Interrelationships of Chloride, Bicarbonate, Sodium, and Hydrogen Transport in the Human Ileum

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ABSTRACT Using a triple-lumen constant perfusion system, the following observations were made in normal subjects. First, chloride, bicarbonate, and sodium were found to exhibit net movement across ileal mucosa against electrochemical gradients. Second, during perfusion with a balanced electrolyte solution simulating plasma, the ileum generally absorbed, but sometimes secreted fluid. A reciprocal net movement of chloride and bicarbonate was noted when sodium movement was zero. Increasing rates of sodium absorption were associated with decreasing bicarbonate secretion rates and finally bicarbonate absorption. Even when bicarbonate was absorbed ileal contents were alkalinized (by contraction of luminal volume). Third, net chloride movement was found to be sensitive to bicarbonate concentration in ileal fluid. For instance, chloride was absorbed from solutions containing 14 or 44 mEq/liter of bicarbonate, but was secreted when ileal fluid contained 87 mEq/liter of bicarbonate. Fourth, when chloride-free (sulfate) solutions were infused, the ileum absorbed sodium bicarbonate and the ileal contents were acidified. Fifth, when plasma-like solutions were infused, the potential difference (PD) between skin and ileal lumen was near zero and did not change when chloride was replaced by sulfate in the perfusion solution.

These results suggest that ileal electrolyte transport occurs via a simultaneous double exchange, Cl/HCO_3 and Na/H. In this model neither the anion nor the cation exchange causes net ion movement; net movement results from the chemical reaction between hydrogen and bicarbonate. No other unitary model explains all of the following observations: (*a*) human ileal transport in vivo is essentially nonelectrogenic even though Na, Cl, and HCO_3 are transported against electrochemical gradients, (*b*) the ileum can secrete as well as absorb, (*c*) ileal contents are alkalinized during absorption of or during secretion into a plasma-like solution, and (*d*) the ileum acidifies its contents when sulfate replaces chloride. Data obtained with a carbonic anhydrase inhibitor support the proposed model.

INTRODUCTION

Current concepts of the mechanisms of ileal electrolyte absorption (1, 2) are derived from both in vitro and in vivo experiments, although in recent years relatively few in vivo studies have been carried out. The most generally held view is that electrolyte absorption in vitro is due to active sodium transport and that the electrical potential so generated is the main force for anion absorption (1). In most species that have been tested, electrolyte absorption in vivo is not associated with a potential difference across ileal mucosa, and this has been attributed to simultaneous active transport of sodium and chloride, both pumps electrogenic but of the same magnitude so that no net potential difference is generated (1). In vivo, concentrations of chloride and bicarbonate vary reciprocally in ileal fluid with their sum equal to approximately 135 mEq/liter; the bicarbonate concentration is generally higher and the chloride concentration lower than their respective plasma levels (1, 2). Bucher, Flynn, and Robinson (3) were apparently the first to suggest that the appearance of bicarbonate in ileal fluid is linked to the absorption of chloride (anion exchange), but it is important to note that reciprocal *net movements* of chloride and bicarbonate do not occur during absorption of balanced electrolyte test solutions but that chloride absorption is always much higher than bicarbonate secretion. Thus, as judged by the rate of bicarbonate secretion, very little chloride could be absorbed by anion exchange.

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We have recently conducted a series of experiments in the human ileum in vivo which are not compatible with these concepts and which have led us to propose a different mechanism for ileal ion transport.

METHODS

The subjects of this study were normal male and female volunteers, aged 21–35 yr. The Ingelfinger triple-lumen perfusion technique was used exactly as described in detail in earlier publications (4). The intestinal test segment was 30 cm and the nonabsorbable marker was polyethylene glycol. Studies were done when the infusion tip of the triple-lumen tube was 200–250 cm from the teeth. Unless otherwise stated, the infusion rate was 10 ml/min.

The mean concentration of solute in the test segment was calculated by averaging the concentration in fluid withdrawn from each end of the test segment. Results are expressed in milliliters of water or milliequivalents of electrolyte absorbed or secreted per hour per 30 cm of ileum. Usually three different test solutions were studied in the same individual on one morning.

Potential difference (PD) between intestinal lumen and abraded skin was measured during perfusion with various test solutions by means of an intraluminal electrode described in an earlier publication from this laboratory (4). PD was measured during perfusion with the identical solutions used for measurement of ion movements and in the same subjects, although the tests were performed on different days.

To validate the use of abraded skin as a reference site PD between skin and peritoneum was measured four times in patients undergoing peritoneal dialysis. With the skin as a reference, peritoneal PD + 2.0, + 0.2, 0.0, and − 0.2 mv in the four studies. Therefore, our results of PD measurements between skin and gut lumen should be approximately the same as between lumen and peritoneum.

Electrolyte and polyethylene glycol (PEG) concentrations were estimated with methods described previously (4). Total carbon dioxide was measured by the microgasometric method using a Natelson microgasometer (Model 600, Scientific Industries, Inc., Springfield, Mass.). pH and P_{CO_2} were measured on an Ultramicro pH/P_{CO_2} gas analyzer (Instrumentation Laboratories, Inc., Lexington, Mass.).

The results are given as mean values ±1 SE of the mean in the tables and figures.

RESULTS

Absorption and secretion rates

Chloride. Ileal chloride absorption at varying chloride concentrations was assessed in nine subjects. Test solutions contained 0, 50, or 100 mEq/liter of chloride as the sodium salt. Sodium and potassium bicarbonate and mannitol were added in amounts required to give each solution a bicarbonate concentration of 30 mEq/liter, a potassium concentration of 5 mEq/liter, and an osmolality of 285 mOsm/kg. As shown in Fig. 1, chloride absorption increased linearly with increasing mean luminal chloride concentration over the range of 10–94 mEq/liter. Absorption occurred against a considerable lumen-to-plasma concentration gradient.

