and intestinal secretions. Only 0.15 l of fluid leaves the intestinal tract each day in feces because of the highly effective water and electrolyte retrieval mechanisms in the small and large intestine. In the jejunum, 4 l of fluid is absorbed daily primarily through nutrient-sodium co-transport mechanisms during the fed state (Fig. 2) and at other times through the sodium-hydrogen exchanger (Fig. 3). In the ileum, sodium and chloride absorption proceeds via the sodium-hydrogen and chloride-bicarbonate exchangers (Fig. 3). Water follows the movement of solute down the osmotic gradient. Thus, quantitatively the small intestine is vitally important in the maintenance of fluid and electrolyte homeostasis. Survival is possible without a colon but not following extensive resection or destruction of the small intestine, again endorsing the central role of the small intestine in fluid homeostasis. Disruption of the ability of the small intestine to effect sodium and fluid retrieval rapidly results in a fluid challenge to the colon that has a limited capacity to compensate for small intestinal losses. Once the colonic threshold for fluid and electrolyte absorption is reached, then diarrhea will occur.

MECHANISMS OF FLUID LOSS IN DISEASE STATES

Increased fluid losses from the small intestine can result either from impaired fluid absorption or increased fluid secretion. A variety of disorders can impair small intestinal fluid absorption including extensive intestinal resection; diseases that affect the mucosal architecture, causing villous atrophy, such as celiac disease and tropical sprue; and primary and secondary motility disorders of the gut that impair intestinal
FIG. 2. Mechanism of solute coupled sodium co-transport in the enterocyte.

FIG. 3. Mechanisms of neutral sodium absorption by the enterocyte.
Table 1. Enteropathy related to intestinal infection

<table>
<thead>
<tr>
<th>Infection</th>
<th>Protozoa</th>
<th>Helminths</th>
<th>Bacteria</th>
<th>Viruses</th>
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<tr>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Rotavirus</td>
<td>Enteric adenoviruses (types 40,41)</td>
<td>Enteric adenoviruses (types 40,41)</td>
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<tr>
<td></td>
<td>Small round structured viruses</td>
<td>Measles virus</td>
<td>Small round structured viruses</td>
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<tr>
<td></td>
<td>Human immunodeficiency virus</td>
<td>?</td>
<td>Human immunodeficiency virus</td>
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</table>

Motility with resulting bacterial overgrowth and intestinal malabsorption. Some intestinal infections also produce enteropathy with an associated reduction in surface area and impaired absorption (Table 1).

However, the most devastating increase in fluid losses occurs as a result of increased intestinal secretion particularly that associated with the release of bacterial enterotoxins into the intestinal lumen (1-3). The classic enterotoxins produced by Vibrio cholerae and enterotoxigenic Escherichia coli (ETEC) promote a secretory process in the small intestine without producing epithelial cell injury. In cholera, for example, fluid losses can be massive and approach 24 L per 24 hours.

A variety of mechanisms can disturb the normal balance between absorption and secretion in the crypt-villus axis. Although in the normal intestine there is net secretion of fluid and electrolytes from the crypts this is balanced by absorption by villous enterocytes.

**INCREASED SMALL INTESTINAL FLUID SECRETION**

**Enterotoxins**

The absorption-secretion relationship however can be disturbed by the action of secretory enterotoxins, by the liberation of endogenous secretagogues such as 5-hydroxytryptamine (5-HT) and inflammatory mediators such as prostaglandins, and by the activation of secretory neural reflexes (Table 2). These secretory mechanisms operate through three major effector processes, namely (i) the small intestinal epithelial cell, (ii) endogenous secretagogues, and (iii) enteric nerves.

Intestinal fluid secretion results predominantly from the active secretion of chloride and bicarbonate ions (1,4). Active chloride ion secretion has several components that together contribute to maintaining chloride ion secretion from the apical membrane of the enterocyte (Fig. 4). The final common secretory pathway is the
TABLE 2. Bacterial enterotoxins

<table>
<thead>
<tr>
<th>Enterotoxin</th>
<th>Signal transduction pathways</th>
<th>Accessory pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholera toxin family</td>
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<tr>
<td>Cholera toxin</td>
<td>cAMP</td>
<td>5-HT, ENS</td>
</tr>
<tr>
<td><em>Escherichia coli</em> LT-I</td>
<td>cAMP</td>
<td>ENS</td>
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<tr>
<td>LT-II</td>
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<td></td>
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<tr>
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<td>cAMP</td>
<td>?</td>
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<tr>
<td><em>Shigella</em> enterotoxin (ShET I + II)</td>
<td>cAMP</td>
<td>?</td>
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<td>Heat stable toxin family</td>
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<tr>
<td><em>E. coli</em> Sta</td>
<td>cGMP</td>
<td>ENS</td>
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<tr>
<td>EAST-1</td>
<td>cGMP</td>
<td>?</td>
</tr>
<tr>
<td><em>Yersinia</em>-ST</td>
<td>cGMP</td>
<td>?</td>
</tr>
<tr>
<td><em>Vibrio cholerae</em> non-01 (NAG)-ST</td>
<td>cGMP</td>
<td>?</td>
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<tr>
<td>Other enterotoxins</td>
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<tr>
<td>Accessory cholera enterotoxin</td>
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<td>?</td>
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<tr>
<td>Zonular occludens toxin</td>
<td>?</td>
<td>Cytoskeleton</td>
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<tr>
<td><em>Clostridium difficile</em> toxin A</td>
<td>Ca++</td>
<td>Cytoskeleton</td>
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cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; ENS, enteric nervous system; 5-HT, 5-hydroxytryptamine.

