

## Effect of luminal and peritubular HCO<sub>3</sub>(-) concentrations and PCO<sub>2</sub> on HCO<sub>3</sub>(-) reabsorption in rabbit proximal convoluted tubules perfused in vitro.

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*J Clin Invest.* 1982;70(3):639-649. <https://doi.org/10.1172/JCI110658>.

### Research Article

The effect of luminal and peritubular HCO<sub>3</sub>(-) concentrations and PCO<sub>2</sub> on HCO<sub>3</sub>(-) reabsorption was examined in rabbit proximal convoluted tubules perfused in vitro. Increasing luminal HCO<sub>3</sub>(-) concentration from 25 to 40 mM without changing either peritubular HCO<sub>3</sub>(-) concentration or PCO<sub>2</sub>, stimulated HCO<sub>3</sub>(-) reabsorption by 41%. When luminal HCO<sub>3</sub>(-) concentration was constant at 40 mM and peritubular HCO<sub>3</sub>(-) concentration was increased from 25 to 40 mM without changing peritubular PCO<sub>2</sub>, a 45% reduction in HCO<sub>3</sub>(-) reabsorption was observed. This inhibitory effect of increasing peritubular HCO<sub>3</sub>(-) concentration was reversed when peritubular pH was normalized by increasing PCO<sub>2</sub>. Passive permeability for HCO<sub>3</sub>(-) was also measured and found to be  $1.09 \pm 0.17 \times 10^{-7}$  cm<sup>2</sup> s<sup>-1</sup>. Using this value, the passive flux of HCO<sub>3</sub>(-) could be calculated. Only a small portion (less than 23%) of the observed changes in net HCO<sub>3</sub>(-) reabsorption can be explained by the passive HCO<sub>3</sub>(-) flux. We conclude that luminal and peritubular HCO<sub>3</sub>(-) concentrations after HCO<sub>3</sub>(-) reabsorption by changing the active H<sup>+</sup> secretion rate. Analysis of these data suggest that both luminal and peritubular pH are major determinants of HCO<sub>3</sub>(-) reabsorption.

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# Effect of Luminal and Peritubular $\text{HCO}_3^-$ Concentrations and $\text{PCO}_2$ on $\text{HCO}_3^-$ Reabsorption in Rabbit Proximal Convolute Tubules Perfused In Vitro

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**A B S T R A C T** The effect of luminal and peritubular  $\text{HCO}_3^-$  concentrations and  $\text{PCO}_2$  on  $\text{HCO}_3^-$  reabsorption was examined in rabbit proximal convoluted tubules perfused in vitro. Increasing luminal  $\text{HCO}_3^-$  concentration from 25 to 40 mM without changing either peritubular  $\text{HCO}_3^-$  concentration or  $\text{PCO}_2$ , stimulated  $\text{HCO}_3^-$  reabsorption by 41%. When luminal  $\text{HCO}_3^-$  concentration was constant at 40 mM and peritubular  $\text{HCO}_3^-$  concentration was increased from 25 to 40 mM without changing peritubular  $\text{PCO}_2$ , a 45% reduction in  $\text{HCO}_3^-$  reabsorption was observed. This inhibitory effect of increasing peritubular  $\text{HCO}_3^-$  concentration was reversed when peritubular pH was normalized by increasing  $\text{PCO}_2$ . Passive permeability for  $\text{HCO}_3^-$  was also measured and found to be  $1.09 \pm 0.17 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ . Using this value, the passive flux of  $\text{HCO}_3^-$  could be calculated. Only a small portion (<23%) of the observed changes in net  $\text{HCO}_3^-$  reabsorption can be explained by the passive  $\text{HCO}_3^-$  flux. We conclude that luminal and peritubular  $\text{HCO}_3^-$  concentrations alter  $\text{HCO}_3^-$  reabsorption by changing the active  $\text{H}^+$  secretion rate. Analysis of these data suggest that both luminal and peritubular pH are major determinants of  $\text{HCO}_3^-$  reabsorption.

## INTRODUCTION

Acid-base factors have been shown to influence renal  $\text{HCO}_3^-$  reabsorption. In clearance studies, Pitts and Lot-speich (1) showed that as blood  $\text{HCO}_3^-$  concentration was progressively increased by  $\text{NaHCO}_3$  infusion, renal

A part of this study was presented at the 8th International Congress of Nephrology, Athens, and was published in abstract form in 1981. 8th International Congress of Nephrology. 41a.

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Received for publication 5 October 1981 and in revised form 20 April 1982.

$\text{HCO}_3^-$  reabsorption exhibited a tendency towards partial saturation. The tendency towards partial saturation is expressed as a threshold in studies where  $\text{NaHCO}_3$  is infused. The threshold was usually observed even when extracellular fluid volume expansion was minimized (2-4) with one exception (5). Two explanations for this tendency towards partial saturation are possible. One is a true saturation of the  $\text{HCO}_3^-$  reabsorptive rate when luminal  $\text{HCO}_3^-$  concentration is increased; the other is an inhibitory effect of increased peritubular  $\text{HCO}_3^-$  concentration that counteracts the stimulatory effect of increasing luminal  $\text{HCO}_3^-$  concentration. The independent effects of luminal and peritubular  $\text{HCO}_3^-$  concentrations on  $\text{HCO}_3^-$  reabsorption have not been examined in clearance and micropuncture studies because luminal and peritubular  $\text{HCO}_3^-$  concentrations are interdependent.

Beside luminal and peritubular  $\text{HCO}_3^-$  concentration, another potential determinant of  $\text{HCO}_3^-$  reabsorption is  $\text{CO}_2$  tension ( $\text{PCO}_2$ ).<sup>1</sup> An independent effect of blood  $\text{PCO}_2$  on  $\text{HCO}_3^-$  reabsorption has been shown by some investigators (6-11), but not by others (12-14).

The main purpose of this study was to examine the independent role of luminal and peritubular  $\text{HCO}_3^-$  concentration,  $\text{PCO}_2$ , and pH in the active and passive component of  $\text{HCO}_3^-$  reabsorption in the proximal convoluted tubule (PCT). We used the in vitro isolated tubule perfusion technique because luminal and peritubular  $\text{HCO}_3^-$  concentrations and  $\text{PCO}_2$  can be changed independently. Our results show that an increase in luminal  $\text{HCO}_3^-$  concentration leads to an increase in  $\text{HCO}_3^-$  reabsorption. Conversely, an increase in peritubular  $\text{HCO}_3^-$  concentration inhibits  $\text{HCO}_3^-$  reabsorp-

<sup>1</sup> Abbreviations used in this paper: G1, G2, G3, group 1, 2, and 3;  $J_{\text{TCO}_2}^{\text{a}}$ , active  $\text{TCO}_2$  flux;  $J_{\text{TCO}_2}^{\text{N}}$ , net total  $\text{CO}_2$  flux;  $J_{\text{HCO}_3}^{\text{p}}$ , passive  $\text{HCO}_3^-$  flux;  $J_{\text{v}}$ , volume flux;  $\text{PCO}_2$ ,  $\text{CO}_2$  tension; PCT, proximal convoluted tubules; PD, potential difference;  $P_{\text{HCO}_3}$ , passive  $\text{HCO}_3^-$  permeability;  $\text{TCO}_2$ , total  $\text{CO}_2$ .

tion. Lastly, raising the  $\text{PCO}_2$  in the lumen and bath stimulates  $\text{HCO}_3^-$  reabsorption. Each of these maneuvers alters primarily the active component of  $\text{HCO}_3^-$  reabsorption. Analysis of the data suggests that the effects of  $\text{HCO}_3^-$  concentration and  $\text{PCO}_2$  are mediated by changes in luminal and peritubular pH.