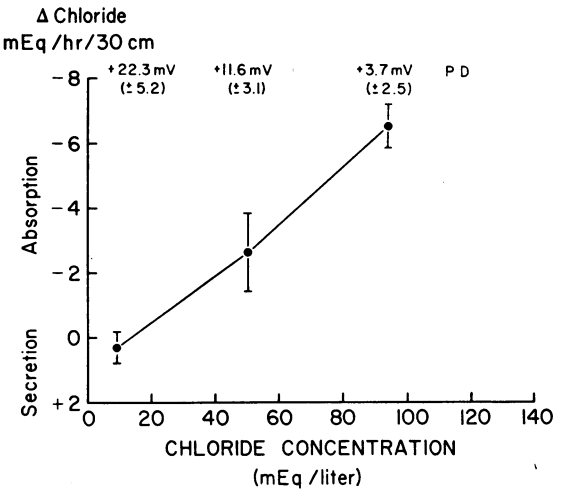

FIGURE 1 Effect of luminal chloride concentration on net chloride movement. Sodium concentration was approximately 25 mEq/liter higher than chloride; bicarbonate concentration was 30 mEq/liter in all studies. The potential difference (PD) between ileal lumen and skin at different sodium chloride concentrations is given at the top of the figure. The sign preceding a PD value indicates the orientation of the potential, + meaning lumen positive compared to skin and − meaning lumen negative compared to skin.

The PD between skin and ileal lumen was measured in seven subjects during perfusion of identical solutions as in the above experiments and the mean values for these PDs are also shown in Fig. 1. As luminal chloride (and sodium) concentration was reduced, the lumen became increasingly electropositive with respect to the skin. Thus chloride absorption occurs against both steep concentration and steep electrical gradients.

Sodium. Ileal sodium absorption, measured during perfusion of the test solutions just described, also occurred against steep concentration gradients, as previously described in detail (4). However, unlike chloride, sodium absorption occurred in the direction of, and not against, an electrical gradient. Utilizing the Nernst equation, it is possible to relate chemical and electrical gradients and to infer whether sodium movement is passive or active, at least as defined by this equation. For instance, in the present studies sodium absorption continued until sodium concentration was reduced to approximately 27 mEq/liter; at lower concentrations, sodium was secreted into the ileal lumen. By the Nernst equation, electrical PD would have to be 43 mv to account for this unequal distribution of sodium ions between ileal lumen and plasma. The observed PD was only approximately 22 mv, indicating that sodium was not distributed passively across the ileal mucosa and that ileal sodium absorption occurs against electrochemical gradients.

TABLE I

Slow Perfusion Studies. Changes in Bicarbonate Concentration Compared with Changes in PEG Concentration and Net Bicarbonate Movement

Study	Bicarbonate concentration at distal aspiration site	Increase in bicarbonate concentration from proximal to distal site	Increase in PEG concentration from proximal to distal site	Δ HCO_3^-*
	mEq/liter	%	%	*mEq/hr per 30 cm*
Infused bicarbonate concentration				
30 mEq/liter				
1	51.7	48.1	17.9	+0.51
2	58.3	52.6	29.4	+0.37
3	31.5	32.3	18.3	+0.29
4	52.2	56.8	35.3	+0.34
5	61.9	43.3	35.3	+0.17
35 mEq/liter				
6	41.6	8.6	36.5	−1.29
7	42.3	23.0	14.9	+0.24
8	41.2	38.8	19.6	+0.27
9‡	47.3	18.3	−21.3‡	+1.65
40 mEq/liter				
10	48.3	17.2	41.8	−0.48
11	50.1	34.7	43.3	−0.22
12	39.1	54.5	7.6	+0.90
13	47.6	37.2	102.4	−0.81

* + sign indicates net secretion, − sign indicates net absorption.

‡ Subject in whom net water movement was into the lumen.

Bicarbonate. Preliminary studies revealed that net bicarbonate movement[1] from balanced electrolyte solutions was small and difficult to measure accurately with the standard perfusion technique. Therefore, experiments were performed at a slow rate of perfusion, 1.7 ml/min, over a long (4 hr) period of time. This slow perfusion accentuates ionic and PEG concentration changes and markedly enhances the accuracy of any given experimental result. The long perfusion period, which was preceded by 1 hr equilibration, precluded more than a single experiment on a given day.

Perfused test solutions contained 30, 35, or 40 mEq/liter of bicarbonate, 105 or 110 mEq of chloride, 135 or 140 mEq of sodium, and 5 mEq/liter of potassium. Results of 13 slow perfusion studies are shown in Table I. In every study bicarbonate concentration increased as fluid passed from proximal to distal aspiration site. Since water absorption occurred as shown by the rise in PEG concentration, some of the increase in bicarbonate concentration was due to a contraction of luminal volume. However, as revealed in the table, per cent rise in bicarbonate concentration between proximal and distal aspiration sites was higher than the per cent rise in PEG concentration in 10 of 13 studies. In these experiments bicarbonate secretion was at least partly responsible for the high concentrations achieved in the ileum. In the three experiments in which bicarbonate absorption was noted, the rise in bicarbonate concentration was due entirely to a shrinkage of ileal volume due to rapid water absorption.

PD was measured four times during slow perfusion of the same test solution. Mean PD was +0.5 ±3.3 mv.

