Ion Transport during Cholera-Induced Ileal Secretion in the Dog

WILLIAM L. MOORE, JR., FRED A. BIEBERDORF, STEPHEN G. MORAWSKI, RICHARD A. FINKELSTEIN, and JOHN S. FORDTRAN

From the Departments of Internal Medicine and Microbiology, The University of Texas (Southwestern) Medical School at Dallas, Dallas, Texas 75235

ABSTRACT To assess the ion transport mechanism by which cholera causes the small bowel to secrete, ion transport rates and electrical potential difference (PD) were determined simultaneously in the normal and choleragen-treated dog ileum in vivo. The results indicate that, during cholera, HCO_3 is actively secreted (i.e., against both an electrical and a concentration gradient); Cl is also actively secreted, against a modest electrochemical gradient. Electrogenic pumping of one or both of these anions is probably responsible for an observed PD change of approximately 13 mv (lumen negative). Na secretion can be accounted for entirely by passive ion movement. K secretion can be partly explained by passive diffusion secondary to the negative intraluminal PD; however, its concentration in the secreted fluid is two to three times higher than expected on the basis of passive forces, suggesting a component of active K secretion. The PD response of the choleragen-treated ileum is normal in response to glucose, but there was no PD response to saline-free mannitol perfusion. This suggests that the normal differential permeability of the ileum to anions and cations may be altered by choleragen, although other explanations of this finding are also possible.

INTRODUCTION

The mechanism by which cholera causes the intestinal mucosa to secrete large volumes of fluid is unknown. Recent experimental results have been reviewed (1, 2); they have focused mainly on Na movement and have shown that fluid production originates in the small intestine, is isotonic to plasma, and is rich in HCO_3 (compared with the bicarbonate concentration normally found in a particular area of the small intestine). The electrical potential difference (PD) across the secreting intestine has not been correlated with ion secretion rates in vivo so that it is not known which ions, if any, are actively secreted and which ions move passively. The possibility of a filtration process has not been completely excluded although there is no direct evidence to support this hypothesis. This paper presents data on ion transport and PD, measured simultaneously, in the dog ileum during choleragen-induced secretion. The ileum was chosen for study because it, unlike the duodenum and jejunum, can develop and sustain large chemical and electrical gradients (3). Electrogenic or nonelectrogenic active transport might thus be easier to appreciate in the ileum than in higher regions of the small intestine.

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METHODS

Studies were performed on dewormed and 18-hr fasted dogs weighing 10–20 kg. Anesthesia was induced by i.v. sodium thiopental and maintained by urethane and chloralose. Respiration was controlled by an animal respirator. At laparotomy a 15–30 cm ileal loop was isolated, the distal end of which was 20 cm from the cecum. Teflon spools were inserted into the ends of the loop and tied in place. Continuity of the remaining ileum was restored by anastomosis. 175 ml of a balanced electrolyte solution (Na 135, K 5, Cl 115, and HCO_3 25 mEq/liter, and 500 mg/100 ml of polyethylene glycol [PEG], a nonabsorbable volume marker) was recirculated through the loop at a pump speed of 6 ml/min. Intraluminal pressure was kept constant at 4–5 cm of H_2O by adjusting the fluid level in a reservoir connected to the distal end of the loop. After a 30-min equilibration period, a 3 ml sample was removed and designated the zero time sample. Hourly samples were then taken for 11 hr.

To measure PD, one end of an agar-KCl bridge was inserted into the distal end of the loop via the reservoir and its connecting tubing; the other end was placed in a beaker of saturated KCl solution. One of a matched pair of calomel half-cell electrodes was placed in the beaker of KCl; the other was placed subcutaneously and the lumen-to-subcutaneous tissue PD measured with a Keithly 600B electrometer (Keithly Instruments, Inc., Cleveland, Ohio). (Intravenous, peritoneal, and subcutaneous reference sites

were compared in three dogs and showed no differences.) PD reported throughout refers to intraluminal potential with respect to subcutaneous tissue.

