The Mechanism of Bicarbonate Secretion in Rabbit Ileum Exposed to Choleragen

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ABSTRACT Bicarbonate may be secreted into the intestinal lumen in cholera because: HCO_3^- ions are transported, or because OH^- ions accumulate and react with dissolved CO_2 to form HCO_3^-. If HCO_3^- ions are transported into the lumen from the interstitial fluid, lumenal Pco_2 should increase ($HCO_3^- \rightleftharpoons OH^- + CO_2$); if OH^- accumulates, Pco_2 should diminish. Net movement of H_2O, and HCO_3^-, and changes in pH and Pco_2 in lumenal fluid were studied in adjacent segments of rabbit ileum in vivo, one of which was exposed to choleragen. 4 h after exposure, segments were drained and infused with gassed Krebs-Henseleit solution whose Pco_2 exceeded arterial Pco_2. After 45 min, fluid was collected anaerobically from control and cholera segments. Among 13 cholera segments, lumenal Pco_2 diminished by a mean of 8.4 torr and was less than femoral arterial blood in six instances. In the paired control segments, mean Pco_2 increased by 4.4 torr, and was always greater than arterial Pco_2. Dilution could not account for the low Pco_2 in cholera segments because in hypertonic solutions that caused water to move into the lumen, the Pco_2 did not differ from control values obtained with isotonic solutions. The results suggest that OH^- accumulation (by addition of OH^- or removal of H^+) causes HCO_3^- secretion in cholera. This does not result from secretion of some other base (e.g., HPO_4^-), because HCO_3^- accounts for most of the base in the lumenal fluid. The Pco_2 changes suggest that OH^- reacts with CO_2 at the cell-lumen interface, but reaction at the cell-interstitial fluid interface cannot be excluded.

INTRODUCTION

Patients with cholera have diarrhea in which massive amounts of water and salt are lost. The concentration of bicarbonate in the fecal fluid may be four times greater than that of the plasma, and the loss of this base results in metabolic acidosis (1). The bicarbonate may accumu-

late in the lumenal fluid of the small intestine because of two processes: either HCO_3^- ions are transported into the lumen, or OH^- ions accumulate in the lumen and react with dissolved CO_2 to form HCO_3^-. By measuring the change in lumenal fluid Pco_2 in the intestine exposed to cholera toxin, it is possible to infer which of these processes predominates: if HCO_3^- ions move into the lumenal fluid, the Pco_2 should increase because HCO_3^- dissociates and shifts the reaction, $OH^- + CO_2 \rightleftharpoons HCO_3^-$, to the left. However, OH^- accumulation in the lumen should reduce Pco_2 as OH^- reacts with CO_2 and removes it from solution. The following studies suggest that in the rabbit ileum exposed to cholera toxin, the latter mechanism predominates.

METHODS

Rabbits of either sex, weighing 2-2.5 kg, were anesthetized by injecting pentobarbital into an ear vein. A femoral vein and femoral artery were cannulated with polyethylene tubing filled with a solution of saline and heparin. Two adjacent segments of ileum, each 20 cm long, were constructed by ligating the proximal ends with black silk after the lumen was washed with saline. A metal cannula attached to a three-way stopcock was then inserted into the distal end of each segment and tied into place. The distal segment terminated about 3 cm from the cecum. The abdomen was then closed with metal clips. A tracheostomy tube was inserted and connected to a respirator.

Study 1: determination of Pco_2 of fluid in ileal segments exposed to choleragen or saline. Choleragen (Wyeth 001, Wyeth Laboratories, Inc., Div. of American Home Products Corp., Philadelphia, Pa.), 2.0 mg, was dissolved in 4 ml of saline and infused into the lumen of one of the ileal segments. The location, i.e., proximal or distal, of the cholera segment was alternated in successive studies. The same volume of saline without choleragen was infused into the "control" segment. 4 h then passed, during which the pH and Pco_2 of femoral arterial blood were periodically determined. After 4 h, the abdomen was opened and fluid was gently expressed from the segments. Fluid was rarely present in the control segment.

