Effects of Extracellular Fluid Volume and Plasma Bicarbonate Concentration on Proximal Acidification in the Rat

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ABSTRACT The effects of systemic bicarbonate concentration and extracellular fluid volume status on proximal tubular bicarbonate absorption, independent of changes in luminal composition and flow rate, were examined with in vivo luminal microperfusion of rat superficial proximal convoluted tubules. Net bicarbonate absorption and bicarbonate permeability were measured using microcalorimetry. From these data, net bicarbonate absorption was divided into two parallel components: proton secretion and passive bicarbonate diffusion.

The rate of net bicarbonate absorption was similar in hydropenic and volume-expanded rats when tubules were perfused with 24 mM bicarbonate, but was inhibited in volume-expanded rats when tubules were perfused with 5 mM bicarbonate. Volume expansion caused a 50% increase in bicarbonate permeability, which totally accounted for the above inhibition. The rate of proton secretion was unaffected by volume expansion in both studies.

The rate of net bicarbonate absorption was markedly inhibited in alkalotic expansion as compared with isohydric expansion. Bicarbonate permeabilities were not different in these two conditions, and the calculated rates of proton secretion were decreased by >50% in alkalosis. Net bicarbonate absorption was stimulated in acidotic rats compared to hydropenic rats. This stimulation was attributable to a 25% increase in the rate of proton secretion.

We conclude that (*a*) proton secretion is stimulated in acidosis, inhibited in alkalosis, and is not altered by volume status; (*b*) bicarbonate permeability is increased by volume expansion but is not altered by increases in plasma bicarbonate concentration; (*c*) when luminal bicarbonate concentrations are similar to those of plasma, net bicarbonate absorption is dominated by proton secretion and is thus sensitive to peritubular bicarbonate concentrations, and insensitive to extracellular fluid volume; (*d*) when luminal bicarbonate concentrations are low and proton secretion is slowed, bicarbonate permeability and thus extracellular fluid volume have a greater influence on net bicarbonate absorption.

INTRODUCTION

Numerous clearance and micropuncture studies have attempted to examine the effects of systemic bicarbonate concentration and ECF[1] volume status on acidification (1–8). In these studies, however, as plasma bicarbonate concentration or ECF volume were altered, there were simultaneous changes in luminal bicarbonate concentration and/or flow rate.

The purpose of the present studies was to examine the independent effects of plasma bicarbonate concentration and ECF volume expansion on proximal tubular bicarbonate absorption. Microperfusion was used to keep luminal bicarbonate concentration and flow rate constant as the animal's plasma bicarbonate concentration and/or ECF volume was altered. As in previous studies, net bicarbonate absorption and bicarbonate permeability were measured, and used to divide net bicarbonate absorption into two compo-

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[1] *Abbreviations used in this paper*: ECF, extracellular fluid; PCT, proximal convoluted tubule.

nents: a proton secretory mechanism in parallel with passive bicarbonate diffusion (9). The results demonstrate that (*a*) proton secretion is stimulated in acidosis, inhibited in alkalosis, and is not altered by volume status; (*b*) bicarbonate permeability is increased by volume expansion but is not altered by increases in plasma bicarbonate concentration; (*c*) when luminal bicarbonate concentrations are similar to those of plasma, net bicarbonate absorption is dominated by proton secretion and is thus insensitive to extracellular fluid volume; (*d*) when luminal bicarbonate concentrations are low and proton secretion is slowed, bicarbonate permeability and thus extracellular fluid volume have a greater influence on net bicarbonate absorption.

METHODS

Experiments were performed using male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 156–325 g. The rats were prepared for microperfusion as previously described (9). 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) to ensure the free drainage of urine. Throughout surgery, rats were infused intravenously with a bicarbonate Ringer's solution (NaCl 105 mM, $NaHCO_3$ 25 mM, Na_2HPO_4 4 mM, KCl 5 mM, $MgSO_4$ 1mM, $CaCl_2$ 1.8 mM) at 1.2 ml/h. Proximal tubular transit time was measured after injection of 0.02 ml of 10% lissamine green dye intravenously, and only those kidneys in which transit time was <13 s were accepted for study. After completion of surgery, rats were divided into four experimental groups.

Hydropenia. The infusion of bicarbonate Ringer's was continued at 1.2 ml/h throughout the experiment. Surgically induced plasma volume losses were not replaced so that a state of marked ECF volume contraction existed (10, 11).

Isohydric volume expansion. The rats were expanded intravenously with 10% body wt bicarbonate Ringer's over ~1 h, followed by a maintenance infusion equal to 3% body wt per h (8).

Alkalotic volume expansion. The rats were infused with a bicarbonate solution ($NaHCO_3$ 180 mM, $KHCO_3$ 30 mM) at the same rates as in group 2. This infusion was chosen so as to avoid changes in plasma K concentration. Plasma K concentration was 5.0±0.1 (n = 20) in this group as compared with 5.0±0.2 (n = 7) in isohydric expansion.

Metabolic acidosis. The rats were infused with 0.5 M NH_4Cl, 2% body wt at 70 μl/min, followed by a maintenance infusion of normal saline at 1.2 ml/h.[2]

Prior to the infusions, plasma samples were obtained for determination of protein concentration (refractometry) and hematocrit. 30 min after maintenance infusions were started, a blood sample was obtained for determination of pH and P_{CO_2} (Corning model 165 blood gas analyzer, Corning Glass Works, Medfield, MA). Subsequent plasma samples were then obtained for determination of protein concentration and hematocrit throughout the experiment.

