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Research Article

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The effect of Na^+ on HCO_3^- absorption is mediated, at least in part, by H^+ secretion, because luminal CO_2 production (manifested by luminal P_{CO_2}) dimished as HCO_3^- absorption decreased. The changes in P_{CO_2} are caused by reaction of H^+ with HCO_3^- in the luminal fluid because luminal P_{CO_2} is augmented by the presence of HCO_3^- and is diminished by addition of phosphate or Tris buffer.

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ABSTRACT An exchange of Na^+ for H^+ has been proposed to explain why jejunal Na^+ absorption is influenced by luminal concentrations of H^+ and HCO_3^-. We studied the influence of luminal Na^+ concentration on net HCO_3^- absorption by perfusing rat jejunum in vivo. When Na^+ was omitted from the perfusion fluid, HCO_3^- absorption diminished by a fixed amount over a range of initial HCO_3^- concentrations of 15 to 80 mM. This change was not caused by alterations in transmural PD or direction of water movement. Because the rate of HCO_3^- absorption decreased as the luminal HCO_3^- concentration lessened, Na^+-dependent HCO_3^- absorption accounted for an increasing percent of total absorption as the luminal concentration of HCO_3^- diminished.

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Whether all H^+ secretion requires luminal Na^+ cannot be determined with these experimental techniques because mucosal permeability to Na^+ and the unstirred layer make it impossible to eliminate Na^+ ions from the luminal cell surface. The nature of the mechanism for HCO_3^- transport that is not sodium dependent remains to be determined.

INTRODUCTION

Parsons proposed that hydrogen ion secretion caused bicarbonate absorption in the rat jejunum, and speculated that H^+ secretion is associated in part with processes of sodium absorption (1). Studies of the perfused human jejunum also suggest that bicarbonate absorption is initiated by an exchange of hydrogen ions for sodium ions. The hydrogen ions react with bicarbonate ions to form carbon dioxide which then diffuses from the lumen (2). Two observations suggest that jejunal sodium absorption is influenced by luminal H^+ concentration: the addition of bicarbonate to saline perfusion solutions reduces the concentration of hydrogen ions and makes it possible for sodium to be absorbed against electrochemical gradients (3), and an increase in hydrogen ion concentration reduces the rate of sodium absorption (4).

If hydrogen and sodium ion transport are mutually dependent, then reduction of luminal sodium concentration should reduce the rate of hydrogen ion transport and bicarbonate absorption. The following studies examine that hypothesis.

METHODS

Male Holtzman rats were fasted overnight and anesthetized by injecting pentobarbital (50 mg/kg) into the peritoneal cavity. A 25-cm segment of proximal jejunum was cannulated at each end, washed with 15 ml of warm saline, and flushed with air. A tracheostomy tube was inserted.

Pairs of solutions were perfused once through the segment with a syringe infusion pump at the rate of 0.41 ml/min during two successive 30-min periods. The input syringe, intestine, and collection syringe attached to the distal cannula formed a closed system and minimized leakage of CO_2. The order of perfusion of the solutions was alternated in successive rats in all studies. Before each perfusion period, the intestinal lumen was washed with the solution to be perfused and was flushed with gas. At the end of each period, collection syringes were removed and capped, and any fluid remaining in the segment was flushed with gas and discarded. After the second period, the jejunum was removed, stripped of mesentery, and weighed.

With the exception of solutions 1 and 10 (Table I), all solutions were gassed with a mixture of O_2 and 5–6% CO_2 (gas) and had an initial pH of about 7.4. Polyethylene-[1,2-^{14}C]glycol ([^{14}C]PEG) [1] 1.25 μCi/dl was used as a

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[1] *Abbreviations used in this paper:* [^{14}C]PEG, polyethylene-[1,2-^{14}C]glycol; gWW, jejunum wet weight in grams.