## METHODS

Isolated segments of the rabbit PCT were dissected and perfused as previously described (15–17). Briefly, kidneys from female New Zealand White rabbits were cut into coronal slices. The PCT were dissected in cooled ( $4^\circ\text{C}$ ) rabbit serum from the midcortex or juxtamedullary cortex. The tubules identified as late PCT by attachment to proximal straight tubules were not used. The dissected tubules were transferred to 1.2-ml temperature-controlled bath. To maintain bath pH constant, bath fluid was continuously changed at a perfusion rate of  $>0.5$  ml/min. Bath pH was continuously monitored during experiments by placing a commercial glass pH electrode (MI-506, Microelectrodes, Inc., Londonderry, NH) close to the tubule. Transepithelial potential difference (PD) was measured using the perfusion pipette as a bridge into the tubular lumen. The perfusate and bath were connected to their respective calomel electrodes by 0.16 M NaCl agarose bridges. The measured PD were corrected for the liquid junction potentials between the NaCl bridge and the perfusate and the protein-containing bath according to the Henderson equation as modified by Barry and Diamond (18).

**Total  $\text{CO}_2$ ( $\text{TCO}_2$ ) flux experiments.** Tubules were perfused at  $38^\circ\text{--}39^\circ\text{C}$ , at a perfusion rate of 13–15 nl/min. The bath solution was rabbit serum (Irvine Scientific, Santa Ana, CA) and the perfusate was an ultrafiltrate of the serum made by low pressure dialysis through Aminco PM-30 membranes (American Instrument Co., Silver Spring, MD).  $\text{TCO}_2$  concentrations of the ultrafiltrate and serum were adjusted either to 25 or 40 mM by adding an isotonic  $\text{NaHCO}_3$  solution (155 mM  $\text{NaHCO}_3$ , 5 mM d-glucose, 5 mM L-alanine, 290 mosmol/kg  $\text{H}_2\text{O}$ ). The osmolality of the perfusate and bath solutions was also adjusted to 290 mosmol/kg  $\text{H}_2\text{O}$  by adding water or NaCl salt. To determine the volume flux ( $J_v$ ) and the net  $\text{TCO}_2$  flux ( $J_{\text{TCO}_2}$ ), the concentrations of [*methoxy-<sup>3</sup>H*]inulin (added to the perfusate as a volume marker) and  $\text{TCO}_2$  were measured in alternating samples of collected fluid (three collections for each in a given experimental period). The bath solution (serum) was preequilibrated with the desired  $\text{PCO}_2$  at  $38^\circ\text{C}$ , stored in a syringe and pumped into the bath at a constant rate. By continuous bath fluid exchange, the monitored bath pH was constant during experiments. The reported bath  $\text{PCO}_2$  was estimated from the Henderson-Hasselbalch equation.

Three protocols were performed. Group 1: The effect of increasing luminal  $\text{HCO}_3^-$  concentration was examined. Bath  $\text{TCO}_2$  concentration and  $\text{PCO}_2$  were maintained constant at 25 mM and 40 mmHg, respectively, and luminal  $\text{TCO}_2$  concentration was increased from 25 to 40 mM. Group 2: The effect of increasing bath  $\text{HCO}_3^-$  concentration was examined. Perfusate  $\text{TCO}_2$  concentration and bath  $\text{PCO}_2$  were kept constant at 40 mM and 40 mmHg, respectively, and bath  $\text{TCO}_2$  concentration was increased from 25 to 40 mM. Group 3: The effect of increasing  $\text{PCO}_2$  was examined. Both luminal and bath  $\text{TCO}_2$  concentrations were 40 mM, and  $\text{PCO}_2$  was changed from 40 to 70 mmHg.

**$\text{HCO}_3^-$  permeability experiments.** In these experiments, artificial solutions were used for the bath and perfusate. The

composition of the control perfusate was, in mM: NaCl, 100;  $\text{NaHCO}_3$ , 40;  $\text{NaHPO}_4$ , 1; KCl, 5;  $\text{MgCl}_2$ , 0.7; d-glucose, 8.3;  $\text{CaCl}_2$ , 1.5. Two bath solutions were used. The control bath solution was in mM: NaCl, 100;  $\text{NaHCO}_3$ , 40;  $\text{NaHPO}_4$ , 1; KCl, 5;  $\text{MgCl}_2$ , 1; d-glucose, 8.3;  $\text{CaCl}_2$ , 3; and dialyzed albumin was added at 6 g/dl. To generate a  $\text{HCO}_3^-$  gradient, 15 mM  $\text{NaHCO}_3$  in the bath solution was replaced by Na isethionate. The transepithelial flux of  $\text{HCO}_3^-$  and  $\text{H}^+/\text{OH}^-$  was inhibited by cooling  $20^\circ\text{C}$  (19, 19) and by adding 0.16 mM ethoxazolamide to the bath (16, 19).<sup>2</sup> Osmolalities of all artificial solutions were adjusted to 290 mosmol/kg  $\text{H}_2\text{O}$ . The perfusion rate was maintained at a slow rate (3–4 nl/min), to maximize the change in  $\text{TCO}_2$  concentration of the collected fluid. Tubules were first perfused at  $38^\circ\text{C}$  for 20 min, then the bath temperature was cooled to  $20^\circ\text{C}$  gradually. In the control period, there was no  $\text{HCO}_3^-$  concentration gradient between lumen and bath. In the experimental period there was a 15 mM  $\text{HCO}_3^-$  concentration gradient from lumen to bath. The  $J_v$  and the  $\text{TCO}_2$  concentration difference between the perfusate and collected fluid were measured in both periods.

**Calculations.**  $J_v$  was calculated as

$$J_v = \frac{V_L}{L} (C_{Lc}^{\text{IN}}/C_{Li}^{\text{IN}} - 1), \quad (1)$$

where  $V_L$  is the collection rate of tubular fluid,  $L$  is the length of the tubule as measured by eye piece micrometer,  $C_{Lc}^{\text{IN}}$  and  $C_{Li}^{\text{IN}}$  are [*methoxy-<sup>3</sup>H*]inulin concentrations of the collected fluid and the initial perfusate, respectively.

$J_{\text{TCO}_2}$  was calculated as

$$J_{\text{TCO}_2}^{\text{N}} = (C_{Li}^{\text{TCO}_2} - C_{Lc}^{\text{TCO}_2})V_L/L + (C_{Li}^{\text{TCO}_2})(J_v), \quad (2)$$

where  $C_{Li}^{\text{TCO}_2}$  and  $C_{Lc}^{\text{TCO}_2}$  are  $\text{TCO}_2$  concentrations of the initial perfusate and collected fluid, respectively.

Passive  $\text{HCO}_3^-$  permeability ( $P_{\text{HCO}_3^-}$ ) may be obtained as

$$P_{\text{HCO}_3^-} = \frac{V_L}{L} \ln \frac{C_{Li}^{\text{HCO}_3^-} - C_B^{\text{HCO}_3^-}}{C_{Lc}^{\text{HCO}_3^-} - C_B^{\text{HCO}_3^-}}, \quad (3)$$

where  $C_{Li}^{\text{HCO}_3^-}$ ,  $C_{Lc}^{\text{HCO}_3^-}$ , and  $C_B^{\text{HCO}_3^-}$  are  $\text{HCO}_3^-$  concentrations of the perfusate, collected fluid, and bath fluid, respectively.<sup>3</sup> Since all solutions were equilibrated with 5%  $\text{CO}_2$  gas, these fluids contained the same amount of dissolved  $\text{CO}_2$ . Therefore, Eq. 3 can be rewritten as

$$P_{\text{HCO}_3^-} = \frac{V_L}{L} \ln \frac{C_{Li}^{\text{TCO}_2} - C_B^{\text{TCO}_2}}{C_{Lc}^{\text{TCO}_2} - C_B^{\text{TCO}_2}}, \quad (4)$$

where  $C_B^{\text{TCO}_2}$  is the  $\text{TCO}_2$  concentration of the bath fluid.

<sup>2</sup> It is possible that there was some passive flux of  $\text{H}^+/\text{OH}^-$  through the paracellular shunt pathway when  $P_{\text{HCO}_3^-}$  was determined. This flux would cause an overestimation of  $P_{\text{HCO}_3^-}$ . The overestimation would be small because the  $\text{H}^+/\text{OH}^-$  flux through the shunt pathway is much smaller than the  $\text{HCO}_3^-$  flux due to its smaller chemical concentration gradient between lumen and bath compared with that of  $\text{HCO}_3^-$  ( $\sim 1/10^6$ ).