A solution of the following composition was then infused into each segment (mM): NaCl 120, KCl 4.5, $NaHCO_3$ 25, Na_2HPO_4 1.8, NaH_2PO_4 0.2, $MgSO_4$ 1.0, mannitol 20. The

TABLE I
Net Movement of H_2O and HCO_3^-, and Final HCO_3^- Concentrations of
Lumenal Fluid of Cholera and Control Ileal Segments

Study no.	ΔH_2O		ΔHCO_3^-		Final lumen HCO_3^- concentration	
	Control	Cholera	Control	Cholera	Control	Cholera
	ml		*μmol*		*mM*	
1	−3*	5	−6	426	43.7	58.9
2	−1	3	61	358	39.8	65.7
3	−2	3	−45	336	28.0	63.2
4	0	5	251	488	62.1	65.8
5	−3	4	−43	443	35.1	68.5
6	−1	1	138	218	51.7	63.2
7	−4	3	−68	340	33.4	62.3
8	1	5	37	291	27.0	43.7
9	0.5	3.5	241	408	55.3	67.6
10	−1	3.5	3	422	30.6	70.0
11	0	3.5	234	361	59.8	62.4
12	−2	4.5	−17	507	33.4	72.1
13	−1	3	142	513	54.3	88.3
Mean	−1.3	3.6	71	393	42.6	65.5
SD	1.5	1.1	116	87	12.6	9.8
P	<0.001		<0.001		<0.001	

* The sign indicates movement into (+) or out of (−) the lumen in the columns showing net movement of H_2O and HCO_3^-.

fluid, equilibrated with 5.4% CO_2-94.6% O_2, had a pH of about 7.4 and an osmolality of 303 mosmol/kg. 7 ml were infused into the control and 4 ml into the cholera segment in order to yield similar quantities at the end of the study period. In other studies 4 ml were infused into each segment; the results were similar regardless of the initial volume in the control segment. After the fluid remained in the segments for 45 min, the abdomen was opened and the fluid was gently expressed into 10-ml syringes. Air introduced into the collected fluid from the dead space of the stopcock and syringe did not exceed 20 μl in volume and could have introduced an error of no more than 0.5% (about 0.2 torr) in the Pco_2 determinations. Gas collected from the segments at the end of the study period was considered to be in equilibrium with the collected fluid. The volume of fluid was measured, and the syringe was capped to prevent loss of CO_2. Samples of blood (4 ml) from the femoral artery and vein were collected in heparinized syringes immediately before and after the 45-min study period, and a sample of portal vein blood was usually obtained by direct puncture after all other specimens had been collected. After the rabbit was sacrificed, the ileal segments were excised, freed of mesentery, and weighed. A standard weight (a hemostat) was then clamped to the segment, which was allowed to stretch by gravity for about 30 s. The length was then measured with a rule calibrated in centimeters.

Study 2. Influence of fluid movement on Pco_2. The lower Pco_2 of the fluid in cholera segments might be caused by simple dilution, because fluid was secreted into the cholera segments and was usually absorbed from the control segments. To determine whether fluid movement contributed to the Pco_2 change, two adjacent ileal segments were pre-

pared in the manner described in study 1, except that choleragen was not added to either segment. To assure that the animals were in approximately the same physiological condition as those of the prior study, 4 h were allowed to pass before the test fluids were infused. Then 4 ml of hypertonic fluid was infused into one of the ileal segments, and 7 ml of isotonic fluid into the other. After 45 min, the segments were drained, and the volume, pH, and Pco_2 of the fluid were measured. Blood was collected in the same manner as in study 1. The isotonic fluid (300 mosmol/kg) was a solution of 160 mM NaCl; the hypertonic fluid was identical to the isotonic fluid except that it was made hypertonic (454 mosmol/kg) by adding mannitol.