Starting 30 min after maintenance infusions were initiated, rat proximal convoluted tubules (PCT) were microperfused at 15 nl/min as previously described (9), using a thermally insulated microperfusion pump (Wolfgang Hampel, Berlin, West Germany). The 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 inserted distally, and a timed collection made. After the collection the tubule was filled with latex. On a subsequent day the kidney was incubated in 6 N HCl at 37°C for 70 min allowing dissection of the latex cast and measurement of the perfused length. Tubules were only accepted that were ≥1 mm in length.

The perfusion solutions used are listed in Table I. In the permeability studies, perfusate 1 was used, which was designed to minimize volume flux and was not gassed. In the absorption studies, perfusates 2 and 3 were used and were gassed with 90% O_2/10% CO_2, in order to achieve a CO_2 tension of ~60–70 mmHg (9). The bicarbonate concentration of perfusate 2 was varied by replacing NaCl with $NaHCO_3$. All perfusion solutions contained 0.05% FD&C green dye No. 3 and exhaustively dialyzed [*methoxy*-^{3}H]inulin.

Analysis. The collected samples were kept in contact with Hepes equilibrated paraffin oil, bubbled with 10% CO_2 (9). The samples were transferred into constant bore tubing for measurement of collected volume. A 25–35-nl aliquot for the absorptive studies, and a 45-nl aliquot for the permeability studies, was then removed for determination of total CO_2 concentration and the remaining fluid was transferred to a vial for liquid scintillation counting. Total CO_2 concen-

TABLE I
Perfusion Solutions

	Solutions*		
	mM		
	1	2	3
NaCl	120	120	145
$NaHCO_3$		24	5
KCl		5	5
$MgSO_4$		1	1
$CaCl_2$	1.5	1.8	1.8
Na_2HPO_4		1	1
Glucose		5	
Alanine		5	
Urea		5	5
Raffinose	55		
Acetazolamide	0.5		

* All perfusion fluids contained 0.05% FD & C green dye No. 3 and [*methoxy*-^{3}H]inulin. Perfusate 1 was not gassed while perfusates 2 and 3 were gassed with 90% O_2/10% CO_2. The bicarbonate concentration of perfusate 2 was varied by replacing NaCl with $NaHCO_3$.

[2] In a previously published paper from this laboratory, using an identical infusion, plasma K concentration increased from 4.9±0.2 mM in control to 5.6±0.2 mM in metabolic acidosis (12).

tration was measured using microcalorimetry (picapnotherm) (13).

Calculations. The perfusion rate (V_O) was calculated as

$$V_O = (I_L/I_O)V_L, \quad (1)$$

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:

$$J_v = (V_O - V_L)/L, \quad (2)$$

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, P_{CO_2}, and bicarbonate concentrations encountered in the absorption experiments, total CO_2 can be considered a reasonable estimate of bicarbonate concentration. In the permeability experiments, however, because dissolved CO_2 concentration was a significant component of total CO_2, bicarbonate concentration was calculated by subtracting the dissolved CO_2 ($0.03 \times P_{CO_2}$) from the measured total CO_2 concentration in the collected fluid (9). For groups 1–3, arterial P_{CO_2} averaged ~36 mmHg in the permeability studies and the plasma-dissolved CO_2 was therefore assumed equal to 1.1 mM. In these three groups, the dissolved CO_2 content of the collected fluid was assumed equal to 1.8 mM. This was based on the arterial P_{CO_2} of 36 mmHg, and a ΔP_{CO_2} (renal cortical P_{CO_2} − arterial P_{CO_2}) of ~25 mmHg, which has been measured in hydropenia (14), and expansion alkalosis.[3] In groups 1–3 the exact magnitude of the correction for dissolved CO_2 was not quantitatively important.[4] However, in group 4 (acidosis), because the bicarbonate concentrations are small, this correction becomes more important. We therefore used the measured arterial P_{CO_2} from each experiment to calculate the plasma-dissolved CO_2. As the exact ΔP_{CO_2} has not been measured in this condition, we chose the lowest value obtained by DuBose et al. (14) of 8 mmHg. Our bicarbonate permeability in acidosis therefore represents a maximum estimate of the true value. The perfused bicarbonate concentration in the permeability studies was assumed equal to zero as there was no bicarbonate added and the solutions were not gassed. 23 measurements of this perfusion fluid revealed [total CO_2] = −0.1±0.1 mM. Net bicarbonate flux ($J^{net}_{HCO_3^-}$) was calculated as:

$$J^{net}_{HCO_3^-} = (C_O V_O - C_L V_L)/L, \quad (3)$$

where C_O and C_L represent the perfused and collected bicarbonate concentrations, respectively.

In the permeability studies the log mean driving force was used to calculate the permeability ($P_{HCO_3^-}$).

$$P_{HCO_3^-} = -J^{net}_{HCO_3^-}/(C_L/\ln(C_P/(C_P - C_L))), \quad (4)$$

where C_P equals the concentration of bicarbonate in the plasma.[5]

In the absorptive studies, net flux ($J^{net}_{HCO_3^-}$) was divided into two components: (*a*) passive bicarbonate diffusion ($J^{pass}_{HCO_3^-}$) and (*b*) proton secretion ($J^{proton}_{HCO_3^-}$),[6] using the equations:

$$J^{net}_{HCO_3^-} = J^{pass}_{HCO_3^-} + J^{proton}_{HCO_3^-}, \quad (5)$$

$$J^{pass}_{HCO_3^-} = P_{HCO_3^-} \times (\bar{C}_L - C_P), \quad (6)$$

where $\bar{C}_L$ equals the arithmetic mean luminal total CO_2 concentration.[7]

Data are presented as mean±SEM and groups are compared by the unpaired two-tailed *t* test, unless otherwise stated.

