Bicarbonate-Water Interactions in the Rat Proximal Convoluted Tubule

An Effect of Volume Flux on Active Proton Secretion

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ABSTRACT   The effect of volume absorption on bicarbonate absorption was examined in the in vivo perfused rat proximal convoluted tubule. Volume absorption was inhibited by isosmotic replacement of luminal NaCl with raffinose. In tubules perfused with 25 mM bicarbonate, as raffinose was increased from 0 to 55 to 63 mM, volume absorption decreased from 2.18 ± 0.10 to 0.30 ± 0.18 to -0.66 ± 0.30 nl/mm·min, respectively, and bicarbonate absorption decreased from 131 ± 5 to 106 ± 8 to 91 ± 13 pmol/mm·min, respectively. This bicarbonate-water interaction could not be attributed to dilutional changes in luminal or peritubular bulk phase bicarbonate concentrations. Inhibition of active proton secretion by acetazolamide abolished the effect of volume flow on bicarbonate absorption, which implies that the bicarbonate reflection coefficient is close to 1 and eliminates the possibility of solvent drag across the tight junction. When the luminal bicarbonate concentration was varied, the magnitude of the bicarbonate-water interaction increased with increasing luminal bicarbonate concentration. The largest interaction occurred at high luminal bicarbonate concentrations, where the rate of proton secretion has been previously shown to be independent of luminal bicarbonate concentration and pH. The results thus suggest that a peritubular and/or cellular compartment exists that limits bicarbonate diffusion, and where pH changes secondary to bicarbonate-water interactions (solute polarization) alter the rate of active proton secretion.

INTRODUCTION

Studies in the in vivo perfused rat proximal convoluted tubule (PCT) have consistently shown that changes in the rate of volume absorption lead to changes in solute flux (6, 17, 29). The mechanism of this solute-solvent interaction has been presumed to be solvent drag through the paracellular pathway. However,

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this is not the only possible mechanism by which solvent flow can affect solute movement.

The rate of solute transport across an epithelium is generally described phenomenologically by the equation

$$J_s = (1 - \sigma)\bar{C}J_v + P(\Delta\mu) + J_{act} \qquad (1)$$

$$\text{Solvent drag} \quad \text{Diffusion} \quad \underset{\text{transport}}{\text{Active}}$$

where σ is the solute reflection coefficient, $\bar{C}$ is the mean solute concentration across the epithelium, J_v is the rate of volume absorption, P is the solute permeability, $\Delta\mu$ is the electrochemical driving force for solute diffusion, and J_{act} is the rate of active transport. The first component of this equation is generally considered the solvent drag component and refers to an effect of volume absorption on solute movement that is independent of active transport or diffusion. In the proximal tubule, such an effect is generally felt to be related to frictional interactions between solute and solvent in the tight junction. Although volume flux is not shown in the second and third components of Eq. 1, it can also affect them. If significant diffusion barriers exist, then the concentrations of solute to which the tight junction and the active transport mechanism are exposed may not be those of the bulk phase fluid. Volume flow through these regions could then modify the local solute concentrations, and secondarily affect the rate of active transport and diffusion. When considered in terms of Eq. 1, this will lead to an apparent effect of volume flux on the solute permeability or on the active transport mechanism. This mechanism will be referred to as solute polarization (37).

The purpose of these studies was to examine the effect of transepithelial volume flux on the components of proximal tubular bicarbonate absorption. The results show that changes in the rate of volume absorption do affect the rate of bicarbonate absorption. This effect is dependent on the presence of active transport and is thus not due to transepithelial solvent drag or diffusion as defined in Eq. 1; rather, it appears to be due to a modification of active proton secretion, secondary to solute polarization. Kinetic analysis of the effect shows that changes in volume flux affect the local peritubular and/or cellular bicarbonate concentration and pH, and secondarily modify the rate of active proton secretion.

METHODS

Experiments were performed using male Wistar rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) weighing 211–280 g. The rats were prepared for microperfusion as previously described (1). Briefly, rats were anesthetized with an intraperitoneal injection of Inactin (100–120 mg/kg) and placed on a heated table that maintained body temperature at 37°C. The right femoral artery was catheterized for monitoring blood pressure and obtaining blood samples. The left kidney was exposed using a flank incision and immobilized in a Lucite cup. The ureter was cannulated (PE-50; Becton, Dickinson & Co., Parsippany, NJ) to ensure the free drainage of urine. Throughout the experiment, rats were infused intravenously with a bicarbonate Ringer's solution (105 mM NaCl, 25 mM NaHCO$_3$, 4 mM Na$_2$HPO$_4$, 5 mM KCl, 1 mM MgSO$_4$, 1.8 mM CaCl$_2$) at 1.6 ml/h.

The proximal tubular transit time was measured following intravenous injection of 0.02 ml of 10% lissamine green dye, and only those kidneys in which the transit time was <12 s were accepted for study. At the completion of surgery, a blood sample was obtained for determination of pH and P_{CO_2} (model 165 blood gas analyzer; Corning Glass Works, Medfield, MA). The rats had normal systemic acid base parameters: pH = 7.38 ± 0.01, P_{CO_2} = 40.4 ± 0.5 mmHg, and [HCO$_3^-$] = 23.4 ± 0.4 mM. Plasma samples were obtained throughout the experiment for determination of total CO_2 concentration.

After completion of surgery, rat PCT were microperfused as previously described (1), using a thermally insulated microperfusion pump (Wolfgang Hampel, Berlin, Federal Republic of Germany). A perfusion pipette was placed into a proximal loop. An oil block was placed proximal to the perfusion pipette and a hole was left for glomerular ultrafiltrate to leak out. A collection pipette was then placed in a late proximal loop, an oil block was inserted distally, and a timed collection was made. After the collection, the tubule was filled with microfil (Canton Biomedical Products, Boulder, CO). On a subsequent day, the kidney was incubated in 6 N HCl at 37°C for 60 min, allowing dissection of the microfil cast and measurement of the perfused length. Only tubules that were ≥1 mm in length were accepted.

