Effect of Luminal and Peritubular HCO_3^- Concentrations and P_{CO_2} on HCO_3^- Reabsorption in Rabbit Proximal Convoluted Tubules Perfused In Vitro

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ABSTRACT The effect of luminal and peritubular HCO_3^- concentrations and P_{CO_2} on HCO_3^- reabsorption was examined in rabbit proximal convoluted tubules perfused in vitro. Increasing luminal HCO_3^- concentration from 25 to 40 mM without changing either peritubular HCO_3^- concentration or P_{CO_2}, stimulated HCO_3^- reabsorption by 41%. When luminal HCO_3^- concentration was constant at 40 mM and peritubular HCO_3^- concentration was increased from 25 to 40 mM without changing peritubular P_{CO_2}, a 45% reduction in HCO_3^- reabsorption was observed. This inhibitory effect of increasing peritubular HCO_3^- concentration was reversed when peritubular pH was normalized by increasing P_{CO_2}. Passive permeability for HCO_3^- was also measured and found to be $1.09 \pm 0.17 \times 10^{-7}$ cm^2 s^{-1}. Using this value, the passive flux of HCO_3^- could be calculated. Only a small portion (<23%) of the observed changes in net HCO_3^- reabsorption can be explained by the passive HCO_3^- flux. We conclude that luminal and peritubular HCO_3^- concentrations alter HCO_3^- reabsorption by changing the active H^+ secretion rate. Analysis of these data suggest that both luminal and peritubular pH are major determinants of HCO_3^- reabsorption.

INTRODUCTION

Acid-base factors have been shown to influence renal HCO_3^- reabsorption. In clearance studies, Pitts and Lotspeich (1) showed that as blood HCO_3^- concentration was progressively increased by $NaHCO_3$ infusion, renal HCO_3^- reabsorption exhibited a tendency towards partial saturation. The tendency towards partial saturation is expressed as a threshold in studies where $NaHCO_3$ 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 HCO_3^- reabsorptive rate when luminal HCO_3^- concentration is increased; the other is an inhibitory effect of increased peritubular HCO_3^- concentration that counteracts the stimulatory effect of increasing luminal HCO_3^- concentration. The independent effects of luminal and peritubular HCO_3^- concentrations on HCO_3^- reabsorption have not been examined in clearance and micropuncture studies because luminal and peritubular HCO_3^- concentrations are interdependent.

Beside luminal and peritubular HCO_3^- concentration, another potential determinant of HCO_3^- reabsorption is CO_2 tension (P_{CO_2}).[1] An independent effect of blood P_{CO_2} on 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 HCO_3^- concentration, P_{CO_2}, and pH in the active and passive component of HCO_3^- reabsorption in the proximal convoluted tubule (PCT). We used the in vitro isolated tubule perfusion technique because luminal and peritubular HCO_3^- concentrations and P_{CO_2} can be changed independently. Our results show that an increase in luminal HCO_3^- concentration leads to an increase in HCO_3^- reabsorption. Conversely, an increase in peritubular HCO_3^- concentration inhibits HCO_3^- reabsorp-

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[1] *Abbreviations used in this paper*: G1, G2, G3, group 1, 2, and 3; $J^A_{TCO_2}$, active TCO_2 flux; $J^N_{TCO_2}$, net total CO_2 flux; $J^P_{HCO_3^-}$, passive HCO_3^- flux; Jv, volume flux; P_{CO_2}, CO_2 tension; PCT, proximal convoluted tubules; PD, potential difference; $P_{HCO_3^-}$, passive HCO_3^- permeability; TCO_2, total CO_2.

tion. Lastly, raising the P_{CO_2} in the lumen and bath stimulates HCO_3^- reabsorption. Each of these maneuvers alters primarily the active component of HCO_3^- reabsorption. Analysis of the data suggests that the effects of HCO_3^- concentration and P_{CO_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°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 CO_2(TCO_2) flux experiments. Tubules were perfused at 38°–39°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). TCO_2 concentrations of the ultrafiltrate and serum were adjusted either to 25 or 40 mM by adding an isotonic $NaHCO_3$ solution (155 mM $NaHCO_3$, 5 mM D-glucose, 5 mM L-alanine, 290 mosmol/kg H_2O). The osmolality of the perfusate and bath solutions was also adjusted to 290 mosmol/kg H_2O by adding water or NaCl salt. To determine the volume flux (J_v) and the net TCO_2 flux ($J^N_{TCO_2}$), the concentrations of [*methoxy*-^{3}H]inulin (added to the perfusate as a volume marker) and 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 P_{CO_2} at 38°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 P_{CO_2} was estimated from the Henderson-Hasselbalch equation.

