Urinary acidification: \( \text{CO}_2 \) transport by the rabbit proximal straight tubule

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MICROPUNCTURE STUDIES have demonstrated that proximal convoluted renal tubules acidify the tubule fluid, reducing its bicarbonate content in rats (15, 23, 27, 30), dogs (5), and monkeys (4). The measurements necessarily limited to the portions of the proximal convoluted tubule on the surface of the kidney since only these were accessible to micropuncture.

Although there have not been direct measurements of proximal straight tubules to determine whether they also acidify, the results of indirect studies are consistent with this possibility. Micropuncture studies in rats (15, 21, 30) and dogs (5) showed that bicarbonate concentration or pH in the early distal tubule was less than or equal to that in the late proximal tubule. Since volume reabsorption in the loop of Henle should of itself increase the bicarbonate concentration and pH of fluid delivered to the early distal tubule (15), Bernstein and Clapp (5) hypothesized that the decrease was due to reabsorption of bicarbonate by the proximal straight tubule or loop of Henle. Malnic and co-workers (11, 21, 22) found that the bicarbonate reabsorption between late proximal and early distal micropuncture sites in rats was inhibited by acetazolamide. Schafer, Troutman, and Andreoli (34) found, in isolated perfused proximal straight tubules, that the chloride concentration increased by 18.5 mM between perfused and collected fluid. They proposed that this reflected an equal decrease in bicarbonate concentration (which they did not measure). Since acetate was added to the perfusate in their studies, it is probable that some of the reabsorbed anion was acetate rather than bicarbonate (32), and it is difficult to be certain how much the bicarbonate concentration actually decreased.

In the present studies the transport of bicarbonate by isolated proximal straight tubules was measured directly. The total \( \text{CO}_2 \) content of the luminal perfusates decreased, reflecting luminal acidification and reabsorption of bicarbonate. In addition, the permeability of the tubules to bicarbonate was estimated from electrical measurements, and transport rates and permeabilities were compared in tubules from superficial and juxtamedullary nephrons.

METHODS

General techniques. Isolated segments of proximal straight tubules from female 1- to 3-kg New Zealand white rabbits were perfused in vitro with the previously described techniques (8, 9).

Segments of tubule of 1-3 mm were transferred to a thermostated perfusion chamber and mounted in concentric glass pipets with an outermost sealing pipet filled with liquid Sylgard (Dow) (10). Measurements of bi-ionic and dilution potentials were made at 25°C following the start of perfusion. At low temperatures there is no fluid absorption or spontaneous potential, which makes it easier to interpret the measured bi-ionic and dilution potentials (34). The bathing medium was then changed to the control bath and slowly heated to 38°C while bubbled continuously with 95% \( \text{O}_2 \)-5% \( \text{CO}_2 \). The inside and outside diameters of each tubule were measured after perfusion was begun. Tubules were perfused with a microperfusion pump (Sage) at rates between 1 and 15 nl/min.

Periods during which three or four samples were collected in an experimental condition were separated by 20-min intervals. Perfusate was collected from under oil at the distal end of the tubule with a calibrated constriction pipet. Each sample was analyzed for total \( \text{CO}_2 \) content immediately so as to minimize any loss of \( \text{CO}_2 \). The total \( \text{CO}_2 \) content of the original perfusates was...
measured in the same manner. The means of the results of the samples in each period were used for the calculations reported in Results.

The composition of the perfusion solutions and bath is listed in Table 1. The control bath was used in all experiments except during the measurement of bi-ionic and dilution potentials. Bicarbonate-containing solutions were equilibrated with 95% O₂-5% CO₂. All solutions were titrated to pH 7.4, and the osmolalities adjusted to 293 mosmol/kg. The total CO₂ content of the bathing media was measured with a Natelson microgasometer following gas equilibration.

Bi-ionic and dilution potentials. The transepithelial potential difference (PD) was measured through the perfusion pipet connected to a calomel half-cell by a 0.16 M NaCl agar bridge (10). The bath was connected to ground through a calomel half-cell with a similar bridge and a precision millivolt reference source (W-P Instruments, model 101). The PD between the lumen and bath was measured with a unity-gain amplifier (Transidyne, Inc., type 561A). Before connecting the tubule the voltage was set to zero with solution I as perfusate and bath using the millivolt reference source to null any small voltages due to electrode asymmetry. The tubule was then perfused at 10 nl/min, and the PD was recorded at room temperature (25°C). Then, the bathing medium was changed to solution II and then to III to obtain bi-ionic and dilution potentials. The modified Henderson equation was used to correct the observed potential for liquid junctional potentials (3), using the limiting equivalent conductances of sodium, chloride, and bicarbonate in water at 25°C for determining the ionic mobilities (31). The calculated liquid junctions (perfusate minus bath) were 1.1 mV for the bi-ionic potentials (solution I vs. II) and 3.5 mV (perfusate minus bath) for the dilution potentials (solution I vs. III). The permeability ratio of sodium to chloride (PNa,cI) was estimated from the dilution potential (soZution Z vs. ZZZ) and 3.5 mV (perfusate minus bath) were 1.1 mV for the bi-ionic potentials (I vs. II) and the PNa,cI using the same equation. The absolute permeability coefficient of bicarbonate was calculated from the ionic permeability ratios and the published values for isotopic chloride permeability in the superficial and juxtamedullary rabbit proximal straight tubules (18).