RESULTS

In previous studies net bicarbonate absorption was divided into two parallel components: (*a*) proton secretion, and (*b*) passive bicarbonate diffusion (9). In this study, in order to assess the effects of ECF volume and plasma bicarbonate concentration on the components of proximal acidification, the effects on net bicarbonate absorption and on bicarbonate permeability were measured.

Effect of volume expansion. To assess the role of ECF volume on proximal bicarbonate absorption, isohydric volume expansion was effected and bicarbonate absorption compared to our previously reported data in hydropenic rats (9). The acid-base parameters of rats in the two groups were similar (Table II). Bicarbonate Ringer's expansion predictably decreased hematocrit and plasma protein concentration (Table II). When tubules were perfused with 24 mM bicarbonate

[3] DuBose, T. D., Jr., and M. S. Lucci. Personal communication.

[4] Since this manuscript was first prepared, measured values for ΔP_{CO_2} have been published (15) that disagree somewhat with those of DuBose et al. (14). However, because the correction for P_{CO_2} is small, there is not a large effect on the results. Bicarbonate permeability would increase by 0.3×10^{-7} in hydropenia, 0.3×10^{-7} in isohydric expansion, and 0.2×10^{-7} in alkalotic expansion if the ΔP_{CO_2} of Gennari et al. is used.

[5] In the calculation of a driving force for bicarbonate diffusion, PD was not included. Using a similar perfusate, Frömter et al. (16) have measured a transepithelial PD of +1.4 mV. With this, electrical driving forces can be calculated for the different groups, and are small compared with the observed chemical driving forces. The log mean driving force was used because bicarbonate diffusion was expected to follow first order kinetics. Bicarbonate diffusion would follow first order kinetics if it occurred as simple diffusion through a pore (i.e., the tight junction). This assumption is not important as there is little difference in the results whether the arithmetic or logarithmic means are used.

[6] In referring to proton secretion we include various possible mechanisms which are not differentiated in this study: Na:H exchange, Cl:OH exchange, and electrogenic proton secretion.

[7] In calculating the driving force for passive bicarbonate diffusion, only the chemical driving force is considered, because the electrical driving force is small. Using a $PHCO_3/PCl$ of 0.44 and a PNa/PCl of 1.22 (17), one can calculate that the effect of chloride-bicarbonate gradients on PD is small. In addition, the effect of any PD on bicarbonate flux would be small because of the low mean bicarbonate concentrations.

TABLE II
Plasma Values

	pH	P_{CO_2}	$[HCO_3^-]$	Protein		Hematocrit	
				Preinfusion	Postinfusion	Preinfusion	Postinfusion
		mmHg	*mM*	*g/dl*		%	
Hydropenia	7.44±0.01	36.3±0.9	24.3±0.5	—	—	—	—
Isohydric expansion	7.46±0.01	35.0±0.6	23.7±0.3	5.1±0.1	3.7±0.1*	48.8±0.6	43.9±0.7*
Alkalotic expansion	7.65±0.01	35.3±0.6	37.1±0.6	5.2±0.1	4.1±0.1*	49.0±0.5	45.9±0.5‡
Acidosis	7.18±0.02	28.5±0.7	9.9±0.4	5.2±0.1	4.7±0.2‡	47.9±0.9	51.7±1.2‡

* $P < 0.001$, preinfusion vs. postinfusion, paired t test.
‡ $P < 0.01$, preinfusion vs. postinfusion, paired t test.

(perfusate 2 in Table I), net bicarbonate absorption in hydropenic and volume-expanded rats was not significantly different: 105±4 pmol/mm · min vs. 107±5 pmol/mm · min, respectively (Table III, Fig. 1).

Bicarbonate permeability was measured by perfusing tubules with a bicarbonate-free perfusate (perfusate 1 in Table I) and measuring bicarbonate appearance. Bicarbonate permeability was $2.6\pm0.3 \times 10^{-7}$ cm^2/s in hydropenia and was increased by 50% to $3.9\pm0.4 \times 10^{-7}$ cm^2/s in volume-expanded rats ($P < 0.05$) (Table IV). From these permeability coefficients and the measured blood to lumen bicarbonate concentration gradients, a rate of passive bicarbonate diffusion can be calculated for the above absorption studies (Eq. 6). The rate of proton secretion can then be calculated as the difference between net bicarbonate absorption and passive bicarbonate diffusion (Eq. 5). Proton secretion was not significantly inhibited by ECF volume expansion: 114±5 pmol/mm · min in hydropenia vs. 126±5 pmol/mm · min in isohydric ex-