Three protocols were performed. Group 1: The effect of increasing luminal HCO_3^- concentration was examined. Bath TCO_2 concentration and P_{CO_2} were maintained constant at 25 mM and 40 mmHg, respectively, and luminal TCO_2 concentration was increased from 25 to 40 mM. Group 2: The effect of increasing bath HCO_3^- concentration was examined. Perfusate TCO_2 concentration and bath P_{CO_2} were kept constant at 40 mM and 40 mmHg, respectively, and bath TCO_2 concentration was increased from 25 to 40 mM. Group 3: The effect of increasing P_{CO_2} was examined. Both luminal and bath TCO_2 concentrations were 40 mM, and P_{CO_2} was changed from 40 to 70 mmHg.

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; $NaHCO_3$, 40; $NaHPO_4$, 1; KCl, 5; $MgCl_2$, 0.7; D-glucose, 8.3; $CaCl_2$, 1.5. Two bath solutions were used. The control bath solution was in mM: NaCl, 100; $NaHCO_3$, 40; Na_2HPO_4, 1; KCl, 5; $MgCl_2$, 1; D-glucose, 8.3, $CaCl_2$, 3; and dialyzed albumin was added at 6 g/dl. To generate a HCO_3^- gradient, 15 mM $NaHCO_3$ in the bath solution was replaced by Na isethionate. The transcellular flux of HCO_3^- and H^+/OH^- was inhibited by cooling 20°C (19, 20) and by adding 0.16 mM ethoxazolamide to the bath (16, 19).[2] Osmolalities of all artificial solutions were adjusted to 290 mosmol/kg H_2O. The perfusion rate was maintained at a slow rate (3–4 nl/min), to maximize the change in TCO_2 concentration of the collected fluid. Tubules were first perfused at 38°C for 20 min, then the bath temperature was cooled to 20°C gradually. In the control period, there was no HCO_3^- concentration gradient between lumen and bath. In the experimental period there was a 15 mM HCO_3^- concentration gradient from lumen to bath. The J_v and the TCO_2 concentration difference between the perfusate and collected fluid were measured in both periods.

Calculations. J_v was calculated as

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

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

$J^N_{TCO_2}$ was calculated as

$$J^N_{TCO_2} = (C^{TCO_2}_{Li} - C^{TCO_2}_{Lc})V_L/L + (C^{TCO_2}_{Li})(J_v), \quad (2)$$

where $C^{TCO_2}_{Li}$ and $C^{TCO_2}_{Lc}$ are TCO_2 concentrations of the initial perfusate and collected fluid, respectively.

Passive HCO_3^- permeability ($P_{HCO_3^-}$) may be obtained as

$$P_{HCO_3^-} = \frac{V_L}{L} \ln \frac{C^{HCO_3^-}_{Li} - C^{HCO_3^-}_{B}}{C^{HCO_3^-}_{Lc} - C^{HCO_3^-}_{B}}, \quad (3)$$

where $C^{HCO_3^-}_{Li}$, $C^{HCO_3^-}_{Lc}$, and $C^{HCO_3^-}_{B}$ are HCO_3^- concentrations of the perfusate, collected fluid, and bath fluid, respectively.[3] Since all solutions were equilibrated with 5% CO_2 gas, these fluids contained the same amount of dissolved CO_2. Therefore, Eq. 3 can be rewritten as

$$P_{HCO_3^-} = \frac{V_L}{L} \ln \frac{C^{TCO_2}_{Li} - C^{TCO_2}_{B}}{C^{TCO_2}_{Lc} - C^{TCO_2}_{B}}, \quad (4)$$

where $C^{TCO_2}_{B}$ is the TCO_2 concentration of the bath fluid.

[2] It is possible that there was some passive flux of H^+/OH^- through the paracellular shunt pathway when $P_{HCO_3^-}$ was determined. This flux would cause an overestimation of $P_{HCO_3^-}$. The overestimation would be small because the H^+/OH^- flux through the shunt pathway is much smaller than the HCO_3^- flux due to its smaller chemical concentration gradient between lumen and bath compared with that of HCO_3^- ($\sim 1/10^6$).

