Effect of Luminal and Peritubular \( HCO_3^- \) Concentrations and \( PCO_2 \) 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 \( PCO_2 \) 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 \( PCO_2 \), 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 \( PCO_2 \), 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 \( PCO_2 \). Passive permeability for \( HCO_3^- \) was also measured and found to be \( 1.09\pm0.17 \times 10^{-7} \text{ cm}^2 \text{s}^{-1} \). 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 NaHCO3 infusion, renal \( HCO_3^- \) reabsorption exhibited a tendency towards partial saturation. The tendency towards partial saturation is expressed as a threshold in studies where NaHCO3 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 (\( PCO_2 \)). An independent effect of blood \( PCO_2 \) 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, \( PCO_2 \), 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 \( PCO_2 \) 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 \( HCO_3^- \) reabsorption.

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1 Abbreviations used in this paper: G1, G2, G3, group 1, 2, and 3; \( J_{PCO_2} \), active \( CO_2 \) flux; \( J_{HCO_3}^- \), net total \( CO_2 \) flux; \( J_{HCO_3}^- \), passive \( HCO_3^- \) flux; \( J_v \), volume flux; \( PCO_2 \), \( CO_2 \) tension; PCT, proximal convoluted tubules; PD, potential difference; \( P_{HCO_3}^- \), passive \( HCO_3^- \) permeability; \( TCO_2 \), total \( CO_2 \).

_Abbreviations used in this paper: G1, G2, G3, group 1, 2, and 3; \( J_{PCO_2} \), active \( CO_2 \) flux; \( J_{HCO_3}^- \), net total \( CO_2 \) flux; \( J_{HCO_3}^- \), passive \( HCO_3^- \) flux; \( J_v \), volume flux; \( PCO_2 \), \( CO_2 \) tension; PCT, proximal convoluted tubules; PD, potential difference; \( P_{HCO_3}^- \), passive \( HCO_3^- \) permeability; \( TCO_2 \), total \( CO_2 \)._
stimulates HCO\textsubscript{3}\textsuperscript{−} reabsorption. Each of these maneuvers alters primarily the active component of HCO\textsubscript{3}\textsuperscript{−} reabsorption. Analysis of the data suggests that the effects of HCO\textsubscript{3}\textsuperscript{−} concentration and PCO\textsubscript{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 juxtaparacellular 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. Transmural pH 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 was 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\textsubscript{2}/TCO\textsubscript{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 Amino-A PM-30 membranes (American Instrument Co., Silver Spring, MD). TCO\textsubscript{2} concentrations of the ultrafiltrate and serum were adjusted either to 0 or 20 mmol/kg H\textsubscript{2}O by adding an isotonic NaHCO\textsubscript{3} solution (155 mM NaHCO\textsubscript{3}, 5 mM D-glucose, 5 mM L-alanine, 290 mosmol/kg H\textsubscript{2}O). The osmolality of the perfusate and bath solutions was also adjusted to 290 mosmol/kg H\textsubscript{2}O by adding water or NaCl salt. To determine the volume flux (J\textsubscript{v}) and the net TCO\textsubscript{2} flux (J\textsubscript{TCO\textsubscript{2}}), the concentrations of [methoxy-\textsuperscript{3}H]inulin (added to the perfusate as a volume marker) and TCO\textsubscript{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 Pco\textsubscript{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 Pco\textsubscript{2} was estimated from the Henderson-Hasselbalch equation.

Three protocols were performed. Group 1: The effect of increasing luminal HCO\textsubscript{3}\textsuperscript{−} concentration was examined. Bath TCO\textsubscript{2} concentration and Pco\textsubscript{2} were maintained constant at 25 mmol/kg H\textsubscript{2}O and 40 mmol/kg H\textsubscript{2}O, respectively, and luminal TCO\textsubscript{2} concentration was increased from 25 to 40 mmol/kg H\textsubscript{2}O. Group 2: The effect of increasing bath HCO\textsubscript{3}\textsuperscript{−} concentration was examined. Perfusion TCO\textsubscript{2} concentration and bath Pco\textsubscript{2} were kept constant at 40 mmol/kg H\textsubscript{2}O and 40 mmol/kg H\textsubscript{2}O, respectively, and bath TCO\textsubscript{2} concentration was increased from 25 to 40 mmol/kg H\textsubscript{2}O. Group 3: The effect of increasing Pco\textsubscript{2} was examined. Both luminal and bath TCO\textsubscript{2} concentrations were 40 mmol/kg H\textsubscript{2}O and Pco\textsubscript{2} was changed from 40 to 70 mmol/kg H\textsubscript{2}O.