TABLE III
Effects of Systemic pH and Volume Status on Bicarbonate Absorption

Condition	n	Length, *mm*	Perfusion rate, *nl/min*	Total CO_2 concentration, *mM*			Volume flux, *nl/mm · min*	Total CO_2 flux, *pmol/mm · min*		
				Perfused	Collected	Mean luminal		Net	Passive	Proton secretion
				24 mM perfusate						
Hydropenia*	8	2.08±0.26	14.88±0.24	24.5±0.3	12.7±2.1	18.6±1.1	1.92±0.11	105±4	−9±1	114±5
Isohydric expansion	9	1.77±0.25	14.29±0.20	23.6±0.7	14.1±1.4	19.2±1.1	2.24±0.16	107±5‡	−18±3	126±5‡
Alkalotic expansion	8	1.92±0.22	14.12±0.36	24.1±0.3	27.6±1.1	25.9±0.5	2.07±0.09	28±8§	−34±3	62±10§
Acidosis	8	1.76±0.14	14.50±0.28	23.6±0.2	7.4±1.4	15.5±0.8	1.87±0.09	149±11‖	7±1	142±11¶
				38 mM perfusate						
Isohydric expansion	6	2.28±0.21	14.23±0.60	36.6±0.2	15.5±3.7	26.1±2.0	2.08±0.12	159±5	4±6	155±6
Alkalotic expansion	9	1.51±0.16	14.15±0.31	39.4±0.5	39.1±1.2	39.3±0.7	1.36±0.30	60±5§	−5±2	64±5§

Net bicarbonate absorption was measured with either the 24 or 38 mM bicarbonate perfusate in the various animal groups. Using the bicarbonate permeabilities of Table IV, net absorption was divided into fluxes related to passive bicarbonate diffusion and proton secretion.
* Previously reported data (9).
‡ NS, vs. hydropenia.
§ $P < 0.001$ vs. isohydric expansion.
‖ $P < 0.002$ vs. hydropenia.
¶ $P < 0.05$ vs. hydropenia.

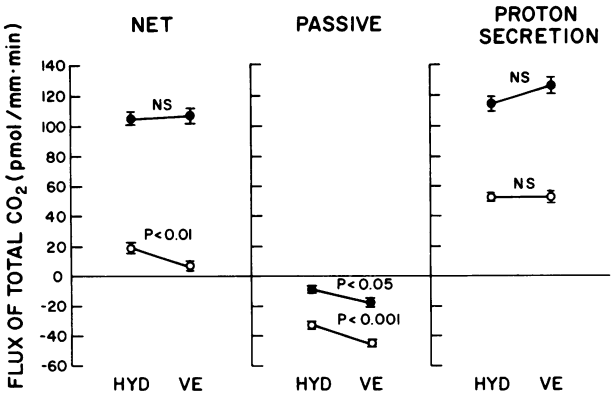

FIGURE 1 Tubules were perfused with 24 mM bicarbonate (closed circles) or 5 mM bicarbonate (open circles) in hydropenic (HYD) and isohydrically expanded (VE) rats. Net bicarbonate absorption, passive bicarbonate diffusion, and active proton secretion are plotted.

pansion (NS) (Table III, Fig. 1). Although bicarbonate permeability was increased by 50% in volume expansion, passive bicarbonate diffusion in both states was small compared to the rates of net absorption (Table III, Fig. 1). This is related to the small magnitudes both of the bicarbonate permeabilities and of the gradients in addition to the large magnitude of proton secretion in these studies. The differences in bicarbonate diffusion observed between the two ECF volume states, therefore, contribute little to net bicarbonate absorption in this setting.

The observed 50% increase in bicarbonate permeability would be expected to have its greatest effect in the end proximal tubule where the low luminal bicarbonate concentrations slow proton secretion and provide a large gradient for bicarbonate back-diffusion (9). To examine this question tubules were perfused with solution 3 (Table I) in hydropenic and volume-expanded animals. This perfusate is similar to that found normally in the late proximal tubule of hydropenic rats in that bicarbonate concentration is 5 mM and organics are absent. The results are shown in Fig. 1. Net bicarbonate absorption was inhibited in volume expanded rats as compared with hydropenic rats: 7±3 vs. 19±3 pmol/mm·min, respectively ($P < 0.01$). This inhibition of net bicarbonate absorption was totally due to the increased rate of bicarbonate back-diffusion in the volume expanded animals (−45±2 pmol/mm·min) as compared to hydropenic animals (−33±1 pmol/mm·min) ($P < 0.001$). The calculated rates of proton secretion were the same in hydropenia (52±3 pmol/mm·min) and isohydric expansion (52±4 pmol/mm·min) (NS). Collected bicarbonate concentrations were higher in isohydric expansion (6.1±0.4 mM) as compared to hydropenia (4.9±0.5 mM), but this was of border line significance ($0.05 < P < 0.1$). Thus, when luminal bicarbonate concentration is low, volume expansion leads to an inhibition of net bicarbonate absorption, which is due totally to the expansion-induced increase in bicarbonate permeability. Volume status does not affect the rate of proton secretion.

In the above studies, when tubules were perfused with solution 2, volume absorption was not affected by isohydric expansion: 1.92±0.11 nl/mm·min in hydropenia vs. 2.24±0.16 nl/mm·min in isohydric expansion (NS). However, when tubules were perfused with solution 3, volume absorption was inhibited by

TABLE IV
Effects of Systemic pH and Volume Status on Bicarbonate Permeability

Condition	n	Length, mm	Bicarbonate concentration, mM			Perfusion rate, nl/min	Bicarbonate secretory flux, pmol/mm·min	Bicarbonate permeability, $\times 10^{-7}$ cm²/s
			Collected	Mean luminal	Plasma			
Hydropenia*	12	1.96±0.17	3.9±0.4	2.0±0.2	21.5±1.0	14.62±0.37	31±4	2.6±0.3
Isohydric expansion	18	1.85±0.15	5.4±0.5	2.8±0.3	23.1±0.5	14.41±0.22	46±5	3.9±0.4‡
Alkalotic expansion	9	1.66±0.16	8.1±1.0	4.2±0.6	37.9±1.0	15.00±0.33	79±6	3.9±0.3§
Acidosis	8	1.91±0.23	1.7±0.3	0.9±0.2	8.7±0.4	14.04±0.44	14±3	3.0±0.5‖

Tubules were perfused in the above conditions with a solution containing zero bicarbonate, and bicarbonate permeability was calculated from the rate of bicarbonate appearance.