The perfusion solutions used are listed in Table I. All perfusion solutions contained 0.05% FD and C green dye No. 3 (Warner-Jenkinson, St. Louis, MO) and exhaustively dialyzed [methoxy-^{3}H]inulin. Perfusion solutions were gassed with 90% O_2/10% CO_2. This gas concentration was used to achieve a P_{CO_2} of 60 mmHg, which has been shown to be present in the renal cortex (14).

Analysis

Perfusate and collected samples were covered with HEPES-equilibrated paraffin oil, bubbled with 10% CO_2 (1). The samples were transferred into constant bore tubing for measurement of collected volume. A 30–60-nl aliquot was then removed for determination of total CO_2 concentration and the remaining fluid was transferred to a vial for liquid scintillation counting. The total CO_2 concentration was measured using microcalorimetry (picapnotherm) (34).

TABLE I
*Perfusion Solutions**

	25C	25R1	25R2	25AC	25AR	5C	5R	60C	60R
	mM				*mM*			*mM*	
NaCl	120	85	80	120	80	140	100	85	45
NaHCO₃	25	25	25	25	25	5	5	60	60
KCl	5	5	5	5	5	5	5	5	5
MgSO₄	1	1	1	1	1	1	1	1	1
CaCl₂	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Na₂HPO₄	1	1	1	1	1	1	1	1	1
Glucose	5	5	5	5	5	5	5	5	5
Alanine	5	5	5	5	5	5	5	5	5
Urea	5	5	5	5	5	5	5	5	5
Raffinose	—	55	63	—	63	—	63	—	63
Acetazolamide	—	—	—	0.5	0.5	—	—	—	—

* All perfusion solutions were gassed with 90% O_2/10% CO_2 and contained 0.05% FD and C green dye No. 3 and exhaustively dialyzed [methoxy-^{3}H]inulin.

Calculations

The perfusion rate (V_O) was calculated as:

$$V_O = (I_L/I_O)V_L, \tag{2}$$

where I_L and I_O represent the inulin concentration in the collected and perfused fluids, respectively, and V_L is the collection rate. Volume flux (J_v) was calculated as:

$$J_v = (V_O - V_L)/L, \tag{3}$$

where L equals the perfused length.

The concentration of total CO_2 was measured in the perfused and collected fluids as well as in plasma samples obtained during the experiment. Total CO_2 includes dissolved CO_2, bicarbonate, and carbonate. At the pH and P_{CO_2} and bicarbonate concentrations encountered in these experiments, total CO_2 can be considered a reasonable estimate of bicarbonate concentration. Net bicarbonate flux ($J_{HCO_3^-}^{net}$) was calculated as:

$$J_{HCO_3^-}^{net} = (C_O V_O - C_L V_L)/L, \tag{4}$$

where C_O and C_L represent the perfused and collected bicarbonate concentrations, respectively. Mean luminal bicarbonate concentration ($\bar{C}_L$) was calculated using the arithmetic mean (1):

$$\bar{C}_L = (C_O + C_L)/2, \tag{5}$$

and the mean bicarbonate concentration ($\bar{C}$) was calculated using the arithmetic mean of plasma (C_P) and mean luminal bicarbonate concentrations:

$$\bar{C} = (\bar{C}_L + C_P)/2. \tag{6}$$

An apparent sieving coefficient (S_i^{app}) can be derived from the first part of Eq. 1:

$$\Delta J_{HCO_3^-} = (1 - \sigma)\bar{C}\Delta J_v = S_i^{app} \bar{C}\Delta J_v. \tag{7}$$

This equation can then be rearranged to yield:

$$S_i^{app} = (\Delta J_{HCO_3^-}/\Delta J_v)/\bar{C}. \tag{8}$$

From this relationship, the apparent sieving coefficient was calculated by dividing the slope of a plot of bicarbonate absorption as a function of volume absorption by the mean bicarbonate concentration:

$$S_i^{app} = \text{slope}/\bar{C}. \tag{9}$$

Data are presented as means ± SEM and groups are compared by the unpaired two-tailed t test. Linear regression was performed by the least-squares method.

RESULTS

Evidence for a Bicarbonate-Water Interaction

In order to determine whether changes in the rate of volume absorption affect bicarbonate absorption, tubules perfused with an ultrafiltrate-like solution containing 25 mM bicarbonate were compared with tubules perfused with a similar solution, except that raffinose replaced NaCl isosmotically (perfusates 25C, 25R1, and 25R2; Table I). As the raffinose concentration was increased from 0 to 55 to 63 mM, the rate of volume absorption decreased progressively from 2.18 ±

