Colonic fluid handling in health & acute diarrhoeal diseases

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The ability of the colonic epithelium to actively absorb sodium against electrochemical gradients confers on it the capacity to dehydrate the faeces. This capacity is of considerable importance in limiting fluid losses in acute diarrhoeal illnesses characterised primarily by small intestinal fluid secretion. Even in these illnesses, colonic absorptive capacity may be altered for various reasons. Loss of colonic absorptive capacity is also responsible for diarrhoea in those illnesses where the pathology is primarily epithelial cell damage in the large bowel. The present review analyses the information available on this subject from studies in patients with acute diarrhoea, and in experimental animals. It also outlines measures that might possibly restore optimal function of the colonic salvage mechanism in acute diarrhoeal disease.

Key words Absorption - colon - diarrhoea - enterotoxins - rehydration - secretion

Diarrhoea is an excessive loss of fluid in the faeces. Fluid loss in acute watery diarrhoea has traditionally been thought to reflect small intestinal malabsorption or secretion. The role of the colon in conservation of fluid and electrolytes by the body has been appreciated only in the last three decades. Until then, the colon was considered to be merely an organ responsible for storage of faeces and ensuring periodic and comfortable evacuation. Studies around this time showed that the colon had a significant capacity to absorb sodium and water, which largely remained unutilized in health. The reserve capacity of the colon to absorb fluid is critical in determining the magnitude of fluid losses in diarrhoeal disease. This review briefly describes the general physiology and significance of transport of fluid and electrolytes by the colon in health and the alterations in these processes that occur in diarrhoeal disease.

Colonic fluid handling in health

Overall movements of fluid and electrolytes: Initial estimates of colonic absorption in health were based on ileostomy losses, which suggested that the colon absorbed about 300-600 ml of water and 40-60 mM of sodium everyday. It was appreciated that patients with ileostomy generally adapted to the loss of the colon, except under conditions of stress such as during diarrhoea or following exposure to heat. Estimates of colonic absorption were revised upward when direct estimates of ileo-caecal flow became available from studies using slow marker perfusion of the terminal ileum in healthy Western subjects. These studies suggested that the colon absorbed about 1400 ml water, 190 mmol sodium and 95 mmol chloride everyday.

When perfused in vivo, the colon is able to absorb sodium from solutions with a sodium concentration as low as 25 mmol/l (i.e., it is able to absorb sodium against steep concentration gradients). Net sodium secretion occurs at luminal sodium concentrations below 25 mmol/l. This conservation of sodium is a
characteristic feature of colonic function in both man and experimental animals. In comparison, the human jejunum ceases to absorb sodium when the luminal concentration is below 133 mmol/l and the human ileum at concentrations below 35 mmol/l. Comparison of the composition of ileostomy fluid and ileocoeal effluent indicates that the ileum is apparently unable to concentrate the luminal contents, the sodium concentration in the ileum not falling below 120 mmol/l. Faecal sodium concentrations on the other hand, range from 25-49 mmol/l. Within the colon, there is segmental heterogeneity of the absorptive processes. Observations in patients with colostomies suggest that the ability to absorb sodium against substantial concentration gradients is primarily a function of the distal colon. The ability of the colon to conserve sodium is enhanced by its response to aldosterone, which increases electrogenic sodium absorption from the colon. There is evidence that the aldosterone effect becomes clinically significant under conditions of negative sodium balance. There is also evidence from experimental animals that angiotensin II can enhance electroneutral NaCl absorption from the colon. Thus the model is that the colon conserves sodium avidly and that this capacity can be altered to suit the needs of the body under adverse conditions.

Under basal conditions, the human colon absorbs about 1.5 lof fluid per day. It is now appreciated that the colon has a considerable reserve capacity to absorb fluid. Perfusion of the whole colon with normal saline at 10 ml/min in healthy southern Indian adults indicated a maximal absorptive capacity of approximately 31 of fluid per day. From these studies, maximal sodium absorption from the colon can be calculated to be of the order of 760 mmol/day and for chloride this value was around 1200 mmol/day. These studies did not utilize luminal short chain fatty acids. In view of the known stimulatory effect of these on sodium absorption it is probable that the maximal absorptive capacity in their presence, would be considerably higher. Perfusion of the colon in normal Western volunteers showed that the colon had a capacity to absorb on an average 5700 ml of fluid, 816 mmol of Na and 44 mmol of K under conditions in which large volumes of additional fluid were infused into the colon in a continuous manner. The capacity of the colon to absorb fluid was reduced by the simultaneous infusion of chenodeoxycholic acid (a bile acid which induces colonic secretion). Also, bolus infusion of 500 ml of fluid into the colon over 60 min (but not 250 ml over 30 min) resulted in loose stool. It was concluded from these studies that the colon has a very large reserve capacity to absorb fluid, and that this capacity may be altered by secretagogues such as chenodeoxycholic acid, and by the total volume of fluid presented.