TABLE I

Composition of Perfusion Fluids (mM)

Solution	Na	K	Choline	Cl	HCO_3	Iseth.	Mann.	Tris	PO_4
1	145	5	—	125	25	—	25		
2	25	5	120	125	25	—	30		
3	145	5	—	125	25	—	80		
4	145	5	—	5	25	120	25		
5	140	5	—	130	15	—	34		
6	—	15	130	130	15	—	41		
7	—	30	120	125	25	—	30		
8	140	5	—	65	80	—	37		
9	—	80	65	65	80	—	37		
10	145	5	—	150	—	—	—		
11	145	5	—	130	20	—	25		
12	145	5	—	70	80	—	28		
13	140	5	—	125	20	—	5	20	
14	140	5	—	125	20	—	35		
15	140	5	—	105	20	—	57		20

nonabsorbable marker to permit calculation of net water movement (5). Except for the hypertonic solution, the osmolality of all perfusion solutions was similar to that of rat plasma, 303–305 mosmol/kg H_2O.

The pH and P_{CO_2} of the luminal fluid were determined soon after collection with a capillary pH electrode and P_{CO_2} electrode designed for small samples (Instrumentation Laboratory, Inc., Lexington, Mass.). Bicarbonate was calculated with the Henderson-Hasselbalch equation using a pK′ of 6.1. Sodium and potassium concentrations were measured with a flame photometer and chloride was determined with a coulometric chloridometer. The concentration of [^{14}C]PEG (counts per minute/milliliter) was measured with a scintillation counter. The osmolality (milliosmoles/kilogram) of perfusion fluids was measured by the method of freezing point depression with an Advanced Osmometer (Advanced Instruments, Needham Heights, Mass.).

"Initial" concentrations of ions and of [^{14}C]PEG were measured in samples of fluid obtained from the input syringes and samples of fluid for "final" determinations were obtained from the collection syringes after each collection period. Fluid remaining in the jejunum was discarded.

Net fluxes of ions and water were calculated as follows:

$$J_{net}\text{ion} = V\left(\text{ion}_f \frac{PEG_i}{PEG_f} - \text{ion}_i\right) gWW^{-1},$$

$$J_{net}H_2O = V\left(\frac{PEG_i}{PEG_f} - 1\right) gWW^{-1}.$$

Ion_i and ion_f are the concentrations of the ion (micromoles/milliliter) measured in the initial and final samples; PEG_i and PEG_f are the specific activities of [^{14}C]PEG (counts per minute/milliliter) measured in the initial and final samples. V is the volume (milliliter) of perfusion fluid pumped into the segment in 30 min; gWW is the wet weight of the jejunum in grams. Thus, J_{net}ion is expressed as micromoles × (30 min)$^{-1}$ × gWW^{-1}, and $J_{net}H_2O$ is expressed as milliliters × (30 min)$^{-1}$ × gWW^{-1}.

Changes in the P_{CO_2} of the luminal fluid are expressed as Δ P_{CO_2} and were calculated by subtracting the initial P_{CO_2} from the final P_{CO_2}, i.e., Δ P_{CO_2} = final P_{CO_2} − initial P_{CO_2}.

Three different groups of six rats each were used to determine transmural electrical potential difference (PD) when the following pairs of solutions were perfused (Table I): *Na^+ 145* vs. *Na^+ 25* (solutions 1 and 2), *isotonic* vs. hypertonic (solutions 1 and 3), and *Na^+ 145* vs. *Na^+ isethionate* (solution 1 and 4). A group of eight rats was used to determine PD when solutions with and without bicarbonate were perfused (solutions 1 and 10). Bridges of saturated KCl in agar were used. One bridge contacted the fluid perfusing the intestinal lumen and the other was placed in the peritoneal cavity. Electrical contact with a sensitive voltmeter was made through calomel half-cells. The voltmeter was read every 3 min for 30 min and the mean of the 10 values was calculated. The studies of PD and transport were not performed concurrently.

The signs preceding the net flux measurements indicate movement into (+) or out of (−) the lumen.

The statistical significance of differences between means was determined with the *t* test for paired samples.

RESULTS

Study 1: effect of reduction in sodium concentration of perfusion fluid (Table II)

Perfusion solutions 1 and 2 (Table I). N = 6. To determine the effect of luminal sodium concentration on bicarbonate absorption, the jejunum was perfused with two solutions whose mean measured sodium concentrations were 143 mM and 30 mM. When the sodium concentration was reduced, less HCO_3^- was absorbed,[2] pH did not fall as far, and P_{CO_2} rose less. Sodium was secreted instead of absorbed, chloride secretion increased, and potassium absorption was enhanced. There was also an apparent absorption of anions in excess of cations,

[2] In this discussion, absorption denotes a net loss from the lumen, and secretion denotes a net increase in the lumen.