The volume of fluid from the ileal segments was measured to the nearest 0.5 ml with the calibrated collection syringe. Fluid pH and Pco_2 were measured with an Instrumentations Laboratory, Inc., (Lexington, Mass.) ultramicro pH and Pco_2 electrode system. The Pco_2 meter was standardized after every determination and the pH meter was standardized frequently. Samples of fluid for pH determination were obtained directly from the syringe immediately after the cap was removed by dipping the plastic aspiration tube at least ½ in into the fluid. The fluid was then aspirated into the pH electrode until it overfilled the cuvette of pH-sensitive glass. After 30 s, the pH reading was made to the nearest 0.01 unit. After the pH cuvette was filled, a small amount of fluid was expressed from the syringe containing the ileal fluid; the cuvette of the CO_2 electrode was then filled until fluid appeared at the exit port. The Pco_2 reading was made exactly 2 min after the sample was introduced. Under these conditions, the mean difference (±SD) between eight duplicate Pco_2

determinations was 0.7 torr (±0.6), and of the pH determinations was 0.01 (±0.01). Bicarbonate concentrations were calculated with the Henderson-Hasselbalch equation with a pK' of 6.1. The net movement of bicarbonate was calculated as follows: $J_{net}HCO_3^- = V_f C_f - V_i C_i$. V and C represent volume and concentration, and the subscripts f and i stand for final and initial.

To determine whether HCO_3^- accounted for the base in the lumenal fluid from the study segments, the concentration of base in samples of lumenal fluid was measured by back titration. To 1.00 ml of lumenal fluid was added 1.00 ml of 0.100 M HCl and 5 ml of distilled water. The mixture was stirred with a magnetic bar for several min to facilitate the loss of CO_2, and was then titrated to pH 7.00 with 0.100 M NaOH. The HCO_3^- concentration of the fluid was calculated with the Henderson-Hasselbalch equation from measurement of total CO_2, made with the Natelson Microgasometer (Scientific Industries, Inc., Queens Village, N. Y.), and pH.

Statistical significance of mean differences was assessed with the Student t test for paired samples.

RESULTS

Study 1: determination of P_{CO_2} of fluid in ileal segments exposed to choleragen or saline. The control and cholera segments were of similar length. The mean (±SD) length of segments exposed to choleragen was 20.8 cm (±2.6); segments exposed to saline measured 20.7 cm (±3.1). Although there was considerable variation, the rabbits were generally in a state of mild

TABLE II

P_{CO_2} of Fluid from Cholera and Control Segments Compared with P_{CO_2} of Initial Infusion Fluid, Mean Arterial P_{CO_2}, and P_{CO_2} of Portal Vein Blood Sampled at End of Study Period

Study no.	Final cholera	Final control	Initial infusion	Mean arterial	Final portal vein
			torr		
1	26.4	33.3	36.6	21.0	29.2
2	22.9	34.8	36.1	23.8	40.0
3	29.0	44.5	38.4	25.8	36.7
4	29.5	38.4	37.9	27.4	32.7
5	26.1	44.4	34.7	34.4	37.9
6	21.0	36.7	34.1	16.4	26.0
7	24.3	35.9	33.3	23.5	29.8
8	28.3	42.0	35.5	24.5	34.1
9	25.8	35.0	34.2	29.3	38.1
10	39.5	48.7	35.8	32.2	39.3
11	32.9	47.6	36.6	37.8	42.8
12	25.7	41.3	41.1	35.2	37.4
13	30.0	45.4	36.4	33.1	44.7
Mean	27.8	40.6	36.2	28.0	36.1
SD	4.7	5.2	2.1	6.3	5.5

Final cholera vs. mean arterial, NS; final cholera vs. initial infusion, $P < 0.001$; final cholera vs. final control, $P < 0.001$; final control vs. mean arterial $P < 0.001$.
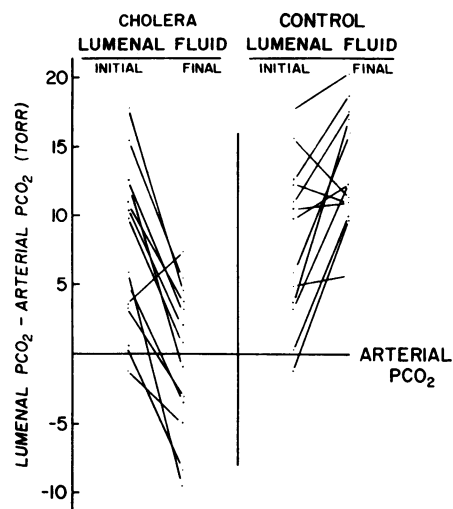
FIGURE 1 Changes in P_{CO_2} of lumenal fluid in cholera and control segments relative to mean arterial P_{CO_2}. The horizontal line represents mean arterial P_{CO_2}. Points above the line represent lumenal P_{CO_2} values greater than mean arterial P_{CO_2}. In 6 of 13 studies, P_{CO_2} of lumenal fluid in cholera segments diminished to values less than mean arterial P_{CO_2}.