One of the major stimuli for sodium absorption in the mammalian colon are the short chain fatty acids (SCFA) - acetate, propionate and butyrate - which are produced in the colon by bacterial fermentation of unabsorbed carbohydrate. SCFA have been shown to enhance sodium absorption from the normal human colon both during in vivo perfusion of the whole colon and in a system where the intact human colon was perfused in vitro. In the latter, SCFA increased sodium absorption approximately five-fold over control. Of the SCFA, butyrate is more effective than acetate or propionate in enhancing sodium absorption. The recognition of SCFA-linked sodium absorption as an important absorptive mechanism has implications for diarrhoeal disease as will be discussed subsequently.

In vitro mechanisms of electrolyte transport: Fluid absorption from the colon is secondary to active absorption of sodium (and chloride). Similarly fluid secretion is primarily linked to active chloride secretion. The last decade has seen the elucidation of these processes at the membrane level, and knowledge of the molecular regulation of these is currently being obtained. The maximum information has been obtained from studies in flux chambers in which colonic membranes are mounted in vitro and unidirectional fluxes studied under voltage clamp conditions. This information has been supplemented by studies using membrane vesicles and intact colonocytes. All the studies together have shown that there is considerable heterogeneity across animal species in the importance of individual absorptive mechanisms in the colon. Further there is a regional heterogeneity with different transport processes assuming importance in the proximal and in the distal colon. There is also heterogeneity in distribution of the transport processes along the crypt-surface axis of the colonic mucosa. It is generally presumed that
secretory processes in the colon are localized to the crypt epithelium, while absorptive processes are localized to the surface epithelium. However, recent studies involving perfused isolated colonic crypts suggest that crypts are able to absorb as well as secrete under different conditions. ACTIVE processes for sodium absorption in the human colon can be studied at the level of the apical and basal membranes of the colonocyte. At the apical membrane, entry of sodium into the cell (which has a low intracellular sodium) is composed of two processes - electrogenic sodium absorption which occurs through sodium channels and is predominant in the distal colon, and electroneutral coupled NaCl absorption which involves coupled Na-H and CI-HCO₃ exchanges and is predominant in the proximal colon. The exchanges involved in these transport processes have been identified in apical membrane vesicles isolated from colonic epithelial cells. The Na channels are inhibitable by 10⁻⁴ amiloride while neutral NaCl absorption is inhibited by either 10⁻³ amiloride or by cyclic AMP. Sodium exit from the cell occurs along the basolateral membrane, and is energy-dependent extrusion by the sodium pump - Na-K-ATPase. Sodium movement across the tight junctions of the colon is minimal under either basal conditions or after exposure to secretagogues. SCFA enhance neutral NaCl absorption by simultaneous linkage of three inwardly directed transport mechanisms - SCFA-HCO₃ exchange, Cl-SCFA exchange and Na-H exchange. Each of these exchanges has been identified in the apical membrane vesicles from colonic epithelial cells. There is also some (and so far unconfirmed) evidence that SCFA may stimulate electrogenic sodium absorption in the human infant colon. Studies using human colon suggest that about half of the sodium transport in the proximal as well as the distal colon is attributable to electroneutral NaCl absorption. In the distal colon, most of the remaining sodium absorption occurs through amiloride-inhibitable sodium channels. The nature of the transport process accounting for the remaining sodium transport in the proximal colon remains unknown, but may be an amiloride-resistant sodium channel.

Potassium transport across the colon is characterised by both active absorption and active secretion. Active absorption occurs through a K-H ATPase which is electroneutral, sodium-independent, and sensitive to ouabain and vanadate. Potassium secretion occurs through K-channels in the apical membrane which appear to be induced by aldosterone, cyclic AMP or epinephrine.