Relation between water and ion absorption rates during perfusion with a balanced electrolyte solution

Because of the high degree of accuracy of the slow perfusion studies, these experiments were chosen to examine the relation between sodium and anion movements in individual perfusion periods. These results are

[1] Difficulties arise in deciding which of several mechanisms is responsible for bicarbonate movement. Removal of bicarbonate from the lumen could be achieved by absorption of bicarbonate ions or by secretion of hydrogen ions. The identical effect to hydrogen secretion could be produced by hydroxyl ion absorption from water which would leave an excess of hydrogen ion in the lumen. Bicarbonate accumulation could occur by reversal of any of these processes. Unless otherwise specified the terms bicarbonate "absorption" and "secretion" are used in the loose sense to indicate removal from or accumulation within the lumen achieved by one of the above processes.

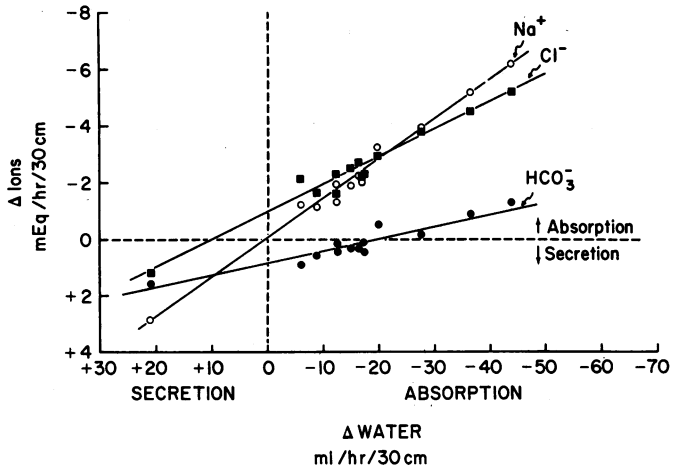

FIGURE 2 Relation of ion and water movements in slow (1.7 ml/min) perfusion studies. Regression lines were drawn by method of least squares. *P* values for difference between chloride and sodium and sodium and bicarbonate movements at zero water flow were < 0.001 and < 0.001, respectively.

shown in Fig. 2, where sodium, chloride, and bicarbonate movements are related to water movement. Mean luminal sodium concentration was 137 (range 125–143), mean potassium concentration was 4.9 (4.5–6.0), and mean chloride concentration was 97 (87–108) mEq/liter. Mean bicarbonate concentration varied between 32.2 and 52.6 mEq/liter, depending on the rate of water absorption and bicarbonate secretion. Several conclusions can be drawn from the data and regression lines shown in Fig. 2. First, it is clear that zero sodium absorption is associated with zero water movement which is compatible with the thesis that water movement is a passive consequence of net solute movement (1). Second, when water and sodium absorption are zero, bicarbonate is secreted and chloride is absorbed at approximately equal rates. This is suggestive of an

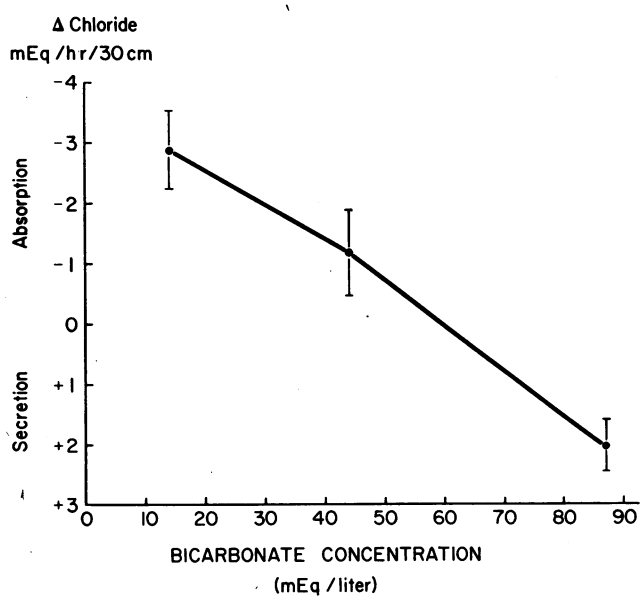

FIGURE 3 Effect of luminal bicarbonate concentration on net chloride movement. Chloride and sodium concentrations were constant at 40 and 135 mEq/liter, respectively; sulfate was added for ionic balance, and all test solutions were isotonic.

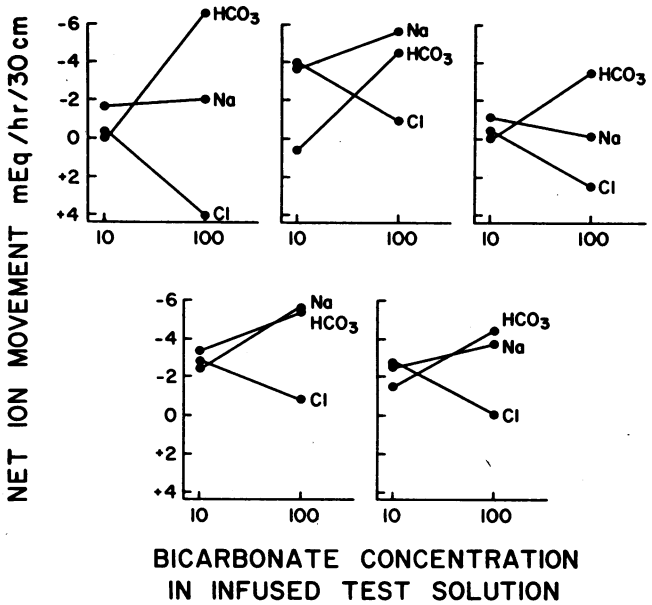

FIGURE 4 Ion movement at different luminal bicarbonate concentrations. The infusion rate for these studies was 5 ml/min. Actual mean concentrations in the test segment were sodium 136 and 137, chloride 42 and 60 and bicarbonate 13 and 81 mEq/liter, respectively, for the low and high bicarbonate solution. Negative values indicate absorption, positive values indicate secretion.

anion exchange. Third, increasing sodium absorption is associated with decreasing bicarbonate secretion, until, at an absorptive rate for sodium of 3.0 mEq/hr per 30 cm, net absorption of bicarbonate occurs. Finally, in one study all ions and water were secreted; thus, the normal ileum does not always absorb but has the capacity to secrete as well.