TABLE II

Effect of Low Luminal Sodium Concentration on the Net Movement of Electrolytes and Water, and the Magnitude of Luminal P_{CO_2} and Transmural Electrical Potential Difference (PD) (N = 6)

Dependent variable	Mean Na^+ concentration of perfusion fluid		P
	143 mM	30 mM	
HCO_3^-*			
μmol	−105±13	−84±22	<0.05
mM	20.4±0.7	21.9±0.8	<0.05
pH			
Initial	7.42±0.02	7.44±0.01	<0.01
Δ	−0.30±0.02	−0.24±0.02	<0.01
P_{CO_2}			
Initial *mm Hg*	38±1	38±1	NS
Δ *mm Hg*	15±2	11±3	<0.05
H_2O			
ml	−0.28±0.20	0.24±0.26	<0.001
PD			
mV	−5.2±1.6	3.5±1.0	<0.001
Na^+			
μmol	−102±15	119±21	<0.001
mM	143±1	30±1	<0.001
K^+			
μmol	0.47±1.75	−7.28±1.98	<0.01
mM	5.1±0.1	4.7±0.1	<0.001
Cl^-			
μmol	24.1±26.5	109±27.3	<0.001
mM	128±2	128±1	NS

* For measurements of net movement and electrolytes and water, signs indicate movement into (+) or out of (−) the lumen. Rates of net transport of water and electrolytes are expressed as the quantity × $(30\ min)^{-1}$ × (gram wet wt of intestine)$^{-1}$. The mean concentration of ions for the perfusion period is expressed in millimoles/liter. Values shown are the mean±SD. The sign of the PD is the polarity of the jejunal lumen.

and this is probably because choline is not accounted for in the table. The charge balances suggest that appreciable amounts of choline were absorbed.

When the luminal Na^+ concentration was reduced, two factors that could influence net movement of HCO_3^- and H^+ changed significantly: the transmural PD became 8.7 mV more positive in the lumen, and the net movement of water was into the lumen. The influence of these two factors on bicarbonate absorption was estimated in studies 2 and 3.

Study 2: effect of direction of water movement (Table III)

Perfusion solutions 1 and 3 (Table I). N = 6. When the osmolality of the perfusion we increased from 305 mosmol/kg to 350 mosmol/kg by adding mannitol, water moved into rather than out of the lumen, and the mean rate of movement of water into the lumen was greater than when the low sodium solution was perfused in study 1. However, the direction of water movement did not significantly affect the net movement of ions, the PD, or the final P_{CO_2} of the perfusion fluid.

Study 3: effect of transmural PD (Table IV)

Perfusion solutions 1 and 4 (Table I). N = 6. When the solution containing sodium isethionate was perfused, the luminal PD was 7.5 mV more negative than

TABLE III

Effect of Direction of Water Movement on the Net Movement of Electrolytes and Water, and the Magnitude of Luminal P_{CO_2} and Transmural Electrical Potential Difference (PD). (N = 6) Initial Osmolalities: Isotonic = 305 mosmol/kg; Hypertonic = 350 mosmol/kg

Dependent variable	Perfusion fluid		P
	Isotonic	Hypertonic	
H_2O*			
ml	−0.18±0.22	0.49±0.18	<0.001
HCO_3^-			
μmol	−130±21	−125±19	NS
mM	21.4±1.3	21.2±0.8	NS
pH			
Initial	7.47±0.01	7.46±0.01	NS
Δ	−0.33±0.06	−0.33±0.07	NS
P_{CO_2}			
Initial *mm Hg*	37±1	37±1	NS
Δ *mm Hg*	14±3	13±5	NS
PD			
mV	−6.2±2.1	−6.0±2.3	NS
Na^+			
μmol	−80.8±39.0	−63.4±34.7	NS
mM	143±1	140±1	<0.001
K^+			
μmol	1.26±1.15	2.56±1.34	NS
mM	5.1±0.1	5.0±0.1	<0.05
Cl^-			
μmol	37.9±33.4	46.5±29.7	NS
mM	127±1	124±1	<0.001

* See footnote to Table II.

when sodium chloride was used. Sodium absorption decreased, chloride was secreted, and water moved into the lumen. However, the change in PD did not alter net movement of HCO_3^-, the change in pH, or the Δ P_{CO_2}.