100 μg of choleragen (4) was added at zero time in the "cholera" studies. In two control studies, 100 μg of choleragenoid, a natural, nontoxic toxoid of the cholera enterotoxin (4), was added at zero time. In three control experiments nothing was added.

Arterial blood pressure, pH, P_{CO_2}, and serum concentrations of Na, K, Cl, HCO_3, and serum osmolality were monitored hourly. Electrolytes were measured by standard techniques, PEG by the method of Hyden (5), osmolality by freezing point depression, and pH and P_{CO_2} by a Duomatic analyzer (Instrumentation Laboratory, Inc., Watertown, Mass.). Net water and solute movement was calculated by standard techniques (6). PEG recovery, measured at the end of the 7th and 11th hr in each study, was 100.5 ±1.9% in the control and 99.6 ±0.9% in the choleragen-treated dogs. Sections of control and choleragen-treated ileal loops, taken at the end of each study, revealed no histologic differences.

RESULTS

A typical control experiment is shown in Fig. 1. Water absorption continued throughout the first 7 hr of recirculation. The Na concentration of the recirculated fluid was between 130 and 135 mEq/liter (serum Na 150); the Cl concentration fell to 85 mEq/liter (serum Cl 120–126). The HCO_3 concentration rose from 21 to 52 mEq/liter (serum HCO_3 20–22). The K concentration rose from 4 to 5.5 mEq/liter (serum K 3.5–4). The PD was between +7 and +4 mv during the first 6 hr of recirculation. Addition of glucose (in an amount calculated to achieve a 50 mM glucose solution) at the 7th hr caused the PD to become −2.0 mv. Isotonic mannitol was perfused during the 8th hr, and the PD became +23 mv. A return to the electrolyte solution cause the PD to return to its original level, and during a final 2 hr period of recirculation, water absorption continued. Mean results in five similar studies, including net ion movement and osmolality at each hour, are shown in Table I.

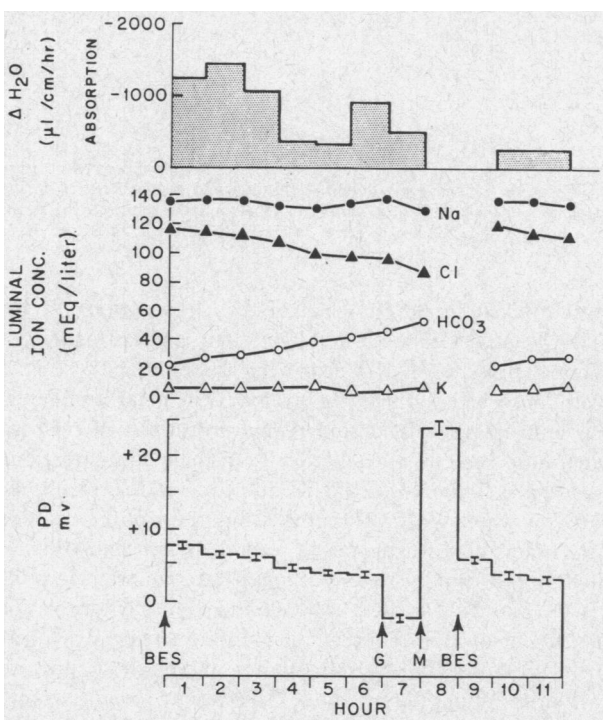

FIGURE 1 Representative experiment in a control dog. A balanced electrolyte solution (BES) was recirculated for the first 7 hr. Glucose (G) was added to BES at beginning of 7th hr (glucose concentration approximately 50 mmoles/liter). During the 8th hr, the loop was rinsed with isotonic mannitol (M). During the 9th hr, the loop was rinsed with BES. Recirculation of BES was resumed at the beginning of the 10th hr. The PD values are the mean ±1 SE of observations made every 2 min.