<sup>3</sup> In this equation transtubular PD was assumed to be zero. In these experiments, the active transport PD was inhibited by cooling and the biionic diffusion PD was also close to zero because isethionate permeability is close to the  $\text{HCO}_3^-$  permeability (21). Therefore, the transtubular PD was a Donnan PD of  $\sim +1.5$  mV. This PD would cause a 3% underestimation of  $P_{\text{HCO}_3^-}$ . No correction was done for this small underestimation.

If  $P_{HCO_3^-}$  is determined, the passive  $HCO_3^-$  flux ( $J_{HCO_3^-}^p$ ) can be estimated according to the equation:

$$J_{HCO_3^-}^p = P_{HCO_3^-} \left[ (C_{Lm}^{HCO_3^-} - C_B^{HCO_3^-}) - \frac{F}{RT} PD \frac{C_{Lm}^{HCO_3^-} + C_B^{HCO_3^-}}{2} \right], \quad (5)$$

where  $C_{Lm}^{HCO_3^-}$  is the mean luminal  $HCO_3^-$  concentration (arithmetic mean), and F, R, and T have their usual meaning.

Measurement of  $TCO_2$  concentrations of the perfusate, collected fluid, and bath fluid were performed by microcalorimetry (22). The tubular fluid was collected under  $CO_2$  equilibrated oil to minimize the  $CO_2$  loss.

A mean value for  $J_v$  and  $J_{TCO_2}^N$  was determined from the individual collections during each experimental period in a given tubule. The data are expressed as mean  $\pm$  SEM ( $n$ , number of tubules). The Student's *t* test for paired or unpaired data was used to determine statistical significance.

## RESULTS

**Effect of increasing luminal  $HCO_3^-$  concentration on  $J_{TCO_2}^N$ .** To determine the effect of increasing luminal  $HCO_3^-$  concentration on  $J_{TCO_2}^N$ , tubules were first perfused with 25 mM  $HCO_3^-$  perfusate (control). After a 30-min equilibration period, samples were collected, and the perfusate was changed to 40 mM  $HCO_3^-$  perfusate. After a 20-min equilibration period, samples were again collected (experimental). During these two periods (control vs. experimental), bath pH and perfusion rate were kept constant (bath pH:  $7.42 \pm 0.02$  vs.  $7.41 \pm 0.02$ ; perfusion rate:  $13.8 \pm 0.5$  vs.  $14.4 \pm 0.8$  nl/min). Bath  $TCO_2$  concentration was also constant at  $25.9 \pm 0.2$  mM during the periods. In two tubules the order of the perfusion was reversed, but the results were similar; therefore, all data are combined and shown in Fig. 1 and Table I, group 1 (G1). When the perfusate  $TCO_2$  concentration was  $24.4 \pm 0.4$  mM, the collected fluid  $TCO_2$  concentration was  $16.0 \pm 1.4$  mM, and  $J_{TCO_2}^N$  was  $95.9 \pm 12.8$  pmol  $mm^{-1} min^{-1}$ . When the perfusate  $TCO_2$  concentration was increased to  $38.0 \pm 0.6$  mM, the collected fluid  $TCO_2$  concentration was  $26.5 \pm 1.6$  mM, and  $J_{TCO_2}^N$  increased to  $135.1 \pm 14.6$  pmol  $mm^{-1} min^{-1}$  ( $P < 0.001$ ). These results show that when the perfusate  $TCO_2$  concentration is increased by 56%,  $J_{TCO_2}^N$  increases by 41%.

**Effect of increasing bath  $HCO_3^-$  concentration on  $J_{TCO_2}^N$ .** In this set of experiments there were three experimental periods. First, bath  $TCO_2$  concentration was  $26.1 \pm 0.3$  mM (precontrol), then it was increased to  $40.9 \pm 0.5$  mM (experimental). Finally, bath  $TCO_2$  concentration was reduced to the precontrol value (postcontrol). The  $TCO_2$  concentration of the perfusate and the bath  $PCO_2$  were kept constant at  $39.4 \pm 0.5$  mM and 40 mmHg, respectively. These results are summarized in Fig. 2 and Table I (G2). In the control period, the bath pH was  $7.40 \pm 0.04$ , and  $J_{TCO_2}^N$  was  $138.8 \pm 8.7$  pmol  $mm^{-1} min^{-1}$ . When the bath  $HCO_3^-$  concentration was increased, the bath pH was alkalin-

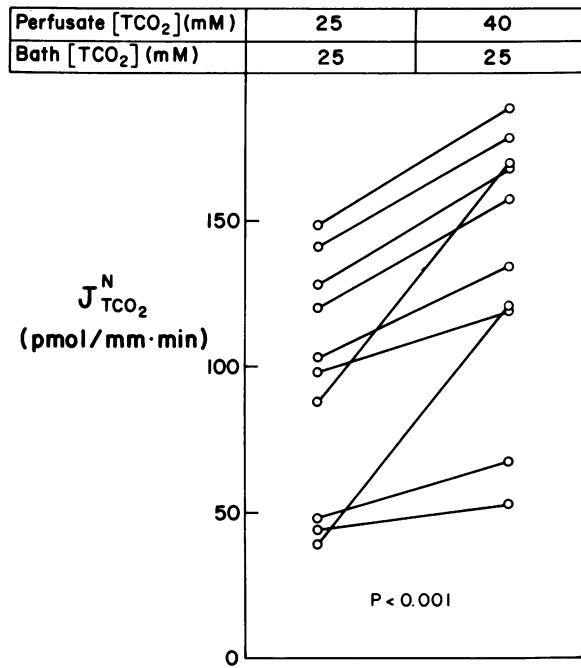


FIGURE 1 Effect of increasing luminal  $HCO_3^-$  concentration on  $HCO_3^-$  reabsorption. Perfusate  $TCO_2$  concentration was selectively increased from 25 to 40 mM, while the bath  $TCO_2$  concentration and pH were maintained constant.  $J_{TCO_2}^N$  increased from  $95.9 \pm 12.8$  to  $135.1 \pm 14.6$  pmol  $mm^{-1} min^{-1}$ .

ized to  $7.65 \pm 0.03$  and  $J_{TCO_2}^N$  decreased to  $76.3 \pm 9.3$  pmol  $mm^{-1} min^{-1}$  ( $P < 0.001$ ).  $J_{TCO_2}^N$  recovered close to the precontrol value ( $118.1 \pm 7.9$  pmol  $mm^{-1} min^{-1}$ ) in the postcontrol period.

**Effect of increasing bath  $PCO_2$ .** Our observation that  $J_{TCO_2}^N$  is suppressed by increasing bath  $HCO_3^-$  concentration may be due to either the high bath  $HCO_3^-$  concentration or to bath alkalinity. To distinguish between these possibilities, the bath pH was altered by changing bath  $PCO_2$  (Fig. 3 and Table I [G3]). The  $TCO_2$  concentrations of the perfusate and bath were constant at  $39.6 \pm 0.6$  and  $40.6 \pm 0.2$  mM, respectively. The bath  $PCO_2$  was increased from  $39.4 \pm 0.4$  to  $71.3 \pm 0.4$  mmHg. As a result, the bath pH was reduced from  $7.62 \pm 0.01$  to  $7.37 \pm 0.02$ . As shown in Fig. 3,  $J_{TCO_2}^N$  increased from  $62.7 \pm 9.0$  to  $98.2 \pm 11.8$  pmol  $mm^{-1} min^{-1}$  (56% increase,  $P < 0.005$ ) in response to the change in  $PCO_2$ .