Study 4: how omission of Na^+ from luminal fluid affects bicarbonate absorption when initial HCO_3^- concentration of luminal fluid is varied (Table VA, B, and C)

Perfusion solutions 5 and 6, 1 and 7, 8 and 9 (Table I). N = 8 for each pair of perfusion fluids. When sodium was omitted from the perfusion fluid, sodium diffused into the lumen and raised the concentration in luminal fluid to about 10 mM whether the initial concentration of HCO_3^- was 15 mM (Table VA), 25 mM (Table VB), or 80 mM (Table VC). Regardless of the initial HCO_3^- concentration, HCO_3^- absorption decreased by a constant amount, about 30 μmol × (30 min)$^{-1}$ × gWW^{-1}. In addition, the Δ P_{CO_2} and Δ pH were less in the solutions from which Na^+ was omitted.

When the initial lumen HCO_3^- concentration was 25 mM (Table VB), the Δ P_{CO_2} was larger than when the initial concentration of HCO_3^- was either 15 mM (Table VA) or 80 mM (Table VC). The reasons for this are not clear, but they do not affect the validity of the conclusions which are based on data with groups rather than between groups.

TABLE IV

Effect of Change in Transmural Electrical Potential Differences Magnitude of Luminal P_{CO_2}. (N = 6) PD was Changed by Substituting Isethionate for Chloride in the Perfusion Fluid

Dependent variable	Perfusion fluid: Chloride	Perfusion fluid: Isethionate	P
PD			
mV	−6.9±2.1	−14.4±2.7	<0.02
HCO_3^-*			
μmol	−125±9	−125±17	NS
mM	21.8±1.9	23.1±1.9	<0.001
pH			
Initial	7.43±0.01	7.46±0.02	<0.02
Δ	−0.33±0.04	−0.33±0.05	NS
P_{CO_2}			
Initial *mm Hg*	41±2	41±3	NS
Δ *mm Hg*	15±3	14±3	NS
H_2O			
ml	−0.34±0.24	0.10±0.12	<0.01
Na^+			
μmol	−98.6±39.9	−2.4±16.1	<0.001
mM	143±1	144±1	<0.05
K^+			
μmol	0.47±2.25	4.54±3.57	NS
mM	5.1±0.1	5.1±0.1	NS
Cl^-			
μmol	−22.7±25.0	134±21	<0.001
mM	126±1	5.6±1	<0.001

* See footnote to Table II.

TABLE V

Effect of Low Luminal Sodium Concentration on the Net Movement of Bicarbonate, and on the Change in pH and P_{CO_2} of Perfusion Fluid When the Initial HCO_3^- Concentration was 15 mM (A), 25 mM (B), and 80 mM (C)

(A)

(N = 8) Dependent variable	Mean luminal Na concentration: 138±2 mM	Mean luminal Na concentration: 9.3±1.4 mM	Δ	P
HCO_3^-*				
μmol	−71.6±11.6	−40.8±8.3	30.8	<0.001
mean *mM*	12.4±0.5	13.3±0.4	0.9	<0.01
pH				
Initial	7.19±0.02	7.20±0.01	—	NS
Δ	−0.28±0.05	−0.20±0.04	0.08	<0.001
P_{CO_2}				
Initial *mm Hg*	40.4±0.9	40.5±0.7	—	NS
Δ *mm Hg*	9.5±1.4	7.6±1.6	1.9	<0.05

(B)

	Mean luminal Na concentration: 143±1 mM	Mean luminal Na concentration: 8.9±1.4 mM		
HCO_3^-				
μmol	−124±27	−94±19	30	<0.05
mean *mM*	20.9±0.9	22.9±0.8	2	<0.001
pH				
Initial	7.43±0.01	7.46±0.01	—	<0.02
Δ	−0.34±0.07	−0.27±0.04	0.07	<0.01
PCO_2				
Initial *mm Hg*	39.0±0.7	39.1±0.6	—	NS
Δ *mm Hg*	16.5±4.8	13.7±4.9	2.8	<0.01

(C)

	Mean luminal Na concentration: 138±1 mM	Mean luminal Na concentration: 11.1±2 mM		
HCO_3^-				
μmol	−174±36	−145±32	29	<0.05
Mean *mM*	73.5±1.9	74.7±2.2	1.2	<0.05
pH				
Initial	7.86±0.01	7.88±0.01	—	<0.01
Δ	−0.14±0.02	−0.12±0.03	0.02	<0.05
P_{CO_2}				
Initial *mm Hg*	42.1±1.3	42.4±0.6	—	NS
Δ *mm Hg*	6.5±2.0	5.4±1.8	1.1	<0.05

* See footnote to Table II.