**HCO\textsubscript{3}\textsuperscript{−} 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\textsubscript{3}, 40; NaHPO\textsubscript{4}, 1; KCl, 5; MgCl\textsubscript{2}, 0.7; d-glucose, 8.3; CaCl\textsubscript{2}, 1.5. Two bath solutions were used. The control bath solution was in mM: NaCl, 100; NaHCO\textsubscript{3}, 40; NaHPO\textsubscript{4}, 1; KCl, 5; MgCl\textsubscript{2}, 1; d-glucose, 8.3, CaCl\textsubscript{2}, 3, and dialyzed albumin was added at 6 g/dl. To generate a HCO\textsubscript{3}\textsuperscript{−} gradient, 15 mmol NaHCO\textsubscript{3} in the bath solution was replaced by Na\textsubscript{2}HPO\textsubscript{4} and H\textsuperscript{+}/OH\textsuperscript{−} was inhibited by cooling 20°C (19, 20) and by adding 0.16 mM ethoxazolamide to the bath (16, 19).\textsuperscript{2} Osmolarities of all artificial solutions were adjusted to 290 mosmol/kg H\textsubscript{2}O. The perfusion rate was maintained at a slow rate (3-4 nl/min), to maximize the change in TCO\textsubscript{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\textsubscript{3}\textsuperscript{−} concentration gradient between lumen and bath. In the experimental period there was a 15 mmol HCO\textsubscript{3}\textsuperscript{−} concentration gradient from lumen to bath. The J\textsubscript{v} and the TCO\textsubscript{2} concentration difference between the perfusate and collected fluid were measured in both periods.

**Calculations.** J\textsubscript{v} was calculated as

\[
J_v = \frac{V_v}{L} (C_{L}^{\text{HCO}_3^-}/C_{B}^{\text{HCO}_3^-} - 1),
\]

where V\textsubscript{v} is the collection rate of tubular fluid, L is the length of the tubule as measured by eye piece micrometer, C\textsubscript{L} and C\textsubscript{B} are [methoxy-\textsuperscript{3}H]inulin concentrations of the collected fluid and the initial perfusate, respectively. J\textsubscript{TCO\textsubscript{2}} was calculated as

\[
J_{TCO_2}^N = (C_{L}^{TCO_2} - C_{B}^{TCO_2})V_v/L + (C_{L}^{TCO_2} - C_{B}^{TCO_2})J_v,
\]

where C\textsubscript{L} and C\textsubscript{B} are TCO\textsubscript{2} concentrations of the initial perfusate and collected fluid, respectively.

**Passive HCO\textsubscript{3}\textsuperscript{−} permeability (P\textsubscript{HCO\textsubscript{3}}) may be obtained as**

\[
P_{HCO_3^{-}} = \frac{V_v}{L} \ln \frac{C_{CO_2}^{\text{HCO}_3^-}}{C_{CO_2}^{\text{HCO}_3^-} - C_{CO_2}^{\text{HCO}_3^-}} ,
\]

where C\textsubscript{CO\textsubscript{2}} and C\textsubscript{H\textsubscript{CO\textsubscript{3}}\textsuperscript{−}} are HCO\textsubscript{3}\textsuperscript{−} concentrations of the perfusate, collected fluid, and bath fluid, respectively.\textsuperscript{3} Since all solutions were equilibrated with 5% CO\textsubscript{2} gas, these fluids contained the same amount of dissolved CO\textsubscript{2}. Therefore, Eq. 3 can be rewritten as

\[
P_{HCO_3^{-}} = \frac{V_v}{L} \ln \frac{C_{CO_2}^{TCO_2} - C_{CO_2}^{TCO_2}}{C_{CO_2}^{TCO_2} - C_{CO_2}^{TCO_2}} ,
\]

where C\textsubscript{CO\textsubscript{2}} is the TCO\textsubscript{2} concentration of the bath fluid.

