The Role of the Colon

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The colon is an organ that, for long, was considered to be responsible merely for the storage of waste material, and its disposal at a convenient time as feces. Over the past three decades, there has been a re-examination of its function in human physiology, and the colon is now understood to play a significant role in the conservation of fluid and energy by the human body. Within the past decade there has been an explosion of information regarding the mechanisms of nutrient and fluid transport in the colon, which impacts our understanding of their significance and regulation. This chapter will review current information regarding the overall processes of absorption and secretion from the colon, the underlying mechanisms and their regulation in health, and specific alterations in disease. It will also examine the special importance of the colon in rehydrative strategies to combat diarrhea.

OVERVIEW OF COLONIC FLUID AND ION TRANSPORT IN HEALTH

One of the principal physiologic roles of the colon is to absorb fluid and electrolytes. Estimates of colonic absorption in health were initially based on studies of fluid losses from ileostomy patients (1), which suggested that the colon absorbed approximately 300 to 600 ml of water and 40 to 60 mmol of sodium everyday. However, due to small intestinal adaptation, these values underestimated normal colonic absorption. Subsequent measurement of ileocecal flow in healthy adults, using slow marker perfusion, suggested that the normal colon absorbed approximately 1500 ml water, 190 mmol sodium, and 95 mmol chloride every day (2). When perfused in vivo, the colon was able to absorb sodium from solutions with sodium concentration as low as 25 mmol/l (3). By contrast, perfusion studies in humans show that sodium absorption in the jejunum ceased below a luminal sodium concentration of 133 mmol/l, while in ileum it ceased below a concentration of 35 mmol/l (4). Examination of ileostomy effluent and ileocecal aspirates indicates that the luminal sodium concentration in the ileum does not fall below 120 mmol/l, whereas fecal sodium concentrations range from 25 to 49 mmol/l, indicating that the colon has the ability to concentrate the luminal contents and to conserve sodium. The ability of the colon to absorb sodium and water is of physiologic significance.
Subjects with ileostomies are well adapted under normal circumstances, but may become dehydrated when exposed to heat, low sodium diet or during diarrhea. The ability of the colon to respond to aldosterone (5) and to the renin-angiotensin system (6) allows it to further conserve sodium and fluid in the adaptation of the body to dehydration.

Short-chain fatty acids (SCFA)—of which acetate, propionate and butyrate are the most abundant in the colon—are produced in the colon by bacterial fermentation of unabsorbed carbohydrate. These are rapidly absorbed from the colon, but in addition to their own absorption they also stimulate sodium absorption from the colon. Enhancement of sodium absorption in the human colon by SCFA has been shown using both in vivo perfusion of the colon (7), and in a system where the intact entire human colon was perfused in vitro (8). In the latter, SCFA increased sodium absorption approximately fivefold over control, but the increase during in vivo perfusion was more modest. Thus, SCFA-linked sodium absorption is an important mechanism of ion transport in the colon. Over the past decade, there has been considerable advance in our knowledge of the mechanisms and regulation of SCFA-linked sodium absorption from the colon.

CELLULAR AND MOLECULAR BASIS FOR COLONIC ELECTROLYTE TRANSPORT

Sodium absorption from the colon occurs as a result of active transport. Active processes also exist for chloride absorption and for bicarbonate secretion in the human colon. The bulk of potassium movement in the colon is probably passive and potential-dependent, but active processes for both absorption and secretion contribute to overall transport. Fluid movement in the colon is passive, and its overall direction and magnitude depends on the balance between the absorptive and secretory processes. The proteins involved in active transport of sodium and other ions from the colon have recently been characterized (9). Elucidation of these processes at the membrane level has been supplemented by molecular studies that give a clearer understanding of their regulation. There is considerable heterogeneity across animal species in the importance of individual absorptive mechanisms in the colon. Further, there are regional differences in transport processes within the colon, with different mechanisms assuming importance in the proximal and distal colon (10). The processes responsible for active ion transport are generally presumed to have a spatial distribution along the crypt-surface axis of the colon, with secretory processes being localized to the crypt epithelium, and absorptive processes to the surface epithelium. However, such a distinction may not be absolute, and has been challenged by the recent findings that isolated colonic crypts can absorb fluid (11), and that active chloride secretion can also originate from the surface epithelium (12,13).

Active sodium absorption is the basic process that drives water absorption from the colon. It is dependent upon the presence of Na\(^+\),K\(^+\)-ATPase in the basolateral membrane of colonic epithelial cells, which actively pumps sodium out of the cell
lowering intracellular sodium concentration. This process, which involves expenditure of energy, provides the driving force for sodium uptake from the lumen of the colon into the cell across the apical membrane. Sodium movement across the tight junctions of the colon (i.e., passive absorption) is minimal under basal conditions. Sodium absorption may be accompanied by chloride absorption, consisting of entry of chloride into the cell via apical transporters, and its exit across the basolateral membrane via Cl$^-$ channels or Cl$^-$-HCO$_3^-$ exchange. Water absorption from the colon is believed to be passive, and in the colon is largely secondary to sodium absorption. Water movement may either occur through paracellular pathways or through the cells via aquaporin (AQP) water channels present in both luminal and basolateral membranes (14). The role of the several different colonic AQP in the absorption of water continues to be explored. In knockout mice lacking AQP4, colonic osmotic water permeability was reduced by 50%, but theophylline-induced secretion was not impaired (15). On the other hand, in AQP3 knockout mice, intestinal water transport was essentially unaffected (16).

Sodium entry into the cell occurs across the apical membrane via sodium channels (electrogenic absorption) or via Na$^+$-H$^+$ exchange (electroneutral absorption). The major transporter responsible for electronegenic sodium absorption is the epithelial sodium channel, ENaC, which is expressed in the apical membrane of surface epithelial cells in the colon. ENaC consists of three different subunits—α, β, and γ—and is sensitive to inhibition by low concentrations (<10$^{-4}$ M) of amiloride. Electroneutral sodium absorption occurs through the Na$^+$-H$^+$ exchangers (NHE), of which three isoforms are expressed in the colon. NHE1 is expressed in the basolateral membrane, and has a role in intracellular pH regulation rather than in sodium absorption. NHE2 and NHE3 are both expressed on the luminal membrane of colonic epithelial cells and are both involved in active sodium absorption. Differences in expression of NHE2 and NHE3 in the colon may underlie inter-species differences in sodium absorptive pathways (17). Yet another NHE has been identified in the apical membrane of crypt epithelial cells, which is Cl$^-$ dependent (18). Bicarbonate and SCFA can both independently stimulate electroneutral sodium chloride absorption from the colon (19). HCO$_3^-$-dependent NaCl absorption appears due to the coupling of Na$^+$-H$^+$ with Cl$^-$-HCO$_3^-$ exchange, brought about by change in the intracellular pH. This process is inhibited by high concentrations of amiloride (10 to 3 M or higher) and by cyclic adenosine-3',5'-monophosphate (cAMP). HCO$_3^-$-dependent NaCl absorption probably involves NHE3 because this transport process is absent in epithelia that lack NHE3 (17). Inhibition of HCO$_3^-$-dependent NaCl absorption by cAMP appears to depend on the presence of an NHE regulatory factor (NHERF) that interacts with NHE3. The role of NHERF has not been directly demonstrated in colonic tissue, but has been inferred from studies in other epithelia (20). On the other hand, SCFA-linked NaCl absorption is a complex process that occurs through the simultaneous operation of three different ion exchangers in the luminal membrane of the colonocyte (Fig. 1). These are Na$^+$-H$^+$ exchange, SCFA$^-$.HCO$_3^-$ exchange that allows entry of SCFA$^-$ into the cell with movement of HCO$_3^-$ into the lumen, and SCFA$^-$.Cl$^-$ exchange that recycles SCFA$^-$ into the lumen in exchange for Cl$^-$ (21). The net
Conceptual diagram of active Na\(^+\) and Cl\(^-\) transport in mammalian colonic epithelium. Possible transporters involved are shown, but not all are present in the same cell (e.g., rabbit distal colon lacks Na\(^+\)—H\(^+\) exchange and Cl\(^-\)—HCO\(_3^-%\) exchange, while rat distal colon lacks Na\(^+\) channel). Na\(^+\)—H\(^+\) exchange may be coupled to Cl\(^-\)—HCO\(_3^-%\) exchange, or independently coupled to SCFA\(^-\)—HCO\(_3^-%\) and Cl\(^-\)—SCFA\(^-\) exchanges (Rajendran and Binder, 1994). Butyrate absorption into the cell may occur via non-ionic diffusion, and dissociation of butyrate within the cell may increase intracellular H\(^+\) ([H\(^+\)]) with stimulation of Na\(^+\)—H\(^+\) exchange. Butyrate—HCO\(_3^-%\) exchange (Mascolo et al., 1991) across the apical membrane will also increase [H\(^+\)] and stimulate Na\(^+\)—H\(^+\) exchange across the apical membrane. Increased [H\(^+\)] will also inhibit Na\(^+\) absorption via Na\(^+\) channels (Chaffant et al., 1999). Cl\(^-\) secretion involves Cl\(^-\) channels at the apical membrane, and Na\(^+\)—K\(^+\)—2Cl\(^-\) cotransporter activity and sodium pump activity in the basolateral membrane. Inhibition of Cl\(^-\) secretion by butyrate may occur at any of these levels. Butyrate also inhibits cAMP generation by colonic epithelium (Krishnan et al., 1999). *Potential levels at which butyrate may potentiate (+) or inhibit (−) processes that eventually affect net Na\(^+\) transport. From Vidyasagar et al. (17).
result of these three exchanges is that Na\(^+\) and Cl\(^-\) move into the cell in exchange for H\(^+\) and HCO\(_3^-\). Unlike HCO\(_3^-\)-dependent NaCl absorption, SCFA-linked NaCl absorption is not inhibited, and may in fact be upregulated, by cAMP (17,22). The current evidence indicates that SCFA-linked sodium absorption may use either NHE2 or NHE3, depending on the specific tissue being examined (17,23). Studies using human colon suggested that about half of the active sodium transport in the proximal as well as distal colon was attributable to electroneutral NaCl absorption (24,25). In the distal colon, the remaining half occurred through amiloride-inhibitable sodium channels. The nature of the process accounting for the remaining sodium absorption from the proximal colon remains unclear, but amiloride-resistant sodium channels may be involved. These studies were carried out before the recognition of SCFA-dependent NaCl absorption, and the contribution of the latter process in quantitative terms to sodium and chloride absorption from the normal human colon is unclear.