As already mentioned, PD during perfusion with the test solutions used in these experiments was +0.5 ±3.3 mv.

Effect of bicarbonate on chloride movement

If, as suggested by the data presented in Fig. 2, chloride/bicarbonate exchange does occur, net movement of one anion might be influenced by the luminal concentration of the other. A series of perfusion experiments was therefore performed in which chloride concentration was maintained at 40 mEq/liter while bicarbonate concentration was varied between 14 and 87 mEq/liter. Sodium concentration was 135 mEq/liter in each solution, the missing anions being provided by

TABLE II

PD in Four Subjects in Whom Sodium Concentration Was Constant While Anion Concentrations Were Varied. In These Four and One Other Subject the Effect of Glucose on PD is Shown in the Last Column. Solutions Containing Sulfate Also Contained Sufficient Mannitol to Make Them Isotonic with Plasma

Infused solutions*	Na^+ = 135 Cl^- = 40 HCO_3^- = 10 SO_4^- = 45	Na^+ = 135 Cl^- = 40 HCO_3^- = 100 SO_4^- = 0	Na^+ = 135 Cl^- = 110 HCO_3^- = 30 SO_4^- = 0	Na^+ = 135 Cl^- = 110 HCO_3^- = 30 Glucose = 50
	(n = 4)	(n = 4)	(n = 4)	(n = 5)
Mean PD	+0.8	+1.8	+0.5	−7.2
±1 SE	±3.1	±3.8	±3.3	±1.7

* Each solution contained 5 mEq/liter of potassium.

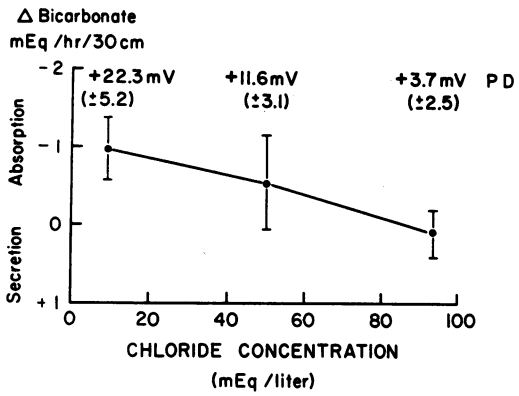

FIGURE 5 Effect of luminal chloride concentration on bicarbonate movement. Luminal sodium paralleled the chloride concentration but was approximately 25 mEq/liter higher at each of the three points. Luminal bicarbonate concentration was constant (24.6–27.7 mEq/liter), regardless of sodium chloride concentration.

sulfate. Isotonicity was maintained with mannitol. 10 subjects were studied with each of the three test solutions.

As shown in Fig. 3, luminal bicarbonate concentration influenced chloride movement despite the maintenance of a constant chloride concentration. When the mean luminal bicarbonate concentration was 14 mEq/liter, chloride was absorbed against a concentration gradient. However, chloride absorption was reduced at a bicarbonate concentration of 44 mEq/liter; and when mean bicarbonate concentration was 87 mEq/liter, chloride was secreted.

Five additional studies were performed to examine more closely the net movements of chloride and bicarbonate as bicarbonate concentration was varied. In order to increase accuracy the perfusion rate was reduced from the standard rate of 10 ml/min to 5 ml/min, and each study period lasted 2 hr instead of 1 hr. Test solutions had a chloride concentration of 40 mEq/liter and bicarbonate concentration was either 10 or 100 mEq/liter. Sodium concentration was 135 mEq/liter in both solutions with the missing anion being provided by sulfate. As before, isotonicity was maintained with mannitol.

As shown in Fig. 4, raising the bicarbonate concentration of the infused solution from 10 to 100 mEq/liter caused chloride absorption to be reduced or chloride secretion to be induced, and the change in net bicarbonate movement was approximately the same as the change in chloride movement, except in those subjects in whom sodium absorption was different in the two studies. These experiments confirm a reciprocal net movement of chloride and bicarbonate as luminal bicarbonate concentration is varied.

Measurements of PD were made during perfusion of solutions with varying anion concentrations while sodium concentration was maintained at 135 mEq/liter. Table II demonstrates that PD does not change significantly when chloride and bicarbonate concentrations are varied reciprocally or when both are largely replaced by sulfate. Therefore, the changes in chloride movement induced by altering luminal bicarbonate concentration occur in the absence of change in PD.

Effect of chloride concentration on bicarbonate movement

The effect of varying (sodium) chloride concentration on ileal bicarbonate movement was assessed in 10 subjects. Luminal contents had a constant bicarbonate concentration (24.6–27.7 mEq/liter) and osmolality was made constant at 285 mOsm/kg by addition of appropriate amounts of mannitol to the test solutions. As shown in Fig. 5, bicarbonate was secreted at a slow rate when luminal chloride concentration was 94 mEq/liter. However, bicarbonate was absorbed when chloride concentration was lowered to 50 and 10 mEq/liter.