TABLE II

Bicarbonate Data

Per-fusate	n	Length	Perfusion rate	Total CO$_2$ concentration (mM)				Volume flux	Total CO$_2$ flux
				Perfused	Collected	Mean lu-minal	Plasma		
		mm	*nl/min*					*nl/mm·min*	*pmol/mm·min*
				25 mM Perfusate					
25C	10	2.43±0.18	17.28±0.20	25.7±0.1	10.7±1.1	18.2±0.5	26.3±1.0	2.18±0.10	131±5
25RI	6	2.37±0.32	16.40±0.29	24.4±0.3	9.9±1.6	17.2±0.9*	26.6±0.1	0.30±0.18	106±8‡
25RII	5	2.28±0.25	15.30±0.60	26.0±0.1	11.6±1.6	18.8±0.8*	26.9±0.8	−0.66±0.30	91±13ǁ
				25 mM + Acetazolamide perfusate					
25AC	4	3.16±0.53	16.49±0.19	25.0±0.1	25.4±0.2	25.2±0.1	23.3±0.8	0.69±0.17	15±6
25AR	9	2.51±0.37	16.18±0.22	25.3±0.1	20.6±0.5	23.0±0.2ǁ	27.0±0.3	−0.89±0.09	13±2*
				5 mM Perfusate					
5C	5	2.41±0.27	16.76±0.18	5.9±0.1	5.3±0.5	5.6±0.3	24.8±0.4	2.15±0.17	15±2
5R	5	2.13±0.20	16.32±0.41	5.7±0.1	4.0±0.5	4.9±0.3*	25.9±0.8	−0.13±0.16	12±2*
				60 mM Perfusate					
60C	11	2.20±0.26	15.43±0.36	59.8±0.1	34.0±3.1	47.3±1.7	28.1±0.5	1.07±0.24	212±8
60R	8	2.39±0.22	15.83±0.43	59.5±0.1	31.6±2.2	45.6±1.1*	25.6±0.8	−0.50±0.11	169±5ǁ

* NS vs. control.
‡ $P < 0.025$ vs. control.
§ $P < 0.005$ vs. control.
ǁ $P < 0.001$ vs. control.

0.10 to 0.30 ± 0.18 to −0.66 ± 0.30 nl/mm·min (Table II). This decreased rate of volume absorption was associated with a steady decrease in the rate of net bicarbonate absorption from 131 ± 5 to 106 ± 8 to 91 ± 13 pmol/mm·min, respectively (Table II).

Fig. 1 shows a plot of the relationship between the rate of bicarbonate absorption and the rate of volume absorption in these tubules. This relationship is described by the equation $J_{HCO_3^-} = 14.3\,J_v + 100.7$ ($r = 0.71$, $P < 0.001$). The slope of this regression, 14.3, is an index of the magnitude of the bicarbonate-water interaction. Using a mean bicarbonate concentration of 22.5 mM (calculated from Eq. 6), the apparent sieving coefficient is found to be 0.64 (Eq. 9).

Inhibition of volume absorption can dilute luminal bulk phase bicarbonate and concentrate peritubular capillary bulk phase bicarbonate. Previous studies (1, 3, 7, 19, 27, 28, 31, 33) have shown that either of these effects will lead to inhibition of bicarbonate absorption. It is unlikely that inhibition of volume absorption will concentrate peritubular capillary bulk phase bicarbonate, as changes in volume absorption of 2 nl/mm·min are small compared with plasma flow rates of 75–100 nl/min. Fig. 2 shows that inhibition of volume absorption did not dilute the luminal fluid bulk phase bicarbonate concentrations when examined as a function of tubular length. This can also be seen in Table II, where the mean luminal bicarbonate concentrations are similar in the three groups. Thus, the bicarbonate-water interaction is not due to an effect on bulk phase bicarbonate concentration or pH.

Dependence of Bicarbonate-Water Interaction on Active Transport

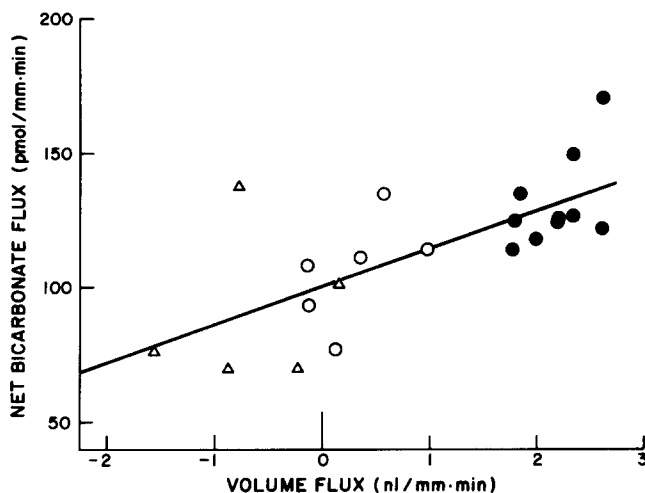In the next set of studies, the effect of inhibition of active proton secretion on

FIGURE 1.   Rate of bicarbonate absorption is plotted as a function of the rate of volume absorption in tubules perfused with 25 mM bicarbonate. Perfusates contained 0 (closed circles), 55 (open circles), and 63 mM raffinose (open triangles). The line is defined by the equation $J_{HCO_3^-} = 14.3 J_v + 100.7$ ($r = 0.71$).

the bicarbonate-water interaction was examined. Tubules were perfused with perfusates similar to those of the previous study, but containing 0.5 mM acetazolamide (perfusates 25AC and 25AR; Table I). This concentration has previously been shown to inhibit 80–100% of active proton secretion in the in vivo perfused rat PCT (7, 24, 26).