Chloride entry across the luminal membrane occurs through Cl\(^-\)-HCO\(_3^-\) exchange and Cl\(^-\)-OH\(^-\) exchange mediated through the proteins anion exchanger type 1 (AE1) and DRA (down-regulated in colonic adenoma) (26,27). Basolateral anion exchange occurs through AE1 and AE2. Basolateral exit of chloride from colono-cytes into the bloodstream may also involve Cl\(^-\) channels and a KCl cotransporter. Active transport mechanisms for potassium absorption exist in the colon, but in health, the bulk of potassium transport occurs passively, in a potential-dependent manner. Active absorption occurs through two K\(^+\),H\(^+\)-ATPases, one that is ouabain-sensitive, and the other which is insensitive to ouabain (28,29). The former occurs in surface epithelium, while the latter is present in both surface and crypt epithelium. Potassium that is taken up from the luminal side is released to the blood side through K\(^+\) channels or KCl cotransport.

Absorptive mechanisms have been examined until now, and this section will briefly describe secretory mechanisms that exist in the colon. Under resting conditions, there is limited secretion from the colonic mucosa, which consists of KCl secretion into the lumen. When exposed to bacterial enterotoxins or other secretagogues, this becomes a pronounced secretion of both KCl and NaCl. As in the case of ion absorption, the polarized distribution of transport proteins accounts for secretory processes. Secretory cells contain potassium and chloride channels in their luminal membranes, leading to KCl secretion. Active chloride secretion by the colono-cytes is accompanied by passive paracellular movement of sodium from the serosal side to the lumen, leading to secretion of NaCl. The chloride channel in the luminal membrane is formed predominantly by the cystic fibrosis transmembrane conductance regulator (CFTR) protein (30). On their basolateral membranes, secretory cells contain Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporters (NKCC) that take up chloride from the serosal side together with sodium and potassium. Potassium recycles via basolateral K\(^+\) channels, while Na\(^+\),K\(^+\)-ATPase recycles Na\(^+\) across the basolateral membrane and provides the active driving force for secretion (31). The basolateral K\(^+\) channels (cAMP- or Ca\(^2+\)-activated) are important in maintaining a hyperpolarized
membrane voltage to provide the electrical driving force for Cl\textsuperscript{−} secretion. Loperamide, a widely used anti-diarrheal drug, has been shown to block basolateral K\textsuperscript{+} channels in colonocytes, possibly explaining its anti-diarrheal effect (32). A number of different secretagogues, acting through diverse second messengers, activate colonic NaCl and KCl secretion. The NKCC type I is present in the colon, and is controlled by intracellular chloride, phosphorylation and by the actin cytoskeleton. This transporter is crucial to chloride secretion, and is inhibited by the loop diuretic bumetanide. CFTR, which is the chloride channel that is defective in cystic fibrosis, is the major chloride channel involved in both cAMP and Ca\textsuperscript{2+}-activated secretion in the adult human colonic mucosa (33). Neither secretagogue evokes secretion in mice without functional CFTR (34). It is activated predominantly by protein kinase A (PKA), but also by other pathways including protein kinase C, Ca\textsuperscript{2+}-calmodulin-dependent kinase, and cGMP-dependent kinase. CFTR also acts as a regulator of other ion conductances participating in colonic electrolyte transport. In mouse models of cystic fibrosis, the rotavirus toxin NSP4, which induces Ca\textsuperscript{2+}-mediated chloride secretion and severe diarrhea in infants and young animals, does so even in CF knockout mice (35). This suggests the presence of non-CFTR Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels (CACC), which are expressed in an age-dependent manner. A number of other Cl\textsuperscript{−} channels have been described in the colon, including intermediate conductance outwardly rectifying Cl\textsuperscript{−} channels, the CIC family of Cl\textsuperscript{−} channels, and endogenous antimicrobial peptides or cryptdins that seem to be able to form chloride channels in colonic epithelial cells (9,36). The role of each of these types of chloride channel in human disease, their role in developing human fetal or infant colon, and their possible role in pathogenesis of diarrhea caused by toxins or enteric viruses all need to be clarified.

Potassium secretion occurs through K\textsuperscript{+} channels in the apical membrane, which appear to be induced by aldosterone, cAMP, and epinephrine. The effect of aldosterone seems to be restricted to the surface epithelium, while cAMP-mediated potassium secretion is located equally in crypts and surface epithelium (37). In addition, carbachol-induced potassium secretion has been described. Potassium secretion becomes clinically important in acute or chronic secretory diarrhea involving the colon.

Bicarbonate secretion also occurs in the colon in close association with chloride secretion. This process is responsible for maintaining the acid microclimate of the mucosal surface in the colon. Bicarbonate is taken up into the colonocyte through Na\textsuperscript{+}-HCO\textsubscript{3}\textsuperscript{−} cotransporters on the basolateral membrane, the existence of which is currently only inferred. It is also generated within cells from CO\textsubscript{2} produced during oxidative metabolism. It may exit the cell into the lumen through Cl\textsuperscript{−}-HCO\textsubscript{3}\textsuperscript{−} exchange, Cl\textsuperscript{−} channels or SCFA\textsuperscript{−}-HCO\textsubscript{3}\textsuperscript{−} exchange. In addition to basal secretion, bicarbonate secretion can be induced by cAMP, cGMP and Ca\textsuperscript{2+}. Induced bicarbonate secretion is generally bumetanide-resistant (38), signifying that ion flux through the basolateral NKCC is not involved. However, studies in isolated perfused colon crypts suggest the existence of bumetanide-sensitive bicarbonate secretion, which appears to occur primarily via an apical membrane Cl\textsuperscript{−} channel, and not through Cl\textsuperscript{−}-HCO\textsubscript{3}\textsuperscript{−} exchange (39).
THE ROLE OF THE COLON

Physiologically, the most important regulatory mechanism of sodium absorption from the colon is its response to aldosterone, which increases electrogenic sodium absorption. Since the principal function of colonic absorption is to conserve salt and water, the aldosterone effect becomes clinically significant under conditions of negative sodium balance. Glucocorticoid and mineralocorticoid receptors are present on both surface and crypt epithelial cells of proximal and distal colon. These steroids exert differential effects on sodium absorption in the colon (9,40). Low-dose glucocorticoids induce electroneutral sodium absorption, while high doses induce both electroneutral and electrogenic absorption, especially in the distal colon. By contrast, aldosterone inhibits electroneutral sodium absorption and induces electrogenic sodium absorption, by activating the transcription of ENaC β and γ subunits, the α subunit being expressed constitutively (41). Early aldosterone action (1 to 3 hours) is mediated by the enzyme serum and glucocorticoid regulated kinase (SGK) (42), while its late effects are mediated by induction of ENaC and upregulation of the activity of Na⁺,K⁺-ATPase. Angiotensin II enhances electroneutral sodium absorption from the colon of experimental animals (6). Sodium depletion down-regulates both NHE2 and NHE3 isoforms in the distal colon, but seems to upregulate them in the proximal colon (43). The Cl⁻-dependent NHE is also upregulated by sodium depletion (18).