An additional series of studies were performed to confirm the effect of bath  $PCO_2$  (Table II). The  $TCO_2$  concentrations of the perfusate and bath fluid were constant at  $25.2 \pm 0.3$  and  $25.3 \pm 0.1$  mM, respectively. The bath  $PCO_2$  was increased from  $39.1 \pm 1.0$  to  $73.2 \pm 0.7$  mmHg, and bath pH was reduced from  $7.41 \pm 0.01$  to  $7.14 \pm 0.01$ .  $J_{TCO_2}^N$  increased from  $92.6 \pm 6.6$  to  $104.1 \pm 6.3$  pmol  $mm^{-1} min^{-1}$  (12% increase,  $P < 0.005$ ) in re-

TABLE I  
Effects of Lumen and Bath  $\text{HCO}_3^-$  Concentrations and Bath  $\text{pCO}_2$  on  $\text{J}_{\text{TCO}_2}^N$

Condition	TCO <sub>2</sub> concentrations			$\text{J}_{\text{TCO}_2}^N$ $\text{pmol mm}^{-1} \text{min}^{-1}$	
	mM		$\text{mmHg}$		
	Bath	Perfusate			
G1 (n = 10)	Control	25.9±0.2	24.4±0.7	39.4±1.8	95.9±12.8
	Experimental	25.9±0.2	38.0±0.6	39.7±1.7	135.1±14.6
	MPD				39.2±7.6
	P				<0.001
G2 (n = 10)	Control	26.1±0.3	39.4±0.5	42.7±3.9	133.3±7.5
	Experimental	40.9±0.5	39.4±0.5	38.6±3.2	76.3±9.5
	MPD				-56.9±6.7
	P				<0.001
G3 (n = 7)	Control	40.6±0.2	39.6±0.3	39.4±0.4	62.7±9.0
	Experimental	40.6±0.2	39.6±0.3	71.8±0.4	98.2±11.8
	MPD				35.5±6.5
	P				<0.005

Values are mean±SEM. MPD, mean paired difference.

sponse to the change in  $\text{PCO}_2$ . This result showed that the effect of bath  $\text{PCO}_2$  in acidic range (7.15–7.4) is smaller than that in alkalotic range (7.4–7.6). This result is qualitatively in agreement with the results of

Jacobson (11). He perfused PCT with 25 mM perfusate and bath fluid and found a 40–60% increase in  $\text{HCO}_3^-$  reabsorption by increasing  $\text{PCO}_2$ . His stimulation of  $\text{HCO}_3^-$  reabsorption is larger than ours. The

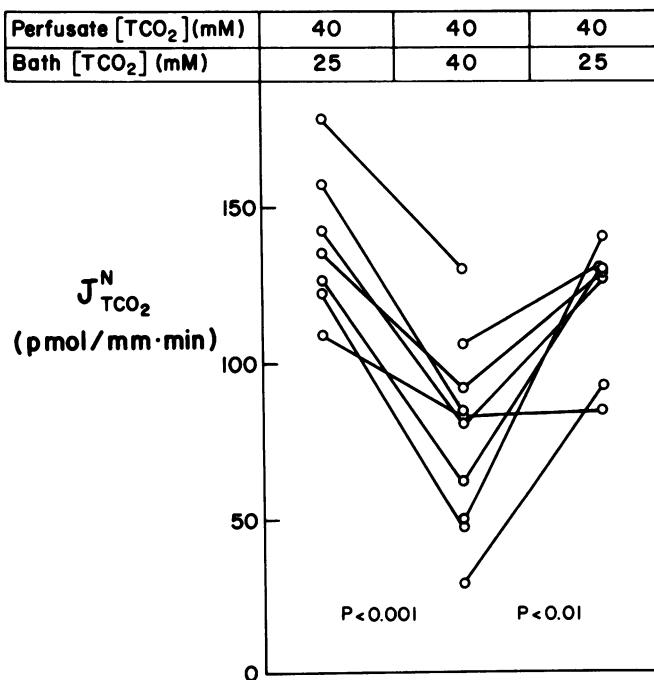


FIGURE 2 Effect of increasing bath  $\text{HCO}_3^-$  concentration on  $\text{HCO}_3^-$  reabsorption. Bath  $\text{TCO}_2$  concentration was selectively increased from 25 to 40 mM, while the bath  $\text{PCO}_2$  and perfusate  $\text{TCO}_2$  concentration were maintained constant at 40  $\text{mmHg}$  and 40 mM, respectively.  $\text{J}_{\text{TCO}_2}^N$  decreased from  $138.8 \pm 8.7$  to  $76.3 \pm 9.3 \text{ pmol mm}^{-1} \text{ min}^{-1}$  and recovered to  $118.1 \pm 7.9 \text{ pmol mm}^{-1} \text{ min}^{-1}$  when the bath  $\text{TCO}_2$  concentration was again returned to 25 mM.

Bath $[TCO_2]$ (mM)	40	40
Bath $pCO_2$ (mmHg)	40	70
Bath pH	7.6	7.4

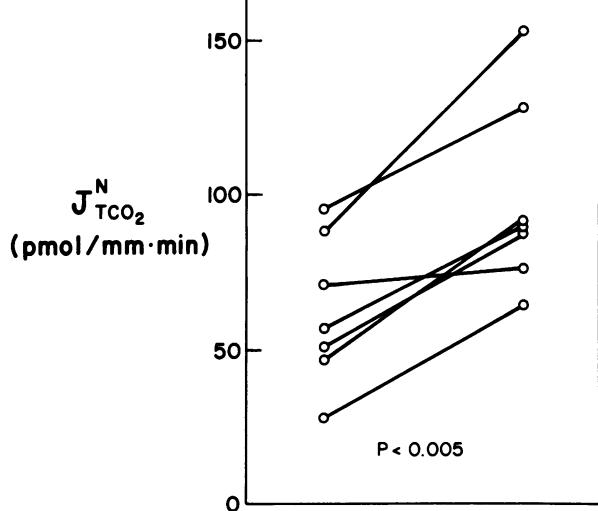


FIGURE 3 Effect of increasing bath  $PCO_2$  on  $HCO_3^-$  reabsorption. Perfusate and bath  $TCO_2$  concentration were maintained constant at 40 mM. Bath  $PCO_2$  was increased from 40 to 70 mmHg and in response to this change, bath pH was reduced from 7.6 to 7.4.  $J_{TCO_2}^N$  increased from  $62.7 \pm 9.0$  to  $98.2 \pm 11.8 \text{ pmol mm}^{-1} \text{ min}^{-1}$ .

reason for this discrepancy is not clear, but could be related to his higher  $PCO_2$ . In his study the bath pH was reduced from 7.4 to 7.08 suggesting  $PCO_2$  was increased from 40 to 87 mmHg.

$HCO_3^-$  permeability. Measurements of  $P_{HCO_3^-}$  are shown in Table III. In these studies the perfusate  $HCO_3^-$  concentration was 40 mM and the bath  $HCO_3^-$  concentration was 25 mM. The direction of the  $HCO_3^-$  concentration gradient is similar to that used to examine the effect of increasing luminal  $HCO_3^-$  con-

centration. To inhibit transcellular transport processes, the bath temperature was maintained at 20°C and 0.16 mM ethoxazolamide was added to the bath. To establish that transcellular transport was inhibited, we perfused the first four tubules shown in Table III with the 40 mM  $HCO_3^-$  perfusate and bath solution. Both  $J_v$  and the  $TCO_2$  concentration difference between the perfusate and collected fluid were not different from zero,  $-0.05 \pm 0.04 \text{ nl mm}^{-1} \text{ min}^{-1}$  and  $-0.49 \pm 0.50 \text{ mM}$ , respectively. These results confirm the absence of an active transport contribution to the measured  $P_{HCO_3^-}$ . When the bath  $HCO_3^-$  concentration was reduced to 25 mM, a significant reduction in  $TCO_2$  concentration of collected fluid was observed ( $\Delta TCO_2$ :  $3.97 \pm 0.62 \text{ mM}$ ,  $n = 7$ ). From these results,  $HCO_3^-$  permeability calculated using Eq. 4 was  $1.09 \pm 0.17 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ . The permeability per surface area was  $1.68 \pm 0.25 \times 10^{-5} \text{ cm s}^{-1}$ . The conversion was calculated on the basis of the measured mean tubular diameter of  $20.1 \pm 0.4 \mu\text{m}$ ,  $n = 7$ .