* Previously reported data (9).

‡ $P < .05$ vs. hydropenia.

§ NS vs. isohydric expansion.

‖ NS vs. hydropenia.

isohydric expansion: 2.59±0.11 nl/mm · min in hydropenia vs. 1.93±0.11 nl/mm · min in isohydric expansion ($P < 0.001$). Cogan et al. and Berry and Cogan found that reduction in peritubular protein concentration in vivo and in vitro specifically inhibited NaCl absorption but did not inhibit absorption of bicarbonate or organic solutes (8, 18). In the tubules perfused with solution 2, it could be calculated that ~50% of the volume absorption was related to sodium bicarbonate, glucose, and alanine absorption, thus making it difficult to detect an effect on volume absorption. In the tubules perfused with solution 3, >95% of the volume absorption was related to NaCl, and thus the effect of volume expansion was able to be detected. Previously, Morgan and Berliner perfused proximal tubules with normal saline and found a similar inhibition of volume absorption with ECF volume expansion (19).

Effect of metabolic alkalosis. To investigate the independent effect of peritubular alkalemia on proximal bicarbonate absorption, rats were studied during alkalosis induced by systemic bicarbonate infusion, and compared with isohydrically expanded rats. The intravenous infusions were at equal rates and the changes in plasma protein concentration indicate that similar degrees of volume expansion were induced in the two groups (Table II). The bicarbonate infusion led to a marked metabolic alkalosis (Table II). When tubules were perfused with 24 mM bicarbonate (perfusate 2), the rate of net bicarbonate absorption in alkalosis was 28±8 pmol/mm · min, which was markedly depressed compared to that in isohydric expansion (107±5 pmol/mm · min) ($P < 0.001$) (Table III). When tubules were perfused with 38 mM bicarbonate, the rate of net bicarbonate absorption in alkalotic expansion (60±5 pmol/mm · min) was again markedly suppressed compared to that in isohydric expansion (159±5 pmol/mm · min) ($P < 0.001$) (Table III). In addition, bicarbonate absorption was lower in tubules perfused with 38 mM bicarbonate in alkalotic rats compared with tubules perfused with 24 mM bicarbonate in isohydric rats: 60±5 vs. 107±5 pmol/mm · min, respectively ($P < 0.001$). Because we have previously shown that independent increases in luminal bicarbonate concentration stimulate bicarbonate absorption (9), these results suggest that peritubular alkalemia exerts an even stronger independent inhibitory effect on bicarbonate absorption. This inhibitory effect of increased plasma bicarbonate concentration on bicarbonate absorption overwhelms the stimulatory effect of increased luminal bicarbonate concentration.

The inhibition of bicarbonate absorption induced by peritubular alkalemia could be related to either an effect on proton secretion or an effect on passive bicarbonate diffusion. An effect on passive bicarbonate diffusion could in turn be related to an increase in bicarbonate permeability or to an alteration in the driving force for bicarbonate diffusion. To resolve these alternatives we used two approaches, both of which led to similar conclusions. First, we measured bicarbonate permeability (Table IV). This value was $3.9\pm0.3 \times 10^{-7}$ cm^2/s in alkalotic expansion, which was not different from the value in isohydric expansion ($3.9\pm0.4 \times 10^{-7}$ cm^2/s). Thus, peritubular pH does not independently affect bicarbonate permeability. In addition, bicarbonate permeability is small, so that passive bicarbonate diffusion accounts for only a small part of the difference in net bicarbonate absorption between alkalotic and isohydric expansion (20 and 9% with the 24 and 38 mM perfusates, respectively, Table III). Thus, the major effect in the depression of net bicarbonate absorption relates to a >50% inhibition in the rate of proton secretion during alkalosis (Table III). This inhibition of proton secretion occurred when tubules were perfused with either 24 mM bicarbonate (62±10 pmol/mm · min in alkalotic expansion vs. 126±5 pmol/mm · min in isohydric expansion, $P < 0.001$) or 38 mM bicarbonate (64±5 pmol/mm · min in alkalotic expansion versus 155±6 pmol/mm · min in isohydric expansion, $P < 0.001$).

Our second approach involved reversing the passive bicarbonate concentration gradients. If the inhibition of bicarbonate absorption induced by peritubular alkalemia was related to an increase in bicarbonate permeability, alkalosis would be expected to stimulate net bicarbonate absorption in the presence of a reversal of physiologic passive bicarbonate gradients (i.e., lumen bicarbonate concentration higher than plasma bicarbonate concentration). Tubules in alkalotic rats were perfused with 94.1±0.8mM bicarbonate and the results were compared with tubules in isohydrically expanded rats perfused with 79.7±0.9 mM bicarbonate (Table V). Bicarbonate concentration gradients favoring passive diffusive absorption were similar in the two studies (44.9±1.5 mM in isohydric expansion and 41.4±1.6 mM in alkalotic expansion, NS). In spite of this, the rate of net bicarbonate absorption was lower in alkalotic expansion (175±14 pmol/mm · min) compared to isohydric expansion (258±15 pmol/mm · min) ($P < 0.002$). These results again represent a 50% inhibition of active proton secretion in alkalotic expansion (Table V). Therefore, the inhibitory effect of alkalosis on proximal acidification is attributable predominantly to decreased proton secretion.