A variety of other regulatory mechanisms influences colonic sodium absorption. Changes in the intracellular concentrations of sodium and chloride during absorption negatively regulate the activity of ENaC (44). This negative regulatory mechanism appears to be defective in Liddle’s disease (45). Electroneutral sodium absorption may also be acutely regulated in response to some G protein-linked receptors, tyrosine kinase-coupled receptors, and protein kinases. Activation of protein kinase C, Ca²⁺/calmodulin-dependent kinase, and cAMP inhibit NHE3, while stimulation of adrenergic α1 or β2 receptors activates NHE3 (9,20). There is some controversy with regard to the effect of cAMP on sodium channel activity. In the human and rat colon, activation of chloride secretion by cAMP was paralleled by inhibition of amiloride-sensitive sodium transport indicating inhibition of ENaC (9,46). However, ENaC from guinea pig colon was activated by cAMP (47). Stimulation of purinergic receptors by extracellular nucleotides is known to inhibit electrogenic sodium absorption, but functional studies of human colon have not found any evidence for purinergic receptors in man (9).

The regulation of colonic secretion occurs through endocrine, paracrine, autocrine, immunologic and neuronal stimuli. In addition, luminally derived compounds including food-derived components, bile acids, and bacterial toxins, may induce secretion. Secretagogues include acetylcholine, vasoactive intestinal polypeptide, PGE2, leukotrienes, bradykinin, and several other hormones. The secretory effect of hormones and neurotransmitters is balanced by the inhibitory effects of neuropeptides, endogenous opioids, norepinephrine, growth hormones, and others, which reduce intracellular cAMP levels or act via phosphatidylinositols (33). There is extensive cross-talk
between these various regulators of secretion. Secretion is elicited via increases in cAMP, cGMP, Ca\(^{2+}\), and also other mediators such as diacylglycerol and PKC. Of the important neurotransmitters, VIP acts through cAMP, while acetylcholine acts through Ca\(^{2+}\). cAMP primarily targets PKA, and may also directly affect CFTR. CFTR chloride channels are activated by PKA-dependent phosphorylation and binding of ATP (48). Exocytosis of CFTR from an intracellular pool may also contribute to cAMP-dependent activation of Cl\(^{-}\) secretion in the colonic mucosa. Simultaneously, there is inhibition of Na\(^{+}\) absorption via NHE3. As noted previously, inhibition of ENaC by CFTR in the presence of cAMP (a phenomenon not observed in all species) completes the picture, and converts the absorptive cell into a secretory cell. cGMP induces Cl\(^{-}\) secretion by stimulation of CFTR through protein kinase G type II. Nitric oxide increases intracellular cGMP in the intestine and leads to secretion (49), but a similar role for the colon has not yet been described. Ca\(^{2+}\) activates colonic secretion due to activation of basolateral K\(^{+}\) channels, and has not been clearly shown to activate CFTR or other Cl\(^{-}\) channels (50). Adenosine nucleotide-mediated intestinal secretion is known, but the importance of this pathway in the colon remains to be demonstrated. Adenosine A2b receptors have been shown on both poles of T84 colon cancer cells, and may be activated to increase intracellular cAMP (51). Proteinase-activated receptor type 2 (PAR2) has recently been shown to be present on the basolateral aspect of human colon epithelium and activation of these receptors by tryptic enzymes may activate increase in intracellular Ca\(^{2+}\) with chloride secretion. This type of secretion may be important in inflammatory bowel disease (52). SCFA have been shown to inhibit secretion induced in the colon by cAMP and by cGMP, both in vivo and in vitro (17,53–56). The basis of this inhibition remains to be understood, but this finding has clinical application in the management of diarrhea in enteric infections.

In addition to the various influences discussed previously, there are also proabsorptive neuroendocrine stimuli such as opiates and enkephalins that act to stimulate absorption of ions and fluid under appropriate conditions. Considerable information is available on these pathways and stimuli with regard to the small intestine (57), but relatively little information is available regarding their presence or importance in the colon. Racecadotril or acetorphan, an enkephalinase inhibitor that raises endogenous enkephalin levels in the intestine, has found use as a proabsorptive agent that is effective in the treatment of diarrhea (58), and is believed to act mainly by enhancing small intestinal absorption of fluid.

**ALTERATIONS OF COLONIC FLUID AND ELECTROLYTE TRANSPORT IN DISEASE**

Altered colonic transport of fluid and electrolytes is an important aspect of the pathophysiology of several diseases affecting man, and knowledge of the specific alterations and their causation will help to target therapy. Particularly in the colon, such therapy may include dietary manipulations, since colonic function is influenced
to a considerable extent by luminal factors including dietary constituents. This section will detail specific alterations in colonic fluid and electrolyte transport that have been noted in disease.

**Acute Diarrhea**

**Cholera**

Cholera is considered the prototype toxigenic diarrhea, and cholera toxin (CT) the prototype enterotoxin. The effects of CT on intestinal absorption and secretion through increased intracellular cAMP are well known. CT directly affects ion transport in the colon, inducing net secretion of water and potassium and grossly reducing net absorption of sodium and chloride in experimental animals. Perfusion of the colon in adults with cholera has indicated a significant defect of colonic fluid and ion absorption in these patients (59). Studies in animals in vivo indicated that a considerable proportion of the intestinal secretion induced by cholera toxin was due to its effect on local neural reflexes, but the entire pathway has been elucidated only in recent years. The reflex appears to be set off by release of 5-HT from enterochromaffin cells, which stimulate 5-HT3 and/or 5-HT4 receptors on a cholinergic afferent neuron, with the reflex then passing through an interneuron with substance P as the neurotransmitter, and a VIPergic efferent neuron, to release VIP, which in turn activates adenylate cyclase in the enterocyte (57). The importance of neural reflexes in the induction of colonic secretion by cholera toxin was demonstrated recently by a study in which instillation of cholera toxin into a ligated ileal loop induced colonic secretion (60). *Vibrio cholerae* also produces other enterotoxins including accessory cholera enterotoxin and zonula occludens toxin, which are both known to alter transport properties of the epithelium in the small intestine. However their effect on the colon, as well as their contribution overall to the fluid secretory diarrhea of cholera, remains unknown.

**Rotavirus Diarrhea**

There are no direct studies on colonic absorption in rotavirus diarrhea. Data on colonic function is available from an early study in pigs infected with transmissible gastroenteritis virus, the counterpart of the human rotavirus in pigs. In this study, it was noted that infant animals less than 3 days old often had clinical diarrhea, while older animals did not have diarrhea despite abnormal handling of fluid by the small bowel in both age groups (61). In the immature animals, fluid malabsorption was noted in both small and large intestine, while the older animals manifested fluid malabsorption only in the small intestine, but absorbed fluid normally in the large intestine. This was accompanied by a failure of colonic fermentation in the young animals, while older animals fermented carbohydrate normally to SCFA in the colon. It was concluded that rotavirus diarrhea resulted in young animals from a deficiency in their capacity to ferment carbohydrates to SCFA in the colon. Interest in the
pathogenesis of rotavirus diarrhea has been rekindled by the recent demonstration of an enterotoxin (NSP4) produced by this virus (35), as well as the finding that neural reflexes are important in causing the fluid secretion and diarrhea of rotavirus infection (62). The role of the colon in secretion induced by NSP4 or by local neural reflexes has not been examined.

**Shigellosis**

Colonic fluid and electrolyte movement is altered in experimental shigellosis. Infection of monkeys with *Shigella flexneri* 2a resulted in net colonic malabsorption or secretion, with evidence of severe histologic damage (63). More recently, perfusion studies in adults with *Shigella* dysentery indicated that ileocecal flow rates were normal in this illness, while perfusion of the colon indicated minimal net water secretion, 2.4 ml/hour, accompanied by diminished net absorption of sodium and chloride, associated with net secretion of potassium and bicarbonate (64). A number of putative mechanisms could explain the decrease of colonic absorptive capacity in shigellosis, including epithelial cell damage, and the effects of cytokines and mucosal inflammation. Shiga toxin, which interferes with ribosomal protein synthesis, targeted the crypt cells of the rat distal colon and increased rather than decreased active NaCl absorption (65). The effect on the colon of *Shigella* enterotoxin, which is also produced by these organisms (66), is unknown.