This result confirms previous studies (20, 21, 24–27) that have shown that the PCT is permeable to  $HCO_3^-$ . Our  $P_{HCO_3^-}$  value is in good agreement with the values reported by Holmberg et al. (20), Alpern et al. (24), and Warnock and Yee (21). On the other hand, it is about one-tenth of the value reported by Lang et al. (25) and is about one-fifth of the value of Frömter (26). The cause of these differences among reported  $P_{HCO_3^-}$  values is not clear. One possibility may be methodological. In the studies where similar  $P_{HCO_3^-}$  values were reported (20, 24, and this study), microcalorimetry was used to measure the  $HCO_3^-$  concentration of the collected fluid. On the other hand, Lang et al. (25) used a microadaptation of the Astrup method to measure the  $HCO_3^-$  concentration of the collected fluid, and Frömter (26) calculated  $P_{HCO_3^-}$  by measuring the NaCl and  $NaHCO_3$  dilution PD and the isotopic permeabilities for  $Na^+$  and  $Cl^-$ .

*Calculation of the passive  $HCO_3^-$  flux.* We have demonstrated that increasing the luminal  $HCO_3^-$  concentration increases  $J_{TCO_2}^N$  (Fig. 1) and that increasing the peritubular  $HCO_3^-$  concentration decreases  $J_{TCO_2}^N$  (Fig. 2). These changes in  $J_{TCO_2}^N$  may be caused by a passive  $HCO_3^-$  flux ( $J_{HCO_3^-}^P$ ), since PCT are permeable to  $HCO_3^-$  (20, 21, 24–27 and Table III).  $J_{HCO_3^-}^P$  can be estimated using the measured  $P_{HCO_3^-}$ , PD, and  $HCO_3^-$  concentrations according to Eq. 5. Once  $J_{HCO_3^-}^P$  is obtained,  $HCO_3^-$  reabsorption can be viewed as a pump-leak system, and  $J_{TCO_2}^N$  can be corrected for  $J_{HCO_3^-}^P$  to yield the active  $TCO_2$  flux ( $J_{TCO_2}^A$ ):

$$J_{TCO_2}^A = J_{TCO_2}^N - J_{HCO_3^-}^P \quad (6)$$

According to this definition,  $J_{TCO_2}^A$  represents the active transcellular flux of  $HCO_3^-$  and is generally believed to be due to active  $H^+$  secretion (28, 29). Therefore,

TABLE II  
Effect of Bath  $pCO_2$  on  $J_{TCO_2}^N$

Bath $pCO_2$	Bath pH	$J_{HCO_3^-}^P$
mmHg		$\text{pmol mm}^{-1} \text{ min}^{-1}$
39.1 $\pm$ 1.0	7.41 $\pm$ 0.01	92.6 $\pm$ 6.6
73.2 $\pm$ 0.7	7.14 $\pm$ 0.01	104.1 $\pm$ 6.3
MPD ( $n = 5$ )		11.5 $\pm$ 1.8 ( $P < 0.005$ )

Perfusate and bath  $TCO_2$  concentrations were  $25.2 \pm 0.3$  and  $25.3 \pm 0.1 \text{ mM}$ , respectively. MPD, mean paired difference.

TABLE III  
Passive Permeability of  $\text{HCO}_3^-$  in PCT

40 mM $\text{HCO}_3^-$ perfusate:40 mM $\text{HCO}_3^-$ bath					40 mM $\text{HCO}_3^-$ perfusate:25 mM $\text{HCO}_3^-$ bath						
Tubular length	Perfusion rate	$J_v$	Collected* $\text{TCO}_2$		$\Delta \text{TCO}_2 \dagger$	Perfusion rate	$J_v$	Collected* $\text{TCO}_2$		$\Delta \text{TCO}_2$	$P_{\text{HCO}_3}$
			mm	nl $\text{min}^{-1}$				mm	nl $\text{min}^{-1}$		
1.80	2.91	-0.12	41.03	-0.20	2.83	0.03	35.52	5.31	1.05		
1.90	3.57	-0.12	42.44	-1.61	3.52	-0.11	34.43	6.40	1.69		
1.90	2.89	0.04	39.21	0.75	3.32	0.09	35.35	4.61	1.14		
1.60	3.87	-0.01	39.4	-0.91	2.87	0.09	34.06	4.24	1.21		
1.25					5.17	-0.01	35.42	2.99	1.46		
1.50						4.38	0.12	37.43	2.28	0.71	
2.10						3.84	0.16	39.01	1.95	0.34	
Mean	1.72	3.34	-0.05	40.47	-0.49	3.70	0.05	35.89	3.97	1.09	
$\pm \text{SEM}$	0.11	0.26	0.04	0.78	0.50	0.32	0.03	0.66	0.62	0.17	

\*  $\text{TCO}_2$  concentration of collected fluid.

†  $\text{TCO}_2$  concentration difference between perfusate and collected fluid.

Experiments were performed at 20°C and in the presence of 0.16 mM bath ethoxazolamide.

$J_{\text{TCO}_2}^A$  can be regarded as an estimate of active  $\text{H}^+$  secretion rate.

The results of the calculations of  $J_{\text{HCO}_3^-}^P$  and  $J_{\text{TCO}_2}^A$  are summarized in Table IV. In group 1, when the perfusate and bath contained 25 mM  $\text{HCO}_3^-$ ,  $J_{\text{HCO}_3^-}^P$  was  $-2.7 \text{ pmol mm}^{-1} \text{ min}^{-1}$  due to  $\text{HCO}_3^-$  entry into the lumen because the mean luminal  $\text{HCO}_3^-$  concentration

was less than the bath concentration. When the perfusate  $\text{HCO}_3^-$  concentration was increased to 40 mM, the mean luminal  $\text{HCO}_3^-$  concentration was higher than the bath concentration and  $J_{\text{HCO}_3^-}^P$  was  $6.3 \text{ pmol mm}^{-1} \text{ min}^{-1}$ . As a result, the net change in  $J_{\text{HCO}_3^-}^P$  was  $9.0 \text{ pmol mm}^{-1} \text{ min}^{-1}$ . This change accounts for only 23% of the observed change in  $J_{\text{TCO}_2}^N$  (39.2 pmol  $\text{mm}^{-1} \text{ min}^{-1}$ , Table I). This analysis shows that in group 1 most (77%) of the change in  $J_{\text{TCO}_2}^N$  is due to an increase in  $J_{\text{TCO}_2}^A$ . The same analysis was performed in groups 2 and 3, and the contributions of  $J_{\text{HCO}_3^-}^P$  were smaller in these groups than in group 1 (11% in group 2, and 4% in group 3). Therefore, the observed changes in  $J_{\text{TCO}_2}^N$  are due mainly to changes in  $J_{\text{TCO}_2}^A$ , the active  $\text{H}^+$  secretion rate.

TABLE IV  
Effects of Lumen and Bath  $\text{HCO}_3^-$  Concentrations and Bath  $\text{pCO}_2$  on  $J_{\text{HCO}_3^-}^P$  and  $J_{\text{TCO}_2}^A$

Condition*	$J_{\text{HCO}_3^-}^P$	$J_{\text{TCO}_2}^A$	$\text{pmol mm}^{-1} \text{ min}^{-1}$	
			Control	Experimental
G1 (n = 10)	Control	$-2.7 \pm 0.6$	$98.7 \pm 13.2$	
	Experimental	$6.3 \pm 0.8$	$128.8 \pm 15.1$	
	MPD	$9.0 \pm 0.8$	$30.1 \pm 7.7$	
	P	$<0.001$	$<0.005$	
G2 (n = 10)	Control	$6.7 \pm 0.3$	$126.6 \pm 7.7$	
	Experimental	$0.2 \pm 0.7$	$76.2 \pm 9.7$	
	MPD	$-6.5 \pm 0.5$	$-50.4 \pm 6.8$	
	P	$<0.001$	$<0.001$	
G3 (n = 7)	Control	$1.9 \pm 0.8$	$60.8 \pm 9.7$	
	Experimental	$0.4 \pm 0.8$	$97.7 \pm 12.3$	
	MPD	$-1.4 \pm 0.3$	$36.9 \pm 6.7$	
	P	$<0.005$	$<0.005$	

\* For experimental condition, see Table I. In G1, luminal  $\text{TCO}_2$  concentration was increased; in G2, bath  $\text{TCO}_2$  concentration was increased; in G3, bath  $\text{pCO}_2$  was increased.  $J_{\text{TCO}_2}^A$  was calculated as  $J_{\text{TCO}_2}^A = J_{\text{TCO}_2}^N - J_{\text{HCO}_3^-}^P$ .  $J_{\text{TCO}_2}^N$  values are given in Table I.