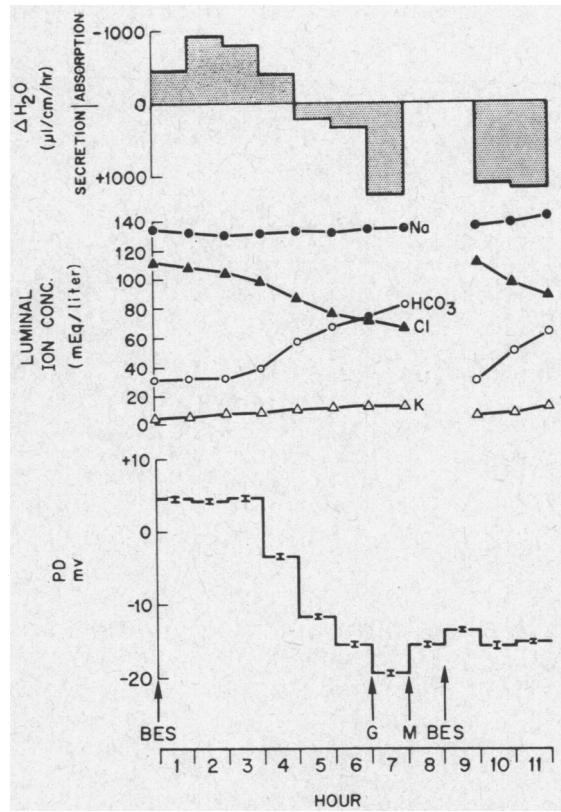

FIGURE 2 Representative experiment in a choleragen-treated dog. See legend in Fig. 1 for explanation of symbols.

Fig. 2 shows a typical study when choleragen was added to the perfusion fluid. Water was absorbed during the first 4 hr, and secretion was observed thereafter. The Na concentration was about 135 mEq/liter in the recirculated fluid compared with serum Na of 143–148. The Cl concentration fell from 110 to 65 mEq/liter (serum Cl 108–120). The HCO_3 concentration rose from 29 to 80 mEq/liter (serum HCO_3 19–21). K con-

TABLE I

Mean Results in Control and

	Blood or serum				Luminal contents, PD, and transport rates							
	Initial		Final		hr 1		hr 2		hr 3		hr 4	
	Control	Cholera	Control	Cholera	Control	Cholera	Control	Cholera	Control	Cholera	Control	Cholera
ΔH_2O					−1158	−770	−858	−513	−779	+ 55	−465	+151
					±150	±178	±180	±585	±143	±186	±83	±137
Osmolality	304	302	312	308	264	268	266	270	266	273	269	276
	±5	±5	±3	±4	±2	±4	±3	±2	±3	±2	±5	±2
Na concentration	146	141	145	141	135	132	134	131	132	131	131	132
	±1	±1	±3	±1	±1	±2	±2	±2	±3	±2	±4	±2
ΔNa					− 142	−115	−123	− 67	−108	+ 14	− 62	+ 26
					±21	±25	±26	±37	±20	±24	±12	±19
K concentration	3.3	2.9	3.6	3.3	5.3	7.3	5.6	8.0	6.0	9.6	6.5	10.6
	±0.2	±0.2	±0.2	±0.1	±0.1	±1.7	±1.0	±1.6	±0.2	±2.1	±0.3	±2.2
ΔK					− 3.4	− 1.4	− 2.7	+ 1.0	− 2.6	+ 5.0	− 1.3	+ 4.5
					±1.0	±0.6	±1.1	±1.4	±0.9	±0.6	±0.2	±1.7
Cl concentration	117	106	117	110	112	103	110	97	107	90	102	85
	±2	±3	±3	±4	±2	±5	±2	±4	±3	±5	±5	±4
ΔCl					− 132	−108	−104	− 74	−102	− 18	− 57	− 6
					±19	±23	±23	±28	±21	±2	±12	±18
HCO_3 concentration	20	19	18	18	28	36	30	40	32	49	36	57
	±1	±1	±2	±1	±1	±4	±1	±4	±2	±5	±1	±5
ΔHCO_3					− 9	− 11	− 20	+9	− 12	+ 36	− 6	+ 33
					±4	±3	±4	±13	±5	±7	±2	±8
PD					+ 2.5	+ 0.8	+ 2.6	− 2.1	+ 2.3	− 6.0	+ 1.9	− 8.8
					±1.6	±1.7	±1.4	±2.7	±1.2	±2.7	±1.0	±1.8
pH	7.45	7.40	7.39	7.37								
	±0.02	±0.02	±0.01	±0.02								
P_{CO_2}	33	34	31	35								
	±2	±2	±2	±2								