[3] 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 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 $P_{HCO_3^-}$. No correction was done for this small underestimation.

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

$$J^P_{HCO_3^-} = P_{HCO_3^-}\left[(C^{HCO_3^-}_{Lm} - C^{HCO_3^-}_{B}) - \frac{F}{RT} PD \frac{C^{HCO_3^-}_{Lm} + C^{HCO_3^-}_{B}}{2}\right], \quad (5)$$

where $C^{HCO_3^-}_{Lm}$ 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 $J^N_{TCO_2}$ was determined from the individual collections during each experimental period in a given tubule. The data are expressed as mean±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 $J^N_{TCO_2}$. To determine the effect of increasing luminal HCO_3^- concentration on $J^N_{TCO_2}$, 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±0.02 vs. 7.41±0.02; perfusion rate: 13.8±0.5 vs. 14.4±0.8 nl/min). Bath TCO_2 concentration was also constant at 25.9±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±0.4 mM, the collected fluid TCO_2 concentration was 16.0±1.4 mM, and $J^N_{TCO_2}$ was 95.9±12.8 pmol mm^{-1} min^{-1}. When the perfusate TCO_2 concentration was increased to 38.0±0.6 mM, the collected fluid TCO_2 concentration was 26.5±1.6 mM, and $J^N_{TCO_2}$ increased to 135.1±14.6 pmol mm^{-1} min^{-1} ($P < 0.001$). These results show that when the perfusate TCO_2 concentration is increased by 56%, $J^N_{TCO_2}$ increases by 41%.

Effect of increasing bath HCO_3^- concentration on $J^N_{TCO_2}$. In this set of experiments there were three experimental periods. First, bath TCO_2 concentration was 26.1±0.3 mM (precontrol), then it was increased to 40.9±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 P_{CO_2} were kept constant at 39.4±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±0.04, and $J^N_{TCO_2}$ was 138.8±8.7 pmol mm^{-1} min^{-1}. When the bath HCO_3^- concentration was increased, the bath pH was alkalinized to 7.65±0.03 and $J^N_{TCO_2}$ decreased to 76.3±9.3 pmol mm^{-1} min^{-1} ($P < 0.001$). $J^N_{TCO_2}$ recovered close to the precontrol value (118.1±7.9 pmol mm^{-1} min^{-1}) in the postcontrol period.

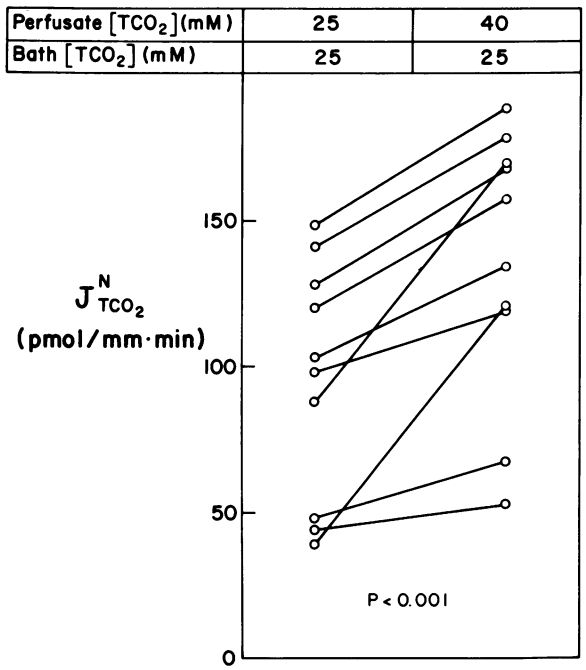

FIGURE 1 Effect of increasing luminal HCO_3^- concentration on HCO_3^- reabsorption. Perfusate TCO_2 concentration was selectively increased from 25 to 40 mM, while the bath TCO_2 concentration and pH were maintained constant. $J^N_{TCO_2}$ increased from 95.9±12.8 to 135.1±14.6 pmol mm^{-1} min^{-1}.

Effect of increasing bath P_{CO_2}. Our observation that $J^N_{TCO_2}$ 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 P_{CO_2} (Fig. 3 and Table I [G3]). The TCO_2 concentrations of the perfusate and bath were constant at 39.6±0.6 and 40.6±0.2 mM, respectively. The bath P_{CO_2} was increased from 39.4±0.4 to 71.3±0.4 mmHg. As a result, the bath pH was reduced from 7.62±0.01 to 7.37±0.02. As shown in Fig. 3, $J^N_{TCO_2}$ increased from 62.7±9.0 to 98.2±11.8 pmol mm^{-1} min^{-1} (56% increase, $P < 0.005$) in response to the change in P_{CO_2}.