\textsuperscript{2} It is possible that there was some passive flux of H\textsuperscript{+}/OH\textsuperscript{−} through the paracellular shunt pathway when P\textsubscript{HCO\textsubscript{3}} was determined. This flux would cause an overestimation of P\textsubscript{HCO\textsubscript{3}}. The overestimation would be small because the H\textsuperscript{+}/OH\textsuperscript{−} flux through the shunt pathway is much smaller than the HCO\textsubscript{3}\textsuperscript{−} flux due to its smaller chemical concentration gradient between lumen and bath compared with that of HCO\textsubscript{3}\textsuperscript{−} (∼1/10\textsuperscript{6}).

\textsuperscript{3} In this equation transtubular PD was assumed to be zero. In these experiments, the active transport PD was inhibited by cooling and the bionic diffusion PD was also close to zero because isethionate permeability is close to the HCO\textsubscript{3}\textsuperscript{−} permeability. Therefore, the transtubular PD was a Donnan PD of ∼+1.5 mV. This PD would cause a 3% underestimation of P\textsubscript{HCO\textsubscript{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:

\[
J_{HCO_3}^p = P_{HCO_3} \left( \frac{C_{HCO_3}^L - C_{HCO_3}^C}{R \cdot D \cdot P_{CO_2}} \cdot \frac{C_{HCO_3}^C + C_{HCO_3}^L}{2} \right),
\]

where \( C_{HCO_3}^L \) 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 \( J \) and \( J_{HCO_3}^p \) 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 \( J_{TCO_2}^p \). To determine the effect of increasing luminal \( HCO_3^- \) concentration on \( J_{TCO_2}^p \), 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 TCO2 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 \( J_{TCO_2}^p \) 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 \( J_{TCO_2}^p \) 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%, \( J_{TCO_2}^p \) increases by 41%.

Effect of increasing bath \( HCO_3^- \) concentration on \( J_{TCO_2}^p \). 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 \( Pco_2 \) 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 \( J_{TCO_2}^p \) 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 \( J_{TCO_2}^p \) decreased to 76.3±9.3 pmol mm⁻¹ min⁻¹ (\( P < 0.001 \)). \( J_{TCO_2}^p \) recovered close to the precontrol value (118.1±7.9 pmol mm⁻¹ min⁻¹) in the postcontrol period.

Effect of increasing bath \( Pco_2 \). Our observation that \( J_{TCO_2}^p \) 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±0.6 and 40.6±0.2 mM, respectively. The bath \( Pco_2 \) 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, \( J_{TCO_2}^p \) 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 \( Pco_2 \).

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±0.3 and 25.3±0.1 mM, respectively. The bath \( Pco_2 \) 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. \( J_{TCO_2}^p \) 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 HCO₃⁻ Concentrations and Bath pCO₂ on J₇TCO₂

<table>
<thead>
<tr>
<th>Condition</th>
<th>Bath pCO₂</th>
<th>Bath TCO₂</th>
<th>Per fusate TCO₂</th>
<th>J₇TCO₂ (pmol mm⁻¹ min⁻¹)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>mM</td>
<td>mmHg</td>
<td>mM</td>
<td></td>
</tr>
<tr>
<td>G1 (n = 10)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>25.9±0.2</td>
<td>24.4±0.7</td>
<td>39.4±1.8</td>
<td>95.9±12.8</td>
</tr>
<tr>
<td>Experimental</td>
<td>25.9±0.2</td>
<td>38.0±0.6</td>
<td>39.7±1.7</td>
<td>135.1±14.6</td>
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<tr>
<td>MPD</td>
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<td></td>
<td></td>
<td>39.2±7.6</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>G2 (n = 10)</td>
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<td></td>
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</tr>
<tr>
<td>Control</td>
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<td>42.7±3.9</td>
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<tr>
<td>Experimental</td>
<td>40.9±0.5</td>
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<td>38.6±3.2</td>
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<td>-56.9±6.7</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>G3 (n = 7)</td>
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<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>40.6±0.2</td>
<td>39.6±0.3</td>
<td>39.4±0.4</td>
<td>62.7±9.0</td>
</tr>
<tr>
<td>Experimental</td>
<td>40.6±0.2</td>
<td>39.6±0.3</td>
<td>71.3±0.4</td>
<td>98.2±11.8</td>
</tr>
<tr>
<td>MPD</td>
<td></td>
<td></td>
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<td>35.5±6.5</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