MPD, mean paired difference.

## DISCUSSION

The main purpose of this study was to examine the direct effect of changing the  $\text{HCO}_3^-$  concentrations of the perfusate and bath on  $\text{HCO}_3^-$  reabsorption in the PCT. For this purpose, the in vitro isolated tubule perfusion technique is suitable, because the bath and the perfusate  $\text{HCO}_3^-$  concentrations can be changed independently. Factors encountered in in vivo studies that affect  $\text{HCO}_3^-$  reabsorption, such as the expansion of extracellular fluid, alteration in plasma  $\text{K}^+$  concentration, changes in peritubular pressures and flows, and humoral factors, are excluded in this technique. In addition, bath pH can be monitored directly.

### Effect of acid-base factors on $\text{HCO}_3^-$ reabsorption.

We observed independent effects of luminal and peritubular  $\text{HCO}_3^-$  concentrations and  $\text{pCO}_2$  on  $\text{HCO}_3^-$

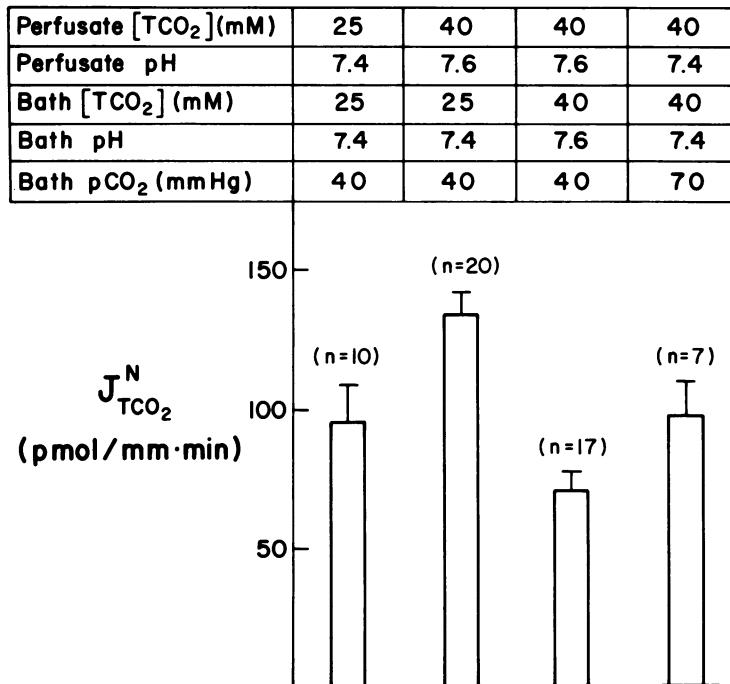


FIGURE 4 Summary of the effect of luminal and peritubular acid-base factors on  $HCO_3^-$  reabsorption. Column 1: control; both luminal and peritubular pH were 7.4. Column 2: increasing luminal  $TCO_2$  concentration and pH stimulated  $J_{TCO_2}^N$ . Column 3: increasing bath  $TCO_2$  concentration and pH markedly reduced  $J_{TCO_2}^N$ . Column 4: correction of bath pH by increasing bath  $PCO_2$ -stimulated  $J_{TCO_2}^N$ . A comparison between columns 1 and 3 shows the combined effect of increasing luminal and bath  $TCO_2$  concentration simultaneously. A comparison between columns 1 and 4 suggests the importance of luminal and bath pH as the determinants of  $HCO_3^-$  reabsorption.  $J_{TCO_2}^N$  were  $95.9 \pm 12.8$ ,  $134.2 \pm 8.0$ ,  $70.7 \pm 6.7$ , and  $98.2 \pm 11.8$  pmol  $mm^{-1} min^{-1}$  in columns 1, 2, 3, and 4, respectively.

reabsorption. Our results are summarized in Fig. 4. At the top of this figure perfusate  $TCO_2$  concentration, perfusate pH, bath  $TCO_2$  concentration, bath pH, and bath  $PCO_2$  are shown. The perfusate pH was calculated assuming that the luminal  $PCO_2$  was equilibrated with the bath  $PCO_2$  (30).<sup>4</sup> The perfusate  $TCO_2$  concentration and pH can be regarded as indices of luminal  $TCO_2$  concentration and pH.<sup>5</sup> The first column is the control. Both luminal and peritubular pH were 7.4. In the second column, the luminal pH was increased by increas-

ing luminal  $HCO_3^-$  concentration, and an increase in  $J_{TCO_2}^N$  was observed. In the third column, the bath pH was increased by increasing peritubular  $HCO_3^-$  concentration, and a marked inhibition of  $J_{TCO_2}^N$  was obtained. In the fourth column, the bath pH was reduced by increasing  $PCO_2$ , and an increase in  $J_{TCO_2}^N$  was observed.

Our results show a stimulatory effect of increasing luminal  $HCO_3^-$  concentration on  $HCO_3^-$  reabsorption (compare columns 1 and 2 in Fig. 4). When the luminal  $HCO_3^-$  concentration was increased from 25 to 40 mM without changing the peritubular  $HCO_3^-$  concentration, net  $HCO_3^-$  reabsorption and  $H^+$  secretion were stimulated (Figs. 1 and 4; Tables I and IV). Other investigators using the *in vivo* microperfusion technique in the rat PCT have found similar results. Malnic and Mello-Aires (31) have shown that  $HCO_3^-$  reabsorption does not saturate when the luminal  $HCO_3^-$  concentration is increased up to 60 mM without changing the peritubular  $HCO_3^-$  concentration. Alpern et al. (32) have also observed that  $HCO_3^-$  reabsorption increases linearly up to a mean luminal  $HCO_3^-$  concen-

<sup>4</sup> Taking  $CO_2$  permeability ( $10^{-4} \text{ cm}^2 \text{ s}^{-1}$ ) determined by Schwartz (30) and perfusion rate of  $13 \text{ nl min}^{-1}$ ,  $PCO_2$  gradient that exists at the tip of perfusion pipette dissipates to only 1% of the original value when tubular fluid flows 0.1 mm tubular length.

<sup>5</sup> It could be more appropriate to use a mean luminal  $TCO_2$  concentration and pH. Mean luminal  $TCO_2$  concentrations were 3–7 mM lower than perfusate  $TCO_2$  concentrations and mean luminal pH were 0.1–0.8 less than perfusate pH in those four groups. Such corrections do not alter the analysis significantly, thus, we chose to use perfusate  $TCO_2$  concentration and pH as indices of luminal  $TCO_2$  concentrations and pH.

tration of 45 mM. Our results in the rabbit confirm these results in the rat and demonstrate that increasing luminal  $\text{HCO}_3^-$  concentration stimulates  $\text{HCO}_3^-$  reabsorption. This conclusion contrasts with the observation of a threshold at a filtered bicarbonate concentration of 20–30 mM in clearance studies (1–4). The difference between clearance studies and these *in vivo* and *in vitro* perfusion studies may be explained by alterations in peritubular environment (see below).