An additional series of studies were performed to confirm the effect of bath P_{CO_2} (Table II). The TCO_2 concentrations of the perfusate and bath fluid were constant at 25.2±0.3 and 25.3±0.1 mM, respectively. The bath P_{CO_2} was increased from 39.1±1.0 to 73.2±0.7 mmHg, and bath pH was reduced from 7.41±0.01 to 7.14±0.01. $J^N_{TCO_2}$ increased from 92.6±6.6 to 104.1±6.3 pmol mm^{-1} min^{-1} (12% increase, $P < 0.005$) in re-

TABLE I

Effects of Lumen and Bath HCO_3^- Concentrations and Bath pCO_2 on $J^N_{TCO_2}$

	Condition	TCO_2 concentrations		Bath pCO_2	$J^N_{TCO_2}$
		Bath	Perfusate		
		mM		*mmHg*	*pmol mm^{-1} min^{-1}*
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	71.3±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 P_{CO_2}. This result showed that the effect of bath P_{CO_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 HCO_3^- reabsorption by increasing P_{CO_2}. His stimulation of HCO_3^- reabsorption is larger than ours. The

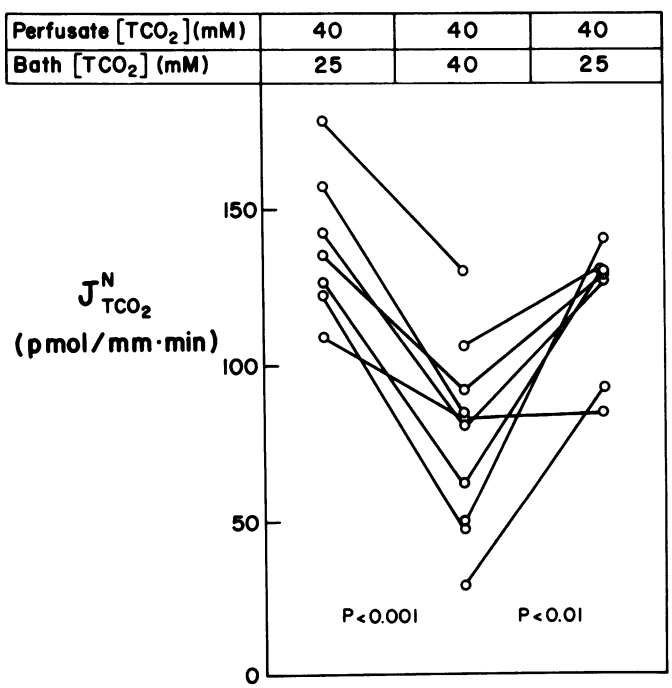

FIGURE 2 Effect of increasing bath HCO_3^- concentration on HCO_3^- reabsorption. Bath TCO_2 concentration was selectively increased from 25 to 40 mM, while the bath P_{CO_2} and perfusate TCO_2 concentration were maintained constant at 40 mmHg and 40 mM, respectively. $J^N_{TCO_2}$ decreased from 138.8±8.7 to 76.3±9.3 pmol mm^{-1} min^{-1} and recovered to 118.1±7.9 pmol mm^{-1} min^{-1} when the bath TCO_2 concentration was again returned to 25 mM.