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

Response to the change in PCO₂. This result showed that the effect of bath PCO₂ 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 HCO₃⁻ reabsorption by increasing PCO₂. His stimulation of HCO₃⁻ reabsorption is larger than ours. The

![Figure 2](image_url)

**Figure 2.** Effect of increasing bath HCO₃⁻ concentration on HCO₃⁻ reabsorption. Bath TCO₂ concentration was selectively increased from 25 to 40 mM, while the bath PCO₂ and perfusate TCO₂ concentration were maintained constant at 40 mmHg and 40 mM, respectively. J₇TCO₂ 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 TCO₂ concentration was again returned to 25 mM.
reason for this discrepancy is not clear, but could be related to his higher pCO₂. In his study the bath pH was reduced from 7.4 to 7.08 suggesting pCO₂ was increased from 40 to 87 mmHg.

**HCO₃⁻ permeability.** Measurements of P_{HCO₃} are shown in Table III. In these studies the perfusate HCO₃⁻ concentration was 40 mM and the bath HCO₃⁻ concentration was 25 mM. The direction of the HCO₃⁻ concentration gradient is similar to that used to examine the effect of increasing luminal HCO₃⁻ 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 HCO₃⁻ perfusate and bath solution. Both J, and the TCO₂ concentration difference between the perfusate and collected fluid were not different from zero, -0.05±0.04 nmol 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_{HCO₃⁻}. When the bath HCO₃⁻ concentration was reduced to 25 mM, a significant reduction in TCO₂ concentration of collected fluid was observed (∆ TCO₂; 3.97±0.62 mM, n = 7). From these results, HCO₃⁻ permeability calculated using Eq. 4 was 1.09±0.17 × 10⁻² cm² s⁻¹. The permeability per surface area was 1.68±0.25 × 10⁻³ cm s⁻¹. The conversion was calculated on the basis of the measured mean tubular diameter of 20.1±0.4 μm, n = 7.

This result confirms previous studies (20, 21, 24–27) that have shown that the PCT is permeable to HCO₃⁻. Our P_{HCO₃} 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 from Frömter (26). The cause of these differences among reported P_{HCO₃} values is not clear. One possibility may be methodological. In the studies where similar P_{HCO₃} values were reported (20, 24, and this study), microcalorimetry was used to measure the HCO₃⁻ concentration of the collected fluid. On the other hand, Lang et al. (25) used a microadaptation of the Astrup method to measure the HCO₃⁻ concentration of the collected fluid, and Frömter (26) calculated P_{HCO₃} by measuring the NaCl and NaHCO₃ dilution PD, and the isotopic permeabilities for Na⁺ and Cl⁻.

**Calculation of the passive HCO₃⁻ flux.** We have demonstrated that increasing the luminal HCO₃⁻ concentration increases J⁰_{TCO₂} (Fig. 1) and that increasing the peritubular HCO₃⁻ concentration decreases J⁺_{TCO₂} (Fig. 2). These changes in J⁰_{TCO₂} may be caused by a passive HCO₃⁻ flux (Jₚ_{HCO₃}), since PCT are permeable to HCO₃⁻ (20, 21, 24–27 and Table III). Jₚ_{HCO₃} can be estimated using the measured P_{HCO₃}, PD, and HCO₃⁻ concentrations according to Eq. 5. Once Jₚ_{HCO₃} is obtained, HCO₃⁻ reabsorption can be viewed as a pump-leak system, and J⁺_{TCO₂} can be corrected for Jₚ_{HCO₃} to yield the active TCO₂ flux (J_{TCO₂}):  

\[ J_{TCO₂} = J_{HCO₃} - J_{TCO₂} \]  

(6)

According to this definition, J_{TCO₂} represents the active transcellular flux of HCO₃⁻ and is generally believed to be due to active H⁺ secretion (28, 29). Therefore,
TABLE III
Passive Permeability of HCO₃⁻ in PCT