TABLE VI

Effect of 25 mM HCO_3^- in Perfusion Fluid on the Magnitude of Luminal P_{CO_2} (N = 8)

Dependent variable	Initial HCO_3^- concentration 0 mM	25 mM	P
P_{CO_2}			
Initial *mm Hg*	Nil	Nil	—
Δ *mm Hg*	30±2	36±2	<0.01

Study 5: effect of HCO_3^- in perfusion fluid on P_{CO_2} and PD (Table VI)

Perfusion solutions 1 and 10 (Table I). N = 8. When the perfusion fluid contained an initial HCO_3^- concentration of 25 mM, the increase in P_{CO_2} was 6 mm Hg larger than when HCO_3^- was omitted. The mean PD (±SD) in eight rats when solutions with and without HCO_3^- were perfused were −4.7 mV (±2.5), and −5.4 mV (±2.3). The differences were not significant.

Study 6: effect of enhanced HCO_3^- absorption on P_{CO_2} (Table VII)

Perfusion solutions 11 and 12 (Table I). N = 8. When the initial concentration of HCO_3^- was increased from 20 mM to 80 mM, net bicarbonate absorption more than doubled. However, there was no significant difference in the Δ P_{CO_2} in the two solutions.

Study 7: effect of buffering on HCO_3^- absorption and P_{CO_2} (Tables VIII and IX)

Perfusion solutions Tris study 13 and 14 (Table I). N = 8. Phosphate study 14 and 15. N = 12. As expected, when the perfusion fluid was buffered with Tris (Table VIII) or phosphate (Table IX), the pH fell less than in the unbuffered fluid. Buffering with Tris reduced HCO_3^- absorption by 18%, but the reduction was not significant when phosphate was used. In both studies, however, buffering significantly reduced the Δ P_{CO_2}.

DISCUSSION

Our studies demonstrate that a reduction in the concentration of sodium in fluid perfusing the lumen of the rat jejunum decreases HCO_3^- absorption by a constant amount, and that this decrease is not caused by concurrent changes in PD or water movement. The associated changes in P_{CO_2} of the perfusion fluid suggest that the rate of HCO_3^- absorption decreases because the rate of H^+ secretion diminishes when the luminal Na^+ concentration is reduced. The data supporting these conclusions are discussed below.

Effect of sodium, net water movement, and PD. When luminal sodium concentration was reduced, hydrogen ions accumulated in the lumen at a reduced rate, and bicarbonate absorption diminished (Table II). However, there were significant changes in two other factors that could have influenced the net movement of H^+ and HCO_3^-: the PD became 8.7 mV more positive in the lumen, and water moved into, rather than out of, the lumen. To estimate the influence of net water movement on transport of HCO_3^-, a hypertonic solution was circulated through the lumen (Table III). Although the hypertonic solution caused a change in water movement greater than that induced by the low sodium solutions, the net movement of HCO_3^- was unaffected. Hence, the net movement of water could not explain the reduction in HCO_3^- absorption when the luminal sodium concentration was decreased. The influence of PD was more difficult to determine because it was not possible to induce PD changes of similar polarity and magnitude without reducing the intraluminal sodium concentration. We could, however, study the effect of changes of similar magnitude but opposite polarity. When the lumen was perfused with a solution containing sodium isethionate instead of sodium chloride, the lumen became more negative by 7.5 mV (vs. 8.7 mV more positive with low sodium solution). The net movement of potassium, a cation that is transported passively, reflected these changes. Net movement of potassium into the lumen diminished when the lumen became positive (Table II), and increased when the lumen became negative (Table IV). However, net movement of HCO_3^- did not change significantly when the lumen became more negative, so the effect on HCO_3^- movement was not caused by changes in water movement or PD; the low luminal sodium concentration itself must have directly affected the processes that govern HCO_3^- absorption.