compensated metabolic acidosis: the mean (±SD) pH and bicarbonate concentration of femoral arterial blood sampled in 13 rabbits immediately before and after the study period was 7.37 (±0.08) and 18.2 mM (±6.8).

As expected, the net movement of water was into the lumen of the cholera segments, and out of the lumen in the control segments (Table I). Significantly more HCO_3^- accumulated in the cholera than in the control segments, and in every instance, the final concentration of lumenal HCO_3^- in the cholera segments exceeded that in the paired control.

The mean P_{CO_2} of the fluid infused into the cholera and control segments (initial infusion) was similar to the mean P_{CO_2} of portal vein blood, and with one exception, was always greater than the mean P_{CO_2} of femoral arterial blood (Table II). By the end of the 45-min test period, the lumenal P_{CO_2} in the cholera and control segments differed significantly: the mean P_{CO_2} of cholera fluid declined to that of arterial blood, and the mean P_{CO_2} of control fluid increased (Fig. 1).

In the lumenal fluid, the magnitude of the initial P_{CO_2} influenced its final value (Fig. 1). Among the 13 cholera segments, the final lumenal P_{CO_2} fell below that of arterial blood in six studies. The initial P_{CO_2} values in five of those studies clustered most closely to that of femoral arterial blood. To put it another way, if the initial P_{CO_2} of the lumenal fluid of the cholera segment was near that of arterial blood, the final P_{CO_2} value was usually less than that of arterial blood.

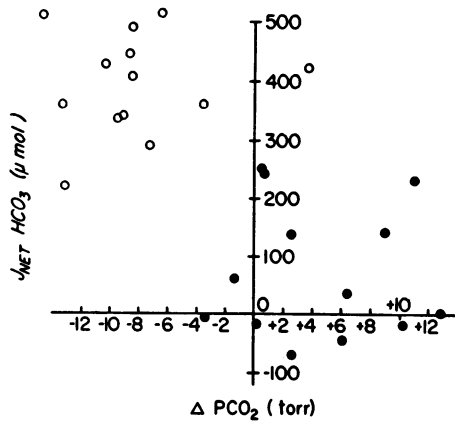Lumenal P_{CO_2} diminished by a mean of 8.4 torr in

FIGURE 2 The relationship between $J_{net}HCO_3^-$ (vertical axis) and the change in lumenal P_{CO_2} (final P_{CO_2} — initial P_{CO_2}) in cholera and control segments.

TABLE IV

PCO2 of Fluid from Ileal Segments Containing Isotonic or Hypertonic Fluid Compared with PCO2 of Initial Infusion Fluid, Mean Arterial PCO2, and PCO2 of Portal Vein Blood Sampled at End of Study Period

Study no.	Isotonic		Hypertonic		Mean arterial	Final portal vein
	Initial	Final	Initial	Final		
			torr			
1	31.7	34.8	32.1	32.3	27.1	30.7
2	30.4	24.0	31.6	29.6	26.4	31.7
3	30.0	34.5	31.6	40.5	29.5	35.0
4	34.0	29.2	32.1	22.3	29.9	33.1
5	32.6	37.2	31.6	40.4	32.3	40.5
6	30.8	40.7	31.1	37.9	29.9	39.3
7	31.6	27.1	33.1	31.4	25.7	34.2
8	32.3	47.6	32.6	41.5	38.1	40.5
Mean	31.7	34.4	32.0	34.5	29.9	35.6
SD	1.3	7.7	0.6	6.7	4.0	4.0

Final isotonic vs. mean arterial, $P < 0.05$; Final hypertonic vs. mean arterial, $P = 0.05$; Final isotonic vs. final hypertonic, NS.

cholera segments. However, in control segments, the mean final P_{CO_2} was 4.4 torr higher than the initial infusion value, and in no instance was the final P_{CO_2} less than that of arterial blood.