**FIG. 4.** A model of Cl⁻ ion secretion. At least four components are involved in the secretions of Cl⁻ ions, including (i) the cAMP-activatable Cl⁻ channel, the **cystic fibrosis transmembrane regulator** (CFTR); (ii) the Na⁺-K⁺-Cl⁻ co-transporter; (iii) Na⁺-K⁺-ATPase; and (iv) the K⁺ channel.
chloride channel. This channel is created by a transmembrane protein, the cystic fibrosis transmembrane regulator, the structural abnormalities of which are now known to be responsible for the disease cystic fibrosis. Phosphorylation of the transmembrane chloride channel protein results in the opening of the channel, which occurs under the influence of cyclic adenine monophosphate (cAMP), cyclic guanine monophosphate (cGMP), and probably calcium, through the action of specific protein kinases. There is an electric driving force for chloride extrusion because the interior of the cell is electronegative (−40 to −60 mV) relative to the extracellular environment. This is sufficient to offset the usual concentration difference for chloride, the intracellular concentrations of which are generally lower than the extracellular concentration.

On the basolateral membrane there are three other components of the system: (i) the sodium pump, (ii) the sodium-potassium-chloride co-transporter, and (iii) potassium-selective channels. The sodium pump maintains a low intracellular sodium concentration relative to the extracellular sodium concentration, providing a driving force for the entry of chloride by co-transport with sodium. The co-transporter is electroneutral transporting two chloride ions for one sodium and one potassium ion. The potassium-selective channels enable potassium that has entered via the sodium pump and co-transporter to return to the extracellular fluid. The paracellular pathways allow the exit of sodium to maintain electroneutrality, followed by fluid, which completes the secretory process.

This chloride secretory process can be activated by the direct effect of enterotoxins, by the liberation of endogenous secretagogues by enterotoxins, from infiltrating inflammatory cells, or by subepithelial neurons that terminate at the basolateral membrane forming the neurosecretory component of an intestinal neuronal reflex.

**Enterotoxins and the Enterocyte**

Cholera toxin (CT) is the most potent bacterial secretory toxin in the small intestine. CT consists of one A subunit (29 kDa) and five B subunits (10.4 kDa). The secretory activity of this toxin relates to the enzymatic activity of the A1 subunit of CT that is a nicotinamide adenine dinucleotide-dependent ribosyl transferase. This covalently links adenosine diphosphate—ribose to a guanine nucleotide-binding protein (G protein), which activates Gs, the catalytic unit of the enzyme adenylate cyclase. This leads to an increase in intracellular cAMP, which through a series of intermediate steps results in phosphorylation of the transmembrane chloride channel protein and opening of the channel that permits chloride ion secretion. *E. coli* heat-labile toxins (LT1 and LT2) are a group of proteins that are closely related structurally, functionally, and immunologically to CT (1,4,5). Like CT, *E. coli* LT has the A and B subunit structure and activates adenylate cyclase. Other bacterial enteropathogens produce LT-like toxins including *Salmonella typhimurium*, *Salmonella enteritidis*, *Aeromonas* sp., and *Plesiomonas* sp.

*E. coli* also produce a group of low-molecular-weight enterotoxins that are heat-stable (ST) (4,6). ST differs from LT and CT in that it activates guanylate cyclase
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with an associated increase in the intracellular cGMP. Unlike LT and CT, there is
no lag phase before secretion is initiated. ST binds to a receptor on the apical
membrane of the enterocyte, which is directly linked to guanylate cyclase. CT and
LT, however, require internalization and transcellular trafficking before the Aβ cata-
lytic subunit can activate adenylate cyclase, which is located on the basolateral
membrane. Heat-labile toxins are also produced by other enteric pathogens including
Yersinia enterocolitica, Vibrio cholerae non-O1, and enteroaggregative E. coli,
which produces enteroaggregative E. coli heat-stable toxin 1 (EAST-1).

It is now evident that Vibrio cholerae produces two additional toxins, the zonula
occludens toxin (ZOT) and accessory cholera enterotoxin (ACE), in addition to CT.
ZOT increases the permeability of the small intestinal mucosa by opening intracellu-
lar type junctions. ZOT has a molecular weight of 10 to 30 kDa, is heat-labile and
protease-sensitive, and has a reversible action in vitro (7-9). ACE increases short-
circuit current in Ussing chambers and causes fluid secretion in ligated rabbit ileal
loops (10). The predicted protein sequence of ACE shows striking similarity to
eukaryotic ion-transporting adenosine triphosphatases (ATPases), including the cys-
tic fibrosis transmembrane regulator. The gene encoding ACE is located immediately
upstream of the genes encoding ZOT and CT. These genes are all located on a
dynamic sector of the V. cholerae chromosome that can be regarded as virulence
cassette.

Rotavirus also produces an enterotoxin that is able to induce chloride secretion.
The enterotoxin is known as nonstructural glycoprotein 4 (NSP4) (11-13). NSP4 and
an active peptide corresponding to NSP4 residues 114 to 135 mobilize intracellular
calcium and induce secretory chloride currents when added exogenously to intestinal
cells or the intestinal mucosa. It has not been clearly established however as to how
important the enterotoxin activity is in the production of diarrhea because rotavirus
also produces a marked reduction in villous height following destruction of villus
epithelial cells that have been invaded by a virus.