In tubules perfused with 25 mM bicarbonate plus acetazolamide, the rate of volume absorption was 0.69 ± 0.17 nl/mm·min and the rate of bicarbonate absorption was 15 ± 6 pmol/mm·min (Table II). When luminal NaCl was
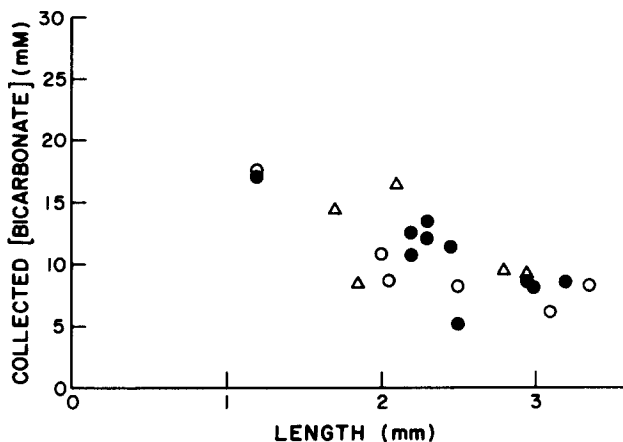

FIGURE 2.   Collected fluid bicarbonate concentration is plotted as a function of tubular length in tubules perfused with 25 mM bicarbonate. Perfusates contained 0 (closed circles), 55 (open circles), and 63 mM raffinose (open triangles).

partially replaced with 63 mM raffinose, the rate of volume absorption decreased to -0.89 ± 0.09 nl/mm·min, but the rate of bicarbonate absorption was unaffected (13 ± 2 pmol/mm·min, Table II). If solvent drag (as defined phenomenologically by Eq. 1) had occurred, the apparent sieving coefficient measured in the presence of active transport (0.64) would have predicted a 25 pmol/mm·min decrease in the rate of bicarbonate absorption to -10 pmol/mm·min.

Thus, inhibition of active proton secretion prevents the effect of volume flux on bicarbonate flux. This finding suggests that a change in volume absorption is not altering bicarbonate absorption by an effect on either the passive processes of solvent drag or diffusion (as defined in Eq. 1), and instead is, in some manner, altering the rate of active proton secretion.

Effect of Luminal Bicarbonate Concentration on the Magnitude of the Bicarbonate-Water Interaction

In previous studies (1–3), the relation between the rate of active proton secretion and the mean luminal bicarbonate concentration was used to examine the mechanism by which various factors modulate bicarbonate absorption (see Discussion). The purpose of the next set of studies was to examine the effect of luminal bicarbonate concentration on the magnitude of the bicarbonate-water interaction. Tubules were therefore perfused with 5 (perfusates 5C and 5R; Table I) and 60 mM (perfusates 60C and 60R; Table I) bicarbonate.

As is shown in Table II, changes in volume absorption did not significantly affect the rate of bicarbonate absorption in tubules perfused with 5 mM bicarbonate. Raffinose addition caused volume absorption to decrease from 2.15 ± 0.17 to -0.13 ± 0.16 nl/mm·min, but bicarbonate absorption was unaffected (15 ± 2 vs. 12 ± 2 pmol/mm·min). When the rate of bicarbonate absorption is examined as a function of the rate of volume absorption, the relationship is: $J_{HCO_3^-} = 1.6 J_v + 11.5$ ($r = 0.41$, NS; Fig. 3).

In tubules perfused with 60 mM bicarbonate, the effect of volume absorption on bicarbonate absorption was large. The addition of raffinose to the luminal perfusate caused volume absorption to decrease from 1.07 ± 0.24 to $-0.50 \pm$
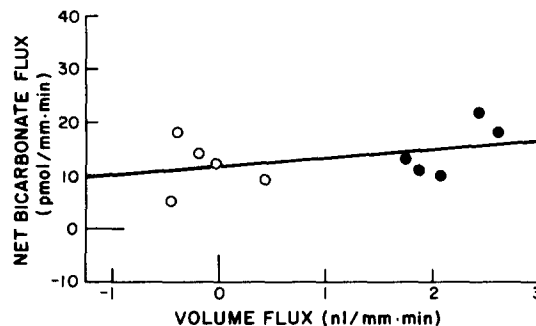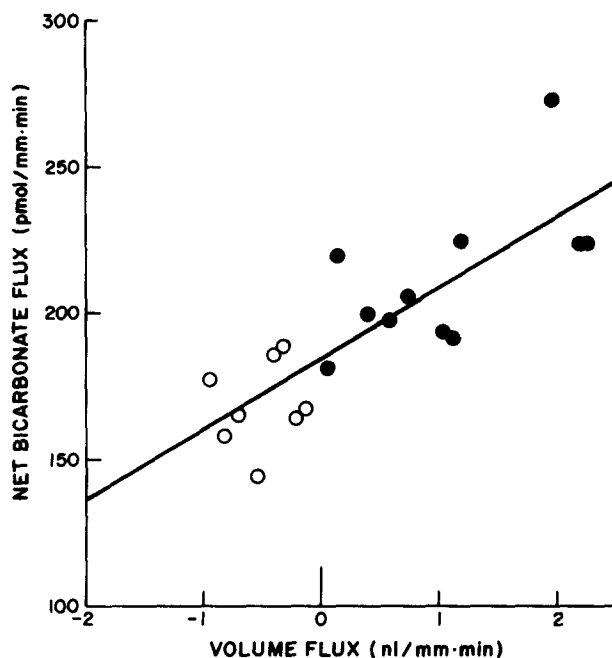

FIGURE 3.   Rate of bicarbonate absorption is plotted as a function of the rate of volume absorption in tubules perfused with 5 mM bicarbonate. Perfusates contained 0 (closed circles) and 63 mM raffinose (open circles). The line is defined by the equation $J_{HCO_3^-} = 1.6 J_v + 11.5$ ($r = 0.41$).

FIGURE 4.   Rate of bicarbonate absorption is plotted as a function of the rate of volume absorption in tubules perfused with 60 mM bicarbonate. Perfusates contained 0 (closed circles) and 63 mM raffinose (open circles). The line is defined by the equation $J_{HCO_3^-} = 24.1\,J_v + 183.7$ ($r = 0.81$).