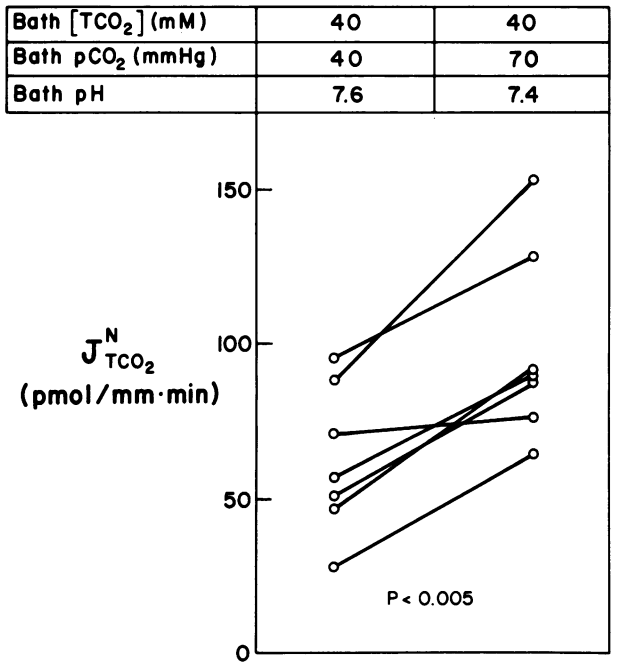

FIGURE 3 Effect of increasing bath P_{CO_2} on HCO_3^- reabsorption. Perfusate and bath TCO_2 concentration were maintained constant at 40 mM. Bath P_{CO_2} was increased from 40 to 70 mmHg and in response to this change, bath pH was reduced from 7.6 to 7.4. $J^N_{TCO_2}$ increased from 62.7±9.0 to 98.2±11.8 pmol mm^{-1} min^{-1}.

reason for this discrepancy is not clear, but could be related to his higher P_{CO_2}. In his study the bath pH was reduced from 7.4 to 7.08 suggesting P_{CO_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^- concentration. 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±0.04 nl mm^{-1} min^{-1} and −0.49±0.50 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 (Δ TCO_2: 3.97±0.62 mM, n = 7). From these results, HCO_3^- permeability calculated using Eq. 4 was 1.09±0.17 × 10^{-7} cm^2 s^{-1}. The permeability per surface area was 1.68±0.25 × 10^{-5} cm s^{-1}. The conversion was calculated on the basis of the measured mean tubular diameter of 20.1±0.4 μm, n = 7.

TABLE II
Effect of Bath pCO_2 on $J^N_{TCO_2}$

Bath pCO_2	Bath pH	$J^N_{TCO_2}$
mmHg		*pmol mm^{-1} min^{-1}*
39.1±1.0	7.41±0.01	92.6±6.6
73.2±0.7	7.14±0.01	104.1±6.3
MPD (n = 5)		11.5±1.8 (P < 0.005)

Perfusate and bath TCO_2 concentrations were 25.2±0.3 and 25.3±0.1 mM, respectively. MPD, mean paired difference.

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

$$J^A_{TCO_2} = J^N_{TCO_2} - J^P_{HCO_3^-}. \quad (6)$$

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

TABLE III
Passive Permeability of HCO_3^- in PCT

	40 mM HCO_3^- perfusate:40 mM HCO_3^- bath					40 mM HCO_3^- perfusate:25 mM HCO_3^- bath				
	Tubular length	Perfusion rate	J_v	Collected* TCO_2	ΔTCO_2‡	Perfusion rate	J_v	Collected* TCO_2	ΔTCO_2	P_{HCO_3}
	mm	*nl min^{-1}*	*nl mm^{-1} min^{-1}*	*mM*		*nl min^{-1}*	*nl mm^{-1} min^{-1}*	*mM*		*10^{-7} cm^2 s^{-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
±SEM	0.11	0.26	0.04	0.78	0.50	0.32	0.03	0.66	0.62	0.17

* TCO_2 concentration of collected fluid.
‡ 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.

$J^A_{TCO_2}$ can be regarded as an estimate of active H^+ secretion rate.

The results of the calculations of $J^P_{HCO_3^-}$ and $J^A_{TCO_2}$ are summarized in Table IV. In group 1, when the perfusate and bath contained 25 mM HCO_3^-, $J^P_{HCO_3^-}$ was −2.7 pmol mm^{-1} min^{-1} due to HCO_3^- entry into the lumen because the mean luminal HCO_3^- concentration was less than the bath concentration. When the perfusate HCO_3^- concentration was increased to 40 mM, the mean luminal HCO_3^- concentration was higher than the bath concentration and $J^P_{HCO_3^-}$ was 6.3 pmol mm^{-1} min^{-1}. As a result, the net change in $J^P_{HCO_3^-}$ was 9.0 pmol mm^{-1} min^{-1}. This change accounts for only 23% of the observed change in $J^N_{TCO_2}$ (39.2 pmol mm^{-1} min^{-1}, Table I). This analysis shows that in group I most (77%) of the change in $J^N_{TCO_2}$ is due to an increase in $J^A_{TCO_2}$. The same analysis was performed in groups 2 and 3, and the contributions of $J^P_{HCO_3^-}$ were smaller in these groups than in group 1 (11% in group 2, and 4% in group 3). Therefore, the observed changes in $J^N_{TCO_2}$ are due mainly to changes in $J^A_{TCO_2}$, the active H^+ secretion rate.