Endogenous Secretagogues

The secretory effects of CT cannot be explained solely in terms of activation of
enterocyte adenylate cyclase. It is now clear that CT secretion also involves the
endogenous intestinal secretagogues 5-HT and prostaglandin E2 (PGE2). 5-HT is a
potent intestinal secretagogue, and there is compelling evidence that it is involved
in CT-induced intestinal secretion (14). Enterochromaffin cells, the main reservoir
of 5-HT in the body become depleted of 5-HT and 5-HT can be found in the intestinal
lumen after exposure to CT (15-18). The effects of CT can be substantially reduced
in the presence of 5-HT tachyphylaxis in denervated rat small intestine. Further
evidence to support a role for 5-HT has been provided by in vitro studies in Ussing
chambers and by in situ perfusion in rats small intestine. These studies have shown
that 5-HT2 and, probably more important, 5-HT3 receptor antagonists can markedly
reduce and even reverse the secretory state induced by CT (19,20). Although E. coli
LT closely resembles CT in structure and mode of action, it does not release 5-HT
from mammalian intestine and thus it must be concluded that LT acts through a 5-HT-independent pathway (21).

Prostaglandin E (PGE) has also been implicated in CT-induced secretion. CT increases PGE synthesis and release in mammalian intestine and CT-induced fluid secretion can be inhibited by indomethacin, the non-selective cyclooxygenase inhibitor (16,17). Recent studies using selective cyclooxygenase-1 (COX-1) and COX-2 inhibitors clearly demonstrated that CT induces COX-2 mRNA expression and COX-2 protein levels and that CT-induced secretion could be inhibited by COX-2 inhibitors NS-398 and DFU but not by the COX-1 inhibitor SC-560 or dexamethasone (22). These observations indicate that CT selectively induces PGE production by stimulating COX-2 synthesis. In addition to 5-HT and PGE and other prostaglandins, there is a substantial consortium of other potential endogenous secretagogues within the intestine. Substance P has been implicated in CT-induced secretion (23) and also in the pathogenesis of Clostridium difficile toxin A–induced secretion (24,25).

Nitric oxide (NO) is not clearly established as an intestinal secretagogue as it probably has a role in both absorptive and secretory processes (26). There is some evidence to suggest however that NO may play a role in CT induced secretion (27). The nitric oxide synthase (NOS) inhibitor, L-NAME dose dependently inhibited CT-induced secretion. Interestingly the NO precursor l-arginine also reduces CT secretion when administered parenterally, although the effect was less marked than that with l-NAME. These apparently discordant results may be explained by the proposed dual role for NO in absorptive and secretory processes with actions both on mesenteric blood flow and the enteric nervous system.

**Enteric Nerves**

The enteric nervous system (ENS) functions independently of the central nervous system but is linked to it through parasympathetic and sympathetic afferent and efferent neurons that assemble centrally as the central autonomic neural network. Cell bodies of enteric neurons are grouped together as ganglia connected by bundles of nerve processes, which constitute two major plexuses, the myenteric plexus and the submucosal plexus. The myenteric plexus is predominantly involved in the motor control of the gut but in addition provides secretory motor innervation to the mucosa. The submucosal plexus innervates the gut epithelium and has a central role in the control of secretory processes.

Neurons within the ENS are classified as intrinsic afferent neurons, interneurons, and motor or secretory neurons. The ENS functions through a variety of established and putative neurotransmitters, more than 20 of which have been proposed (Table 3). Two major morphologic forms of neuron have been identified in ENS; namely, Dogiel type I, which has a single, long axon and numerous short club-shaped dendrites; and Dogiel type II neurons, which are multi-polar with many long smooth processes.

Activation of enteric nerves by electric field stimulation showed a role for the ENS in intestinal ion transport processes (28). Further studies indicated that intestinal
secretion related to luminal distension and feeding were mediated at least in part by the ENS, involving the neurotransmitters substance P and acetylcholine and also the release of 5-HT from enterochromaffin cells (29,30). The role of NO as a neurotransmitter in modulating absorptive and secretory processes remains controversial. Neural pathways have been implicated in a variety of intestinal bacterial and viral infections including those due to *V. cholerae*, ETEC, *C. difficile*, and rotavirus (31). There is increasing evidence that the secretory effects are produced at least in part via a neuronal reflex arc.

The first indication that CT-induced secretion involved the ENS were in situ experiments in cat intestine in which CT secretion was inhibited 60% to 70% by the neurotoxin, tetrodotoxin, the nicotinic receptor antagonist hexamethonium and lidocaine, a local anesthetic. It was uncertain as to which component of the enteric nervous system was involved. However further experiments in Ussing chambers using "stripped" mucosa, which is largely devoid the myenteric plexus, responded poorly to CT. In addition lidocaine and tetrodotoxin inhibited CT secretion when applied to the serosal surface of the intestine; localization studies confirmed that lidocaine had diffused as far as the myenteric plexus and possibly the submucosal plexus but not into the sub-epithelial zone. Chemical ablation studies with benzalkonium chloride that effectively destroys the myenteric plexus again confirmed its role in cholera secretion.

Current evidence suggests therefore that the neuronal reflex involves a sensory afferent type II neurone which is probably cholinergic, and interneuron in the myenteric plexus which has substance P as the neurotransmitter and a secretory type I neuron, which is likely to have vasoactive intestinal polypeptide as a neurotransmitter.