Effect of metabolic acidosis. Since peritubular alkalemia inhibited proximal tubular bicarbonate absorption, we studied rats made acutely acidotic by ammonium chloride infusion (Table II) to examine

TABLE V
Effect of Alkalosis on Bicarbonate Absorption in the Presence of High Luminal Bicarbonate Concentration

	Isohydric expansion		Alkalotic expansion
n	10		9
Perfusion rate, *nl/min*	14.74±0.32		14.90±0.32
Length, *mm*	1.45±0.13		1.64±0.15
Perfused [tCO_2], *mM*	79.7±0.9		94.1±0.8
Collected [tCO_2], *mM*	54.0±2.1		73.7±1.3
Mean luminal [tCO_2], *mM*	66.9±1.2		83.9±0.7
Plasma [tCO_2], *mM*	22.0±0.6		42.5±1.4
Gradient, *mM*	44.9±1.5	(NS)	41.4±1.6
Net tCO_2 flux, *pmol/mm · min*	258±15	($P < 0.002$)	175±14
Passive HCO_3^- flux, *pmol/mm · min*	103±3	(NS)	95±4
Proton secretion, *pmol/mm · min*	154±17	($P < 0.003$)	80±13

Tubules were perfused in isohydrically and alkalotically expanded animals with solutions of high bicarbonate concentration. The perfusate bicarbonate concentrations were chosen such that the concentration gradients would be reversed (lumen greater than plasma) and of equal magnitude.

whether bicarbonate absorption was stimulated when compared to normal hydropenic rats. Net bicarbonate absorption from a 24-mM bicarbonate perfusate (solution 2) was increased (149±11 pmol/mm · min) compared to normal hydropenic rats (105±4 pmol/mm · min) ($P < 0.002$) (Table III). Again, this increase could be related to a stimulation of proton secretion or to a more favorable gradient for bicarbonate diffusion. To resolve these possibilities, bicarbonate permeability was measured in metabolic acidosis. Due to the lack of knowledge of the exact renal P_{CO_2} in this condition, we were only able to estimate a maximum bicarbonate permeability (see Methods). This was $3.0\pm0.5 \times 10^{-7}$ cm^2/s, and was not statistically different from that measured in hydropenia (Table IV). Using this maximal permeability, there was an increase in passive bicarbonate efflux in metabolic acidosis, but a stimulation of proton secretion was also observed. Proton secretion increased by 25% from 114±5 pmol/mm · min in hydropenia to 142±11 pmol/mm · min in metabolic acidosis ($P < 0.05$) (Table III).

DISCUSSION

Previously, we have described bicarbonate absorption as consisting of two components in parallel: (*a*) proton secretion, which displayed saturation kinetics with respect to luminal bicarbonate concentration; and (*b*) a small bicarbonate leak (9). In the present study we have examined the independent effects of altered plasma bicarbonate concentration and ECF volume expansion on these components of acidification.

Effect of ECF volume expansion on proximal bicarbonate absorption. Clearance studies in dogs, rats, and man have all demonstrated that ECF volume expansion inhibits fractional bicarbonate reabsorption (3–6, 8). A prevalent view has been that this inhibition occurs in the proximal tubule and is related to increased bicarbonate back leak (20). Levine et al. (7) used free-flow micropuncture in the rat to show that ECF volume expansion inhibited fractional bicarbonate reabsorption in the PCT (7). These authors found a good correlation between the effects of volume expansion on bicarbonate and water reabsorption and therefore postulated that both effects were related to an altered peritubular capillary protein concentration. Cogan et al. (8) however, using free-flow micropuncture found that fractional bicarbonate reabsorption in the PCT of the rat did not correlate with peritubular protein concentration, but rather with single nephron glomerular filtration rate. These authors therefore postulated that the decreased fractional bicarbonate reabsorption seen in volume-expanded states was related to changes in filtered load rather than to changes in peritubular protein concentration. In agreement with this proposal, Berry and Cogan using the isolated perfused rabbit PCT, found that bath protein removal did not inhibit bicarbonate absorption despite a significant depression in NaCl and volume absorption (18).

To further investigate the specific effect of changes in ECF volume and peritubular protein concentration on proximal acidification in the absence of luminal flow changes, we studied the effects of volume expansion on bicarbonate permeability and net bicarbonate absorption at constant luminal perfusion rates. As pre-

viously described (9), bicarbonate permeability was measured by perfusing tubules with a bicarbonate-free solution containing acetazolamide and raffinose, and measuring bicarbonate appearance. Bicarbonate permeability was 50% higher in volume expansion than in hydropenia. This finding is consistent with the results of Boulpaep, Grandchamp and Boulpaep, and Seely, who found that paracellular resistance was decreased by ECF volume expansion in vivo (21–23). Berry and Cogan, however, found that bicarbonate permeability was not affected by bath protein removal in the in vitro perfused rabbit proximal tubule (18). Berry has also found that bath protein removal in vitro does not alter transepithelial specific resistance (24). It therefore appears that the paracellular effects of ECF volume expansion in vivo on bicarbonate permeability and electrical resistance are not observed with bath protein removal in vitro.

Although we found that bicarbonate permeability was 50% larger in volume expansion as compared to hydropenia, it was small in both conditions. As such its effects on net bicarbonate absorption were small. When tubules were perfused with 24 mM bicarbonate we were unable to detect an effect on net bicarbonate absorption. When tubules were perfused with 5 mM bicarbonate, there was an easily detectable inhibition of net bicarbonate absorption that was totally attributable to the effect of volume expansion on bicarbonate permeability and was small (12 pmol/mm · min). In both series, the calculated rates of proton secretion were not affected by volume status.