TABLE IV
Effects of Lumen and Bath HCO_3^- Concentrations and Bath pCO_2 on $J^P_{HCO_3^-}$ and $J^A_{TCO_2}$

	Condition*	$J^P_{HCO_3^-}$	$J^A_{TCO_2}$
		pmol mm^{-1} min^{-1}	
G1 (*n* = 10)	Control	−2.7±0.6	98.7±13.2
	Experimental	6.3±0.8	128.8±15.1
	MPD	9.0±0.8	30.1±7.7
	P	<0.001	<0.005
G2 (*n* = 10)	Control	6.7±0.3	126.6±7.7
	Experimental	0.2±0.7	76.2±9.7
	MPD	−6.5±0.5	−50.4±6.8
	P	<0.001	<0.001
G3 (*n* = 7)	Control	1.9±0.8	60.8±9.7
	Experimental	0.4±0.8	97.7±12.3
	MPD	−1.4±0.3	36.9±6.7
	P	<0.005	<0.005

* For experimental condition, see Table I. In G1, luminal TCO_2 concentration was increased; in G2, bath TCO_2 concentration was increased; in G3, bath pCO_2 was increased. $J^A_{TCO_2}$ was calculated as $J^A_{TCO_2} = J^N_{TCO_2} - J^P_{HCO_3^-}$. $J^N_{TCO_2}$ 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 HCO_3^- concentrations of the perfusate and bath on HCO_3^- reabsorption in the PCT. For this purpose, the in vitro isolated tubule perfusion technique is suitable, because the bath and the perfusate HCO_3^- concentrations can be changed independently. Factors encountered in in vivo studies that affect HCO_3^- reabsorption, such as the expansion of extracellular fluid, alteration in plasma 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 HCO_3^- reabsorption. We observed independent effects of luminal and peritubular HCO_3^- concentrations and P_{CO_2} on HCO_3^-

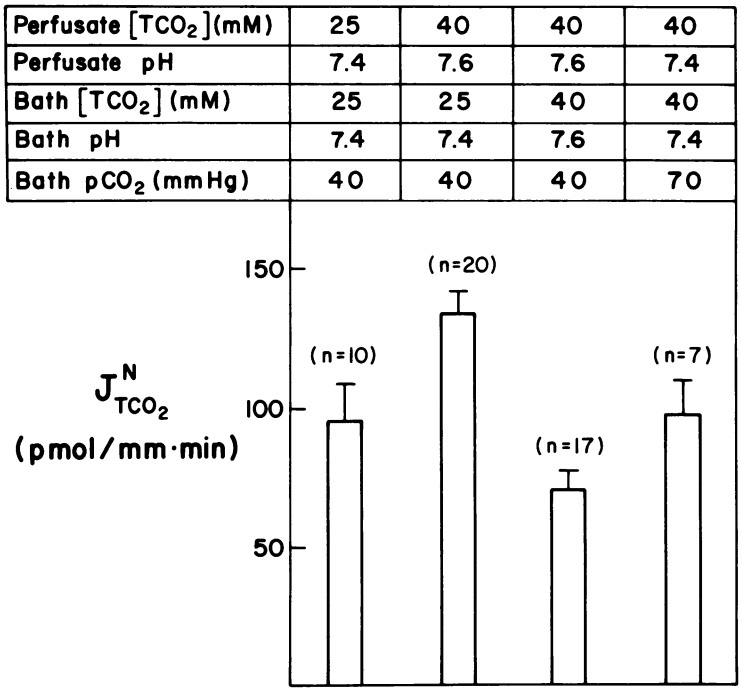

FIGURE 4 Summary of the effect of luminal and peritubular acid-base factors on HCO_3^- reabsorption. Column 1: control; both luminal and peritubular pH were 7.4. Column 2: increasing luminal TCO_2 concentration and pH stimulated $J^N_{TCO_2}$. Column 3: increasing bath TCO_2 concentration and pH markedly reduced $J^N_{TCO_2}$. Column 4: correction of bath pH by increasing bath P_{CO_2}-stimulated $J^N_{TCO_2}$. 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. $J^N_{TCO_2}$ were 95.9±12.8, 134.2±8.0, 70.7±6.7, and 98.2±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 P_{CO_2} are shown. The perfusate pH was calculated assuming that the luminal P_{CO_2} was equilibrated with the bath P_{CO_2} (30).[4] The perfusate TCO_2 concentration and pH can be regarded as indices of luminal TCO_2 concentration and pH.[5] The first column is the control. Both luminal and peritubular pH were 7.4. In the second column, the luminal pH was increased by increasing luminal HCO_3^- concentration, and an increase in $J^N_{TCO_2}$ was observed. In the third column, the bath pH was increased by increasing peritubular HCO_3^- concentration, and a marked inhibition of $J^N_{TCO_2}$ was obtained. In the fourth column, the bath pH was reduced by increasing P_{CO_2}, and an increase in $J^N_{TCO_2}$ 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-