<table>
<thead>
<tr>
<th>Tubular length</th>
<th>Perfusion rate</th>
<th>Jₜ</th>
<th>Collected*</th>
<th>∆TCO₂</th>
<th>∆TCO₂m</th>
</tr>
</thead>
<tbody>
<tr>
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<td>nl min⁻¹</td>
<td>nl mm⁻¹ min⁻¹</td>
<td>mM</td>
<td>mM</td>
<td>10⁻⁷ cm² s⁻¹</td>
</tr>
<tr>
<td>1.80</td>
<td>2.91</td>
<td>-0.12</td>
<td>41.03</td>
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<td>1.90</td>
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<td>42.44</td>
<td>-1.61</td>
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<td>1.90</td>
<td>2.89</td>
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<td>39.21</td>
<td>0.75</td>
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<tr>
<td>1.60</td>
<td>3.87</td>
<td>-0.01</td>
<td>39.4</td>
<td>-0.91</td>
<td>4.24</td>
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<tr>
<td>1.25</td>
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<td>1.50</td>
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<td></td>
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<td>2.28</td>
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<td>2.10</td>
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<tr>
<td>Mean</td>
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<td>3.34</td>
<td>-0.05</td>
<td>40.47</td>
<td>-0.49</td>
</tr>
<tr>
<td>±SEM</td>
<td>0.11</td>
<td>0.26</td>
<td>0.04</td>
<td>0.78</td>
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</tbody>
</table>

* TCO₂ concentration of collected fluid.
1 TCO₂ concentration difference between perfusate and collected fluid.
Experiments were performed at 20°C and in the presence of 0.16 mM bath ethoxazolamide.

JₜCO₂ can be regarded as an estimate of active H⁺ secretion rate.

The results of the calculations of JₜHCO₃ and JₜA HCO₃ are summarized in Table IV. In group 1, when the perfusate and bath contained 25 mM HCO₃, JₜHCO₃ was -2.7 pmol mm⁻¹ min⁻¹ due to HCO₃ entry into the lumen because the mean luminal HCO₃ concentration was less than the bath concentration. When the perfusate HCO₃ concentration was increased to 40 mM, the mean luminal HCO₃ concentration was higher than the bath concentration and JₜHCO₃ was 6.3 pmol mm⁻¹ min⁻¹. As a result, the net change in JₜHCO₃ was 9.0 pmol mm⁻¹ min⁻¹. This change accounts for only 23% of the observed change in JₜCO₂ (39.2 pmol mm⁻¹ min⁻¹, Table I). This analysis shows that in group 1 most (77%) of the change in JₜCO₂ is due to an increase in JₜHCO₃. The same analysis was performed in groups 2 and 3, and the contributions of JₜHCO₃ were smaller in these groups than in group 1 (11% in group 2, and 4% in group 3). Therefore, the observed changes in JₜHCO₃ are due mainly to changes in JₜCO₂, the active H⁺ secretion rate.

TABLE IV
Effects of Lumen and Bath HCO₃⁻ Concentrations and Bath pCO₂ on JₜHCO₃ and JₜCO₂

<table>
<thead>
<tr>
<th>Condition*</th>
<th>JₜHCO₃</th>
<th>JₜCO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol mm⁻¹ min⁻¹</td>
<td>pmol mm⁻¹ min⁻¹</td>
</tr>
<tr>
<td>G1 (n = 10)</td>
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</tr>
<tr>
<td>Control</td>
<td>-2.7±0.6</td>
<td>98.7±13.2</td>
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<tr>
<td>Experimental</td>
<td>6.3±0.8</td>
<td>128.8±15.1</td>
</tr>
<tr>
<td>MPD</td>
<td>9.0±0.8</td>
<td>30.1±7.7</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>G2 (n = 10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6.7±0.3</td>
<td>126.6±7.7</td>
</tr>
<tr>
<td>Experimental</td>
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</tr>
<tr>
<td>MPD</td>
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</tr>
<tr>
<td>P</td>
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<td>&lt;0.001</td>
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<tr>
<td>G3 (n = 7)</td>
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<td></td>
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<tr>
<td>Control</td>
<td>1.9±0.8</td>
<td>60.8±9.7</td>
</tr>
<tr>
<td>Experimental</td>
<td>0.4±0.8</td>
<td>97.7±12.3</td>
</tr>
<tr>
<td>MPD</td>
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<td>36.9±6.7</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