* A balanced electrolyte solution was recirculated for the first 6 and final 2 hr. During hr 7 glucose was added. Isotonic mannitol was perfused during hr 8. The loop was rinsed with the electrolyte solution during hr 9. Units are μl/cm per hr for ΔH_2O. μEq/cm per hr for Δ ion movement, mOsm/kg for osmolality, mv for PD, and mm Hg for P_{CO_2}. A minus sign denotes absorption, a positive sign denotes secretion. Values given are the mean ±1 SE of five control and six choleragen-treated dogs.

centration rose from 5 to 13 mEq/liter (serum K 3.0–3.9). PD was approximately +4 mv for the first 3 hr, and fell thereafter, reaching a level of −16 mv in the 6th hr. Addition of glucose caused the PD to become more negative to −20 mv. Perfusion of isotonic mannitol caused the PD to return to the level observed with the electrolyte solution. (Thus, the PD response of the choleragen-treated ileum is normal in response to glucose but abnormal in response to isotonic mannitol perfusion.) When a balanced electrolyte solution was rinsed through the loop during the 9th hr, the PD was −14 mv; during 2 final hr of recirculation, fluid secretion continued, and the PD was −16 mv.

Mean results in six similar studies are shown in Table I. Choleragen caused a secretion of all measured electrolytes, and the osmolality of recirculated fluid remained less than that of serum.

DISCUSSION

When a balanced electrolyte solution was recirculated for a period of hours through the normal ileum, the fluid developed a lower Na and Cl concentration than the serum levels of these electrolytes. Absorption of Na and Cl associated with a fall in their concentrations in luminal fluid and a PD near zero means that these ions were absorbed against their electrochemical gradients. By contrast, the HCO_3 and K concentrations in recirculated fluid rose to levels higher than their concentrations in plasma. This rise could be due to a relatively higher rate of water absorption than the absorption rate of HCO_3 and K, with the result that their concentration in unabsorbed fluid rises. Although active transport of HCO_3 and K has not been excluded, no evidence for movement of these ions against an electrochemical gradient was observed in our studies. Addition of glucose to luminal fluid caused the PD to become negative and perfusion of isotonic mannitol caused the PD to become markedly positive (compatible with a Na diffusion potential). These results in normal dogs are similar to those obtained in the human ileum studied by a perfusion technique and are in accord with an anion and cation exchange model proposed for normal ileal absorption (7). The results are also compatible with the model of Shultz and Curran wherein electrogenic Na