Omitting Na^+ from the perfusion fluid diminished the rate of HCO_3^- absorption by a constant amount of about 30 μmol × (30 min)$^{-1}$ regardless of whether the initial

TABLE VII

Effect of Increased Net Movement of HCO_3^- on the Magnitude of Luminal P_{CO_2} (N = 8)

Dependent variables	Initial HCO_3^- concentration 20 mM	80 mM	P
HCO_3^-*			
μmol	−78.5±7.8	−167±46.8	<0.01
P_{CO_2}			
Initial *mm Hg*	42±1	41±1	NS
Δ *mm Hg*	8±3	11±2	NS

* See footnote to Table II.

luminal HCO_3^- concentration was 15, 25, or 80 mM (Table V). However, the percent of HCO_3^- absorption that was Na^+ dependent increased from 17% to 43% as the initial HCO_3^- concentration was reduced from 80 mM to 15 mM and overall HCO_3^- absorption rate diminished. Thus, the mechanism of HCO_3^- absorption that requires Na^+ becomes relatively more important as the concentration of HCO_3^- in the lumen decreases below that of the plasma. Is 30 μmol $\times$ (30 min)$^{-1}\times$ gWW^{-1} the maximal rate of Na^+-dependent HCO_3^- absorption? Probably not, because the unidirectional flux of Na^+, ($J_{sm}Na^+$), into the unstirred layer of fluid adjacent to the mucosa makes it impossible to create a luminal environment that is sodium-free. If sodium could be eliminated completely from the lumen, the reduction in HCO_3^- absorption might be greater.

Could alterations in chloride movement have influenced the movement of hydrogen or bicarbonate ions when the luminal concentration of sodium was reduced? Mechanisms that could link the transport of chloride to the movement of H^+ or HCO_3^- are: (*a*) transport of H^+ and Cl^- into the lumen, or (*b*) exchange of Cl^- for luminal HCO_3^-. When the concentration of sodium in the perfusion fluid was lowered, the blood-lumen concentration gradient of Cl^- remained the same; hence the unidirectional movement of Cl^- into the lumen could have been influenced only by a change in PD (Table II). Increased electropositivity of the lumen should have increased Cl^- flux into the lumen and the net absorption of HCO_3^-. Bicarbonate absorption diminished, however, despite the increased net movement of chloride into the lumen. It seems unlikely that Cl^- transport influenced HCO_3^- absorption, but the possibility cannot be entirely excluded.

Mechanism of action of sodium. By what mechanism did the reduction in sodium concentration alter the movement of hydrogen and bicarbonate ions? Was this caused primarily by a reduction in net movement of bicarbonate ions from the lumen, or by a reduction in net movement of hydrogen ions into the lumen? If bicarbonate leaves the lumen as the HCO_3^- ion rather than as dissolved CO_2, the increase in luminal P_{CO_2} should be less in the solution that has the higher rate of bicarbonate loss, because removal of HCO_3^- ions forces the reaction, $OH^- + CO_2 \leftrightarrows HCO_3^-$, to the right. The ΔP_{CO_2} was higher in the solution that had the higher rate of bicarbonate loss, however, implying that secretion of hydrogen ions into the lumen caused the net loss of bicarbonate, i.e., H^+ ions reacted with HCO_3^- to form CO_2 which diffused from the lumen (Tables II and V).

Alternative explanations for changes in ΔP_{CO_2}. Could some other process have caused these changes in ΔP_{CO_2}? If HCO_3^- ions were transported from the lumen into tissue fluids that were more acid than those of the lumen, the P_{CO_2} of the tissue fluid would increase at a rate faster than P_{CO_2} decreased in the lumen. The tissue CO_2 might then diffuse back into the lumen and increase luminal P_{CO_2}. If such a process is important, enhancement of the mucosa-to-serosa flux of HCO_3^- ($J_{ms}HCO_3^-$) should increase ΔP_{CO_2}. Assuming that the serosa-to-mucosa flux of HCO_3^- ($J_{sm}HCO_3^-$) remains constant when the HCO_3^- concentration of the perfusion fluid is increased from 20 mM to 80 mM, an increase in HCO_3^- absorption must be caused by an augmented $J_{ms}HCO_3^-$. When net HCO_3^- absorption increased from 78.5 to 167 μmol $\times$ (30 min)$^{-1}\times$ gWW^{-1}, ΔP_{CO_2} did not change significantly (Table VII). These findings demonstrate that it is possible to increase HCO_3^- absorption (and $J_{ms}HCO_3^-$) without increasing ΔP_{CO_2}, and suggest that luminal P_{CO_2} is not affected significantly by reactions of HCO_3^- in mucosal tissue fluid. They support the view that the difference in ΔP_{CO_2} seen when low Na solutions