There is now compelling evidence that CT activates this reflex by local release of 5-HT from enterochromaffin cells (32,33). Intraluminal 5-HT concentrations after exposure of intestine to CT closely correlate with the magnitude of intestinal fluid secretion. Pre-treatment of mammalian intestine in vivo with p-chlorophenylalanine results in a marked diminution of the intestinal secretory response to CT. We have recently shown that 5-HT can modulate 5-HT release from enterochromaffin cells.
via 5-HT₃ and 5-HT₄ receptors, which are known to exist on enterochromaffin cells; 5-HT acting via 5-HT₃ receptors inhibits further 5-HT release while 5-HT₄ receptors apparently mediate enhancement of release (34). Thus, autoregulation of enterochromaffin cells by 5-HT is likely to be important both in physiologic and pathophysiologic states.

Further evidence that a 5-HT-initiated neural secretory reflex is important in cholera is derived from pharmacologic inhibition studies in mammalian intestine (35–37). However, the most profound inhibitory effects can only be achieved when the 5-HT antagonist is administered before exposure to CT.

Studies with 5-HT antagonists have been performed in humans but have produced conflicting results. The 5-HT₂ receptor antagonist, ketanserin, given in combination with the 5-HT₃ receptor antagonist, ondansetron, failed to reverse cholera secretion as did the 5-HT₃ receptor antagonist, tropisetron (38). The 5-HT₃ receptor antagonist, alosetron, increased basal sodium and fluid absorption but failed to significantly reduce secretion in a human model of cholera (39). However, more recent studies with the 5-HT₃ receptor antagonist, granisetron, demonstrated reversal of fluid and chloride ion secretion to net absorption (40).

Experiments with substance P antagonists have also confirmed a role for this secretagogue and neurotransmitter in CT secretion (23). Preliminary studies with the peptide antagonist DPRO²-DTRP⁷⁹SP reduced CT-induced secretion but unlike the 5-HT receptor antagonists, failed to convert secretion to absorption. Further studies with a nonpeptide substance P antagonist, SP96, 345, which is highly selective, also confirmed reduction of CT secretion in a dose-dependent manner with a parallel reduction in sodium and chloride ion secretion. This agent however was also not able to reverse secretion to net absorption.

VIP is considered to be an important potential neurotransmitter in the effector limb of the secretory neuronal reflex. The VIP antagonist (4Cl-D-Phe⁶Leu¹⁷) VIP converted fluid secretion in rat jejunum to net absorption (41). Similarly the sigma receptor agonist igmesine which reverses VIP induced increase in short circuit current in mouse ileum mounted in Ussing chambers, reverses CT-induced secretion in rat jejunum in vivo and was effective when given both before and following establishment of the secretory state (42).

These observations are entirely consistent with a neural reflex involving 5-HT neural receptors on an afferent sensory neuron, a cholinergic/substance P-ergic interneuron in the myenteric plexus and a secretory VIPergic and possibly nitrergic neuron in the effector limb of the reflex.

The situation regarding ETEC enterotoxins is less clear. Although there is marked structural homogeneity between CT and LT and both toxins are known to activate adenylate cyclase in enterocytes, there are structural differences. The major difference between the two toxins lies at the cleavage sites between subunit A₁ and A₂. This results in structural differences between the two toxins at the C terminus of the A₂ chain, so that unlike CT in which A₁ and A₂ subunits are covalently linked, this is not the case with LT. This may account for the observation that LT does not release 5-HT from enterochromaffin cells in the small intestine and that the secretory
state induced by this toxin is not inhibitable by 5-HT receptor antagonists (37). Similarly, LT-induced secretion is not inhibited by substance P antagonist (23) although the sigma receptor ligand, igmesine, does inhibit LT secretion (42). Despite these differences between CT and LT, the action of the latter is inhibited by hexamethonium and lidocaine, which supports the view that the ENS is involved in LT secretion. Similarly the secretory activity of ST does appear to involve the ENS because fluid secretion is inhibited by tetrodotoxin, lidocaine, and hexamethonium. However, like LT, 5-HT release from enterochromaffin cells does not appear to be involved.

NO has been implicated in ST induced secretion although neither tetrodotoxin nor the NOS inhibitor L-NAME affect ST-induced changes in short-circuit current in muscle-stripped preparations of pig jejunum and colon. This would support the importance of the myenteric plexus in enterotoxin-induced secretory events.

C. difficile toxin A is a potent cytotoxin and produces cell death and necrosis of intestinal epithelial cells. During natural infection, the colon is the major target. However recent studies indicate that neural mechanisms may be involved in the increased intestinal secretion, which is associated with exposure to this toxin. Pretreatment of rats with the substance P antagonist CP-96, 345 inhibited the secretion and reduced mannitol permeability in ileal loops induced by toxin A. These secretory effects were also inhibited by lidocaine, hexamethonium, and capsaicin, indicating a role for the ENS (24,25). In addition, the antagonist substance P also significantly reduced inflammation in the lamina propria and reduced epithelial cell necrosis. It is proposed that cell products from necrotic enterocytes activate primary afferent neurones containing substance P. Substance P is then thought to promote release of secretagogues and chemoattractants from mast cells that then produce intestinal secretion and recruit neutrophils that will enhance the secretory process still further. Substance P released in the vicinity of submucosal arterioles will produce vasodilatation and enhance neutrophil influx. Thus although C. difficile toxin A has direct cytotoxic effects on intestinal epithelial cells, it would appear that neural reflexes are also involved by enhancing both secretory effects and promoting its role in the pro-inflammatory cascade.