The change in lumenal fluid P_{CO_2} was roughly proportional to the rate and direction of net HCO_3^- movement (Fig. 2). In the cholera segments, where the rate of HCO_3^- secretion was greatest, lumenal fluid P_{CO_2} diminished; in the control segments, where HCO_3^- was either absorbed or was secreted at lower rates, lumenal P_{CO_2} increased.

Among the factors that might reduce the P_{CO_2} of lumenal fluid in the cholera segments is dilution. In study 2, we determined the influence of water movement on the final P_{CO_2} of lumenal fluid.

Study 2: influence of fluid movement on P_{CO_2}. The length of the segments infused with hypertonic fluid was almost identical to that of isotonic segments 21.8 (±2.1) and 21.9 cm (±2.0). The mean pH of the arterial blood was 7.41 (±0.03) and its mean HCO_3^- concentration was 19 mM (±3.3).

There was a net movement of water into the lumen of 1.8 ml (±1.6) in the hypertonic segments whereas in the isotonic segments, 1.4 ml (±1.2) was absorbed (Table III). The quantity and concentration of HCO_3^- that accumulated in the lumenal fluid of isotonic and hypertonic segments did not differ significantly.

Water movement had no significant effect on the

TABLE III

Net Movement of H_2O and HCO_3^-, and Final HCO_3^- Concentrations of Lumenal Fluid that was Initially Either Isotonic or Hypertonic

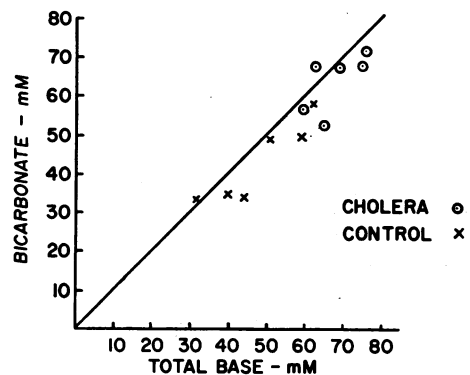| Study no.	ΔH₂O		ΔHCO₃⁻		Final lumen HCO₃⁻ concentration	
	Iso	Hyper	Iso	Hyper	Iso	Hyper
	ml		*μmol*		*mM*	
1	−2.5	0.5	125	148	27.6	32.9
2	−1.5	1.5	144	178	26.2	32.4
3	−1.0	1.0	113	109	18.9	21.7
4	0.5	5.0	182	428	24.2	47.6
5	−0.5	0.0	270	116	41.6	29.1
6	−2.5	2.5	175	339	38.8	52.1
7	−0.5	1.0	305	164	46.9	32.8
8	−3.0	2.5	120	301	30.0	46.3
Mean	−1.4	1.8	179	223	31.8	36.9
SD	1.2	1.6	72	118	9.6	10.5
P	<0.01		NS		NS	

FIGURE 3 Relationship of bicarbonate ion concentration to the concentration of total base in lumenal fluid of cholera and control segments. Bicarbonate accounts for most of the base present.

final P_{CO_2} of lumenal fluid (Table IV). Thus, it is unlikely that the movement of water into the lumen in the cholera segments in study 1 reduced the lumenal P_{CO_2} to values less than that of control segments and femoral arterial blood.

Total base and bicarbonate concentration of lumenal fluid. Bicarbonate accounts for most, but not all, of the total amount of base in the lumenal fluid of cholera and control segments (Fig. 3). The two values that fall to the left of the line of ideal correlation must be explained by experimental error, since bicarbonate content cannot exceed the content of total base.