**Colonic fluid handling in acute diarrhoea**

Mechanisms of secretion in the colon: Active chloride secretion occurs in the small intestine in response to various secretagogues. A response similar to this, although of different magnitude, occurs when the colon is exposed to secretagogues. These processes have been characterised by in vivo perfusion studies in man and experimental animals, by in vitro studies in flux chambers, and in studies using the T84 colonic carcinoma cell line which actively secretes chloride in response to secretagogues. Active electrogenic chloride secretion is thought to occur primarily through the chloride channels in the apical membrane of the crypt cells. The time frame of occurrence of secretion is compatible with insertion of new chloride channels into the apical membrane. Chloride entry into cells occurs across the basolateral membrane via Na-K-2Cl cotransport. The entire process is energy-dependent and involves redistribution of the cytoskeleton. In addition to inducing chloride secretion in crypt cells, secretagogues inhibit neutral NaCl absorption in surface epithelial cells. In the case of cyclic AMP, inhibition of neutral NaCl absorption is thought to be secondary to inhibition of Na-H exchange. The colon is a relatively ‘tight’ epithelium. The permeability of tight junctions is unaffected by choleratoxin, but the recently described zona occludens toxin (zot) may well alter tight junction permeability. An increase in paracellular permeability will by itself only increase bidirectional fluxes of solute, and will not result in net secretion in the absence of a serosal-mucosal hydrostatic pressure gradient. The major role of the paracellular pathway in active colonic secretion is to allow the passage of sodium from the serosal aspect to the lumen in response to electrogenic chloride secretion.

**Overview of the role of the colon in diarrhoeal disease:** In contrast to the wealth of information available about colonic fluid handling in health, there is very little information about the way the colon
handles fluid in acute diarrhoeal disease. Diarrhoea is an excessive loss of fluid in the faeces. For a long time, it was considered that this loss of fluid occurred primarily from the small bowel, where it was considered to be due to either excessive secretion of fluid and electrolytes (as in cholera) or impaired absorption due to reduced villus absorptive area (as in rotavirus diarrhoea). The close interrelationship between colonic absorptive capacity and development of diarrhoea was appreciated relatively recently, leading to the concept of diarrhoea as a "failure of colonic salvage"\textsuperscript{28}. The maximal absorptive capacity of the colon has been calculated to be in the range of 5.5 to 6 l per day based on steady perfusion of fluid into the caecum of volunteers. If this were also true in diarrhoeal disease, then diarrhoea would occur only if the small bowel outflow into the caecum was greater than 6 l per day (i.e., if the capacity of the colon to absorb fluid was exceeded). On the other hand, if colonic absorption was decreased in acute diarrhoeal illness, then diarrhoea would occur with much lower levels of small bowel secretion. This can also happen in diseases like Shigella dysentery where small bowel outflow is essentially normal, but colonic absorption is decreased.

\textbf{Specific alterations in colonic absorption in acute diarrhoeal disease:} Cholera—Watery diarrhoea in cholera reflects massive secretion of water and electrolytes by the small intestinal mucosa. This has been clearly demonstrated by perfusion studies both in animals and in humans. Early studies in dog colon suggested that the colon was not affected by the secretory process in experimental cholera\textsuperscript{29}. Early studies of jejunal and ileal absorption in adult Indians with cholera suggested that the small bowel fluid losses alone could account for faecal losses of fluid\textsuperscript{30}. In a study of 12 adults with cholera, flow rates across the ileocaecal valve were measured and compared with faecal outputs\textsuperscript{31}. These indicated that the colon was absorbing 0.3 ml/min on an average (i.e., 432 ml per day), emphasizing that colonic absorption was negligible compared to the amounts of fluid flowing across the ileocaecal valve into the colon. In these same patients, the colon (all but the last 20 cm) was directly perfused, and revealed net water secretion of 0.03 ml/min. Sodium and chloride absorption from the colon were also significantly decreased in these patients, and potassium secretion was markedly increased. Repeat perfusions performed in five patients during convalescence revealed no change in net water, sodium or chloride transport, although potassium secretion had reversed to net absorption. Similar perfusions have been done in normal southern Indian subjects using a saline solution, and have demonstrated absorption rates of around 2 ml/min for water, 530 μmol/min for sodium, and 846 μmol/min for chloride\textsuperscript{10} (Table).

It is presumed that the defect in colonic absorption observed in patients with cholera is due to the effect of cholera toxin. Cholera toxin induces secretion of fluid and electrolytes in the colon of experimental animals \textit{in vivo}\textsuperscript{23}. This is characterised by net secre-