**Salmonellosis**

Colonic fluid and electrolyte movement is altered in monkeys infected with *Salmonella typhimurium*, and net fluid secretion is found in association with histological colitis (67). As in shigellosis, it is not clear whether the colonic dysfunction results from the effects of mucosal inflammation or from toxins elaborated by the organism.

**Clostridium difficile Colitis**

There is no direct information available regarding colonic absorptive function in colitis caused by *Clostridium difficile*. Of the toxins produced by this organism, toxin A has been shown to cause secretion in the rat colon in vivo (68). Secretion occurred after a long latent period and was accompanied by shedding of surface, but not crypt epithelial cells. Thus, diarrhea could have been due to fluid malabsorption from the colon secondary to damage to surface epithelial cells. The toxin is known to induce dysfunction of the tight junctions after relatively brief exposure (69), but the role of this in altering colonic absorption remains unclear.

**Other Diarrheal Conditions**

Heat-stable enterotoxin (STa), produced by enterotoxigenic *Escherichia coli* responsible for traveler's diarrhea, reduces net water absorption in the colon (70).
Infection with enterohemorrhagic *E. coli* in rabbits induced mucosal changes and inhibited sodium absorption in the colon (71). The role of the enteric nervous system in mediating colonic secretion due to intestinal infection has been further investigated recently. Galanin is found in nerve terminals in the enteric nervous system, and galanin receptors (GALR) are found on enterocytes. Chloride secretion is initiated when colonocytes are stimulated with galanin. It has been shown that bacterial enteropathogens including *S. typhimurium* and *S. flexneri* may mediate colonic secretion by upregulating GALR expression in colonocytes, and that the secretion induced by these organisms could be completely attenuated by eliminating the GALR gene (72).

**Inflammatory Bowel Disease**

Diarrhea is the major symptom of the inflammatory bowel diseases (IBD)—ulcerative colitis and Crohn’s disease. Diarrhea may result from one of several reasons, including fluid malabsorption caused by reduced absorptive area, enhanced secretion by inflammatory mediators, and a leak flux due to an impaired epithelial barrier. The changes in ulcerative colitis have received considerable attention. Electrophysiologic studies have shown a major defect in electrogenic sodium absorption from the involved colon. Recent molecular studies suggest that this is due to reduced activity of apical sodium channels in colonocytes, due to reduced ENaC β and γ subunit expression secondary to inflammation (73). In addition, there is reduced activity of Na⁺,K⁺-ATPase due to reduced expression of the α1 subunit of this protein. Mucosal inflammation has other effects that may impact on colonic transport. Colonocyte expression of the transporter DRA is reduced during intestinal inflammation, which is likely to result in a reduced Cl⁻-HCO₃⁻ exchange and diarrhea (74). Alterations in tight junctions with increased permeability, seen in inflammatory diseases of the colon, are likely to contribute to barrier dysfunction and diarrhea (75). Increased expression of galanin receptors on colonocytes in experimental colitis may reflect a susceptibility to local neural reflexes that induce colonic secretion (76). Tryptases released from mast cells in IBD may provide an additional pathway for colonic secretion by activating PAR2 on the basolateral aspect of colonocytes (52).

**Collagenous Colitis**

Collagenous colitis, a rare cause of secretory diarrhea, is characterized by the presence of a continuous subepithelial collagenous layer that is 25- to 60-μm thick in the colorectal mucosa. Perfusion of the colon in these patients demonstrated net fluid secretion, which was attributed to mucosal overexpression of inducible nitric oxide synthase, with the excessive release of nitric oxide in the lumen (77). Nitric oxide production and fluid secretion could be significantly reduced by topical treatment with N(G)-monomethyl-L-arginine.
Microscopic Colitis

Microscopic colitis is a condition where the colonic mucosa shows diffuse mucosal inflammation in the absence of other evidence of inflammatory bowel disease. Perfusion of the colon of these subjects has demonstrated a defect of net water, sodium, and chloride absorption with net bicarbonate secretion (78). Colonic absorptive properties in these patients differ from ulcerative colitis in that mucosal potential difference was normal and that mucosal permeability was decreased (in contrast to the increased permeability observed in ulcerative colitis). The pathogenesis of these abnormalities remains to be investigated.

Malabsorption Syndromes

Diarrhea is the most common symptom of the malabsorption syndromes, and is secondary to colonic malabsorption and secretion of fluid caused by the action of unabsorbed bile acids and free fatty acids. Hydroxy fatty acids produced by bacterial action on unabsorbed fatty acids in the colon have been shown to induce colonic fluid secretion (79). In southern Indian patients with tropical sprue, water malabsorption in the colon was demonstrated by perfusion of the colon (80). Hydroxy fatty acids were not elevated in the feces of patients with tropical sprue. Fluid malabsorption in the colon of these patients correlated well with the fecal excretion of unsaturated free fatty acids, and was associated with reduced activity of mucosal Na⁺,K⁺-ATPase in the colon (81,82). Strategies to bind free fatty acids in the lumen of the colon are likely to prevent colonic water malabsorption and reduce severity of diarrhea. Medium chain triglycerides are often substituted for long chain triglycerides in the dietary management of patients with malabsorption and short bowel syndrome. It has recently been demonstrated that medium-chain fatty acids are very well absorbed from the colon (83,84), and this introduces a rational basis for the use of such fats in short bowel syndrome.

Enteral Feeds

Diarrhea is a common complication of enteral feeding. Using segmental perfusion of the colon, net secretion of water, sodium, and chloride was noted in the ascending colon of patients in whom low or high load polymeric diets were infused by tube either into the stomach or into the duodenum (85). Net secretion was also noted in the distal colon of patients in whom a high load polymeric diet was infused into the stomach. Infusion of an SCFA solution into the cecum reversed the net secretion to net absorption (86). This observation may be of significance in considering the delivery of newer oral rehydration solutions, such as the energy-dense liquefied meals.

Congenital Chloride Diarrhea

This rare autosomal recessive diarrheal disorder is characterized by profuse watery diarrhea with a high stool chloride concentration (>150 mol/l). A defect in mucosal...
chloride transport has been noted in the ileum and colon of these children (87). Colonic perfusion studies suggested an absence of apical chloride-bicarbonate exchange in the affected segment of bowel. Defective bicarbonate secretion probably results in acidification of the colonic lumen with consequent impairment of sodium-hydrogen exchange. The disease has now been shown to be caused by a mutation in the gene for DRA, the Cl\(^-\)-HCO\(_3\)^- exchanger of the colonocytes (88).

FERMENTATION AND THE CONTRIBUTION OF THE COLON TO ENERGY CONSERVATION

The colon harbors a complex assemblage of microorganisms, which endows great metabolic potential on the large intestine, primarily through its degradative abilities. This bacterial flora behaves as an integral part of the colon in its metabolic activity, and must be considered in any examination of the role of the colon in the utilization and absorption of fluid and nutrients. Fermentation of unabsorbed carbohydrate and protein is one of the major functions of the human colon. This function helps the host to obtain energy from dietary constituents, principally from complex carbohydrates that cannot be hydrolyzed or absorbed in the upper gastrointestinal tract. The rich and diverse anaerobic bacterial flora of the colon breaks down these unabsorbed dietary and endogenous constituents and aids the host in the conservation of energy and nutrients. The principal substrates for utilization by these bacteria are starch and non-starch polysaccharides (89,90). Breakdown of these substrates occurs through an anaerobic process called fermentation. Carbohydrates and proteins are broken down through a variety of intermediates to SCFA, various gases which are either absorbed or excreted in flatus, to ammonia which is either absorbed or used by bacteria for protein synthesis, and to other molecules such as branched chain fatty acids, amines, phenols and other organic acids. SCFA are important in various ways, the bulk of them being absorbed and used for energy production in the liver and other peripheral organs. SCFA are the major source of energy for the colonic epithelial cells or colonocytes, and contribute to lipid and protein synthesis within these cells. Lactate is a major fermentation product of bifidobacteria, streptococci, and lactobacilli. Some gut species such as the propionibacteria can use lactate by the succinate pathway producing propionate and acetate. A number of putatively toxic metabolites, including phenols, indoles, and amines, are produced during protein breakdown in the colon. Production of these noxious substances can be inhibited or repressed by the presence of fermentable carbohydrate in the colonic lumen. In addition, many of these metabolites are detoxified either by the colonocytes or in the liver.