Thus the ENS appears to have a central role in enhancing the secretory effects of some bacterial enterotoxins. Recent work however indicates that this is not limited to bacterial infections since rotavirus infection also appears to involve a secretory neural reflex (43). Rotavirus infection in murine intestine can also be substantially reduced by nerve blocking agents such as hexamethonium, tetrodotoxin, and lidocaine. These observations would indicate that the ENS has a wider role in mediating intestinal secretory processes associated with infections in the small intestine.

INCREASED SMALL INTESTINAL FLUID SECRETION

Mucosal Inflammation

It is now well established that a variety of inflammatory mediators are known to stimulate secretion or to inhibit intestinal absorption directly, although the precise
role in the pathophysiology of infectious diarrhea is still incompletely understood. Studies in experimental porcine cryptosporidiosis, for example, have shown reduced fluid and sodium absorption in vivo and in vitro (44,45). Inhibition of prostaglandin synthesis by indomethacin almost completely reversed the transport abnormalities in vitro, suggesting that mucosal prostaglandins are a major mediator of diarrhea in cryptosporidiosis. Similarly there is preliminary evidence to suggest that 5-HT release is also increased in this infection in humans. Inflammation accompanies many intestinal infections and it is likely that the local release of inflammatory mediators such as histamine, prostaglandins, leukotrienes, kinins, and cytokines during intestinal infection promotes intestinal secretion or inhibit intestinal absorption. Bradykinin for example acts indirectly by liberating arachidonic acid metabolites such as prostaglandins and leukotrienes from inflammatory cells immediately adjacent to the epithelial cell. PGE$_2$ and PGF$_{2a}$ act on receptors on the basolateral membrane of enterocytes, again activating the enzyme adenylate cyclase.

A variety of patterns of inflammation is seen in intestinal infection including (i) intestinal anaphylaxis, (ii) acute inflammation with a mixed inflammatory cell infiltrate including polymorphonuclear leucocytes, and (iii) chronic inflammation, which may be associated with major alterations of villous architecture.

**Intestinal Anaphylaxis**

IgE-mediated immediate hypersensitivity reactions occur in response to antigens from a number of enteropathogens. This process may contribute to intestinal secretion and the production of diarrhea while at the same time assisting in parasite eradication. Immediate hypersensitivity reactions are a consequence of antigen exposure and reaginic antibody production that usually involves IgE but which may also involve IgG$_4$ in humans. These antibodies bind to mast cells, inducing degranulation and release of a variety of inflammatory mediators (Table 4). These mediators have far-reaching effects including fluid and mucus secretion and an increase in vascular permeability. Mast cell products recruit other immunocompetent and inflammatory cells such as eosinophils and neutrophils, which in turn contribute to the inflammatory cascade, particularly the later phases of anaphylaxis. The majority of the experimental work that involves intestinal helminths namely *Nippostrongylus brasiliensis* and *Trichinella spiralis*. A secretory response to a specific antigen following sensitization can be demonstrated by intestinal perfusion in vivo or by using isolated mucosa in Ussing chambers. It has been possible to characterize the secretory process and to demonstrate chloride ion secretion (46,47). It is generally associated with fluid secretion or at least impaired fluid absorption. Experiments incorporating specific or relatively specific mediator inhibitors have shown that a variety of mast cell products and neurotransmitters are involved including histamine, 5-HT, prostaglandins, leukotrienes, platelet-activating factor, and substance P. In human trichuriasis, histamine is released from biopsy specimens from infected individuals (48).
### Acute Inflammation

Acute inflammatory cell infiltrates occur in a variety of intestinal infections although these are most commonly associated with invasive bacterial enteropathogens such as *Salmonella* sp., *Shigella* sp., and *Campylobacter jejuni*. Polymorphonuclear neutrophils predominate in these acute inflammatory processes liberating a variety of intestinal secretagogues including prostaglandins and leukotrienes. Studies with enterohemorrhagic *E coli* O157:H7 in rabbits also implicate neutrophils in the impairment of sodium absorption and the increase in chloride secretion. Pretreatment with a monoclonal antibody against the leukocyte adhesion molecule CD18 prevented histologic damage and tissue infiltration and inhibited the sodium and transport abnormalities (49).

### DECREASED SMALL INTESTINAL FLUID ABSORPTION

Impaired intestinal absorption is a major mechanism of diarrhea caused by infective enteropathogens and is generally accompanied by macroscopic or microscopic injury to the intestine. Impaired intestinal absorption may not only occur as a result of epithelial cell loss and reduced absorptive surface area, but also by alterations in intestinal transit particularly reductions in transit time, which inevitably reduces the contact time for absorption of nutrients, electrolytes, and fluids.

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<table>
<thead>
<tr>
<th>TABLE 4. Mast-cell mediators</th>
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<tbody>
<tr>
<td><strong>Performed in granules</strong></td>
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<tr>
<td>Amines</td>
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<td><strong>Superoxide anions</strong></td>
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<td>TGF-β</td>
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5-HT, 5-hydroxytryptamine; IFN, interferon; IL, interleukin; LT, leukotriene; PG, prostaglandin; TGF, transforming growth factor; TNF, tumor necrosis factor.
Fluid Loss from the Small Intestine

Microvillus Membrane Injury

A variety of bacterial enteropathogens attach to the intestinal microvillus membrane through lectin-mediated mechanisms without damaging the epithelial cells. However, a number of enteropathogens have developed sophisticated molecular techniques for subverting host cell cytoskeletal function to create a domain that is conducive to attachment, colonization, and multiplication.