[4] Taking CO_2 permeability (10^{-4} cm^2 s^{-1}) determined by Schwartz (30) and perfusion rate of 13 nl min^{-1}, P_{CO_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.

[5] 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 HCO_3^- concentration stimulates 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 HCO_3^- concentration on HCO_3^- reabsorption (compare columns 2 and 3 in Fig. 4). A selective increase in the bath HCO_3^- concentration from 25 to 40 mM at constant luminal HCO_3^- concentration and bath P_{CO_2} caused a marked inhibition of HCO_3^- reabsorption and 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 HCO_3^- concentration on HCO_3^- reabsorption. First, Giebisch et al. (33) found that H^+ secretion was reduced when $NaHCO_3$ was infused acutely to obtain a plasma HCO_3^- concentration of 44 mM. Second, Chan and Giebisch (34) observed that increasing peritubular HCO_3^- concentration from 2 to 40 mM inhibited HCO_3^- reabsorption. However, the interpretation of these data as indicating an independent effect of peritubular HCO_3^- concentration is complicated by the presence of extracellular volume expansion in the former and by the presence of an extremely low peritubular P_{CO_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 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, HCO_3^- reabsorption is inhibited by selectively increasing peritubular 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 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 HCO_3^- concentration is masked by the stimulatory effect of increasing luminal HCO_3^- concentration.

This inhibitory effect of increasing bath HCO_3^- concentration can be reversed by raising P_{CO_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 P_{CO_2} on bicarbonate absorption. In early clearance (6–8) and micropuncture (9, 10) studies, a stimulatory effect of respiratory acidosis (high P_{CO_2}) on HCO_3^- reabsorption was demonstrated. Later, Kurtzman (12) pointed out that respiratory acidosis reduces effective plasma volume, itself a strong stimulus to HCO_3^- reabsorption. Reexamination of the effect of respiratory acidosis on HCO_3^- reabsorption in clearance studies (12–14) showed little effect on 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 P_{CO_2} from 45 to 65 mmHg caused a very small increase (12%) in proximal HCO_3^- reabsorption. In contrast to the above clearance and free-flow micropuncture studies, a direct effect of P_{CO_2} on 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 P_{CO_2}, since the P_{CO_2} of the renal cortex is higher than systemic P_{CO_2} (38). Therefore, the renal cortical P_{CO_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 $J^N_{TCO_2}$ is plotted against H^+ ion concentration. Only data where lumen and bath pH are identical are shown. The pH was changed by increasing the bath P_{CO_2} from 40 to 70 mmHg. Increasing H^+ ion concentration from 40 to 72 neq liter^{-1} (pH 7.4–7.15) by increasing P_{CO_2} with 25 mM HCO_3^- in the lumen and bath stimulates $J^N_{TCO_2}$ by 12%; whereas, increasing it from 22 to 40 neq liter^{-1} (pH 7.6–7.4) by increasing P_{CO_2} with 40 mM HCO_3^- in the lumen and bath stimulates $J^N_{TCO_2}$ by 40%. Thus, the stimulatory effect of P_{CO_2} on 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 P_{CO_2} is one of the determinants of 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 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 TCO_2 concentrations were 25 mM (column 1), $J^N_{TCO_2}$ was 96 pmol mm^{-1} min^{-1}. When both TCO_2 concentrations were increased to 40 mM (column 3), $J^N_{TCO_2}$ was 71 pmol mm^{-1} min^{-1}. This comparison shows that when both luminal and peritubular HCO_3^- con-