PD was +22.3 mv (lumen positive) when lumen chloride concentration was 10 mEq/liter, and +3.7 mv when chloride concentration was 94 mEq/liter. Thus ab-

TABLE III

Ion Movement in the Presence and Absence of Chloride (Mean Values ±1 SE). Concentrations Refer to Mean Concentration in Test Segment

	ΔH_2O	[Na]	ΔNa	[K]	ΔK	[Cl]	ΔCl	$[HCO_3]$	ΔHCO_3	P_{CO_2}		
										Infusion	Proximal	Distal
Chloride-free solution $n = 6$	−12.6 ±3.1	142.2 ±0.6	−1.30 ±0.50	5.13 ±0.12	−0.02 ±0.03	11.3 ±2.1	−0.01 ±0.28	15.5 ±1.7	−1.35 ±0.28	53.9 ±1.8	61.8 ±1.1	57.7 ±2.3
Chloride-containing solution $n = 6$	−24.9 ±9.8	141.0 ±0.4	−3.50 ±1.45	5.30 ±0.09	+0.00 ±0.12	98.5 ±2.3	−4.13 ±1.34	34.3 ±2.7	+0.63 ±0.29	52.1 ±2.1	57.4 ±2.6	56.7 ±2.8

sorption of bicarbonate occurred against a PD of 22.3 mv at a chloride concentration of 10 mEq/liter, while at the highest chloride concentration (94 mEq/liter), bicarbonate movement (secretion) was in the direction favored by the small PD gradient (3.7 mv).

These experiments show that net bicarbonate movement is influenced by luminal sodium chloride concentration and that bicarbonate can be absorbed against electrochemical gradients (see footnote 1).

Ion transport during sodium sulfate perfusion

Since the studies recorded in Fig. 5 indicated that bicarbonate can be absorbed against electrochemical gradients when luminal chloride concentration is low, it was decided to study the effect of chloride removal in greater detail, especially in regard to its effect on Na and K movements and on pH and P_{CO_2} of luminal fluid. To make these observations, the ileum was perfused with an electrolyte solution containing 140 mEq/liter Na, 5 mEq/liter K, 25 mEq/liter HCO_3, and no Cl. The missing anion was sulfate, and mannitol was added to bring the final osmolality to 290 mOsm/kg. A control solution had the same bicarbonate, sodium, and potassium concentrations but contained 100 mEq/liter of chloride rather than sulfate. The infused test solutions were bubbled with 5% CO_2 for 30 min before and during the intestinal perfusions.

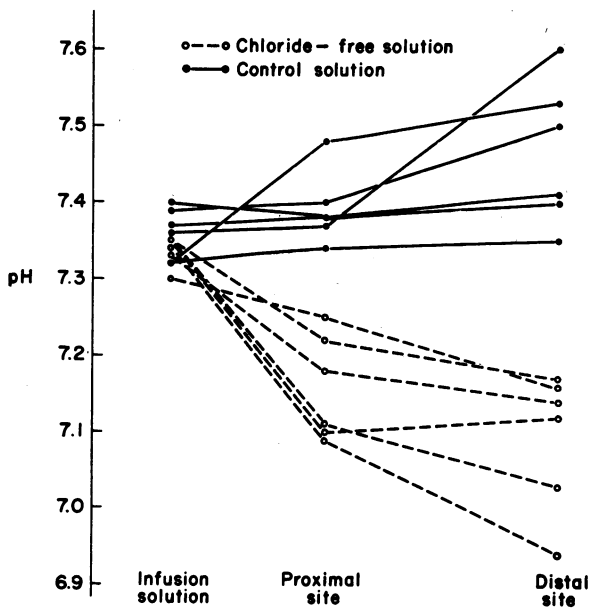

FIGURE 6 Effect of chloride and chloride-free (sulfate) solutions on pH of infused test solution and fluid collected from the proximal and distal collection sites of the three-lumen tube. Test solutions were bubbled with 5% CO_2 before infusion. Sodium, potassium, and bicarbonate concentrations of both test solutions were 140, 5, and 25 mEq/liter, respectively.

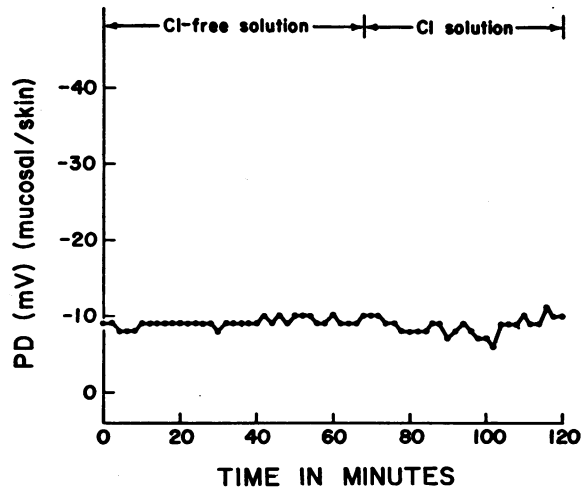

FIGURE 7 Potential difference (lumen to skin) during perfusion with chloride and chloride-free (sulfate) test solutions. All test solutions contained 140, 5, and 25 mEq/liter of sodium, potassium, and bicarbonate, respectively. A positive PD indicates lumen positive with respect to skin; a negative potential means lumen negative with respect to skin.

Table III shows the mean concentration of ions in the test segment and their movements for the two infusion solutions. Substitution of sulfate for chloride was associated with a reduction in sodium absorption from 3.5 to 1.3 mEq/hr. Potassium movement was the same in the presence or absence of chloride. Bicarbonate was secreted in the presence of chloride and absorbed in its absence.