In the context of food and fluid intake, the fermentation products of greatest interest are those arising from carbohydrate fermentation, i.e., SCFA, of which acetate, propionate, and butyrate are the most abundant in the colon. Non-starch polysaccharides (traditionally termed as dietary fiber) and amylase-resistant starch (RS) are not digested in the small intestine, and are the major carbohydrates to reach the colon where they can be fermented. The RS content of many cereals is high, but the highest proportion of RS is found in green bananas and in certain varieties of
maize or corn (90-93). In developing countries with a high intake of starchy foods, the amount of RS available for colonic fermentation is likely to be considerable. The proportions of individual SCFA produced during fermentation depend to an extent on the type of carbohydrate being fermented. Starch fermentations typically are characterized by high levels of butyrate production, whereas more acetate is produced from a more oxidized substrate such as pectin. Interactions between different species of bacteria within the colon also determine the nature of the fermentation product. Lastly, the rate at which a substrate can be broken down to its constituent sugars also affects the end products of fermentation.

SCFA are rapidly absorbed from the colon. At the concentrations found in the healthy colon, absorption probably takes place by unionized diffusion although active mechanisms exist for SCFA absorption. Bicarbonate secretion into the lumen always accompanies SCFA absorption, and occurs in the absence of luminal chloride. It is likely that the luminal bicarbonate appearance is due to SCFA-bicarbonate exchange, a process that has been demonstrated in vitro (94). Fasting portal vein concentration of total SCFA is approximately 150 μmol/l, and increases to 380 μmol/l in the fed state (89). While the ratio of the three major SCFA in the colonic lumen is acetate : propionate : butyrate 57 : 22 : 21, in the portal vein the ratio changes to 71 : 21 : 8. This indicates that most of the butyrate and a small amount of the propionate absorbed are utilized within the colonic mucosa. Indeed, more than 70% of oxygen consumption by isolated colonocytes in vitro is due to butyrate metabolism (95). Carbon dioxide production from butyrate is similar in proximal and distal colon of man, but ketone body production is less in the proximal colon, implying that more butyrate enters the tricarboxylic acid cycle in the distal colon. Besides being the major source of energy for colonocytes, butyrate is also used in the synthesis of lipid and protein by colonocytes. In addition, butyrate regulates growth and differentiation of the colonic mucosa, probably by effects on nucleic acid metabolism (96). Total SCFA concentration in the hepatic vein is only 40% of that in the portal vein, indicating significant hepatic clearance of all three SCFA. Propionate is largely cleared by the liver in man, but its metabolic role remains unclear, although it serves as a gluconeogenic precursor in ruminants (89). Propionate may lower LDL cholesterol and raise HDL cholesterol levels in serum. Free acetate is present in peripheral blood, and is metabolized by cardiac and skeletal muscle and brain. Acetate spares fatty acid oxidation in man. It is a valuable fuel for the tissues and is oxidized as free fatty acids. During periods of low production of acetate from the gut, it can also be endogenously synthesized in liver and muscle. Thus, fermentation in the colon provides fuels for a wide range of body tissues and may be important in a number of metabolic events. It has to be realized that fermentation with metabolism of SCFA is inefficient as a means of energy conservation compared to absorption and metabolism of glucose. A factor of 2 kcal/g of measured dietary fiber has been suggested as the appropriate conversion factor to determine the energy contribution of fiber (97), which compares with the standard Atwater factor of 4 kcal/g total carbohydrate. Western diets probably contain about 30 to 35 g of fermentable carbohydrate per day, consisting of non-starch polysaccharides 12 to 20 g, resistant starch
0 to 40 g, sugars and sugar alcohols 2 to 10 g, oligosaccharides 2 to 6 g, and other substrates (98). Fermentation of unabsorbed carbohydrate may account for only 5% or less of the total daily energy needs in man in the Western world. However, in tropical developing countries with a high intake of cereal-based starchy foods containing resistant starch and a high intake of fruits and vegetables containing non-starch polysaccharides, the contribution of colonic fermentation could be much more significant. In such populations colonic fermentation may, theoretically, liberate up to 540 kcal per day and contribute up to 10% of the daily energy requirements of the host (99). This contribution is of significance in chronically energy deficient populations. Fermentation in the colon is probably also of physiological significance in children and infants, and fermentation of unabsorbed lactose to acetate has been shown in preterm infants, with potential effect on energy and protein requirements (100).

Colonic fermentation may be altered in a number of disease states (101,102). Fermentation may be altered by the administration of antibiotics, due to changes in the bacterial flora, and may predispose to the development of antibiotic associated diarrhea. Surgical diversion of the colon may reduce SCFA generation and predispose to mucosal inflammation in the bypassed colon. SCFA have also been proposed as therapy for a number of diseases including ulcerative colitis, radiation colitis, and pouchitis, but without clear or consistent benefit. Altered colonic fermentation may prolong the course of acute diarrheal illness, and will be discussed in greater detail in the following section.

THE ROLE OF THE COLON IN DIARRHEA AND ORAL REHYDRATION

The central role of colonic absorption in conserving salt and water and in dehydrating the feces has been discussed. Acute diarrheal disease is characterized by an excessive loss of water in feces, leading to dehydration and electrolyte imbalance in untreated patients with severe diarrhea. Mortality from acute diarrhea continues to be moderately high in some developing countries, despite the availability of oral rehydration therapy (ORT). ORT was based on the recognition that glucose stimulates active sodium absorption from the small intestine, and that this process (sodium-glucose cotransport) was not susceptible to inhibition in infective diarrhea. ORT revolutionized the treatment of diarrhea, reducing the mortality of diarrhea by over 90% and saving millions of lives (103). Despite the success of glucose-ORT in preventing deaths from dehydration in diarrhea, it did not significantly reduce either magnitude or severity of diarrhea. Paradoxically, glucose ORT would sometimes increase fecal fluid loss in a treated patient. This led to attempts to improve the absorptive characteristics of oral rehydration solutions, using other substrates such as amino acids, which would augment small intestinal absorption of sodium. The greatest advance in this area was the introduction of hypo-osmolar oral rehydration
solutions, which significantly increased small intestinal absorption and reduced diarrhea (104-106). This section will briefly detail evidence that suggests that the absorptive characteristics of the colon may be advantageously exploited to achieve further reductions of fecal fluid loss in diarrhea.

The colon has a large capacity to absorb fluid, over and above that usually required in health. The importance of this absorptive capacity of the colon can be illustrated by examination of the intestinal fluid fluxes that occur in health and in cholera, the prototype of toxigenic diarrhea. Early studies in cholera, comparing ileocecal flow with fecal output, suggested that the colon would be able to absorb up to approximately 6 l of fluid per day when stressed with a high fluid inflow (107). In studies in Western volunteers, large volumes of fluid were continuously infused into the colon and the absorptive capacity of the colon, extrapolated from these studies, was approximately 5,700 ml water, 816 mmol sodium, and 44 mmol potassium per day (108). In the same studies, infusion of the secretagogue, chenodeoxycholic acid, reduced the capacity of the colon to absorb fluid. The colon was therefore able to absorb approximately 250 ml of fluid per hour, and indeed delivery of 500 ml fluid into the colon over 60 minutes resulted in loose stool. Data obtained by perfusing the colon of healthy volunteers in southern India with normal saline at a flow rate of 10 ml/min (80) could be extrapolated to indicate a maximal absorptive capacity of the colon of approximately 3 liters of fluid per day. The calculated maximal sodium absorption from this study was 760 mmol/day, and of chloride 1,200 mmol/day. The lower maximal absorptive capacity of the colon in healthy Indians compared to Western data may possibly be explained on the basis of epithelial cell dysfunction in the tropics; i.e., a tropical colonopathy (109). The solution used to perfuse the colon in the latter studies was not very physiological, and in particular did not contain SCFA, which are a powerful stimulus to sodium and water absorption from the colon.