*Clostridium parvum* for example adheres intimately to the microvillus membrane, disrupting microvilli at the point of contact (50). *C. parvum* colonizes the small and large intestine in large numbers, particularly in immunocompromised individuals and it is likely therefore that this process can substantially reduce the intestinal surface area available for absorption. The protozoan parasite, *Giardia intestinalis*, also disrupts the microvillus membrane, although again there is no direct evidence that this significantly interferes with intestinal absorption (51).

Enteropathogenic *E. coli* (EPEC), which is an important cause of persistent diarrhea in the infants in the developing world, causes a major disruption of the microvillus membrane, inducing what is now referred to as an attaching and effacing (A/E) cytoskeletal lesion (52,53). A three-stage model has been developed to describe the process. The first stage involves non-intimate attachment that has, until recently, been thought to be mediated by a bundle-forming pilus. However, recent work suggests that the bundle-forming pilus only functions to attach EPEC to each other and that adhesion to the enterocyte is mediated by another filamentous organelle that contains an EPEC secretory protein, EspA, which is exported by a type III secretion system. The second stage involves signal transduction and cytoskeletal rearrangement in the host cell, which is initiated by other secretory proteins, EspB and EspD, which are translocated into the infected host cells. The final stage of intimate bacterial adhesion is accompanied by actin accumulation in the host cell beneath the organism, which is followed by formation of a pedestal that requires an outer membrane protein adhesin, intimin, that binds to a protein receptor (formally HP90). This was originally thought to be a protein of host origin but is now known to be an EPEC-secreted protein known as the translocated intimin receptor (Tir). The genes for EspA, EspB, and EspD and for intimin and Tir are contained in a chromosomal pathogenicity island designated as the locus for enterocyte effacement.

Disruption of Mucosal Architecture

More extensive damage to the small intestine often manifests as partial or subtotal villous atrophy characterized by reduction in villus height and crypt hyperplasia. Obvious damage to surface epithelial cells may also be apparent. This so-called enteropathy is found during infection with a variety of enteropathogens, including rotavirus, *G. intestinalis*, *C. parvum*, *Microsporidium* sp., *Isopora belli*, and *Cyclospora cayetanensis* (54). The mechanisms by which enteropathogens produce villous atrophy are incompletely understood although they can be broadly classified into those associated with (i) direct injury of the epithelial cell, and (ii) indirect...
effects, possibly by immune mechanisms and the liberation of inflammatory mediators including cytokines.

**Direct Epithelial Damage**

Rotavirus directly invades mid-villus enterocytes causing disruption of microvilli and ultrastructural changes within the cell consistent with cytotoxicity (55). Ultimately infected epithelial cells die resulting in varying degrees of villous atrophy. A variety of animal models have been used to study the morphology and pathophysiology of rotavirus infection. Group B rotavirus infection in 8-day-old neonatal rats produces diarrheal illness within 24 hours with clinical recovery in about 7 days. Reduction in villus height precedes diarrhea, occurring at 12 hours in the ileum and 18 hours in the jejunum. A compensatory increase in crypt depth occurs later at 48 to 72 hours. During the first 24 hours there is a net secretory state for fluid at the time when reduction in villus height is maximal. At this time there is also a marked reduction in sodium absorption. Other enteropathogenic viruses including enteric adenovirus serotypes 40 and 41, small round structured viruses, astroviruses, and caliciviruses have broadly similar effects on villus and crypt architecture.

**Immune-Mediated Mucosal Injury**

Varying degrees of villus atrophy also occur in intestinal protozoal infection such as giardiasis, cryptosporidiosis, and microsporidiosis. The mechanisms by which these parasites alter villus morphology are poorly understood (54,56). However, there is evidence in a mouse model of giardiasis that T cells are involved, as athymic nu/nu mice have relatively minor morphologic changes in the small intestine, compared with more severe lesions in conventional, immunocompetent mice (56). Further evidence to support a role for T cells in producing this lesion comes from in vitro studies using human fetal small intestinal explants, in which activation of T cells by a mitogen or anti-CD3 antibody produces reduction in villus height and crypt hyperplasia (57). It has been proposed, therefore, that specific activation of T cells by Giardia antigens might induce a similar lesion in the small intestine. Partial villous atrophy occurs in cryptosporidiosis in animals and humans. The mechanisms involved are poorly understood, but because abnormalities of crypt architecture are often accompanied by mucosal inflammation, it is possible that damage could be mediated by T cell–associated mechanisms.

**Motor Dysfunction of the Intestine**

It seems highly likely that disordered intestinal motility plays a role in the pathogenesis of diarrhea in some intestinal infections. Opiates are the most widely used antidiarrheal preparations, and although antisecretory effects can be demonstrated in model systems, it is generally considered that their main therapeutic benefit lies in their ability to cause profound inhibition of gut motility and intestinal transit.
There have however been few systematic studies of gut motility in intestinal infection. There is some evidence that bacterial enteropathogens and their toxins can induce abnormal patterns of myoelectric activity in the gut, with an increase in distal propulsive activity. Some helminths, such as Trichinella spiralis also stimulate intestinal transit, predominantly by promotion of giant migrating contractions in the small intestine(58).