Our results also show an independent effect of peritubular  $\text{HCO}_3^-$  concentration on  $\text{HCO}_3^-$  reabsorption (compare columns 2 and 3 in Fig. 4). A selective increase in the bath  $\text{HCO}_3^-$  concentration from 25 to 40 mM at constant luminal  $\text{HCO}_3^-$  concentration and bath  $\text{PCO}_2$  caused a marked inhibition of  $\text{HCO}_3^-$  reabsorption and  $\text{H}^+$  secretion (Figs. 2 and 4; Tables I and IV). Two studies in the *in vivo* perfused rat PCT have suggested an effect of peritubular  $\text{HCO}_3^-$  concentration on  $\text{HCO}_3^-$  reabsorption. First, Giebisch et al. (33) found that  $\text{H}^+$  secretion was reduced when  $\text{NaHCO}_3$  was infused acutely to obtain a plasma  $\text{HCO}_3^-$  concentration of 44 mM. Second, Chan and Giebisch (34) observed that increasing peritubular  $\text{HCO}_3^-$  concentration from 2 to 40 mM inhibited  $\text{HCO}_3^-$  reabsorption. However, the interpretation of these data as indicating an independent effect of peritubular  $\text{HCO}_3^-$  concentration is complicated by the presence of extracellular volume expansion in the former and by the presence of an extremely low peritubular  $\text{PCO}_2$ , and thus high pH, in the latter. More recently, Alpern et al. (35) have shown that in the *in vivo* perfused rat PCT, systemic metabolic alkalosis markedly inhibits  $\text{HCO}_3^-$  reabsorption even when the effect of volume expansion is carefully excluded. Our results in the rabbit PCT excluded any possible effects of extracellular volume expansion and maintained peritubular pH in the physiological range (7.4–7.6). Thus, in both the rabbit and the rat PCT,  $\text{HCO}_3^-$  reabsorption is inhibited by selectively increasing peritubular  $\text{HCO}_3^-$  concentration. This observation provides one explanation for the existence of the tendency towards partial saturation in clearance (1–4) and free-flow micropuncture studies (29). The difference between clearance and micropuncture studies and these *in vivo* and *in vitro* perfusion studies is due to the fact that in the former peritubular and filtered luminal  $\text{HCO}_3^-$  concentrations are always equal; increasing one results in an equal increase in the other. Thus, in clearance and micropuncture studies the inhibitory effect of increasing peritubular  $\text{HCO}_3^-$  concentration is masked by the stimulatory effect of increasing luminal  $\text{HCO}_3^-$  concentration.

This inhibitory effect of increasing bath  $\text{HCO}_3^-$  concentration can be reversed by raising  $\text{PCO}_2$  and returning the bath pH to 7.4 (compare columns 3 and 4 in Fig. 4). There has been considerable controversy

regarding the effect of increasing  $\text{PCO}_2$  on bicarbonate absorption. In early clearance (6–8) and micropuncture (9, 10) studies, a stimulatory effect of respiratory acidosis (high  $\text{PCO}_2$ ) on  $\text{HCO}_3^-$  reabsorption was demonstrated. Later, Kurtzman (12) pointed out that respiratory acidosis reduces effective plasma volume, itself a strong stimulus to  $\text{HCO}_3^-$  reabsorption. Reexamination of the effect of respiratory acidosis on  $\text{HCO}_3^-$  reabsorption in clearance studies (12–14) showed little effect on  $\text{HCO}_3^-$  reabsorption if hemodynamic changes were taken into account. In agreement, Cogan (36) showed that using free-flow micropuncture in the rat, increasing systemic  $\text{PCO}_2$  from 45 to 65 mmHg caused a very small increase (12%) in proximal  $\text{HCO}_3^-$  reabsorption. In contrast to the above clearance and free-flow micropuncture studies, a direct effect of  $\text{PCO}_2$  on  $\text{HCO}_3^-$  reabsorption has been demonstrated in the *in vivo* (37) and the *in vitro* (11) PCT where both luminal and peritubular environments are regulated. One explanation for this discrepancy between clearance and free-flow micropuncture studies on the one hand, and microperfusion studies on the other, may be a difference in the *in vivo* peritubular  $\text{PCO}_2$ , since the  $\text{PCO}_2$  of the renal cortex is higher than systemic  $\text{PCO}_2$  (38). Therefore, the renal cortical  $\text{PCO}_2$  examined in clearance and micropuncture studies might be higher and the peritubular pH more acidic compared with the *in vivo* and *in vitro* perfusion studies. Fig. 5 shows that our data support this view. In this figure  $J_{\text{TCO}_2}^N$  is plotted against  $\text{H}^+$  ion concentration. Only data where lumen and bath pH are identical are shown. The pH was changed by increasing the bath  $\text{PCO}_2$  from 40 to 70 mmHg. Increasing  $\text{H}^+$  ion concentration from 40 to 72 neq liter<sup>-1</sup> (pH 7.4–7.15) by increasing  $\text{PCO}_2$  with 25 mM  $\text{HCO}_3^-$  in the lumen and bath stimulates  $J_{\text{TCO}_2}^N$  by 12%; whereas, increasing it from 22 to 40 neq liter<sup>-1</sup> (pH 7.6–7.4) by increasing  $\text{PCO}_2$  with 40 mM  $\text{HCO}_3^-$  in the lumen and bath stimulates  $J_{\text{TCO}_2}^N$  by 40%. Thus, the stimulatory effect of  $\text{PCO}_2$  on  $\text{HCO}_3^-$  reabsorption is larger in the alkalotic range than in the acidic range. In any case, our results confirm the results of the *in vivo* and *in vitro* perfusion studies (11, 37) and show that  $\text{PCO}_2$  is one of the determinants of  $\text{HCO}_3^-$  reabsorption in the PCT.

Further examination of Fig. 4 permits an evaluation of the combined effects of luminal and peritubular acid-base factors on proximal  $\text{HCO}_3^-$  reabsorption. The comparison between the first and third columns is analogous to a comparison between normal acid-base status and acute metabolic alkalosis. When both luminal and peritubular  $\text{TCO}_2$  concentrations were 25 mM (column 1),  $J_{\text{TCO}_2}^N$  was 96 pmol mm<sup>-1</sup> min<sup>-1</sup>. When both  $\text{TCO}_2$  concentrations were increased to 40 mM (column 3),  $J_{\text{TCO}_2}^N$  was 71 pmol mm<sup>-1</sup> min<sup>-1</sup>. This comparison shows that when both luminal and peritubular  $\text{HCO}_3^-$  con-

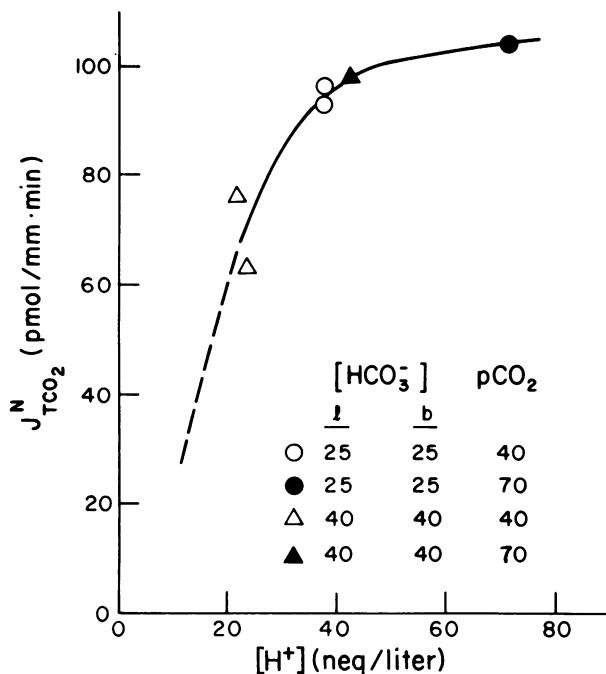


FIGURE 5 Relationship between  $J_N^{HCO_3}$  and  $H^+$  ion concentration of lumen (l) and bath (b). Only data where lumen and bath  $H^+$  ion concentrations were identical are chosen.  $H^+$  ion concentration was changed by altering the bath  $PCO_2$  from 40 to 70 mmHg.

centrations are increased simultaneously at constant  $PCO_2$ , the stimulatory effect of luminal  $HCO_3^-$  concentration is completely abolished by the inhibitory effect of peritubular  $HCO_3^-$  concentration. This comparison again points out that one of the mechanisms for the tendency towards partial saturation of  $HCO_3^-$  reabsorption observed in clearance (1-4) and micropuncture (29) studies appears to be the inhibitory effect of peritubular alkalinity offsetting the stimulatory effect of luminal alkalinity.