The concept of the maximal absorptive capacity of the colon is shown in Fig. 2. Under normal circumstances, the colon is presented with approximately 1,500 ml of fluid per day, of which all but 100 ml are absorbed. When the inflow of fluid into the colon is increased, the healthy colon can compensate by increasing its absorption up to a maximum of approximately 6 l per day. This capacity can potentially be increased by the action of aldosterone and angiotensin II, both of which increase sodium absorption from the colon. In addition, the presence of luminal SCFA should increase this absorptive capacity, although this premise has not been directly tested. Diarrhea will theoretically result only if small bowel fluid secretion occurs in excess of this amount, overwhelming the maximal absorptive capacity of the colon. On the other hand, if colonic absorption is compromised, with combined small and large intestinal dysfunction, diarrhea can occur at lesser levels of small intestinal secretion. Diarrhea may also result solely from changes in colonic absorptive capacity, i.e. with normal amounts of fluid inflow from the small intestine, if the absorptive capacity of the colon is considerably compromised. This latter situation is observed in shigellosis (64), where inflow of fluid from the small intestine was found to be normal, while colonic absorption was completely abolished.
FIG. 2. Conceptual diagram of changes in fluid absorption and secretion in the intestine and colon in acute diarrhea of infectious origin. Panel A represents fluxes in a healthy individual. Endogenous secretion into the intestine of approximately 7,000 ml/day is completely reabsorbed from the intestine, allowing only about 1,500 ml fluid to enter the colon. Most of this is again reabsorbed, and fecal output of water averages about 200 ml per day. Panel B represents fluid fluxes in a patient with severe cholera. Active fluid secretion into the bowel is increased, but the volume of fluid absorbed from the small intestine remains normal due to presence of glucose in the oral rehydration solution. Fluid inflow into the colon is very high, and colonic absorption is virtually absent. Thus, fecal water loss is very high in the first 24 hours of illness. Panel C represents a patient with severe cholera who has been treated with resistant starch or other colonic substrate that allows production of short-chain fatty acids (SCFA). Fecal SCFA, which are grossly diminished in cholera (110), are increased by administration of the starch (117). Despite similar flow into the colon as in B, colonic absorption is stimulated, and secretion inhibited, by the presence of luminal SCFA (110), which increases the maximal absorptive capacity of the colon. The result is that fecal fluid loss is considerably diminished. Panel D represents the situation that obtains in shigellosis, a condition in which colonic mucosal damage is observed, in the absence of changes in the small intestine. Small intestinal absorption, fluid flux, and inflow into the colon are similar to A. However, due to epithelial cell dysfunction in the colon, absorption is markedly diminished (64) and this results in diarrhea due to increased fecal water loss.

Studies carried out in Bangladesh on adult patients with cholera compared the ileocecal flow with fecal output, and showed that the colonic fluid absorption was approximately 20 ml/hour on average, compared to the fasting cecal inflow of approximately 475 ml/hour (59). In the same cholera patients, direct perfusion of the colon indicated that there was no net water absorption from the colon, with minimal net secretion of approximately 2 ml/hour. Although that study did not include healthy volunteers for comparison, data obtained from perfusion of the colon with normal saline in healthy Indian volunteers suggests a maximal colonic absorptive capacity of approximately 250 ml/hour (80). Thus, colonic dysfunction is a significant part of the clinical picture of cholera. SCFA-linked NaCl absorption is an ion absorptive
pathway in the colon that does not appear to be affected in cholera. Cholera toxin did not inhibit colonic SCFA-linked NaCl absorption in rat colon perfused in vivo or in rat and rabbit distal colon mucosa in vitro (17,53). In addition to preservation of SCFA-linked NaCl absorption during CT induced secretion, butyrate (the prototype SCFA) was found to inhibit active Cl- secretion in the colon induced by either cAMP or cGMP (17,54,55). Studies in patients with cholera or non-cholera watery diarrhea revealed that fecal SCFA concentrations were quite low, indicating inadequate SCFA generation in the colon. In these patients, rectal absorption of water and sodium were low compared to healthy control subjects, and could be restored to normal by the presence in the lumen of a mixture of SCFA (110,111). Based on all these observations, it was suggested that butyrate or other SCFA would be a useful addition to the therapy of acute diarrhea to reduce fluid secretion and to improve fluid absorption from the colon. Butyrate is rapidly absorbed from the proximal gastrointestinal tract when given orally, and alternative delivery systems would be necessary for it to reach the colonic lumen. Butyrate is normally generated within the colonic lumen by bacterial fermentation of unabsorbed carbohydrate. Starch is rapidly fermented in the colon (112), and this fermentation results in the preferential generation of butyrate (113). Incubation of starch in vitro with feces from patients with cholera showed that the fecal flora of patients with cholera possessed the ability to generate SCFA (114). Starch that is resistant to amylase digestion in the small intestine (RS) provides a good substrate for colonic bacteria to ferment to SCFA. Cereals such as rice contain significant amounts of RS. Indeed, a meta-analysis of all trials involving the use of rice-based ORS concluded that rice-based ORS was effective in reducing stool output in patients with cholera, but not in infants or children with non-cholera diarrhea (115). It is possible that some of the beneficial effect of rice-based ORS may be derived from production of SCFA from undigested carbohydrate present in the ORS. However, rice-based ORS may be superior to conventional ORS for other reasons including a low osmolarity of the solution, and the presence of a possible anti-secretory factor in rice (104). High amylose maize starch has a high content of RS, which raises fecal butyrate concentrations in normal volunteers after ingestion (116). Administration of this starch to patients with cholera significantly reduced the fecal output, beginning after the first 12 hours in hospital (117). In addition, the starch significantly shortened diarrhea duration in patients with cholera (Fig. 3). The lack of efficacy of rice-based ORS in non-cholera diarrhea in children (115) raises the possibility that SCFA generation in the colon may not be adequate in children with diarrhea. However, perfusion of 14C-glucose into the cecum of malnourished infants with diarrhea has been shown to lead to significant fermentation in many of them (118). There is very limited regarding the use of colonic substrate in the treatment of diarrhea. Partially hydrolyzed guar gum is another substrate that can be fermented to SCFA in the colon. Administration of this gum to children with non-cholera diarrhea led to a substantial reduction of diarrhea duration and a modest reduction in the severity of diarrhea in treated children compared to control subjects (119). In persistent diarrhea in children, green banana (a rich source of resistant
FIG. 3. Fecal concentration and output of short-chain fatty acids (SCFA) increased in patients with cholera who received an amylase-resistant starch along with standard therapy. Standard therapy consisted of glucose—oral rehydration solution (Glucose-ORS) along with early refeeding, while the other two groups received standard therapy along with either rice flour 50 g/l ORS (Rice-ORS), or a maize-derived amylase-resistant starch 50g/l ORS (Starch-ORS). Fecal concentrations and output shown here are after 24 hours of administration of the respective interventions. Fecal SCFA concentration (mmol/l) was higher in patients receiving the amylase-resistant starch, *P = 0.04 and 0.03 compared to glucose-ORS and rice-ORS. There was a trend toward higher fecal SCFA output in starch-ORS patients, **P = 0.06 and 0.07, respectively, compared to glucose-ORS and rice-ORS patients. SCFA are rapidly absorbed from the colon at the site of production, and hence fecal concentrations are not an accurate guide to production in the colon. Nevertheless, these studies show that oral administration of an amylase-resistant starch increased fecal SCFA production even in patients with moderately severe diarrhea from cholera. From Ramakrishna et al. (117).

starch) and pectin have both been used as colonic substrate to provide SCFA. Supplements of cooked and mashed green banana or of pectin led to earlier recovery and increased rates of recovery in treated children compared to conventionally treated controls (120). Fructo-oligosaccharides (FOS), which are not absorbed in the small intestine, provide an alternative colonic carbohydrate substrate. In a study carried out to determine its utility in preventing traveler’s diarrhea, non-significant decreases in diarrhea during and after travel were found in volunteers administered FOS (121). The intention of the study was to prevent diarrhea by altering the colonic bacterial flora, but achievement of this alteration was not directly tested. Diarrhea from colonic secretion sometimes complicates enteral feeding, and this secretion is reversible by luminal SCFA. A trial of pectin supplementation to tube feeds has been reported, which did not identify any obvious beneficial effect of pectin (122). However, the number of patients studied was small and the study was not of adequate power to resolve the issue. Thus, while initial trials are promising, more work remains to be done to identify the nature and concentration of the colonic substrate to be used in therapy of secretory diarrhea. Currently, further studies are under way to determine the place of RS in the therapy of diarrhea.
SUGGESTED DIRECTIONS FOR FUTURE RESEARCH

Further research is necessary to elucidate key aspects of colonic fluid secretion and absorption. In particular it would be useful to define the nature of final common pathways of secretion, including the role of neural pathways and of regulated membrane proteins. The role of CFTR and other membrane proteins in controlling epithelial cell absorptive and secretory function, and the mechanism of the cellular switch from absorption to secretion needs to be elucidated. Regulation of both these processes by luminal constituents, largely derived from dietary ingestion, will need to be defined. The role of specific bacterial species in providing luminal regulators of epithelial cell function in the colon will also aid in this process. Taken together, advances in our knowledge in these apparently diverse areas is likely to result in strategies for targeted intervention in the colon that is likely to impact on the state of our digestive health and overall nutrition, besides providing clinical benefit in disease involving the colon.

REFERENCES


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DISCUSSION

Dr. Abdul Majid Molla: When we're working on the CDL-based oral hydration using rice as starch, we have used all kinds of starches, and in adult cholera, we have found 40% to 50% reduction in the stool output and we used only polysaccharides, not the normal starches, so is it necessary that we have to use the amyloid resistant starch, if we get the same thing by using normal rice starch or any other cereal starch?