There is increasing evidence to suggest that many of the mediators of intestinal secretion such as enterotoxins, prostaglandins, leukotrienes and other mediators of anaphylaxis, also have potent effects on intestinal smooth muscle. One might expect therefore to see transit abnormalities in infective diarrhea due to cholera toxin where there is release of 5-HT, which has effects both on secretion and smooth muscle. Similarly in cytotoxin-mediated inflammatory disorders when there is increased synthesis and release of prostaglandins and leukotrienes, changes in intestinal smooth muscle function would also be expected. Although stimulation of intestinal motility and reduction in transit rates may be beneficial with respect to the eradication of enteropathogens and their toxic products, this will inevitably increase flow rates and decrease contact time, thereby impairing the absorption of nutrients, fluid and electrolytes still further.

REFERENCES

FLUID LOSS FROM THE SMALL INTESTINE


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**DISCUSSION**

*Dr. Abdul Majid Molla:* Now, as we all know, the Blachu and the other centers, especially international centers for diarrheal diseases research, have made extensive trials with anti-secretory drugs with really no advantages shown with any of them, so I think the Blachu has, as a matter of fact, stopped doing such trials. Today you have really introduced some new ideas. Do you suggest any new candidate for clinical trials?

*Dr. Michael Farthing:* If you look at the drugs that have been tried in the past, the studies were not always set up with a highly rational basis. Chlorpromazine and a related drug were tested, but not really backed up by sound laboratory work. Octreotide has been tried but there is little evidence that it is a potent anti-secretory agent in enterotoxin-mediated diarrhea. So the philosophy that we and others have used is to try and identify some useful new targets. Is 5-HT a useful target? I think it potentially is useful but the question that needs to be answered is why cannot we inhibit 5-HT or the effects of 5-HT, once cholera secretion is established. Now that may be partly due to effects on the enterochromaffin cell itself, because we believe that the 5-HT antagonists don't only act on 5-HT receptors on nerves, but they
also act on 5-HT receptors on the enterochromaffin cells. I suspect VIP is a good target. At the moment, we’ve got mainly peptide antagonists of VIP, but I think if we had small molecules that could inhibit the VIP receptor, there may be therapeutic possibilities. Inhibition of substance P is likely to have a relatively weak effect. It may be that we are not going to succeed by hitting one target, but if I were to choose one target, I would choose the secretory limb, the efferent limb of that neural reflex, which is the VIP limb. There are many other receptors on that nerve. If you could activate, for instance, the sigma receptors with a sigma agonist, you might also disable that part of the secretory pathway. So there is hope, but I don’t know of a drug that’s sitting there on the shelf, waiting to be used in humans as yet.

Dr. George Fuchs: I’ve been reading quite a bit lately about this post-viral gastroenteritis, irritable bowel syndrome phenomenon, and I just wondered if you had any comments on how that might provide some insights into some mechanisms of acute infection. It seems like there’s some sort of residual effect on motility and secretion, and it may be that it’s mediated by these neuroendocrine mechanisms. I just wondered what your thoughts were on that.

Dr. Michael Farthing: During the last 10 years, there have been several studies that have shown very clearly that this is a true phenomenon, that you do develop a classic functional bowel disorder following a single acute infection, and there is some science to back it up. One of the things that happens is that the number of enterochromaffin cells in the gut increase after an intestinal infection, and they remain elevated for some months, even up to a year after infection. So I believe this is one possible target. There’s quite a lot of evidence that 5-HT is involved in symptom production in IBS and there is great interest in developing 5-HT drugs to treat IBS. There’s also some evidence that there is increased output of pro-inflammatory cytokines in histologically normal tissue. Now that’s more controversial. I could now imagine a situation in which there could be normal numbers, but activated T-cells that were producing pro-inflammatory cytokines in small amounts locally.

Dr. B. S. Ramakrishna: When you mentioned nerves and secretion, I was a bit puzzled by your omission of pro-absorptive effects mediated through nerves. You’ve done a lot of work on the enkephalin inhibitors and now these are used in the therapy that’s acetorphan or racecadotril. Do we know what happens to these enkephalinergic nerves in diarrhea?

Dr. Michael Farthing: Thank you, Rama. I didn’t touch on this today because of time, but Ramakrishna has pointed out that there are not only these pro-secretory neural reflexes, but there are pro-absorptive neural reflexes involving enkephalin. This has been exploited pharmacologically. Acetorphan was the first drug produced which inhibits the action of enkephalinase that therefore increases the biological half-life of enkephalin; this increases the pro-absorptive side of the absorption and secretion balance and indeed this drug now has been subjected to clinical trial. It’s now called racecadotril. It’s been shown to be effective in children and in adults and has one major advantage in that it affects secretion without altering GI motility. So it acts on the K-opioid receptor, not the μ receptors. It’s the μ receptors that drugs like morphine and codeine act on and these inhibit gut motility. So I think this again is a useful target for the future.

Dr. Anne Ballinger: There were two questions. With the COX-2 inhibitors, do you have to give them before you induce cholera toxin as with 5-HT antagonist? And secondly, in cancer, COX-2 antagonist has been shown to have many other effects than just reducing prostaglandins. So how do the specifics work with receptor antagonists, with prostaglandins, tried in cholera toxin and also shown to have the same effect that the COX-2 antagonist does? Is it a prostaglandin receptor effect?