Fig. 6 shows pH of the infusion solutions and fluid collected at proximal and distal sites under a layer of oil. The pH values of both infusion solutions ranged from 7.30 to 7.40. Whereas the chloride-containing solutions either became more alkaline or did not show a pH change, the pH values of the chloride-free solutions fell. At the proximal collecting site, the pH had fallen to values between 7.09 and 7.25. Between the proximal and distal sites, the pH continued to fall to values between 6.94 and 7.16. As shown in Table III, luminal P_{CO_2} was approximately the same during perfusion of the two test solutions.

As shown in Fig. 7, substitution of sulfate for chloride had no demonstrable effect on PD. Three other studies gave similar results.

Carbonic anhydrase inhibitor

The effects of acetazolamide[2] (200 mg/liter of perfused fluid) on sodium, chloride, and bicarbonate movements were tested during perfusion of a balanced electrolyte solution. As shown in Fig. 8, absorption of sodium and chloride was markedly inhibited by acetazolamide.

[2] Diamox, American Cyanamid Co., Lederle Laboratories Div., Pearl River, N. Y.

There was no consistent effect on net bicarbonate movement which was near zero with and without acetazolamide.

Sodium diffusion potential

As shown in Table II and in Fig. 7, the PD between ileal lumen and skin is not affected by reciprocal variation of chloride and bicarbonate or chloride and sulfate. By contrast, progressive lowering of luminal sodium concentration is associated with a progressive increase in PD with the lumen positive to skin. These results, illustrated in Fig. 9, are compatible with a sodium diffusion potential. Similar results were obtained in the rat by Wright (5) and this conclusion is compatible with PD measurements in man reported by Gustke, Whalen, Geenen, and Soergel (6). As shown in Table II, the average PD across ileal mucosa is close to zero when the ileum is perfused with an electrolyte solution simulating plasma; when glucose is added to such solutions, the PD becomes substantially negative, as previously reported (4).

DISCUSSION

These experiments were designed primarily to elucidate three aspects of ileal transport. First, we measured ion movement and PD as ion concentrations varied in order to establish which ions equilibrate passively and which must be subjected to some special transport process. Second, we determined the interrelationship between the movement of various ions by carefully measuring Na, K, Cl, and HCO_3 movements during perfusion of a balanced electrolyte solution and by assessing the effect of the concentration of one ion on the movement of the others. Finally, by measuring potential difference under

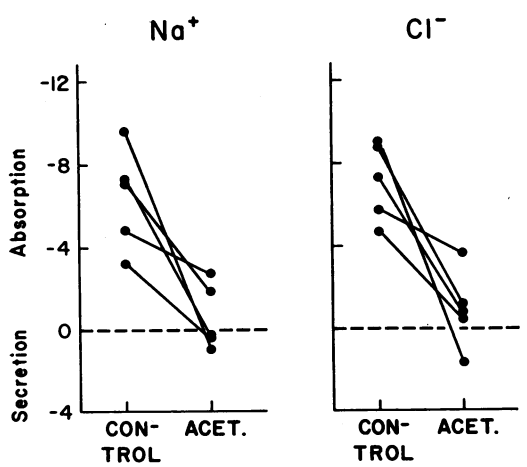

FIGURE 8 Effect of acetazolamide on sodium and chloride movements during perfusion with a balanced electrolyte solution.

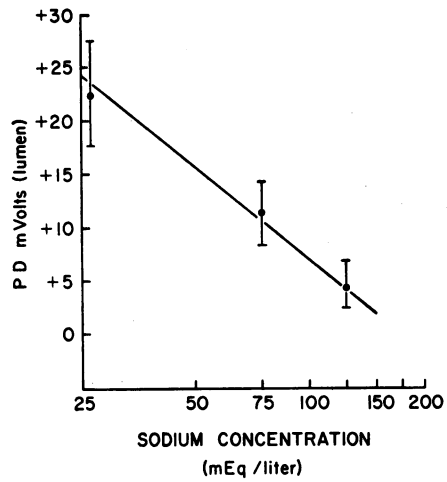

FIGURE 9 Effect of luminal sodium concentration on potential difference (mean ±1 SE) between ileal lumen and skin. Orientation of PD is the same as in Fig. 1.

a variety of experimental conditions, we attempted to determine whether or not ion transport in the ileum in vivo is electrogenic.

Ion movement against electrochemical gradients

The results presented in Fig. 1 provide clear evidence for absorption of chloride against a steep electrochemical gradient. Similarly, the data in Table I suggest that bicarbonate secretion usually occurs against an electrochemical gradient when luminal chloride concentration is approximately 100 mEq/liter. However, when luminal chloride concentration is low, bicarbonate movement in the reverse direction, i.e. absorption, occurs against electrochemical gradients (Fig. 5, Table III). Thus, under different conditions there is evidence for movement of bicarbonate against an electrochemical gradient in either direction across the membrane. In addition to the apparently active nature of these transport processes, it is clear that the concentration of one anion markedly influences movement of the other.

The present results confirm previous investigations reported from this laboratory suggesting that sodium is actively transported in the human ileum (4). In the present studies the orientation of the PD developed across the ileal membrane (lumen positive) favored sodium absorption from ileal solutions having a low sodium concentration; however, the magnitude of this PD could not alone account for the observed concentration gradient against which sodium was absorbed. Furthermore, when 135–140 mM saline solutions plus glucose are perfused, the ileal lumen becomes electronegative (Table II and reference 4); sodium absorption continues under these conditions demonstrating conclu-

sively that sodium can be absorbed against electrochemical gradients. We found no evidence for potassium movement against an electrochemical gradient in the normal human ileum.