*Choleragen-Treated Animals**

Luminal contents, PD, and transport rates												
hr 5		hr 6		hr 7 (Glucose)		hr 8 (Mannitol)		hr 9		hr 10		hr 11	
Control	Cholera	Control	Cholera	Control	Cholera	Control	Cholera	Control	Cholera	Control	Cholera	Control	Cholera
−359	+316	−529	+626	−319	+1272					−259	+1127	−161	+724
±85	±84	±115	±256	±46	±217					±25	±495	±43	±196
270	280	269	285	285	296					268	272	275	282
±5	±3	±6	±3	±12	±6					±1	±2	±2	±3
130	132	130	133	128	135					134	135	135	136
±4	±2	±4	±2	±3	±2					±1	±1	±1	±2
− 51	+ 44	− 66	+ 88	− 55	+ 177					− 34	+ 154	− 20	+104
±14	±13	±14	±34	±8	±33					±4	±71	±7	±30
6.7	12.0	7.0	13.1	7.7	14.3					6.3	7.1	7.6	8.8
±0.5	±2.5	±0.8	±2.5	±0.9	±2.5					±0.5	±0.5	±0.7	±0.8
− 0.8	+ 6.5	− 1.5	+ 10.8	+ 0.4	+ 23.3					+ 2.4	+ 15.3	+ 4.2	+ 11.9
±0.6	±1.5	±1.1	±3.0	±0.7	±3.0					±1.4	±6.0	±3.0	±2.0
97	79	93	75	88	70					113	102	110	93
±6	±5	±8	±5	±6	±4					±2	±1	±3	±1
− 49	+ 3	− 56	+ 35	− 53	+ 58					− 34	+ 73	− 29	+ 42
±16	±9	±9	±17	±9	±1					±8	±37	±6	±13
40	64	44	69	48	77					28	37	33	50
±2	±5	±4	±5	±4	±4					±2	±3	±3	±4
− 2.0	+ 43	+ 9	+ 60	− 4	+ 138					+4	+ 94	+ 15	+ 82
±2	±8	±8	±20	±3	±21					±8	±42	±8	±17
+ 1.5	− 11.0	+ 1.1	− 13.2	− 4.0	− 19.0	+35.0	−15.0	+1.0	−14.0	+ 0.5	− 14.4	0.0	− 15.3
±1.1	±1.4	±0.9	±1.4	±0.8	±1.5	±7.0	±1.0	±1.0	±1.1	±1.2	±1.4	±1.0	±0.7

and Cl absorption occur at equal rates so that no PD develops (8).

As noted previously by Visscher (9), fluid recirculated through the normal dog ileum always developed an osmolality lower than that in plasma; in one of our dogs the osmolality of luminal contents fell to 240 mOsm/kg compared with a blood osmolality of 300 mOsm/kg. These results are compatible with passive water movement secondary to active ion transport and suggest that the permeability of ileal mucosa to bulk water flow is sufficiently low that passive water transport cannot keep pace with active solute absorption.

The ileum exposed to choleragen behaved quite differently than the controls. Fluid secretion usually began in the 3rd hr after choleragen was added to ileal fluid and was associated with the development of a lumen-negative PD of about 13 mv. During choleragen-induced secretion, luminal fluid developed HCO_3 and K concentrations higher than their serum concentrations, while the Cl cncentration fell far below its serum concentration.

As shown by the cholera data in Table I, HCO_3 is secreted against both an electrical and a chemical concentration gradient; and since its concentration in the recirculated fluid rose steadily and to levels higher than serum HCO_3, solvent drag cannot be responsible for its movement against this steep gradient (3, 10). According to standard definitions (10), HCO_3 movement in cholera may therefore be said to be active.[1] By contrast, Na movement can be attributed entirely to passive diffusion in response to the lumen-negative PD.

Cl secretion was against an electrochemical gradient as defined by the Nernst equation (10). This is made evident most clearly in the 10th hr study period. In spite of a PD of −14.4 mv, which would favor absorption, and a lumen Cl concentration close to that in plasma, Cl was secreted. It is unlikely that this resulted from solvent drag (10) since, in the human at least (3), passive permeability in the ileum is so slight that sol-

[1] Secretion of HCO_3 might arise from secretion of HCO_3 ions or absorption of H ions. HCO_3 secretion in the text is used loosely to indicate net accumulation within the lumen achieved by one of these processes.

vent drag is not a significant force for NaCl movement. However, solvent drag might be a more important force in the choleragen-treated ileum since anion and/or cation permeability is possibly altered (see below). It is also possible that the high HCO_3 concentration in luminal fluid may have stimulated passive Cl secretion via an anion exchange (7), but this cannot be the major explanation for Cl secretion since Cl was secreted at a brisk rate in hr 10, when a fresh solution (with a bicarbonate concentration of only 25 mEq/liter) was perfused.