0.11 nl/mm·min[1] and net bicarbonate absorption to decrease from 212 ± 8 to 169 ± 5 pmol/mm·min (Table II). Fig. 4 shows a plot of the rate of bicarbonate absorption as a function of the rate of volume absorption. This relationship is defined by the equation: $J_{HCO_3^-} = 24.1\,J_v + 183.7$ ($r = 0.81$, $P < 0.001$).

Once again, this bicarbonate-water interaction could not be attributed to dilutional effects on bulk phase luminal bicarbonate concentration. Fig. 5 shows that decreases in the rate of volume absorption did not affect the luminal bulk phase bicarbonate concentration in tubules perfused with 60 mM bicarbonate, when examined as a function of tubular length. In addition, Table II shows that the mean luminal bicarbonate concentrations were similar in the two groups.

The slopes of the regression lines relating bicarbonate absorption to volume absorption in Figs. 1, 3, and 4 ($\Delta J_{HCO_3^-}/\Delta J_v$) are a measure of the magnitude of the bicarbonate-water interaction. Fig. 6 shows a plot of these slopes as a function of mean luminal bicarbonate concentration. It can clearly be seen that the magnitude of the bicarbonate-water interaction increased with increasing luminal

[1] When tubules were perfused with 60 mM bicarbonate, the rate of volume absorption was low as compared to tubules perfused with 25 mM bicarbonate. This is similar to results in previous studies (1) and is because the Cl concentration was low in this solution. In studies in which the effect of Cl concentration on Cl flux was measured, a permeability of ~15–20 × 10^{-5} cm/s was obtained (unpublished observations). Similar results have been obtained from tracer Cl permeability measurements (17). A low Cl concentration will therefore lead to Cl secretion, which is responsible for the inhibition of volume absorption.
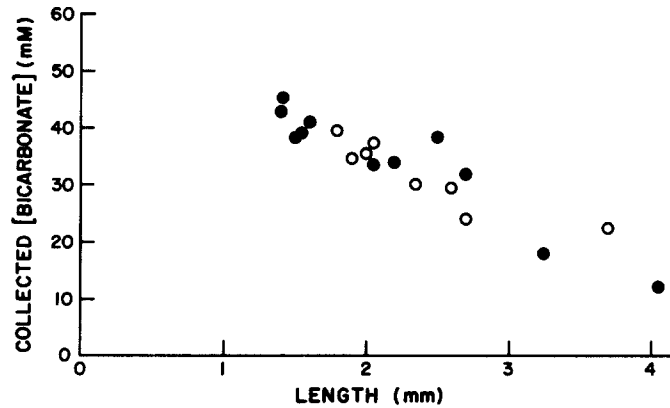
FIGURE 5. Collected fluid bicarbonate concentration is plotted as a function of tubular length in tubules perfused with 60 mM bicarbonate. Perfusates contained 0 (closed circles) and 63 mM raffinose (open circles).
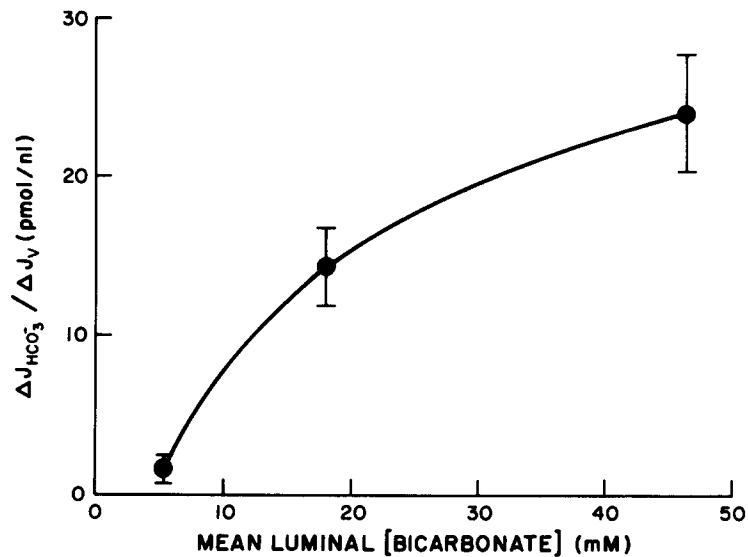

FIGURE 6. Magnitude of effect of changes in volume absorption on bicarbonate absorption is examined as a function of mean luminal bicarbonate concentration. The magnitude of the effect ($\Delta J_{HCO_3^-}/\Delta J_v$) is the slope of the equation relating $J_{HCO_3^-}$ to J_v (see Figs. 1, 3, and 4).

bicarbonate concentration. The significance of this kinetic behavior will be discussed below.

DISCUSSION

Methodology

In these studies, volume absorption was decreased by lowering the luminal NaCl concentration and thus decreasing the rate of NaCl absorption. This method

leads to a change in the rate of volume absorption which is relatively constant along the length of the perfused segment. It is important, however, to consider whether decreases in luminal NaCl concentrations in themselves could decrease the rate of acidification. A decrease in the luminal Cl concentration could affect the rate of acidification if a luminal membrane Cl-hydroxyl exchanger were present (25, 35). The expected effect, however, would be a stimulation of acidification. A decrease in the luminal Na concentration could decrease the rate of Na/H exchange. Chan et al. (8), however, have found that luminal Na concentrations as low as 40 mM do not affect the rate of acidification. In addition, brush border membrane vesicle studies have found that the K_{Na} for the Na/H antiporter is 14 mM, far below the lowest Na concentration used in the present studies (107 mM) (5, 22). Lastly, a decrease in the luminal NaCl concentration would be expected to cause a more lumen-positive potential difference (16). This effect, however, should be <1 mV (16), and because of the low mean bicarbonate concentrations present, it will have minimal effects on acidification (1).