| Table. Comparison of ileo-caecal flow rates and mean net absorption of water, sodium and chloride from the colon in healthy subjects from southern India and in patients with cholera and shigellosis from Bangladesh. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Fasting ileal flow rate ml/min | Water ml/min | Colonic net absorption Sodium μmol/min | Chloride μmol/min |
| Healthy subjects\textsuperscript{10} | 0.67 | 2.02 | 530 | 846 |
| Cholera\textsuperscript{31} | | | | |
| Acute | 7.90 | -0.03 | 100 | 127 |
| Convalescent | 0.80 | 0.33 | 100 | 135 |
| Shigellosis\textsuperscript{32} | | | | |
| Acute | 0.57 | -0.04 | 30 | 25 |
| Convalescent | 0.56 | 0.65 | 75 | 90 |
| Prefix indicates net secretion into the lumen. Superscript numerals refer to the s.no. of the references. |
tion of water, grossly diminished net absorption of sodium and chloride, and by increased net potassium secretion. The latter is presumably due to activation of K secretion by cyclic AMP, although potential-dependent secretion is also likely to contribute. In patients with cholera, net potassium secretion is also likely to be contributed by increased aldosterone levels. In addition to the direct effect of cholera toxin on the colonic epithelium, it has now been demonstrated that neural reflexes are also likely to play a part in the colonic secretion induced by cholera24. In these studies, incubation of small bowel loops with cholera toxin, induced colonic secretion in the rat. Other factors also need to be considered in the causation of colonic secretion in acute diarrhoea. Vitamin A deficiency can potentiate the colonic response to a secretagogue25. This may explain the increased severity of diarrhoeal illness in vitamin A deficient children. Enteral feeding has been shown to induce secretory responses in the colon, related to the site and the load of the diet infused26. This observation may be of significance in considering the delivery of newer oral rehydration solutions, such as the energy-dense liquefied meals.

In the rat colon exposed to cholera toxin in vivo, net secretion of water could be reversed by luminal SCFA, particularly butyrate27. Butyrate also significantly increased net absorption of sodium and chloride from cholera toxin-treated colon. This effect has been reproduced in vitro in rat distal colon mucosa, in which tissue SCFA promoted neutral NaCl absorption even in the presence of cyclic AMP elevation brought about by theophylline37. We have further characterised the effect of cholera toxin on colonic transport in vitro in the colon. In this tissue, exposure of the colon to cholera toxin induced net secretion of both chloride and of sodium in solutions containing Ringer solution (with or without bicarbonate). However, when the bathing solutions contained any of the three common SCFA (acetate, propionate or butyrate or a mixture of all three) net Na and Cl absorption was observed (unpublished observations). These studies suggested that cholera toxin does not inhibit SCFA-linked Na absorption. It is possible that, like glucose-linked sodium absorption in the small bowel, this absorptive pathway can be used therapeutically in patients with diarrhoea.

We have examined rectal absorption of water from saline and an SCFA-containing solution in adult patients with cholera and cholera-like watery diarrhoea and compared them to healthy controls38. The results indicated that net Na absorption from a luminal saline solution was significantly low in cholera, but that sodium absorption from a luminal SCFA-containing solution was apparently normal in these patients. It was also apparent from this study that faecal SCFA excretion was significantly low in the acute phase of diarrhoeal illness. This suggested that if the colonic concentrations of SCFA could be increased in these individuals, sodium absorption from the colon would be enhanced thereby reducing faecal losses of water. By juxtaposing results of colonic perfusion in healthy southern Indians and in Bangladeshi patients with cholera (Table), it is not unreasonable to expect that colonic water absorption could be increased by 2 ml/min (from 0.0 to 2.0 ml/min) by restoring colonic absorption to normal. If this were to happen, it would cut down daily faecal fluid losses by 3 l. In addition to reducing faecal volumes, this would also shorten the duration of diarrhoea, since diarrhoea would stop once small intestinal fluid losses dropped below 3.5 l per day. SCFA can be generated by the faecal flora of patients with cholera when incubated with rice starch in vitro39. Perfusion of 14C-glucose into the caecum of malnourished infants with diarrhoea led to significant fermentation in many of them40, indicating that the bacterial flora are capable of fermenting carbohydrate when presented to the colon. Starch that is resistant to digestion in the small intestine (resistant starch) is a good substrate for colonic fermentation in health. There is now evidence that giving such a resistant starch orally to patients with cholera increases faecal SCFA concentrations and shortens the duration of diarrhoea41. These studies are being pursued to assess whether it is feasible to optimize colonic salvage of fluid and electrolytes in these patients.

b. Other toxigenic diarrhoeas—Colonic function has not specifically been examined in other toxigenic human diarrhoeas. In a study of adult acute watery diarrhoea, patients with stool cultures negative for Vibrio cholerae also showed impaired absorption of sodium from a luminal saline solution placed in the rectum. As in cholera, this impaired absorption was
reversed by luminal SCFA\textsuperscript{24.}