To some extent K secretion can be attributed to the lumen-negative PD, although the K concentration in recirculated fluid (6th hr, for instance) is 2.4 times higher than predicted for passive transport by the Nernst equation (10). This suggests active K transport.

To summarize our interpretation of these experiments, choleragen elicits active HCO_3 and Cl secretion; the transport of one or both of these anions is electrogenic causing the lumen to become negatively charged.[2] All Na secretion is passive in response to the negative intraluminal PD. K is secreted passively in response to the negative PD, but its concentration in the secreted fluid is higher than expected if its movement were entirely passive, suggesting a component of active K secretion. The importance of K secretion is emphasized by the observation that this cation is secreted as early as the 2nd hr, before the onset of H_2O or Na secretion.

The possibility that cholera secretion is caused by filtration related to an increased pressure in the microcirculation and/or an increased mucosal interstitial pressure has been entertained by some previous workers (12). We have previously argued, on theoretical basis, that filtration is an unlikely mechanism for gut secretion in cholera unless permeability of the gut is markedly increased (13), which does not seem to be the case since the ratio of the diffusion rates of urea-^{14}C and arabinose-^{3}H from plasma to gut lumen is not significantly altered during experimental cholera in the dog ileum.[3] Furthermore, it has recently been shown that marked reduction in mesenteric blood flow does not significantly reduce gut secretion in response to cholera toxin (14). This also argues against filtration as an important mechanism of gut secretion in cholera. There are two additional reasons for believing that filtration was not a major factor in the etiology of the secretion that occurred after exposure of the ileal mucosa to choleragen. First, a filtration process could not explain the concentration gradients of HCO_3, Cl, and K, which we observed. Second, a filtration process could not explain the high lumen-negative PD which developed across the ileal mucosa.

Although both HCO_3 and Cl are actively secreted, HCO_3 transport appears to be of greater importance in the generation of fluid production, especially in the first few hours after exposure to choleragen. For instance, HCO_3 is secreted at higher rates and against steeper electrochemical gradients than is Cl, and for the first 2 hr after the onset of fluid secretion, Cl is absorbed rather than secreted (hr 3 and 4, Table I). However, this does not necessarily mean that choleragen stimulates HCO_3 secretion to a greater extent than Cl secretion, since it is possible that normal nonelectrogenic Cl absorption (as NaCl) may have continued during cholera, thus partially obscuring the cholera-induced Cl secretion. The fact that the rate of fall in Cl absorption paralleled the rate of increase in HCO_3 secretion during the early hours of our experiment (Table I) lends weight to this suggestion. Using the 1 hr values in the cholera group (Table I) as the normal absorption rate for these ileal loops and *assuming* this to remain constant for the first 6 hr of the experiment, the calculated anion makeup (total anion concentration assumed to be 140 mEq/liter) of the cholera-induced secretion is 86–98 mEq/liter of Cl and 42–54 mEq/liter of HCO_3. Similar values were calculated for hr 10 and 11, but for hr 7, when glucose was added to luminal fluid, relatively more HCO_3 (66 mEq/liter) and less Cl (74 mEq/liter) was present in this calculated secretion. The significance of this apparent effect of glucose is not clear.

Our finding that the PD becomes negative in the dog ileum during cholera is in disagreement with a recent study by Sachar, Taylor, Saha, and Phillips (15). These workers found jejunal and ileal PD to be normal in seven cholera patients who were rehydrated but still had diarrhea. Unfortunately, water and ion transport rates in the small bowel of these subjects were not studied, and it is not known whether or not the ileal segments under study were actually secreting fluid at the time PD measurements were made. On the other hand, Field, Fromm, Wallace, and Greenough (16) and Moritz, Moore, Grady, and Iber (17) have reported small (3 and 2 mv) lumen-negative transmural PD's in in vivo rabbit small intestine exposed to cholera toxin, and the development of this PD was found to correlate with onset of fluid secretion. Ion flux measurements were not reported so a distinction of which ions are actively and which are passively secreted in the rabbit in vivo is not available from these preliminary reports.