It should be noted that the present results disagree with those of Frömter et al. (17). These authors found no effect of raffinose-induced changes in volume absorption on bicarbonate flux. However, in those studies, the bicarbonate concentration was not measured, but rather was calculated from the Na and Cl concentrations. The interaction measured here was probably too small to detect by such an indirect approach.

Nature of the Bicarbonate-Water Interaction

In these studies, the rate of volume absorption was found to be a determinant of proximal acidification. This could not be attributed to a dilutional effect on the composition of the luminal or peritubular bulk fluid. Most importantly, the bicarbonate-water interaction was dependent on the presence of active proton secretion. When active proton secretion was inhibited by acetazolamide, changes in the rate of volume absorption did not significantly affect the flux of bicarbonate.

As discussed in the Introduction, transepithelial solute transport can be phenomenologically divided into three general components: solvent drag, passive solute diffusion, and active solute transport. In this context, solvent drag refers to an effect of solvent movement on solute flux that is independent of active transport or diffusion. In that the presently observed bicarbonate-water interaction is dependent on the presence of active transport, it cannot be due to this form of solvent drag. Thus, the reflection coefficient for bicarbonate can be assumed to be 1, and the sieving coefficient close to 0.

An alternative mechanism by which volume flux can affect bicarbonate flux is solute polarization (37). Water flux through the epithelium modifies the local bicarbonate concentrations and pH, and secondarily modifies the rate of active proton secretion. The failure to observe a bicarbonate-water interaction in the absence of active transport suggests that this solute polarization is not affecting

the rate of passive bicarbonate diffusion.[2] While this could imply that the solute polarization is not affecting the concentration gradient across the tight junction, it is more likely that this negative result is due to the small bicarbonate permeability in the mammalian PCT (1.5–3.5×10^{-5} cm/s) (1, 9, 21, 31).

Localization of the Bicarbonate-Water Interaction

The present results suggest the presence of a diffusion barrier, where convective interactions are able to alter the local pH and secondarily modify the rate of active proton secretion. Such an interaction could occur in the lumen, the cell, or in a peritubular compartment. In previous studies, when active proton secretion was examined as a function of luminal bicarbonate concentration, it was demonstrated to saturate at high luminal bicarbonate concentrations (1). Thus, at luminal bicarbonate concentrations of >45 mM, the rate of proton secretion was unaffected by further changes in the luminal bicarbonate concentration or pH. These results are plotted as the solid lines in Fig. 7, *a* and *b*. It has also been found (2) that increasing the perfusion rate from 15 to 49 nl/min increased the rate of proton secretion when the mean luminal bicarbonate concentration was low, but not when the concentration was sufficiently high to saturate the system. These results are shown in Fig. 7a. This kinetic behavior suggested the presence of a flow-dependent luminal diffusion barrier (2).

On the other hand, in studies where the effect of peritubular pH on proton secretion was examined (3), the effect occurred at all luminal bicarbonate concentrations and was greatest in magnitude at high luminal bicarbonate concentrations. These results are plotted in Fig. 7b. The solid line represents results in tubules with a peritubular bicarbonate concentration of 24 mM, and the dashed line results in tubules with a peritubular bicarbonate concentration of 37 mM. Thus, changes in peritubular pH modulate the apparent V_{max} of the proton secretory system.

Although the biochemical nature of the observed kinetics is not yet known, they can be used phenomenologically to examine the location of the bicarbonate-water interaction. If the convective interaction occurs in a luminal compartment, modifying local luminal bicarbonate concentration and pH, the effect should be greatest at low luminal bicarbonate concentrations, decrease as luminal bicarbonate concentration increases, and disappear as proton secretion saturates at luminal bicarbonate concentrations above 45 mM. If the interaction occurs in a cellular or peritubular compartment, the effect should increase as the luminal bicarbonate concentration increases and, most importantly, should still be present at luminal bicarbonate concentrations associated with saturation (45 mM).

Fig. 6 shows a plot of the magnitude of the bicarbonate-water interaction as a function of mean luminal bicarbonate concentration. It can be seen that the bicarbonate-water interaction increased as the luminal bicarbonate concentration increased and, most importantly, was present at luminal bicarbonate concentra-

[2] In referring to paracellular diffusion, I have only discussed bicarbonate as a diffusing moiety. Although protons and hydroxyl ions possess high permeability coefficients (20, 32), they exist in low concentrations in vivo and thus they will contribute little to diffusion through the paracellular pathway.
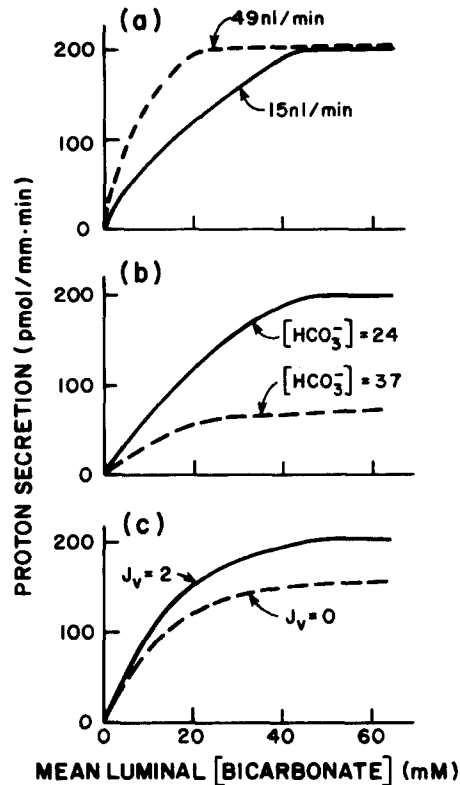
FIGURE 7. The rate of proton secretion is plotted as a function of the mean luminal bicarbonate concentration. (a) The solid line represents tubules perfused at 15 nl/min, and the dashed line represents tubules perfused at 49 nl/min (1, 2). (b) The solid line represents tubules perfused in control animals (plasma bicarbonate concentration = 24 mM), and the dashed line represents tubules perfused in alkalotic animals (plasma bicarbonate concentration = 37 mM) (1, 3). (c) The solid line represents tubules with a J_v of 2 nl/mm·min, and the dashed line represents tubules with a J_v of 0 nl/mm·min. Values are calculated from the intercepts and slopes of Figs. 1, 3, and 4.