Several of the other enterotoxins induce colonic secretion. Escherichia coli heat-stable enterotoxin (STa) induces secretion in the rat colon \textit{in vivo}\textsuperscript{33,42.} Comparison of similar doses in the ileum and the colon revealed that net fluid secretion was much greater in the ileum compared to the colon as the latter had a higher base-line absorption. The experimental data were consistent with the concept that STa-induced diarrhoeal disease resulted from a decreased absorptive capacity of the colon in the face of increased small intestinal fluid secretion. Secretion is due to binding to specific receptors with activation of guanylate cyclase\textsuperscript{42.} In the rat colon \textit{in vivo}, butyrate was able to partially reverse secretion induced by STa\textsuperscript{33.} STa decreased net absorption of sodium and chloride in rat distal colon \textit{in vitro}\textsuperscript{43.} However, net sodium and chloride fluxes were normal in the presence of butyrate in the luminal solution. This suggests that cyclic GMP does not inhibit SCFA-dependent NaCl transport.

\textit{Clostridium difficile} toxin A has been shown to cause secretion in the rat colon \textit{in vivo}\textsuperscript{44.} This occurred after a long latent period, and was accompanied by shedding of surface epithelial cells without damage to the crypt cells. Presumably the net fluid secretion is secondary to damage to surface cells caused by the cytotoxin.

c. Rotavirus diarrhoea—Colonic fluid absorption has not been studied in human rotavirus diarrhoea. Pigs are susceptible to infection with the transmissible gastroenteritis virus, which exactly resembles human rotavirus diarrhoea. In an interesting study of diarrhoea due to this infection in infant and young pigs, it was noted that infant animals (<3 days old) often had clinical diarrhoea while older animals did not have diarrhoea despite abnormal handling of fluid in the small bowel\textsuperscript{45.} It was noted that the young animals had small bowel malabsorption of fluid, and that fluid malabsorption persisted in the large bowel, where SCFA generation was negligible (presumably due to an immature bowel flora). In the older animals, small bowel malabsorption of fluid was compensated by large bowel absorption which correlated with SCFA production in these animals. The conclusion drawn from this study was that colonic conservation of fluid, presumably secondary to SCFA generation, was an important factor determining the occurrence of diarrhoea in infected animals.

d. Shigella dysentery—Colonic fluid and electrolyte movement is also altered in experimental shigellosis\textsuperscript{46.} Infection of monkeys with \textit{Shigella flexneri} 2a resulted in net colonic malabsorption or secretion, with evidence of severe histological damage.

More recently, perfusion studies in 11 adult patients with shigella dysentery\textsuperscript{33} indicated that ileal flow rates (into the cecum) were normal. Perfusion of the entire colon (except the proximal 20 cm) indicated that there was minimal net water secretion (0.04 ml/min) which was accompanied by diminished net absorption of sodium and chloride, associated with net secretion of potassium and bicarbonate. The magnitude of the change in colonic absorption is comparable to that seen in cholera (Table). However, the change is not accompanied by increased ileocaecal flow, which is the major source of the faecal fluid losses in cholera. Repeat studies in 7 convalescent patients showed a significant increase in net absorption of water, sodium and chloride, and a reversal of net potassium secretion to net potassium absorption. It is not known whether the change in colonic absorption in shigellosis is caused by Shiga toxin, by \textit{Shigella} invading and damaging epithelial cells, or by prostaglandins, leukotrienes or cytokines released secondary to mucosal damage. Alterations in fluid handling in shigellosis are not clinically very significant, except in the initial stages in some subjects who have a watery diarrhoea requiring rehydration.

e. Salmonellosis—Monkeys infected with \textit{Salmonella typhimurium} demonstrated net colonic fluid secretion associated with histological colitis\textsuperscript{47.} This infection appears to behave like shigellosis, and similar features can theoretically be expected in other large bowel infections causing diarrhoea in man.

Conclusion

In summary, the reserve capacity of the colon to absorb fluid and electrolytes is an important determinant of faecal fluid losses in diarrhoea. The greatest impact of this capability of the colon is in acute diarrhoeal disease, where for various reasons the absorptive capacity may be altered. The understanding of colonic pathophysiology in acute diarrhoeal
disease gained over the past decade calls for a well-planned and executed attempt to enhance or restore colonic absorptive capacity in these conditions. The expectation from this is a technologically improved therapy which will not only rehydrate, but reduce faecal fluid losses and duration of diarrhoea. It would then remain to test in the field situation whether such a therapy would have universal applicability in the treatment of diarrhoea.

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