The physiologic significance of the volume expansion-induced increase in bicarbonate permeability remains unclear at this time. The effect on net bicarbonate absorption is small when compared with the usual delivery out of the PCT in the plasma replete rat (300 pmol/min) (8). It is therefore unclear whether the small increases in delivery that will result will lead to bicarbonaturia in the absence of an inhibitory effect of volume expansion distal to the PCT or in juxtamedullary nephrons. A change in bicarbonate permeability in the pars recta may also be present and if so could be a more important determinant of bicarbonate excretion.

Effect of plasma bicarbonate concentration and pH on proximal bicarbonate absorption. Results of split droplet (stationary microperfusion) studies have suggested that both peritubular alkalosis and acidosis inhibit proximal tubular acidification in the rat (25–27). The mechanism of this effect has not been elucidated. The purpose of the present studies was to examine whether this inhibition exists in the presence of luminal flow, and to further elucidate the mechanisms involved. In our studies, metabolic alkalosis caused a marked inhibition and metabolic acidosis a stimulation of bicarbonate absorption. The stimulation seen in metabolic acidosis contrasts with the results of the above split droplet studies, but the explanation for the discrepancy is not clear.

The effects of peritubular bicarbonate concentration could be related to effects on active proton secretion or on passive bicarbonate diffusion. An effect on passive bicarbonate diffusion could in turn be related to a change in the bicarbonate concentration gradient or a change in bicarbonate permeability. Bicarbonate permeabilities measured in rats with metabolic alkalosis were similar to those in rats with normal plasma bicarbonate and pH and of similar ECF volume. Therefore, increased peritubular pH did not independently affect bicarbonate permeability. In addition, because bicarbonate permeabilities were small, changes in passive fluxes were inadequate to account for the observed changes in net absorption. Thus, metabolic acidosis stimulated and metabolic alkalosis inhibited active proton secretion. Similar effects of serosal pH on proton secretion have been demonstrated in the rabbit PCT by Sasaki et al. (28) and in the turtle bladder by Cohen and Steinmetz (29).

Whether the observed effects were mediated by changes in plasma pH or bicarbonate concentration is not addressed in these studies. It is, however, probable that the effects were mediated by changes in cell pH. Studies in the turtle bladder (29) and in tubular suspensions (30, 31) have demonstrated that symmetrical pH changes in luminal and serosal fluid lead to cell pH changes in a similar direction. Previous studies in the turtle bladder demonstrated that isolated changes in luminal pH have only a small effect on cell pH (32), thus suggesting that cell pH varies with peritubular pH and may mediate the effect of peritubular pH on acidification.

The mechanism of the effect of peritubular bicarbonate concentration or pH on active proton secretion can be further elucidated from our results. In a previous study, bicarbonate absorption was measured as a function of mean luminal bicarbonate concentration in hydropenic rats (9). The closed circles in Fig. 2 represent the calculated rates of proton secretion in these previous studies. Proton secretion was a saturable function of luminal bicarbonate concentration with a $K_{1/2}$ of ~16 mM and a maximal rate (J_{max}^{proton}) of 200 pmol/mm · min. In the present study, when tubules in alkalotic rats were perfused with 24, 38, and 94 mM bicarbonate, the rates of proton secretion were 62, 64, and 80 pmol/mm · min, respectively. These results are plotted in Fig. 2 as a function of mean luminal bicarbonate concentration (open circles). The inhibitory effect on the rate of proton secretion of a 15-mM in-

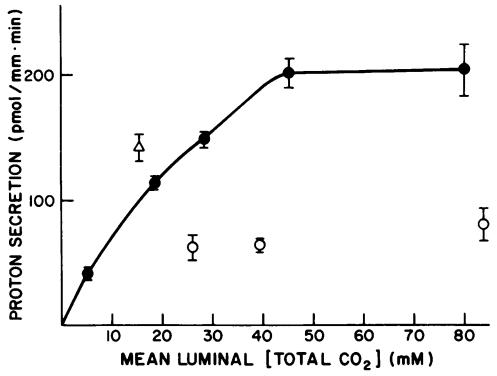

FIGURE 2 Proton secretion is plotted as a function of mean luminal bicarbonate concentration. The closed circles represent our previously reported data in hydropenic rats (9); the open circles represent the data obtained during alkalosis; and the open triangle represents the data obtained during acidosis.

crease in plasma bicarbonate concentration was not counterbalanced by a similar or greater increase in luminal bicarbonate concentration. Cohen and Steinmetz also found that proton secretion in the turtle bladder was more sensitive to serosal pH than to luminal pH (29).

The failure of increasing mean luminal bicarbonate concentration to stimulate proton secretion in alkalosis (Fig. 2) suggests that J_{max}^{proton} has been reached and is decreased from that in isohydric rats. Clearance studies by Monclair et al. (33) and Langberg et al. (34) defined a J_{max} for bicarbonate absorption by varying the filtered load of bicarbonate, and also found that peritubular alkalemia caused a decrease in J_{max}. In the appendix, we present a kinetic model for the Na:H exchanger which is used to explain the mechanistic significance of J_{max}^{proton}. Using the model, it is demonstrated that an alkalosis-induced increase in cell pH would be expected to decrease J_{max}^{proton}. However, the expected magnitude of the change in cell pH is not sufficient to explain the entire observed decrease in J_{max}^{proton}, and thus an alteration in the intrinsic properties of the Na:H exchanger is postulated. This intrinsic change in the Na:H exchanger in alkalosis, could be a decreased number of carriers, or a pH-dependent alteration in the structure and function of the carrier. Acidosis, which stimulated proton secretion (open triangle, Fig. 2), would have the opposite effect. Recent studies in the turtle bladder have suggested that increased CO_2 tension stimulates acidification by increasing the number of proton pumps in the luminal membrane (35).