Interrelation between ion movements

Variation of normal ileal function during balanced electrolyte perfusion. In previous ileal perfusion experiments, wide variation in the rates of sodium and water absorption from day to day in the same subject and between different normal subjects has been noted. Secretion rather than absorption is occasionally observed (unpublished observations of the authors). Similar findings were seen in the slow perfusion studies reported in the present paper, and the high accuracy of these studies precludes experimental error as an explanation for this variation in ileal behavior. Secretion of sodium, chloride, bicarbonate, and water was observed at one end of the scale and absorption of all these at the other. This marked diversity of ileal activity occurred in response to factors not defined in the present study; unknown hormonal or circulatory phenomena may have played a part.

The interrelation between different ion movements at varying rates of absorption or secretion was also of interest. Only when sodium movement was zero did an equimolar anion exchange become obvious. Sodium absorption was associated with a decrease in bicarbonate secretion and at high sodium absorption rates, bicarbonate was absorbed; sodium secretion, on the other hand, was associated with enhanced bicarbonate secretion. Regardless of the rate or direction of fluid movement, ileal fluid becomes alkaline. During fluid secretion, alkalinity results from bicarbonate secretion; during absorption, alkalinity results from water absorption (which causes luminal bicarbonate concentration to rise) and in many instances also from bicarbonate secretion.

Effect of bicarbonate concentration on chloride movement. The data presented in Figs. 3 and 4 clearly demonstrate that chloride movement is dependent upon luminal bicarbonate concentration. This effect was not mediated by a change in PD, as shown in Table II. Although these observations, like the reciprocal anion movement at zero sodium movement during balanced electrolyte perfusion, suggest a chloride/bicarbonate exchange, it is clear that net chloride absorption from physiologic solutions is much greater than net bicarbonate accumulation within the lumen. For instance, when luminal contents were similar in ionic composition to plasma, chloride was absorbed at a rate of 6.5 mEq/hr per 30 cm, mainly as sodium chloride, while bicarbonate was secreted at a rate of only 0.1 mEq/hr per 30 cm (compare Figs. 1 and 5). Thus, that portion of chloride absorption normally mediated via an exchange must be exceedingly small if judged by the net bicarbonate accumulation rate. However, this ignores the possibility that some of the exchanged, i.e. secreted, bicarbonate may have been removed by a separate process, such as hydrogen secretion.

Ileal ion movements during perfusion of chloride-free solutions. The studies described in Table III and Fig. 6 demonstrate that when sodium plus a nonabsorbable anion (sulfate instead of chloride) is perfused, bicarbonate is absorbed and hydrogen ions accumulate in the ileal lumen. Several mechanisms might explain this observation. Active sodium absorption in the absence of an absorbable anion might cause the intraluminal PD to become negative with the result that hydrogen and potassium would be secreted passively. This possibility is unlikely since PD was the same when the ileum was perfused with chloride-containing or chloride-free solutions and since sodium absorption from chloride-free solutions did not stimulate potassium secretion. At least two possible explanations remain. First, a neutral sodium bicarbonate pump might become activated when chloride is removed from the ileal lumen. Second, a sodium-hydrogen exchange carrier could effect sodium bicarbonate absorption without a change in PD. Our data do not, unfortunately, provide a clear indication that either of the last two possibilities is correct. However, two facts are clear. The ileum continues to absorb sodium in the absence of luminal chloride, and sodium absorption under these conditions is associated with bicarbonate absorption and acidification of ileal contents.

Nonelectrogenic nature of ileal ion transport in man

The experiments reported here suggest that ion transport in the normal human ileum in vivo does not generate an electrical potential across ileal mucosa. This statement is based on the following pieces of evidence:

(*a*) when balanced electrolyte solutions are perfused through the human ileum, the average PD between skin and ileal lumen (which should approximate lumen/peritoneum PD as discussed in Methods) is near zero; the lumen is slightly positive as often as it is slightly negative. This observation has also been made in ileum of experimental animals in vivo, although the interpretation was that chloride and sodium were actively absorbed at the same rates by separate (and negating) electrogenic pumps (1).

(*b*) When the human ileum is perfused with a balanced electrolyte solution in which chloride has been replaced by poorly absorbed sulfate, the PD between skin and lumen is the same as when a chloride-containing solution is perfused. This suggests that an electrogenic chloride pump is not negating the potential of an electrogenic sodium pump when chloride-containing so-

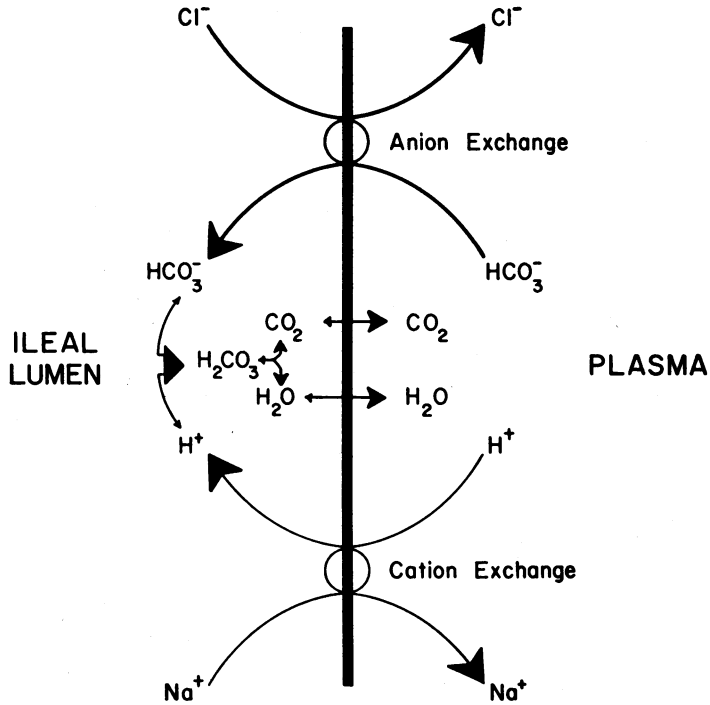

FIGURE 10 A double exchange model that best explains our observations on the relation of different ion movements in the ileum.

lutions are perfused.[3] Sodium (bicarbonate) absorption from a chloride-free solution is not accompanied by potassium secretion which is further evidence against a change in PD since potassium probably equilibrates passively across ileal mucosa (1, 2).