Dr. B. Ramakrishna: Thank you, Professor Molla. I think the point you make is extremely valid. I did not have the time, and I don't think it was my task to review those today. That's why I didn't show those slides, but I've always referred to your studies, where the cereal ORS have shown that there is a significant reduction in stool output, particularly in patients with cholera. There has been a meta-analysis of rice ORS and, if Dr. Bhan were here, he would probably provide insights into this, but essentially what they showed were that in cholera, they significantly improved or hastened recovery from diarrhea, but they were not that effective, about 18% to 20% effective in children with non-cholera diarrhea. Now rice of course, in addition to providing starch which is possibly one of the ways in which it works, but people have also made the observation that there's an anti-secretory factor in rice, which might be responsible.

Dr. S. K. Mittal: Maybe I can add, high pore sporadity of the rice-based ORS might be one of the reasons.

Dr. Abdul Majid Molla: In the rice-based ORS or any other cereal-based ORS there is hypoosmolla. If you use 50 g of rice’s starch, or even 80 g of rice’s starch, the osmolarity is barely 1 in 250 or 240. So in that way there's a tremendous advantage.

Dr. B. Ramakrishna: Absolutely.

Dr. Dilip Mahalanabis: I think I can comment on this just to add to what Majid just said that when you give rice, the issue is, I think Ramakrishna can tell us better because he is an expert on this, with amylase-resistant starch. Any starch which is cooked and ingested is obviously largely digested and is not resistant, but there various physical factors, which can make a starch, even a cooked starch, not digestible and risk the colon. As an example, say if you cook potatoes and keep them in the refrigerator and serve them cold, a significant part of them will be amylase-resistant, will go into the colon and, if you use a rice ORS, if it is served cold and served warm, this might make a difference to the amount of rice, which might enter the polysaccharides and might enter the colon and get fermented into short-chain fatty acids. Now these are the issues, which I discussed in one of the consolidated meetings which Ramakrishna, myself and Endibida had in Malagio. After that, Ramakrishna started this study. So this is the information, which is provided by people who actually do research on this resistant starch and we have a new expert who is here to help us, for your information.

Dr. B. Ramakrishna: I think that’s perfectly correct. A lot of the starch in cereals, depending on the way they’re cooked, can be retrograded so that it is not attacked by amylase, and typically between 10-20% of the starch that we’ve taken is not digested in the small intestine.

Dr. George Fuchs: You mentioned the neuroendocrine pathway that may mediate some effects distal to where the action is in rotavirus. And one of the motivations of our doing some of these banana and pectin studies in Bangladesh in acute diarrhea and persistent diarrhea, as well as shigellosis, was related to an animal study by, I think his name is Rombeau in Philadelphia, who did the recepted model on which he had animals with an ileostomy and
then infused short-chain fatty acids in the colon and saw a decrease in secretion in fluids in the ileostomy output. We had sort of just jumped to doing clinical studies based somewhat on that hypothesis, but I wonder if you are aware, have you done any studies that have further defined that in its potential importance as a mechanism of the efficacy of short-chain fatty acids in the colon?

Dr. B. Ramakrishna: I know John Rombeau. He's a surgeon in Philadelphia and essentially he is looking at the healing of anastomosis of the small intestine. Mark Rolandelli and he infused short-chain fatty acids intravenously and looked at the strength of these anastomoses, both intestinal and colonic, and they looked at mucosal proliferation. I don’t think that they actually looked at intestinal secretion. There is information available from a long time ago of the effects of short-chain fatty acids on fluid or ion transport in the small intestine and the answer is that acetate, to a certain extent, does stimulate sodium absorption from the ileum. We have done studies in the ileum and in the jejunum and diffusion studies in rats and short-chain fatty acids do not have an effect on sodium absorption.

Dr. Roger J. Glass: I have a slightly different question. There’s increased severity of diarrheal disease in very young children and in the elderly, and I was wondering to what extent this might be explained by differences in the colonic absorptive function. In the very young, is there a maturation of these functions in the first 6 to 12 months of life?

Dr. B. Ramakrishna: In terms of the functions, there is no maturation. Again, we have looked at the development of these processes in the infant rat colon and this is published in The Journal of Pediatric Gastroenterology and Nutrition. They have shown that these processes are present at birth. Now what happens is that actually it’s not a maturation of function, it’s a maturation of the bacterial flora, and it’s been shown for instance by Professor Tore Midtvedt in Denmark, I think, and he’s shown that up to an age of 2 years, colonic flora keep on maturing continuously and obviously the more mature they are, the more likely they are to ferment carbohydrates to short-chain fatty acids.

Dr. Anne Ballinger: Do all colonic bacteria ferment carbohydrates, or does it vary depending on the type of bacteria, because for instance in inflammatory bowel disease, it’s suggested that the flora is different from controls. So is there any evidence showing that there are different bacteria from the colonic bacteria?

Dr. B. Ramakrishna: The anaerobic bacteria are responsible for the fermentation. Some of them are better at producing butyrate. A lot of them produce more acetate than butyrate and that would not be desirable from the point of view of colonic physiology. In inflammatory bowel disease, it’s more a change in the aerobic flora, which occurs rather than the anaerobic flora and again, there’s very recent information from the molecular characterization of these from Philippe Pochart and his group in Paris.

Dr. Angha Jayakar: We used to use pectin and kaolin in combination as a substrate for binding many years back. Now in children we recommend this ORS and maybe rice-based ORS. Do you think there’s any role for this pectin kaolin in the process of diarrhea in children, since you mentioned that pectin could be of help?

Dr. B. Ramakrishna: That’s an interesting question. I often wondered about that, because I’m not quite sure what physical form kapectate takes, as it used to be called. I’m not quite sure what the physical form of pectin is, perhaps somebody could elucidate that.

Dr. S. K. Mittal: I don’t know the physical form, but yes pectin was used and now it has been banned by the drug controllers, so we don’t know.

Dr. George Fuchs: In our studies in Dacca, we used pectin that was in a powder form and just put it in the normal diet and we sort of used it as a control, not a true control, but a more pharmacologic dose of potential short-chain fatty acid to compare it with the cooked green
banana group. So it was a sort of a powder. I just want to come back to this effect of instilling short-chain fatty acids on small bowel healing. Has there been any further definition of what that mechanism is, because that’s again one of these examples of a distant effect from where the contact has been made. Has Rimbaud or anybody else done any definition of this?

Dr. B. Ramakrishna: The trouble with butyrate is that affects gene function or gene expression in many ways. A lot of it has to do with histone acetylation. We are in fact looking at the effects of butyrate in inflammatory bowel disease and the effects of it on intestinal inflammation, and it seems to have a whole range of diverse effects and no one’s got the answer at the end.

Dr. Marcello Giovannini: Also for the pediatrician, it is a consideration. Sometimes lactose fermentation produces cholic and this time is used by the cholic in the production of short-chain fatty acids for this reason. When a baby’s breastfed, because one part of lactose is only fermented in the colon, it is useful for a pediatrician to explain to the mother not give drugs and to allow no absorption. I think also of prevention, no good for the baby, no good for the parents, this is the pediatrician’s consideration.

Dr. B. Ramakrishna: I think Jean-Claude Rombaud in Paris has done a lot of work on that. Again, we alluded to this briefly yesterday, that when you give lactose to children who malabsorb lactose, there are two ways in which they behave. Those who have a colonic flora, which ferments the lactose, there are two ways in which they behave. Those who have a colonic flora, which ferments the lactose, get constipation and cholic and distension. The ones who have a colonic flora, which does not ferment the lactose, get diarrhea and there is a kind of distinction here.

Dr. Ashish Baudekar: This is a little different from the understanding of the concept we like as pediatricians. When a child gets diarrhea, we tend to give it food that is basically digested or something that is easy to digest. Well here there is a suggestion that you give something that is slightly more difficult to digest, so that’s a little different from the usual thing that they’re used to dealing with. The second point is regarding the short-chain fatty acid. If the same concept is used in small babies, less than 3 months old, would this excess of short-chain fatty acids cause any problems to the baby? Although you have said that it comes out again, this is still something I want to get clear.

Dr. B. Ramakrishna: Let me take those two questions. Sorry what is that first question again?

Dr. Ashish Baudekar: The usual concept is that we get predigested or easy to digest food.