TABLE VIII

Effect of Buffering with Tris on Net Movement of HCO_3^-, and on Changes in Luminal pH and P_{CO_2} (N = 8)

Dependent variables	Tris Not added	Tris Added	P
HCO_3^-*			
μmol	−82.0±12.7	−67.0±15.6	<0.05
pH			
Initial	7.29±0.01	7.29±0.01	NS
Δ	−0.26±0.04	−0.19±0.05	<0.01
P_{CO_2}			
Initial *mm Hg*	41.6±0.8	42.6±0.8	NS
Δ *mm Hg*	8.1±2.4	5.5±1.7	<0.05

* See footnote to Table II.

TABLE IX

Effect of Buffering with Phosphate on Net Movement of HCO_3^- and on Changes in Luminal pH and P_{CO_2} (N = 8)

Dependent variables	Phosphate Not added	Phosphate Added	P
HCO_3^-*			
μmol	−87.1±9.8	−77.4±13.5	NS
pH			
Initial	7.27±0.0	7.31±0.0	<0.001
Δ	−0.27±0.04	−0.22±0.04	<0.02
P_{CO_2}			
Initial *mm Hg*	42.3±0.1	43.1±0.1	<0.05
Δ *mm Hg*	13.3±2.9	10.1±2.4	<0.05

* See footnote to Table II.

are perfused arises because of reactions in the intestinal lumen, and not because of reactions of HCO_3^- in cells or interstitial fluid.

The effect of intraluminal buffering provides additional evidence that CO_2 is generated in the lumen rather than in the surrounding tissues. Tris buffer reduced the absorption of HCO_3^- (Table VIII). When H^+ ions moved into the luminal fluid, they could interact with either Tris or HCO_3^-. Because fewer H^+ ions reacted with HCO_3^- in the Tris-containing solution, less CO_2 was generated, and less HCO_3^- was absorbed. Phosphate buffer had a similar effect on ΔP_{CO_2}, but did not decrease HCO_3^- absorption significantly (Table IX). The reduction in ΔP_{CO_2} differs from the results of studies in man by Turnberg, Fordtran, Carter, and Rector (2) who found that the ΔP_{CO_2} increased when the perfusion solution was buffered with phosphate. In both studies the P_{CO_2} changes are cited as evidence that hydrogen ions are secreted into the luminal fluid. The reasons for the difference are not clear. Because, in my studies, the reaction of H^+ with HCO_3^- should have come to equilibrium in most of the luminal fluid by the time it entered the distal cannula, I believe that the above explanations for the changes in P_{CO_2} are applicable.

Our view that the differences in ΔP_{CO_2} were caused by intraluminal reactions of HCO_3^- differs from that of Hamilton, Dawson, and Webb, who concluded from their studies in anesthetized dogs that luminal P_{CO_2}, "is not the result of chemical interaction postulated in previous studies, but rather of separate factors of tissue perfusion, CO_2 diffusion, and CO_2 production" (6). It is clear that in the absence of chemical reactions in the lumen, the P_{CO_2} of luminal fluid will equilibrate with that of the adjacent mucosa; hence, factors of tissue perfusion, CO_2 production, and diffusion will determine luminal P_{CO_2}. It is equally clear, however, that reaction of HCO_3^- with H^+ in the lumen could contribute significantly, because luminal P_{CO_2} does not equilibrate instantaneously with tissue P_{CO_2}. In the studies of Hamilton, et al., after gas mixtures with a P_{CO_2} higher than steady-state mucosal P_{CO_2} were infused into the canine intestinal lumen, luminal P_{CO_2} values declined at an exponential rate to steady-state values with a half-time of about 5 min (6). Thus, luminal P_{CO_2} in the steady state might be considerably higher than that of the basal P_{CO_2} of surrounding tissues if CO_2 were being generated in the lumen. In our study (Table VI) and that of Turnberg et al. (2), the addition of bicarbonate to jejunal fluid augmented ΔP_{CO_2}. If that additional CO_2 was not generated in the lumen, it must have been caused by an enhanced rate of aerobic metabolism of mucosal cells, a reduced rate of CO_2 removal by the blood, or reaction of HCO_3^- with H^+ in the fluid of the mucosal cells or interstitium. We have shown that the last possibility is unlikely (Table VII). The contrasting effects on luminal P_{CO_2} of bicarbonate and the two buffers strongly suggest that the differences in ΔP_{CO_2} were not caused by changes in tissue perfusion or cell metabolism, because it is unlikely that tissue perfusion is decreased by bicarbonate and increased by phosphate and Tris, or that cell metabolism is enhanced by bicarbonate and diminished by phosphate and Tris. The changes in ΔP_{CO_2} are readily understood, however, if the reactions that influence luminal P_{CO_2} occur in the lumen.