Dr. Michael Farthing: I don’t think those experiments have been done, so I can’t answer
the second question. In answer to the first question, my recollection is that Beubler used the usual model, which is to give the drug before the exposure.

Dr. Vasant Kumar: I just want to ask one question about the principal problem in cystic fibrosis of chloride transport. Has there been any research on inactivated toxins improving the chloride transport in the management? Do we have gene therapy or the pancreatic transplant therapy? Has there been any research on that?

Dr. Michael Farthing: If the transmembrane regulator is mutated, cholera toxin is not active. There are suggestions that children with cystic fibrosis are protected from the enterotoxin-mediated secretory diarrheas.

Dr. Roger J. Glass: We've heard a lot about chronic diarrheas in the past, and your suggestion of this post-irritable bowel syndrome after an acute infection brings to mind the possibility that some of that could account for the chronic diarrheas. I know in longitudinal studies of children, very often you'll have children who have one infection followed by another, followed by another, which appears to be chronic in time, but in fact is a serial series of infections. Do you think that this explanation that you provided could account for some of the chronic diarrhea? Because if it could, it would provide a means for intervention in therapy that's different from what's currently suggested.

Dr. Michael Farthing: As far as I know, Roger, no one has systematically looked at this, but it would be a relatively simple thing to do, and there are archive specimens of mucosal biopsies from children with persistent diarrhea. I think it's a very plausible hypothesis, and I think it probably should be tested.

Dr. George A Bray: You described a secretory reflex for serotonin with cholera toxin and then you described a blockade by a relatively non-specific blocking agent hexamethonium and lidocaine for a rotavirus, suggesting there was a neural reflex there. Would those agents block the serotonin reflex from the cholera toxin? Do they have any effect on that reflex, as they did on the rotavirus one?

Dr. Michael Farthing: They do, and indeed, they also block the LT and ST effects. That's why we know that LT and ST do involve the enteric nervous system and, in fact, these were the first experiments that were done over a decade ago that began to draw our attention to the enteric nervous system. So you're absolutely right. They are nonspecific, but they are very complete. The great thing about tetrodotoxin is that it works, and if it blocks the effect, you know that nerves are involved.

Dr. Wolf Endres: If we discuss how to prevent or to diminish the loss of fluids from the intestine, what about the loss or the decrease in the loss of toxins? In other words, could there be a disadvantage to block the loss of fluids too much in terms of keeping the toxins in the body?

Dr. Michael Farthing: Theologically, increasing flow in the gut is thought to be beneficial in terms of washing out organisms. It also changes the microenvironment at the mucosal interface changing the pH and ionic environment, which sometimes may be disadvantageous to micro-organisms. It's suggested, however, that the quite pronounced bicarbonate secretion that occurs in cholera is actually beneficial to the organism and creates a better niche in which to multiply. There may be a good effect, in terms of diluting or flushing out organisms, but on the other hand, you may not survive to see the benefit of that, because you've lost 24L of fluid in 24h. My argument would be, that I'd be willing to lose some of the beneficial effects of the increase flow rate for survival, so as not to become so dehydrated or acidotic.

Dr. Suporn Treepongkaruna: You mentioned prostaglandin production in cholera. What is the mechanism for this? And my second question is what is the mechanism of diarrhea caused by the enteropathogenic E.coli?
Dr. Michael Farthing: I don't think we know exactly what the cell of origin for prostaglandin production in cholera is, but I suspect it's through a cyclic-AMP-driven mechanism; there's evidence that prostaglandins are synthesized by epithelial cells as well as other cells in the submucosa. Enteropathogenic E. coli, adhere very closely to the epithelium. They create pedestals and disrupt the microvilli. There is a loss of surface area in the gut during an infection but they also derrange intracellular function in a number of ways. They disrupt the cytoskeleton and might also have effects on secretory processes within the cell. As far as I'm aware, no secretory toxins have been identified.

Dr. S. K. Mittal: Why has nature decided to put so much 5-HT in the intestine? Does it have a role in normal health, in terms of fluid electrolyte homeostasis?

Dr. Michael Farthing: It does. Every time we eat a meal, there'll be a massive release of 5-HT from the gut as part of the normal physiological response to feeding. It increases secretion and helps create an isotonic environment in the gut, so we can absorb and digest. It promotes gut motility and has been shown to be clearly involved in the peristaltic reflex. Thus it's a normal physiological secretory amine and pro-motility agent.

Dr. S. K. Mittal: In acute giardiasis, it has been described many times there will be very severe watery diarrhea and with the ion electrolyte losses nearing almost childhood cholera. What is the mechanism there in Giardia apart from enteropathy?

Dr. Michael Farthing: I don't think anybody's explained it. We certainly know that once an infection becomes established and particularly one that goes into a chronic phase, there are usually some abnormalities of the villus architecture associated with an inflammatory response. The small intestinal biopsies can look very much like celiac disease.

Dr. Dilip Mahalanabis: One question. What is your opinion on NSP4? Are there any therapeutic or vaccine implications?

Dr. Michael Farthing: NSP4 is an interesting molecule. My own personal feeling is that it's not a major contributor to the diarrheal state. It may be important early in infection but I wouldn't derail any of the current vaccination programs to include this non-structural protein. We know that if you create vaccines to the key structural proteins, you can produce a good protection.