Dr. B. Ramakrishna: I think that’s a question of how you look at it. If we look at the colon as a digestive organ, and if we look at the bacterial flora as part of us, then resistant starch is actually a digestible substance. So in that way, you could think of it as an easily digestible substance. In fact, there are lots of benefits from a nutritional point of view, because it’s only about half as efficient as being converted to glucose and being absorbed from the small intestine. So you can take more and still not put on weight and that’s in fact one of the goals of using resistant starch. There’s EURESTA, the European Resistant Starch Association, which is really trying to promote this. Now the second question is whether the flora of young babies is mature enough to ferment the starch to short-chain fatty acid—if they can be fermented. We’ve had this concern about whether it causes acidosis, because a lot of these acids are really good, but butyrate is largely used by the colonic epithelium. About 80% of it, as it goes through a lot of the colon, 80% is metabolized there. Propionate and acetate reach the liver. Propionate is largely metabolized in the liver. Only a little bit of acetate really gets out into the blood stream. So in terms of causing an acidosis, probably it is unlikely, and in our study designs, we would certainly be looking at the blood pH.

Dr. Deba Prasad Banerjee: It is very interesting to note that you have said that there is
some role for green bananas in the control of diarrhea, so I would be very glad if you could tell me the mechanism, how it helps.

Dr. B. Ramakrishna: In this particular instance, we think it acts by providing resistant starch. About 80% of the starch in green bananas is presented in a form, which is not digested by amylase in the small intestine. So all the starch goes to the colon, there the bacteria chews it up and produces short-chain fatty acids and presumably that’s how it works. The way they gave the green bananas is interesting. Basically they took the green banana, they cooked it and mashed it and then fed it to these children with persistent diarrhea.

Dr. Shrichandra Bhawnani: Question number one: Is it possible to ascertain clinically whether the food lost in diarrhea comes from the small intestine or colon, because we have now understood that the strategies, which we take to treat colonic disorders, are quite different from those of the small intestine? Number two, I wanted you to elaborate on chloride-secreting diarrhea and also on potassium-secreting diarrhea.

Dr. B. Ramakrishna: Is it possible to differentiate in clinical practice? I don’t think so. You really need to do studies, where you intubate the intestine like I used to do more than 20 years ago, like Dr. Mahalanabis did 30 to 40 years ago, where we intubate children and see what happens. Other than that, there is no clinical way of differentiating. In practice, it probably doesn’t make a huge difference because any strategy that we use would really try to target both the small intestine and the large intestine. That’s the only one that really makes sense.

Dr. Shrichandra Bhawnani: What about fluid-secretory diarrhea, we wanted you to elaborate on that?

Dr. B. Ramakrishna: Congenital chloride diarrhea, as I mentioned, is a fairly uncommon condition, probably because it is not being recognized very well. You have to remember this condition when mothers present with hydramnios or babies present with intractable or fatal diarrhea, and a lot of the studies have come from Finland. Essentially, they pinpointed the genetic effect, but I was mentioning that there are studies from Saudi Arabia and Kuwait which show that it’s there also, so I don’t see why it’s not here in our population?

Dr. Abdul Majid Molla: Intercloide diarrhea cases in the Middle Eastern countries probably are the highest in the world now with a rate of 1 in 5,000. It used to be considered 1 in 9,000 in Finland, but it is higher is Kuwait. The polyhydramnios is a very common presentation and the diarrhea right from the back is a very common presentation. It’s the only presentation.

Dr. B. Ramakrishna: So please all be aware and keep looking out for it. The polyhydramnios is secondary to the congenital chloride diarrhea.

Dr. Michael J. Farthing: Just a comment on the last question. I think you can get some indication clinically as to whether the small intestine is involved, if there is very high volume diarrhea. Exclusively colonic diarrheas are usually lower volume. If diarrhea exceeds 2 to 3 l, it is likely that the small intestine is involved. I also want to make a comment about George’s remark about short-chain fatty acids and their potential trophic benefit. In short bowel syndrome, one of the reasons it’s said you get better adaptation if the colon is in situ, is because you have that colonic generator for short-chain fatty acids, which then enter the circulation and promote adaptation in the retained small intestine.

Dr. Sanath P. Lambadasuriya: A child with classical secondary lactose intolerance has profuse water stools that are very acidic and even the buttocks get excoriated. You said there are these colonic organisms, that the residual carbohydrate is metabolized and absorbed and that, if there are no organisms, that it produces diarrhea. This is a bit contradictory. How do you explain it?

Dr. B. Ramakrishna: I’m not quite sure you’re right about the acidic part of it. I mean,
obviously in clinical practice, when you have carbohydrate malabsorption, you have more acidic stools, but that’s probably because just only a part of it is being fermented. To the best of our knowledge, there is no defect in absorption of short-chain fatty acids in these individuals. There are a lot of possible transport processes, but if you actually look in vivo, the main mechanism through which they get in is probably just non-ionic diffusion.

Dr. Veena Kalra: How would you conceptualize the role of probiotics and the generation of the short-chain fatty acids in the gut, if there was concurrent use?

Dr. B. Ramakrishna: I think we’re really excited about probiotics. We’d certainly like to use them in conjunction with all this. Now the only thing about probiotics is, in addition to doing all this, they probably also influence immune responses in the intestine and affect cytokine release from epithelial cells from the lamina propria immune cells and so on. So a lot of interaction’s going on and I don’t think we’ve really begun to understand what happens with probiotics.

Dr. Suporn Treepongkaruna: I refer to your study about short-chain fatty acid. Do you also study the capacity of the colon for absorption of short-chain fatty acids? How much can the colon absorb, how much still remains in the lumen and what will happen, if there is too much short-chain fatty acid in the lumen of the colon?

Dr. B. Ramakrishna: These kinds of studies have largely been done in healthy people. The average colon produces, we don’t really know how much it produces, perhaps about 200 to 800-mmol of short-chain fatty acids a day. More than 90% of it could be absorbed from the colon.

Dr. Thomais Karagiozoglou Lampoudi: Do we know, if we have any information about the different pattern of short-chain fatty acids produced by different strains of probiotics?

Dr. B. Ramakrishna: With regard to the ones, which are commercially available, we don’t have the information, but with regard to different bacterial species, yes, we know the patterns of short chain fatty acids which are produced.

Dr. Malathi Sathiasekaran: The absorption of water is different in different regions of the colon, but is the intestine adaptation as good as in the small bowel? Suppose you dissect the left side of the colon, would the right take over as well?

Dr. B. Ramakrishna: I think it does. It compensates to a large extent. That’s from clinical experience, and if you look at people who’ve had this, a lot of these studies have been done by people like John Cummings and others, where they’ve looked at people with colostomies, sigmoidostomies versus transverse colostomies versus ileostomies or cecostomies and have shown that it does compensate largely.

Dr. S. K. Mittal: I come back to the rice-based cereal. One problem is that we find that the processed rice cereal available for infant feeding doesn’t seem to work like the wild rice we are used to using, if we prepare it for ORS. Do you think there is a reason for this? Is it the rice cereals?

Dr. B. Ramakrishna: There’s a clinical observation, which is that processed rice cereals do not work as well as rice gruel or rice kanji. I don’t really know, perhaps Dilip would like to comment.

Dr. Dilip Mahalanabis: We really do not know how this commercially processed rice is prepared.

Dr. S. K. Mittal: Do you agree with this observation? That is the point first.

Dr. Dilip Mahalanabis: We have not done any studies. We cannot say. It’s very difficult to say off hand, whether it works or not. These commercial cereal preparations have so many other things added to them. I guess they’re not pure rice cereals.

Dr. S. K. Mittal: The other thing I was asking you about is that you are now saying amylase
is resistant starch. We know studies by Emmanuel Lebenthal, which are saying amylase is rich rice cereal and they have shown that it is a very effective oral reaction solution.

Dr. B. Ramakrishna: Now, regarding Emmanuel Lebenthal, there are some studies where he looked at energy-dense starches. Basically he took starches and predigested them with amylase. The rationale for this goes back to studies a long time ago, which showed that pancreatic function was not mature in infants, so it took a few months for the pancreatic exocrine function to mature. So he said let's pre-digest the starch and in doing so, what he actually got was really energy-dense porridge. So I believe this had several effects. One is that it suddenly improved small intestinal absorption. I think it probably improved the nutritional status of these children, so definitely there is a role for that.

Dr. Dilip Mahalanabis: Well, I just want to say that this approach has some potential danger in creating a potentially hyperosmolar preparation for infants, and osmotic problems could arise from this approach.

Dr. S. K. Mittal: Sir, may I differ from you. We have, I also used this amylase-rich rice and dahls mix in persistent diarrhea and found extremely good results, because it makes energy dense.

Dr. Dilip Mahalanabis: If you add dahl it makes it less of an osmotic problem. You might have saved it from being hyperosmolar, but if we just take a lot of amylase and just digest it and give it to diarrhea kids?

Dr. S. K. Mittal: We use only 1g/100 mmol.