tions of >45 mM. These results are plotted in a manner similar to that of the previous studies (1–3) in Fig. 7c. The rates of proton secretion were calculated at J_v = 0 and 2 nl/mm·min using the intercepts and slopes of Figs. 1, 3, and 4. The rate of active proton secretion was calculated by correcting rates of net bicarbonate absorption for calculated rates of passive bicarbonate diffusion using a bicarbonate permeability of 3.5 × 10^{-5} cm/s (1). Once again, it can be seen that the effect increases as the luminal bicarbonate concentration increases and is present at luminal bicarbonate concentrations of >45 mM. This kinetic behavior is not consistent with an interaction in a luminal diffusion barrier and is more consistent with an interaction in the cell and/or in a peritubular compartment.

Model

The present results can now be assimilated into a model. Protons are actively transported across the luminal membrane by a Na/H antiporter. The rate of this antiporter is dependent on both luminal and cellular pH in the vicinity of the antiporter (4, 5). Proton secretion leads to the local accumulation of protons in the lumen and hydroxyl ions in the cell, which must be carried away from the membrane. Although protons and hydroxyl ions possess large diffusion coefficients, their concentrations in vivo are extremely small (10^{-8}–10^{-7} M), such that there will be little diffusion of these species. These acid and base equivalents leave the vicinity of the luminal membrane by combining with buffers that exist in concentrations of 10^{-3}–10^{-2} M and can thus diffuse in greater amounts. As bicarbonate is the most prevalent buffer in the in vivo proximal tubule, it will be quantitatively the most important diffusing species. The presence of carbonic anhydrase in the cytoplasm allows rapid interconversion of hydroxyl and bicarbonate ions.

If diffusion of bicarbonate is restricted at some point in its path from the luminal membrane to the peritubular capillary, then significant concentration gradients can exist. Volume flux can then lower the bicarbonate concentration by dilution and convectively transport bicarbonate away from the membrane. Once again, the rate of convection of a solute is related to the mean concentration of the solute. Since protons and hydroxyl ions exist in small concentrations in vivo, there will be very little convection of these moieties. Buffers, on the other hand, exist in higher concentrations and can thus be moved convectively. Since bicarbonate is the most prevalent buffer in vivo, it will have the highest rate of convection. Removal of bicarbonate ions from the vicinity of the antiporter will by mass balance cause hydroxyl ions to combine with CO_2 to form bicarbonate. This will then by mass balance cause water to dissociate to form protons and hydroxyl ions. The net effect will be an increased proton concentration, a lower pH, and stimulation of the Na/H antiporter.

It is possible to estimate the magnitude of solute polarization that is required to cause the observed changes by comparing the effects of peritubular bicarbonate concentration (Fig. 7b) and changes in volume flux (Fig. 7c) on proton secretion rate. In tubules perfused with 60 mM bicarbonate, increasing peritubular bicarbonate concentration by 13 mM inhibits proton secretion by 130 pmol/mm·min (3). Inhibiting volume absorption from 1.07 to −0.50 nl/mm· min inhibits proton secretion by only 43 pmol/mm·min, which by interpolation would be equivalent to a change in the peritubular bicarbonate concentration of 4 mM. Since we do not know the exact location of the interaction, it is not possible to be more quantitatively specific at this time. It is worth noting, however, that for the change in bicarbonate absorption to be accounted for by an effect of solute polarization on passive bicarbonate absorption alone, the lateral intercellular space bicarbonate concentration would have to change by 27 mM (using a bicarbonate permeability of 3.5×10^{-5} cm/s). This explains the absence of an observed effect on passive diffusion when active transport was inhibited.

In order for bicarbonate-water interactions to modify the local pH in a cellular or peritubular compartment, bicarbonate diffusion must be restricted. If diffu-

sion is rapid, all solute concentration gradients will be collapsed by diffusion and local solute concentrations will be unaffected by volume flux. Because the entire proximal tubule epithelial wall is only ~10 μm thick, it has been assumed that significant diffusion barriers would be unlikely. This line of reasoning, however, assumes a limitation on the degree to which diffusion can be restricted in these compartments. Until we know the limits of this restriction, it is difficult to exclude a diffusion barrier on a morphologic basis.

In considering the possible locations for the bicarbonate-water interaction, one needs to consider the possible diffusion resistances in series with active proton secretion. As stated above, the Na/H antiporter on the luminal membrane ejects protons from the cell into the luminal fluid, and thus bicarbonate ions, formed in the cell, must diffuse across the cytoplasm to the basolateral membrane. The bicarbonate ion next diffuses across a conductance pathway in the basolateral membrane (18). As 90% of the basolateral membrane is lateral (36), most of the bicarbonate will exit into the lateral intercellular space, where it will then have to diffuse out. The bicarbonate must then diffuse across the basement membrane, across the renal interstitium, and into the peritubular capillary. Thus, the resistances in series with the active transport step, where bicarbonate-water interactions could occur, include: the cytoplasm, the basolateral membrane, the lateral intercellular space, the basement membrane, and the renal interstitium. There are presently no data available to distinguish between these possibilities.