While the results presented here obviously cannot be extrapolated directly to secretion induced by cholera toxin in the duodenum and jejunum, the mechanisms

[2] A change in tissue resistance could change the absolute level of transmembrane PD without a change in ion transport (11). However, an alteration in resistance could not change the orientation of PD from plus in controls to strongly minus in cholera.

[3] Unpublished data from our laboratory.

proposed for the ileum are compatible with previous observations made in the upper small bowel, provided account is taken of the normal difference in ion transport and permeability between the upper and lower small intestine. Thus, although fluid collected from the duodenum and jejunum of cholera-treated animals has a HCO_3 concentration only slightly higher than plasma (1, 2, 18), such levels are much higher than the HCO_3 concentration of fluid which is allowed to equilibrate with the normal upper small bowel (19). The failure of HCO_3 concentration in jejunal fluid to rise to the high concentrations noted when the ileum is exposed to cholera toxin might be explained by H secretion, which we believe occurs normally in the proximal small intestine (20). If this normal mechanism persisted during cholera, HCO_3 secretion in response to cholera would be partially dissipated by its reaction with H. Furthermore, active Cl absorption does not normally occur in the proximal small bowel (3, 20), so persistence of normal absorption mechanisms would not result in a chloride impoverishment of jejunal contents. Hence, it is not surprising that jejunal fluid during cholera has Cl and HCO_3 concentrations close to that of plasma (1, 2, 18). Finally, steep concentration gradients are unlikely under any circumstances across the upper small bowel because of the high degree of its passive permeability to water, Na, Cl, and K (3). On the other hand, the proximal small bowel is relatively impermeable to HCO_3 (3), so that concentration gradients might be generated were this ion to be actively secreted; as already noted, however, the normal process of H secretion might partially mask HCO_3 secretion.

Active HCO_3, Cl, and K secretion cannot be explained readily in terms of an alteration of normal ion transport. One model of normal ileal transport does not include anything about active HCO_3 movement (8); another predicts and explains active HCO_3 and Cl secretion under some circumstances, but this model involves a coupled anion and cation exchange (7), and thus, does not explain the generation of a PD by ion transport. Neither model explains active K secretion. It seems likely, therefore, that cholera toxin either initiates a new transport system that is not present normally or stimulates a transport system that normally is small and undetectable.

Recently Field et al. (16) and Al-Awqati, Cameron, Field, and Greenough (21) have published abstracts dealing with the effect of cholera toxin on ion transport by in vitro rabbit and human ileal mucosa, respectively. They concluded that ileal fluid loss resulted primarily from stimulation of active Cl secretion. There was apparently no evidence of active HCO_3 or K secretion. There thus may be a discrepancy between the effects of cholera toxin in vitro and in vivo.

Normally, when isotonic mannitol is perfused through the ileum the PD becomes strongly lumen positive; this has been interpreted to mean that ileal mucosa is more permeable to cations than to anions (7). A remarkable finding of our cholera experiments is that mannitol perfusion does not alter PD. This suggests either that ileal mucosa during cholera loses its selective permeability to anions and cations or that the ileal permeability barrier is exposed only to the secreted fluid and does not "see" the low level of Na and Cl in the luminal contents during mannitol perfusion. If failure of the PD to respond to mannitol indicates an abnormality in ion permeability, this might be caused by enhanced anion or decreased cation permeability; either could theoretically play a role in the pathogenesis of cholera.

The significance of the PD response to glucose during cholera is not clear since the mechanism of this response in the normal ileum is controversial (7).

A final point of interest is the observation that luminal contents remained hypotonic to serum during choleragen-induced secretion. While the data are most likely explained by delayed osmotic adjustment of the slightly hypotonic fluid originally recirculated, the possibility of a hypotonic secretion cannot be ruled out. Hypotonic secretion could result from a special arrangement of membranes in series as described by Patlak, Goldstein, and Hoffman (22).

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