It is also interesting to compare the first and fourth columns in Fig. 4. In these two experimental conditions, the perfusate and bath  $HCO_3^-$  concentrations and the  $PCO_2$  were different, but the perfusate and the bath pH were the same. Observed  $J_N^{HCO_3}$  was essentially the same (96 vs. 98 pmol  $mm^{-1} min^{-1}$ ). This observation suggests that the luminal and peritubular pH, not  $HCO_3^-$  concentration or  $PCO_2$ , are the major determinants of  $HCO_3^-$  reabsorption. In this regard, Mello-Aires and Malnic (37) have suggested that peritubular pH per se affects  $H^+$  secretion in the rat PCT. They perfused the peritubular capillary with phosphate buffers of varying pH, and observed a higher  $H^+$  secretion rate at pH 7.4 than at pH 8.5.

**Mechanism and model of  $HCO_3^-$  reabsorption in the PCT.** The current view of the acidification mecha-

nism in the PCT (39) is that  $H^+$  is secreted into the lumen via a  $Na^+-H^+$  antiporter located in the luminal membrane. When  $H^+$  is secreted,  $OH^-$  is formed inside the cell that reacts with  $CO_2$  to form  $HCO_3^-$ . The exact mechanism by which  $HCO_3^-$  exits from the cell is controversial, but one hypothesis is that  $HCO_3^-$  diffuses out of the cell through the basolateral membrane down its electrochemical gradient. The rate of  $H^+$  secretion is equal to the rate of  $HCO_3^-$  diffusion out of the cell. According to this model,  $HCO_3^-$  reabsorption may be regulated by at least two limiting steps:  $Na^+-H^+$  exchange at the luminal membrane and the  $HCO_3^-$  exit step at basolateral membrane. The acid-base factors examined in this study could regulate  $HCO_3^-$  reabsorption at either or both of these two limiting steps.

An increase in luminal  $HCO_3^-$  concentration raises the luminal pH and reduces the  $H^+$  concentration gradient against which the  $Na^+-H^+$  antiporter operates. Consequently,  $H^+$  secretion should increase. Associated with this, there would be an increase in  $HCO_3^-$  production within the cell and an increase in the driving force for  $HCO_3^-$  exit across the basolateral membrane. Our result showing that increasing luminal  $HCO_3^-$  concentration stimulates  $HCO_3^-$  reabsorption is in good agreement with this model prediction and suggests that the  $H^+$  concentration gradient between lumen and cell may be an important regulating mechanism of the  $Na^+-H^+$  antiporter.

In contrast, an increase in peritubular  $HCO_3^-$  concentration might reduce the electrochemical driving force for  $HCO_3^-$  exit across the basolateral membrane. As a consequence, intracellular  $HCO_3^-$  concentration and pH would be increased and the driving force for  $H^+$  secretion across the luminal membrane would be reduced. Thus, the overall rate of acidification would be slowed. Alternatively, it may be possible that peritubular pH affects the  $HCO_3^-$  exit step by changing the basolateral membrane properties ( $HCO_3^-$  permeability or basolateral membrane PD). In this regard, Biagi et al. (40) have observed recently that peritubular pH affects the basolateral membrane PD by changing the basolateral membrane permeability for  $K^+$ . Their results show that peritubular alkalinity causes hyperpolarization of the basolateral membrane PD. Therefore, when peritubular  $HCO_3^-$  concentration is increased, the electrochemical driving force for  $HCO_3^-$  diffusion across the basolateral membrane might be constant. In other words, the reduction in the chemical  $HCO_3^-$  concentration gradient might be counterbalanced by an increase in the electrical driving force.<sup>6</sup>

<sup>6</sup> Their result in rabbit PCT showed that the basolateral membrane PD is -51 mV at a peritubular pH of 7.4. It hyperpolarized to -60 mV when the peritubular pH was alkalinized to 7.6. (In their study this effect was examined

If the electrochemical gradient for  $\text{HCO}_3^-$  diffusion across the basolateral membrane is unchanged, then a reduction in the  $\text{HCO}_3^-$  permeability of the peritubular membrane caused by peritubular alkalinity may be the explanation for the observed decrease in  $\text{HCO}_3^-$  diffusion out of the cell.

The inhibition of  $\text{HCO}_3^-$  reabsorption induced by increasing bath  $\text{HCO}_3^-$  concentration cannot be attributed specifically to either peritubular  $\text{HCO}_3^-$  concentration or peritubular pH. However, our data suggest that peritubular pH is more important than peritubular  $\text{HCO}_3^-$  concentration.  $\text{HCO}_3^-$  reabsorption was stimulated when peritubular alkalinity was corrected by increasing  $\text{PCO}_2$  (Fig. 3). Further analysis of this effect, shown by a comparison of the first and fourth columns in Fig. 4, shows that peritubular pH rather than  $\text{HCO}_3^-$  concentration is the principal determinant of  $\text{HCO}_3^-$  reabsorption.

The precise mechanism by which a selective increase in  $\text{PCO}_2$  at constant luminal and peritubular  $\text{HCO}_3^-$  concentrations stimulates  $\text{HCO}_3^-$  reabsorption is not clear. An increase in  $\text{PCO}_2$  should affect luminal, intracellular, and peritubular pH (30, 41), and therefore precise measurements of the PD across the basolateral membrane and the intracellular pH (or  $\text{HCO}_3^-$  concentration) are necessary before it is possible to locate the effect of  $\text{PCO}_2$ . The  $\text{PCO}_2$  effect could be mediated by changes in the appropriate driving forces for the  $\text{Na}^+/\text{H}^+$  antiporter and/or by changes in the basolateral membrane exit step. Alternatively,  $\text{PCO}_2$  could have a direct effect on the  $\text{Na}^+/\text{H}^+$  antiporter and/or the basolateral membrane permeability for  $\text{HCO}_3^-$ .

In summary, our data suggest that (a) increasing luminal  $\text{HCO}_3^-$  concentration stimulates  $\text{HCO}_3^-$  reabsorption by increasing the  $\text{H}^+$  secretion rate secondary to a more favorable  $\text{H}^+$  concentration gradient; (b) increasing peritubular  $\text{HCO}_3^-$  concentration reduces  $\text{HCO}_3^-$  reabsorption by decreasing the  $\text{HCO}_3^-$  exit across the basolateral membrane; (c) increasing  $\text{PCO}_2$  stimulates  $\text{HCO}_3^-$  reabsorption; and (d) peritubular pH may be regarded phenomenologically as a determinant of  $\text{HCO}_3^-$  reabsorption.

only in the proximal straight tubule, therefore the same response in the PCT is assumed.) Intracellular  $\text{HCO}_3^-$  concentration may be calculated from the intracellular pH measurement by Struyvenberg et al. (41). Based on their data, the calculated intracellular  $\text{HCO}_3^-$  concentration is 22 and 23 mM when peritubular  $\text{HCO}_3^-$  concentration is 25 mM and 40 mM, respectively. The electrochemical driving force for  $\text{HCO}_3^-$  diffusion across the basolateral membrane is  $-51 \text{ mV} + 60 \log 25/22 = -48 \text{ mV}$  at a peritubular  $\text{HCO}_3^-$  concentration of 25 mM, and is  $-60 \text{ mV} + 60 \log 40/23 = -46 \text{ mV}$  at a peritubular  $\text{HCO}_3^-$  concentration of 40 mM.

## ACKNOWLEDGMENTS

The authors would like to thank Dr. David G. Warnock for assistance with the microcalorimetry.

This investigation was supported by U. S. Public Health Service research grant AM27045 and RO 1-AM 26142 from the National Institute of Arthritis, Metabolism, and Digestive Disease. Dr. Sasaki was the recipient of a fellowship grant from the National Kidney Foundation.

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