It is, however, interesting to compare the results of studies that have examined the effect of transepithelial volume flow on solute flux in the rat PCT perfused in vivo and in the rabbit PCT perfused in vitro. Studies in the in vivo perfused rat PCT have consistently found solute-solvent interactions. Bomsztyk and Wright (6) found that changes in volume flux induced by luminal mannitol altered the fluxes of Na, Cl, K, and Ca with sieving coefficients of 0.45, 0.45, 0.85, and 0.85, respectively. Rector et al. (29), using a similar method, found a sieving coefficient for NaCl of 0.42. Frömter and colleagues (17) found sieving coefficients for Na and Cl of 0.30 and 0.57, respectively. In addition, Frömter and Gessner (15) obtained a lumen-positive streaming potential in the rat proximal convoluted tubule, which suggests solvent drag. The streaming potential, however, may represent an altered diffusion potential secondary to altered solute concentrations in the lateral intercellular space, rather than a true streaming potential.

Similar studies in the in vitro perfused rabbit PCT have found no effect of volume movement on solute flux. Jacobson et al. (23) found that raffinose-induced changes in volume flow did not affect Cl or bicarbonate fluxes in the presence or absence of active transport. Similarly, Corman and DiStefano (10) found that raffinose- or mannitol-induced changes in volume movement did not lead to any osmolar flux. In addition, these authors found no streaming potential (10).

It is not clear whether the difference between the results in rats and rabbits is due to a species difference or to a difference in techniques (in vitro vs. in vivo preparation). The present results, however, suggest that the difference is due at least in part to a significant cellular and/or peritubular diffusion barrier in the

in situ rat proximal tubule that is not present in the in vitro perfused rabbit proximal tubule. In support of this is the finding that butanol permeability (a measure of diffusion barriers in series with a membrane) is 5.0×10^{-3} cm/s in the in vitro perfused rabbit PCT (C. A. Berry, personal communication), but only 2.1×10^{-3} cm/s in the in vivo perfused rat PCT (unpublished observations).

One possible explanation for the difference between the results in these two preparations is that the renal interstitium is the site of the diffusion barrier and is removed in the in vitro perfused tubule preparation. In support of an interstitial diffusion barrier are data of Frömter et al. (16), who found that luminal perfusion with high K concentrations will depolarize cells from a neighboring unperfused tubule. This implies that a concentration gradient can be maintained between the renal interstitium and the capillary, and thus that a diffusion barrier exists.

In summary, volume flux out of the lumen is able to dilute and convectively "carry" base equivalents in the form of bicarbonate and other alkaline buffers away from the luminal membrane. This will acidify the local area and secondarily stimulate the rate of the Na/H antiporter. The exact location of this diffusion barrier where the convective interaction takes place is not elucidated from the present results, and could be the cell cytoplasm, the basolateral membrane, the lateral intercellular space, the basement membrane, or the renal interstitium. It is worth noting that although these results show that the quantitatively most important interaction is on the peritubular side of the luminal membrane, they do not rule out a small additional effect on a luminal diffusion barrier (2).

Physiologic Significance

The epithelial diffusion barrier demonstrated in these studies has significance with respect to water movement, solute movement in general, and acidification. As stated above, the presently observed diffusion barrier can account for the solute-solvent interactions observed by Rector et al. (29), Bomsztyk and Wright (6), and Frömter et al. (17). The effects observed by these investigators can be explained by alterations in local solute concentrations within the epithelium (solute polarization) and thus do not necessarily indicate solvent drag by water movement through the paracellular pathway.

In addition, the present studies may have relevance to the mechanism by which solute transport leads to water movement. Dainty (12) has pointed out that when osmotic water permeability is measured by imposing an osmotic gradient, the presence of a diffusion barrier or unstirred layer will lead to solute polarization and secondarily to an underestimation of the permeability. In addition, as initially proposed by Curran and MacIntosh (11) and Diamond and Bossert (13), the presence of an intraepithelial diffusion barrier will allow intraepithelial hypertonicity, which can provide the driving force for water movement. Recently, Sackin and Roth (30) have found an increased NaCl concentration in the lateral interspace of the *Ambystoma* proximal tubule. Intraepithelial hypertonicity can lead to greater rates of water movement than would be calculated from knowledge of the measured water permeability and the measured osmotic gradient (plasma minus luminal).

The present results are also relevant to the control of acidification. Extracel-

lular fluid volume expansion leads to a small inhibition of proximal tubular bicarbonate absorption. This inhibition has been attributed to a 50% increase in bicarbonate permeability (3). The bicarbonate-water interaction demonstrated in the present paper provides a second mechanism for the effect of extracellular fluid volume status on the rate of proximal bicarbonate absorption. Inhibition of NaCl and volume absorption by volume expansion would secondarily inhibit active proton secretion.

Chan and co-workers (9) have recently examined the effect of peritubular protein on the rate of bicarbonate and water transport in the rat proximal tubule. These authors found the effect on Cl and water transport to be far greater than the effect on acidification. They did, however, find a small but significant inhibition of acidification that could not be attributed totally to an increased bicarbonate permeability and backleak (inhibition was found even when the capillary contained no bicarbonate). If one considers just their studies where lumen and capillary were perfused with 25 mM bicarbonate and the capillary protein concentration was varied, the relationship between the rates of bicarbonate and volume absorption was linear: $J_{HCO_3^-} = 16.0 J_v + 100.6$ (9). This regression is very similar to that which was observed in Fig. 1 of this paper ($J_{HCO_3^-} = 14.3 J_v + 100.7$). This similarity suggests that most of the inhibition of bicarbonate absorption observed by Chan and co-workers was secondary to the observed inhibition of volume absorption.

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