* For experimental condition, see Table I. In G1, luminal TCO₂ concentration was increased; in G2, bath TCO₂ concentration was increased; in G3, bath pCO₂ was increased. JₜHCO₃ was calculated as JₜHCO₃ = JₜCO₂ - JₜHCO₃. JₜHCO₃ 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 HCO₃⁻ concentrations of the perfusate and bath on HCO₃⁻ reabsorption in the PCT. For this purpose, the in vitro isolated tubule perfusion technique is suitable, because the bath and the perfusate HCO₃ concentrations can be changed independently. Factors encountered in in vivo studies that affect HCO₃⁻ 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 HCO₃⁻ reabsorption. We observed independent effects of luminal and peritubular HCO₃ concentrations and PCO₂ on HCO₃
Reabsorption. Our results are summarized in Fig. 4. At the top of this figure perfusate TCO₂ concentration, perfusate pH, bath TCO₂ concentration, bath pH, and bath PCO₂ are shown. The perfusate pH was calculated assuming that the luminal PCO₂ was equilibrated with the bath PCO₂ (30). The perfusate TCO₂ concentration and pH can be regarded as indices of luminal TCO₂ concentration and pH. 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 HCO₃⁻ concentration, and an increase in J₈TCO₂ was observed. In the third column, the bath pH was increased by increasing peritubular HCO₃⁻ concentration, and a marked inhibition of J₈TCO₂ was obtained. In the fourth column, the bath pH was reduced by increasing PCO₂, and an increase in J₈TCO₂ was observed.

Our results show a stimulatory effect of increasing luminal HCO₃⁻ concentration on HCO₃⁻ reabsorption (compare columns 1 and 2 in Fig. 4). When the luminal HCO₃⁻ concentration was increased from 25 to 40 mM without changing the peritubular HCO₃⁻ concentration, net HCO₃⁻ 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 HCO₃⁻ reabsorption does not saturate when the luminal HCO₃⁻ concentration is increased up to 60 mM without changing the peritubular HCO₃⁻ concentration. Alpern et al. (32) have also observed that HCO₃⁻ reabsorption increases linearly up to a mean luminal HCO₃⁻ concen-

<table>
<thead>
<tr>
<th>Perfusion [TCO₂] (mM)</th>
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<th>40</th>
<th>40</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfusion pH</td>
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<td>7.6</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>Bath [TCO₂] (mM)</td>
<td>25</td>
<td>25</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Bath pH</td>
<td>7.4</td>
<td>7.4</td>
<td>7.6</td>
<td>7.6</td>
</tr>
<tr>
<td>Bath pCO₂ (mmHg)</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>70</td>
</tr>
</tbody>
</table>

![Figure 4](image-url)
diation 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 Pco$_2$ 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, Giebiesh 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 Giebiesh (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 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 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 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 Pco$_2$ on bicarbonate absorption. In early clearance (6–8) and micropuncture (9, 10) studies, a stimulatory effect of respiratory acidosis (high Pco$_2$) 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 Pco$_2$ 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 Pco$_2$ 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 Pco$_2$, since the Pco$_2$ of the renal cortex is higher than systemic Pco$_2$ (38). Therefore, the renal cortical 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$_{\text{CO}_2}$ 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 Pco$_2$ from 40 to 70 mmHg. Increasing H$^+$ ion concentration from 40 to 72 neq liter$^{-1}$ (pH 7.4–7.15) by increasing Pco$_2$ with 25 mM HCO$_3^-$ in the lumen and bath stimulates J$_{\text{CO}_2}$ by 12%; whereas, increasing it from 22 to 40 neq liter$^{-1}$ (pH 7.6–7.4) by increasing Pco$_2$ with 40 mM HCO$_3^-$ in the lumen and bath stimulates J$_{\text{CO}_2}$ by 40%. Thus, the stimulatory effect of Pco$_2$ 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 Pco$_2$ 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$^{\text{lum}}_{\text{CO}_2}$ was 96 pmol mm$^{-1}$ min$^{-1}$. When both TCO$_2$ concentrations were increased to 40 mM (column 3), J$^{\text{lum}}_{\text{CO}_2}$ was 71 pmol mm$^{-1}$ min$^{-1}$. This comparison shows that when both luminal and peritubular HCO$_3^-$ con-
centrations are increased simultaneously at constant 
PHCO2, the stimulatory effect of luminal HCO3- concentration is completely abolished by the inhibitory effect of peritubular HCO3- concentration. This comparison again points out that one of the mechanisms for the tendency towards partial saturation of HCO3- 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 HCO3- concentrations and the PCO2 were different, but the perfusate and the bath pH were the same. Observed JHCO3- was essentially the same (96 vs. 98 pmol mm-1 min-1). This observation suggests that the luminal and peritubular pH, not HCO3- concentration or PCO2, are the major determinants of HCO3- 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 HCO3- reabsorption in the PCT. The current view of the acidification mech-