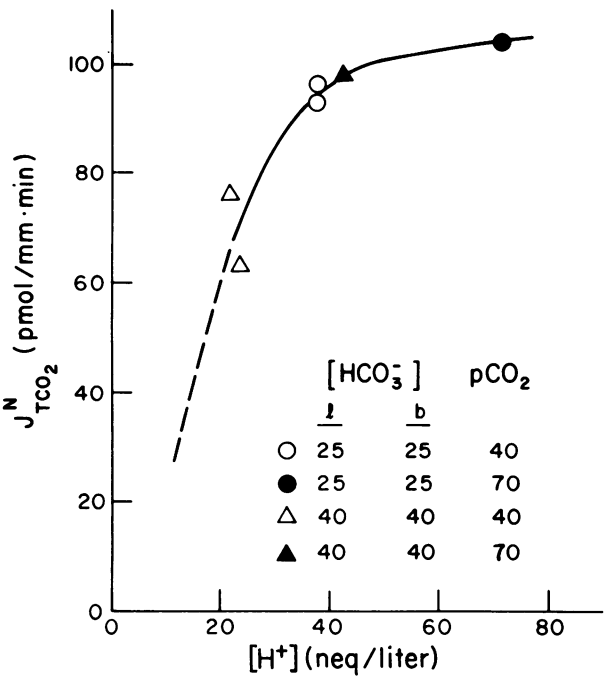

FIGURE 5 Relationship between $J^N_{TCO_2}$ and H^+ ion concentration of lumen (l) and bath (b). Only data where lumen and bath H^+ ion concentrations were identical are chosen. H^+ ion concentration was changed by altering the bath P_{CO_2} from 40 to 70 mmHg.

centrations are increased simultaneously at constant P_{CO_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 P_{CO_2} were different, but the perfusate and the bath pH were the same. Observed $J^N_{TCO_2}$ 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 P_{CO_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 mechanism 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.[6]

[6] Their result in rabbit PCT showed that the basolateral membrane PD is −51 mV at a peritubular pH of 7.4. It hyperpolaralized to −60 mV when the peritubular pH was alkanized to 7.6. (In their study this effect was examined

If the electrochemical gradient for HCO_3^- diffusion across the basolateral membrane is unchanged, then a reduction in the HCO_3^- permeability of the peritubular membrane caused by peritubular alkalinity may be the explanation for the observed decrease in HCO_3^- diffusion out of the cell.

The inhibition of HCO_3^- reabsorption induced by increasing bath HCO_3^- concentration cannot be attributed specifically to either peritubular HCO_3^- concentration or peritubular pH. However, our data suggest that peritubular pH is more important than peritubular HCO_3^- concentration. HCO_3^- reabsorption was stimulated when peritubular alkalinity was corrected by increasing P_{CO_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 HCO_3^- concentration is the principal determinant of HCO_3^- reabsorption.

The precise mechanism by which a selective increase in P_{CO_2} at constant luminal and peritubular HCO_3^- concentrations stimulates HCO_3^- reabsorption is not clear. An increase in P_{CO_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 HCO_3^- concentration) are necessary before it is possible to locate the effect of P_{CO_2}. The P_{CO_2} effect could be mediated by changes in the appropriate driving forces for the Na^+-H^+ antiporter and/or by changes in the basolateral membrane exit step. Alternatively, P_{CO_2} could have a direct effect on the Na^+-H^+ antiporter and/or the basolateral membrane permeability for HCO_3^-.

In summary, our data suggest that (*a*) increasing luminal HCO_3^- concentration stimulates HCO_3^- reabsorption by increasing the H^+ secretion rate secondary to a more favorable H^+ concentration gradient; (*b*) increasing peritubular HCO_3^- concentration reduces HCO_3^- reabsorption by decreasing the HCO_3^- exit across the basolateral membrane; (*c*) increasing P_{CO_2} stimulates HCO_3^- reabsorption; and (*d*) peritubular pH may be regarded phenomenologically as a determinant of HCO_3^- reabsorption.

only in the proximal straight tubule, therefore the same response in the PCT is assumed.) Intracellular HCO_3^- concentration may be calculated from the intracellular pH measurement by Struyvenberg et al. (41). Based on their data, the calculated intracellular HCO_3^- concentration is 22 and 23 mM when peritubular HCO_3^- concentration is 25 mM and 40 mM, respectively. The electrochemical driving force for HCO_3^- diffusion across the basolateral membrane is -51 mV $+ 60 \log 25/22 = -48$ mV at a peritubular HCO_3^- concentration of 25 mM, and is -60 mV $+ 60 \log 40/23 = -46$ mV at a peritubular HCO_3^- concentration of 40 mM.

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