DISCUSSION

Bicarbonate may accumulate in a body fluid because HCO_3^- ions enter or because OH^- ions accumulate and react with the CO_2 present, i.e., $OH^- + CO_2 \rightleftharpoons HCO_3^-$. If these reactions occurred in an intestinal lumen whose mucosa was impermeable to CO_2, one could infer which of these two mechanisms predominated by determining the difference between lumenal P_{CO_2} values measured at the beginning and end of the period of HCO_3^- accumulation: transport of HCO_3^- ions into the fluid would increase the lumenal P_{CO_2} by partial dissociation of HCO_3^- into CO_2 and OH^-, and accumulation of OH^- would reduce the P_{CO_2} by removing CO_2 from solution as the two combined to form HCO_3^-. In our studies, however, the mucosa was permeable to CO_2, so the final P_{CO_2} of the lumenal fluid could have been affected not only by chemical reactions within the lumenal fluid, but by the P_{CO_2} of fluid in the surrounding mucosa. The P_{CO_2} of fluid in mucosal cells is influenced by the interstitial fluid (ISF)[1] and the rate of cell CO_2 production. The basal value of cell P_{CO_2} is determined by the P_{CO_2} of the ISF; the cell adds to this basal value of cell P_{CO_2} by contributing metabolic CO_2, or subtracts from the basal value by exporting CO_2 at a rate faster than it is produced. In our studies, the final P_{CO_2} of lumenal fluid in the cholera segment decreased significantly, and was lower than arterial P_{CO_2} in 6 of 13 studies. It follows that such a reduction could have been caused primarily by a reduction in the P_{CO_2} of the ISF, cell, or lumenal fluid.

ISF P_{CO_2}. If HCO_3^- ions were transported from the ISF across the cell into the lumenal fluid (Fig. 4A), the P_{CO_2} of the ISF could be reduced to values less than arterial blood, i.e., loss of HCO_3^- would shift the reaction, $OH^- + CO_2 \rightleftharpoons HCO_3^-$, to the right. By solving a set of simultaneous equations (Appendix I), it is possible to predict how much the P_{CO_2} would diminish if HCO_3^- were transported from the ISF at maximal rates. Given that the initial HCO_3^- concentration of ISF was

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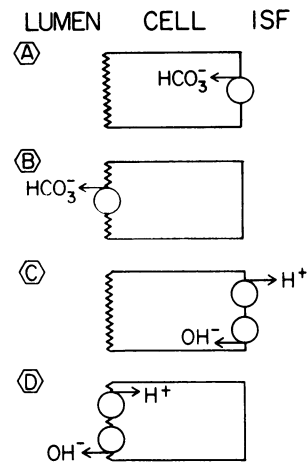[1] *Abbreviation used in this paper:* ISF, interstitial fluid.

FIGURE 4 Possible mechanisms of bicarbonate secretion. (A) HCO_3^- ion pump at the cell-ISF membrane; (B) HCO_3^- ion pump at the cell-lumen membrane; (C) an ion pump at the cell-ISF membrane that either exports H^+ ions or imports OH^- ions; (D) an ion pump at the cell-lumen membrane that either imports H^+ ions or exports OH^- ions.

18 mM (the mean measured value of arterial HCO_3^-), a reduction in HCO_3^- concentration to 1 mM would reduce the ISF P_{CO_2} by 1.1 torr. The entry of HCO_3^- ions into the lumenal fluid in an amount that could increase the HCO_3^- concentration from 25 mM to 65 mM (the initial and final mean concentrations of lumenal HCO_3^- in the cholera segment) would augment lumenal P_{CO_2} by 7.1 torr in a closed system. The net effect would be to increase lumenal P_{CO_2} by 6 torr. These calculations do not take into account cell CO_2 production, which would minimize any reduction in P_{CO_2} in the ISF. Hence, transport of HCO_3^- ions from the ISF could not explain the observed reduction in P_{CO_2} of lumenal fluid in the cholera segments. These calculations would also apply if a pump for HCO_3^- ions was located at the cell-lumen membrane (Fig. 4B), as long as the ultimate source of HCO_3^- ions was the ISF.