Contribution of H^+ secretion to HCO_3^- absorption. What percent of HCO_3^- absorption is caused by H^+ secretion? The answer to this question may depend on the luminal concentration of HCO_3^-. When the initial HCO_3^- concentration of luminal fluid was increased from 20 to 80 mM, the rate of HCO_3^- absorption more than doubled, but ΔP_{CO_2} did not increase significantly ($P > 0.1$). This suggests that H^+ secretion is not the only mechanism of HCO_3^- absorption at the higher luminal HCO_3^- concentration, for, if it were, the ΔP_{CO_2} might have been greater in the perfusion fluid with the higher rate of HCO_3^- absorption. It is clear that H^+ secretion causes some HCO_3^- absorption at higher luminal concentrations of HCO_3^- because omission of Na^+ from the perfusion solution reduces CO_2 production (ΔP_{CO_2}) and absorption of HCO_3^- (Table VC). When the initial luminal HCO_3^- concentration was 80 mM, omission of Na^+ from the perfusion fluid reduced HCO_3^- absorption by 17%. Hence, at least 17% of HCO_3^- absorption is caused by H^+ secretion (assuming that the effect of Na^+ omission is mediated entirely by a reduction in H^+ secretion). With the available data, there is no way to estimate the maximal contribution of H^+ secretion to HCO_3^- absorption. In what other ways might HCO_3^- be absorbed? Passive diffusion of HCO_3^- may contribute to absorption when HCO_3^- concentration in the lumen exceed those of plasma, but the failure of changes in PD to affect the HCO_3^- movement provides some evidence against it. Perhaps the magnitude of change in PD was too small (about 8 mV) to effect a change in HCO_3^- net movement that could be detected in our studies. These questions cannot be answered with the available data.

The addition of HCO_3^- to a saline perfusion solution caused no significant change in PD, implying that the processes that cause HCO_3^- absorption do not generate a PD. What are examples of such a system? No PD would be generated if Cl^- accompanies H^+ into the lumen, or if Na^+ leaves as H^+ enters. Our studies and those of Turnberg et al. (2) support the latter mechanism, but they do not exclude the possibility that secretion of

H^+ and Cl^- accounts for a fraction of HCO_3^- absorption that may not be Na^+ dependent.

CONCLUSIONS

(*a*) When the concentration of HCO_3^- in the lumen is equal to or less than plasma, H^+ secretion causes some, and perhaps all, HCO_3^- absorption.

(*b*) Some H^+ secretion requires Na^+ in the lumen. Perhaps all H^+ secretion requires luminal Na^+, but this cannot be determined with the techniques used in this study because the diffusion of Na^+ into the lumen makes it impossible to create a luminal fluid that is free of Na^+.

(*c*) The rate of HCO_3^- absorption that is Na^+ dependent is constant regardless of whether the initial HCO_3^- concentration of the perfusion fluid is smaller, larger, or equal to that of plasma. However, as the initial HCO_3^- concentration of perfusion fluid is reduced, the percent of HCO_3^- absorption that is Na^+ dependent increases because the rate of total HCO_3^- absorption decreases.

(*d*) The nature of the mechanism for HCO_3^- transport that is not Na^+ dependent remains to be determined.

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