(*c*) In a low resistance epithelium in which net ion fluxes are small compared to unidirectional fluxes, sodium transport might be electrogenic but produce a low potential that could not be distinguished experimentally from zero. (We probably could not pick up a PD change less than 2–3 mv by our system.) Fortunately, however, we have some idea of the magnitude of PD that an electrogenic sodium pump would have to generate in order to account for the bicarbonate concentration gradient that is developed across ileal mucosa during perfusion of chloride-free solutions. As shown in Table III, the mean bicarbonate concentration in the study segment fell to 15.5 mEq/liter. The average concentration of bicarbonate at the end of the study segment was 13 mEq/liter. To account for this chemical gradient on an electrical basis would require a PD of 17 mv, assuming a plasma bicarbonate concentration of 25 mEq/liter. A PD change (as chloride is replaced by sulfate) of this magnitude would be easily detectable. Since no PD change at all could be observed, an electrically neutral mechanism must be postulated for sodium bicarbonate absorption under these experimental conditions.

The PD change observed when the sodium chloride concentration of ileal contents was progressively lowered was clearly related to sodium and not to chloride concentration, i.e., a sodium diffusion potential. These results suggest a much higher permeability of ileal mucosa to sodium than to anions and are consistent with the finding that the maximum electrochemical gradients against which sodium can be absorbed is much smaller than that against which chloride can be absorbed. This may also help explain why glucose added to electrolyte solutions perfusing the human ileum causes the lumen to become electrically negative even though sodium absorption is not increased (4). Glucose enhances ileal water absorption, and its effect on PD might be due to a streaming potential (7). This possible mechanism for the glucose effect has not, to our knowledge, been ruled out in vitro.

[3] This analysis assumes equal passive permeability of the ileum to sulfate and chloride. Only if the ileum were more permeable to sulfate than to chloride would the observed results be compatible with electrogenic sodium transport (sulfate diffusion potential cancels sodium transport potential). Had the lumen become negative under these experimental conditions, this might indicate either electrogenic sodium transport or a higher ileal permeability to chloride than sulfate (chloride diffusion potential).

A model for ion transport in the ileum

Any complete model for ileal transport must take into account the ability of the ileum to secrete as well as absorb, the interrelations of ionic movements at different rates of absorption or secretion, the effect of bicarbonate concentration on chloride movement, the bicarbonate absorption and acidification of luminal contents when the ileum is perfused with sodium plus a nonabsorbable anion, and the nonelectrogenic nature of ion transport. The simplest model which fits our data and observations is one in which a chloride-bicarbonate exchange is linked with a sodium-hydrogen exchange.

According to this double exchange hypothesis, illustrated in Fig. 10, neither the anion nor the cation exchange brings about net ion movement with the result that an electrical potential difference is not generated. Net movement observed by measuring the disappearance or accumulation of solute is the consequence of the chemical reaction between hydrogen and bicarbonate to form water and CO_2. If the rate of the anion and cation exchange is exactly equal, the model functions as a NaCl pump and observed bicarbonate movement is zero; on the other hand, if the rate of the two exchanges is not equal, net bicarbonate movement will be observed. For instance, when chloride absorption exceeds sodium absorption, more bicarbonate than hydrogen would enter the lumen resulting in net bicarbonate secretion. Conversely, when sodium absorption exceeds chloride absorption, more hydrogen than bicarbonate enters the lumen resulting in net bicarbonate absorption. Ileal secretion can occur by reversing the usual direction of the two exchange mechanisms.[4]

Since the rate of movement of sodium and chloride is often different and since sodium is absorbed even when chloride is completely replaced by sulfate, it is clear that the two exchange processes can operate independently of each other. However, independent cation or anion exchange would cause rapid development of steep ion concentration gradients across ileal mucosa and this presumably creates a passive functional link between the two exchanges so that activity in one exchange process tends to be followed by similar activity in the other.

The inhibition of sodium and chloride absorption by the carbonic anhydrase inhibitor, acetazolamide, also lends support to the model proposed in Fig. 10. Inhibition of carbonic anhydrase, which is known to be present in the ileum of laboratory animals (8, 9), would be expected to inhibit sodium and chloride absorption if, in the proposed model, hydrogen and/or bicarbonate exchanged for sodium and chloride are derived from carbon dioxide and water under the influence of carbonic anhydrase. These experiments cannot be used as conclusive proof for the model, however, since it is possible that acetazolamide exerts its effect directly on sodium and chloride absorption and not via inhibition of carbonic anhydrase (10).

On the basis of our data, it is not possible or necessary to specify the location of the exchange processes; these could reside on one or more of the several membranes that separate luminal fluid from plasma.

Although we are well aware that we have not presented definitive proof for this hypothesis, it is worth noting that we and many others have been unable to design or suggest an alternate unitary model that satisfactorily explains our experimental observations.

[4] Alternately, secretion might be explained by a separate double exchange carrier system, oriented to secrete sodium and chloride in exchange for absorbed hydrogen and bicarbonate. This separate system might be located in the same or in different cells of the intestinal mucosa. Secretion would result when the magnitude of transport in this second system was greater than that of the NaCl absorption oriented double exchange.

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