ism 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 CO2 to form HCO3-. The exact mechanism by which HCO3- exits from the cell is controversial, but one hypothesis is that HCO3- diffuses out of the cell through the basolateral membrane down its electrochemical gradient. The rate of H+ secretion is equal to the rate of HCO3- diffusion out of the cell. According to this model, HCO3- reabsorption may be regulated by at least two limiting steps: Na+-H+ exchange at the luminal membrane and the HCO3- exit step at basolateral membrane. The acid-base factors examined in this study could regulate HCO3- reabsorption at either or both of these two limiting steps.

An increase in luminal HCO3- 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 HCO3- production within the cell and an increase in the driving force for HCO3- exit across the basolateral membrane. Our result showing that increasing luminal HCO3- concentration stimulates HCO3- 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 HCO3- concentration might reduce the electrochemical driving force for HCO3- exit across the basolateral membrane. As a consequence, intracellular HCO3- 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 HCO3- exit step by changing the basolateral membrane properties (HCO3- 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 HCO3- concentration is increased, the electrochemical driving force for HCO3- diffusion across the basolateral membrane might be constant. In other words, the reduction in the chemical HCO3- concentration gradient might be counterbalanced by an increase in the electrical driving force.6

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6 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 alkalanized to 7.6. (In their study this effect was examined...
If the electrochemical gradient for HCO₃⁻ diffusion across the basolateral membrane is unchanged, then a reduction in the HCO₃⁻ permeability of the peritubular membrane caused by peritubular alkalinity may be the explanation for the observed decrease in HCO₃⁻ diffusion out of the cell.

The inhibition of HCO₃⁻ reabsorption induced by increasing bath HCO₃⁻ concentration cannot be attributed specifically to either peritubular HCO₃⁻ concentration or peritubular pH. However, our data suggest that peritubular pH is more important than peritubular HCO₃⁻ concentration. HCO₃⁻ reabsorption was stimulated when peritubular alkalinity was corrected by increasing Pco₂ (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 HCO₃⁻ concentration is the principal determinant of HCO₃⁻ reabsorption.

The precise mechanism by which a selective increase in Pco₂ at constant luminal and peritubular HCO₃⁻ concentrations stimulates HCO₃⁻ reabsorption is not clear. An increase in Pco₂ 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 HCO₃⁻ concentration) are necessary before it is possible to locate the effect of Pco₂. The Pco₂ 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, Pco₂ could have a direct effect on the Na⁺-H⁺ antiporter and/or the basolateral membrane permeability for HCO₃⁻.

In summary, our data suggest that (a) increasing luminal HCO₃⁻ concentration stimulates HCO₃⁻ reabsorption by increasing the H⁺ secretion rate secondary to a more favorable H⁺ concentration gradient; (b) increasing peritubular HCO₃⁻ concentration reduces HCO₃⁻ reabsorption by decreasing the HCO₃⁻ exit across the basolateral membrane; (c) increasing Pco₂ stimulates HCO₃⁻ reabsorption; and (d) peritubular pH may be regarded phenomenologically as a determinant of HCO₃⁻ reabsorption.

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