Cell P_{CO_2}. Cellular P_{CO_2} could be reduced if HCO_3^- ions were synthesized in the cell by reaction of OH^- with CO_2 at the cell-ISF membrane (Fig. 4C). For this process to reduce lumenal P_{CO_2}, the rate of OH^- generation must exceed the rate of cell CO_2 production and reduce the intracellular P_{CO_2} to values less than that of the lumen. Carbon dioxide would diffuse into the cell and lumenal P_{CO_2} would fall as HCO_3^- was synthesized. It can be shown that the subsequent movement of the HCO_3^- ions into the lumen would not increase lumenal P_{CO_2} sufficiently to counteract the P_{CO_2} reduction (See Appendix II).

Lumen P_{CO_2}. Lumenal P_{CO_2} would also diminish if HCO_3^- ions were synthesized on the lumenal side of the cell-lumen membrane (Fig. 4D). As OH^- accumulated

in the lumen, the CO_2 from the lumenal fluid and adjacent cell would react to form HCO_3^-. For lumenal fluid P_{CO_2} to fall below arterial P_{CO_2}, the rate of generation of OH^- would have to exceed the rate of cell CO_2 production. The P_{CO_2} of lumenal fluid would fall below arterial P_{CO_2} more readily if the site of HCO_3^- synthesis is the cell-lumen membrane, because the lumenal fluid and neighboring cytoplasm would be depleted of CO_2 before the CO_2 from more distal sites was used. If the cell-ISF membrane were the site of synthesis, however, the ISF and basal cytoplasm would be the nearest CO_2 source, and it is less likely that lumenal CO_2 would be depleted. For these reasons I favor the model shown in Fig. 4D, although it is clear that Fig. 4C cannot be excluded.

Alternative explanations for the reduction in P_{CO_2} in cholera fluid are less acceptable. If the mucosa sieved out the CO_2 as water and dissolved CO_2 moved from cell (or ISF) to lumen, lumenal P_{CO_2} would decrease. To determine whether such sieving occurs, we induced water movement into the lumen with a hypertonic solution (Table III) and found that the lumenal P_{CO_2} was no less than if an isotonic fluid were being absorbed (Table IV). Although this suggests that sieving does not occur, the study is an imperfect control for two reasons: first, the cell membrane was not exposed to cholera toxin and might have different pore characteristics; and second, in cholera fluid may move into the lumen through crypt cells (2), whereas in the hypertonic studies, water movement may be mostly through villus cells (3). If the route of water movement differs, sieving characteristics may differ. For these reasons, one cannot exclude the possibility that CO_2 sieving causes the low lumenal P_{CO_2} in the cholera fluid. In view of the high tissue permeability of CO_2, however, sieving seems unlikely

Bicarbonate is the major base that accumulates in the lumen. When the concentrations of HCO_3^- and total base are compared in the cholera and control segments, HCO_3^- accounts for most of the base present (Fig. 3). This implies that transport of OH^- ions into the lumen (or H^+ ions out) was the primary event in HCO_3^- secretion, for if OH^- ions had accumulated in the lumen because of the secretion of another basic salt, such as HPO_4^-, HCO_3^- would have accounted for a much smaller fraction of the total base.

Hydroxyl ions could have accumulated in ileal fluid either because OH^- ions enter or because H^+ ions exit (Fig. 4C, D). Although there is an obvious precedent for a hydrogen ion pump in gastric mucosa, there are no compelling reasons to exclude a hydroxyl ion pump in ileal mucosa.

Turnberg, Bieberdorf, Morawski, and Fordtran (4) have proposed that hydrogen ions are secreted into the human ileum as sodium ions are absorbed. A reduction in the rate of H^+ secretion could explain why the final P_{CO_2} of lumenal fluid in cholera segments was less than that of control segments, but it could not explain why the final P_{CO_2} was less than the P_{CO_2} of arterial blood. Our results could be explained by a "reversal" of such a sodium-hydrogen exchange mechanism, however (4).

Although considerable quantities of mucus are secreted into the cholera segments, it is unlikely that the mucus reduced the lumenal P_{CO_2} for two reasons: most of the mucus was drained from the cholera segments before the start of the 45-min study period; and the mucus was probably in equilibrium with ambient CO_2 before it was expelled into the lumen.