The results of these and our previous studies can be used to understand how the proximal tubule responds to systemic alkalosis and acidosis. Numerous clearance studies have examined the effect of acutely increasing plasma bicarbonate concentration on bicarbonate absorption (1, 3–5). As plasma bicarbonate concentration is increased from subnormal values, fractional reabsorption remains 100% until the threshold is reached and bicarbonaturia occurs. Our previous studies have demonstrated that isolated increases in luminal bicarbonate concentration will lead to linear increases in bicarbonate absorption up to mean luminal concentrations of 45 mM (9). The present studies demonstrate that increases in plasma bicarbonate concentration >25 mM will independently inhibit proximal acidification and thus prevent bicarbonate absorption from increasing linearly with load. This should lower fractional bicarbonate absorption in expanded alkalemic states, and lead to the observed bicarbonaturia.

In metabolic acidosis the distal nephron must increase ammonium and titratable acid excretion in order to increase bicarbonate regeneration. It is therefore important to minimize bicarbonate delivery from the proximal tubule such that required distal bicarbonate reclamation is small. Cogen et al. (8) have measured end-proximal total CO_2 concentrations in metabolic acidosis of 1.6 mM (equivalent to bicarbonate concentrations <0.5 mM). The ability of the proximal tubule to achieve these low bicarbonate concentrations and pH is determined by a balance between proton secretion and bicarbonate back leak. Because our bicarbonate permeability in acidosis is a maximum number, we cannot rule out a decrease in bicarbonate permeability in this condition. However, the stimulation of proton secretion that we observed will also contribute to the ability of the proximal tubule to maintain low luminal bicarbonate concentrations and thus limit distal bicarbonate delivery.

On the basis of these and previous results (9), proximal acidification can be considered as consisting of two components in parallel: proton secretion and passive bicarbonate diffusion. Proton secretion is stimulated by increases in luminal bicarbonate concentration, inhibited by increases in plasma bicarbonate concentration, and not affected by ECF volume status. The rate of passive bicarbonate diffusion is determined in part by the bicarbonate permeability, which is increased by ECF volume expansion and is not affected by increasing plasma bicarbonate concentration. When luminal bicarbonate concentrations are similar to those of plasma, net bicarbonate absorption is dominated by proton secretion, and is therefore sensitive to changes in pH, but insensitive to changes in ECF volume. When luminal bicarbonate concentrations are low, proton secretion is slowed and the gradient for bicarbonate back-diffusion increases. Here, bicarbonate

permeability and ECF fluid volume will have a greater effect on net bicarbonate absorption.

APPENDIX

Proton secretion across the luminal membrane of the proximal tubule is effected by a Na:H exchanger (36–39), which has been thought to transport one sodium ion for one proton. The rate of proton secretion (J^{proton}) can, therefore, be described by the following equation:

$$J^{proton} = k_1[Na^+]_L[H^+]_C - k_{-1}[Na^+]_C[H^+]_L,$$

where $[Na^+]_L$ and $[Na^+]_C$ are the sodium concentrations in the lumen and cell, respectively, $[H^+]_L$ and $[H^+]_C$ are the hydrogen ion concentrations in the lumen and cell, respectively, and k_1 and k_{-1} are rate constants. If one assumes that $[Na^+]_L$ and $[Na^+]_C$ are constant in the present experiments, new rate constants, k'_1 and k'_{-1} can be defined such that:

$$J^{proton} = k'_1[H^+]_C - k'_{-1}[H^+]_L.$$

In previous studies, the rate of proton secretion was examined as a function of luminal bicarbonate concentration (9). As luminal bicarbonate concentration was increased, the rate of proton secretion reached a maximum (J^{proton}_{max}). According to the kinetic model presented above, J^{proton}_{max} would occur when $[H^+]_L$ is small such that $k'_{-1}[H^+]_L$ is approximately zero. J^{proton}_{max} would then be equal to $k'_1[H^+]_C$, and represent the unidirectional proton flux. This model would therefore predict that a decreased $[H^+]_C$ in alkalosis would lead to a decrease in J^{proton}_{max}, as was seen in these studies.

The model would also predict that the change in J^{proton}_{max} would be proportional to the change in $[H^+]_C$ if k'_1 remained constant. Using the measured plasma bicarbonate concentrations (Table II), it can be calculated that the peritubular hydrogen ion concentration in the alkalotic animals is ~35% lower than in the isohydric animals. In this study, $[H^+]_C$ was not measured, but the data of Struyvenberg et al. (31) suggest that a 35% reduction in peritubular hydrogen ion concentration would only lead to an 11% reduction in $[H^+]_C$. As this 11% reduction in $[H^+]_C$ cannot account for the 50% reduction in J^{proton}_{max} (Fig. 2), k'_1 must be decreased in alkalosis. These results would therefore suggest that increased intracellular pH affects the rate of proton secretion by two mechanisms: (*a*) changing the chemical driving force for protons ($\downarrow[H^+]_C$), and (*b*) altering the intrinsic properties of the Na:H exchanger ($\downarrow k'_1$).

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