In the control segments, the final P_{CO_2} of lumenal fluid was higher than the initial P_{CO_2} in 11 of 13 studies, and in no instance was the final P_{CO_2} less than that of arterial blood (Fig. 1). Several factors may have contributed to the increase in lumenal P_{CO_2}. First, in most studies, water was absorbed more rapidly than HCO_3^-; the resultant rise in HCO_3^- concentration would have shifted the reaction, $OH^- + CO_2 \rightleftharpoons HCO_3^-$, to the left and increased P_{CO_2}. Second, because of the lower rate of HCO_3^- synthesis and secretion by control segments, less CO_2 derived from cell metabolism may have been used in HCO_3^- synthesis, allowing intracellular P_{CO_2} to rise. Carbon dioxide from the cells would then have diffused into the lumenal fluid and caused P_{CO_2} to increase. Third, there may be concurrent secretion of bicarbonate and H^+ ions as suggested by Turnberg et al. (4). Fourth, the possibility that HCO_3^- ions are transported into the lumen of control segments cannot be excluded.

The recent demonstrations that the effects of cholera toxin may be mediated by cyclic AMP (5) suggest that the mechanism of bicarbonate secretion observed in the cholera segments is normally present in the cell and is either activated or accelerated by a chain of events initiated by contact of the mucosa with toxin. If that is true, OH^- ion accumulation in the cell or lumen may be the normal mechanism of bicarbonate secretion in the small intestine.

APPENDIX I

With a computer program described by Haglund, Moss, and Flynn (6), it is possible to predict the magnitude of changes that occur in P_{CO_2}, H^+, and HCO_3^- concentrations when the concentrations of HCO_3^- or H^+ are changed. The program is an iterative solution of a set of simultaneous equations that characterize the system at equilibrium. The following constants were used in the calculation.

$$[H^+][OH^-] = K_w = 2.388 \times 10^{-14} = C_2$$

$$\frac{[H^+][HCO_3^-]}{[CO_2]} = K_I = 7.9 \times 10^{-7} = C_3$$

$$\frac{[H^+][CO_3^{--}]}{[HCO_3^-]} = K_{II} = 6.6 \times 10^{-11} = C_4.$$

Equilibrium conditions were changed by substituting appropriate values in the equation for charge balance (C_1) and mass balance (C_6), where $C_1 = [H^+] - [OH^-] - [HCO_3^-] - 2[CO_3^{--}]$, and $C_6 = [CO_2] + [HCO_3^-] + [CO_3^{--}]$. In the pH range of 7–8.3 that characterized the fluids in these studies, the values of H^+, OH^-, and CO_3^{--} were smaller than CO_2 and HCO_3^- by a factor of at least 10^2, and were not included in the calculations for C_1 and C_6.

APPENDIX II

The average wet weight of ileal segments was 4.6 g. A high estimate of the volume of intracellular mucosal water may be obtained by assuming that 50% of the total wet weight is composed of intracellular water, or 2.3 ml/segment. The average volume of lumenal fluid in the cholera segments was 5.7 ml during the 45-min study period. If the mucosal cell mass is a closed system with an intracellular milieu similar to arterial plasma, and the intracellular OH^- concentration was increased by 1 mM by transport at the cell-ISF membrane, the intracellular concentration of HCO_3^- would increase by 0.6 mM (from 25 to 25.6 mM) and intracellular P_{CO_2} would decrease from 40 to 14 torr. If the freshly synthesized HCO_3^- then moved from the cells into the 5.7 ml of lumenal fluid, the HCO_3^- concentration of lumenal fluid would increase by 0.24 mM and cause a rise in lumenal P_{CO_2} too trivial to be measured by our methods. In response to the marked reduction in intracellular P_{CO_2}, however, CO_2 would diffuse from the lumen into the cells and lumenal P_{CO_2} would diminish. In that manner, synthesis of HCO_3^- at the cell-ISF membrane could reduce the lumenal fluid P_{CO_2}.

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