Picapnotherm. Total CO₂ contents of perfusates and standards were measured with a microcalorimeter (Picapnotherm) developed by Gerald G. Vurek of the Laboratory of Technical Development, National Heart and Lung Institute (38). The apparatus measures the heat released when CO₂ reacts with LiOH (6):

\[
2\text{LiOH} + \text{CO}_2 \rightarrow \text{Li}_2\text{CO}_3 + \text{H}_2\text{O} \quad (I)
\]

\[
\Delta H = -8.96 \times 10^4 \text{J/mol CO}_2
\]

The measured quantity (total acid-releasable CO₂) is the sum of bicarbonate, carbonate, carbonic acid, and dissolved CO₂ in the sample. Since carbonic acid and carbonate concentrations are trivial at physiologic pH, they will be neglected in what follows. The Picapnotherm has a sensitivity of less than 10 pmol and responds linearly over the range of total CO₂ concentrations used.

Transport calculations. The net rate of transport of CO₂ from lumen to bath (Jb) was calculated as:

\[
Jb = (C_0 V_0 - C_l V_L)/L \quad (2)
\]

where L is the length of the tubule, V₀ and Vₐ are the rates of flow of perfusate, and C₀ and Cₐ are the total CO₂ concentrations at the beginning and end of the tubule, respectively. Since V₀ = Vₛ + JₛL, where Jₛ (nl/mm·min) is the rate of absorption of fluid

\[
Jb = (C_0 - C_l)V_L/L + J_lC_l \quad (3)
\]

Measurements of CO₂ transport were also made with Cₐ equal to zero so that the net transport was from bath to lumen (Jₘ). For the latter

\[
J_m = -V_L C_l/L \quad (4)
\]

Statistics. The data for each tubule for each experimental condition are presented as the means of three or four collections. The results are expressed as means ±SE. Statistical analyses were done with paired or unpaired-t tests, as indicated in the text.

### RESULTS

Absorption of total CO₂. The total CO₂ concentration of the collected fluid was less than that of the perfusate when the tubules were perfused with solution II or IV (bicarbonate of 25 mM), as illustrated in Fig. 1. Considering only the slowest flow rate for each tubule, the average total CO₂ concentration in the collected fluid was 16.8 mM (n = 10) when the tubules were perfused at Vₛ/L = 1.3 nl/mm·min. The perfused length of these tubules averaged 2.4 ± 0.2 mm. Solid lines connect sets of collections from individual tubules perfused at different rates. There was a decline in the total CO₂ concentration when the perfusion rate was slowed (i.e., transit

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**TABLE 1. Composition of perfusion solutions and bath**

<table>
<thead>
<tr>
<th>Perfusion Solutions</th>
<th>Control Bath</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>NaCl</td>
<td>150</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>25</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>2.5</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>1.2</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>2.0</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.5</td>
</tr>
<tr>
<td>Alamine</td>
<td>6.0</td>
</tr>
<tr>
<td>Na lactate</td>
<td>4.0</td>
</tr>
<tr>
<td>Na₃ citrate</td>
<td>1.0</td>
</tr>
<tr>
<td>Albumin</td>
<td>6.0</td>
</tr>
<tr>
<td>Mannitol</td>
<td>135</td>
</tr>
</tbody>
</table>

Perfusion solutions and control bath are in mM unless otherwise noted. * Sufficient NaCl added for isosmolality with perfusates I–V. † g/dl.

1 When Vₛ is great enough so that Cₐ is close to zero, Jₘ calculated in this manner is the flux of total CO₂ from bath to lumen. Since at the perfusion rates used in these experiments Cₐ ≈ 2.5 mM, the actual flux of total CO₂ from bath to lumen is greater than Jₘ.
FIG. 1. Total CO₂ concentration in fluid collected from rabbit proximal straight tubules. Perfusate and bath contained 25 mM bicarbonate at pH 7.4. Solid lines connect collections made from same tubule at different perfusion rates. Open circles represent tubules perfused with solution II, which contained no organic solutes. Solid circles represent tubules perfused with solution IV, which contained glucose, lactate, alanine, and citrate.

time prolonged). Presumably the fall in the total CO₂ concentration of the perfusates at slow rates was due to the reabsorption of bicarbonate accompanying acidification of the tubule fluid.

Back-leak of total CO₂. Total CO₂ appeared in the collected fluid when the perfusate was initially free of total CO₂ (solution I or III), as illustrated in Fig. 2. Considering only the slowest flow rate for an individual tubule, the transepithelial entry of total CO₂ into the lumen resulted in a mean concentration of total CO₂ in collected fluid of 10.7 mM (n = 14 tubules) when the collection rate was ≤1.3 nl/mm·min. The average perfused length of these tubules was 2.2 ± 0.1 mm. Solid lines in Fig. 2 connect the collections made from the same tubule at different flow rates. In 12 out of 15 tubules there was an increase in the total CO₂ concentration of the collected perfusate when the perfusion rate was reduced. Evidently, CO₂ and/or bicarbonate leaked across the tubule epithelium under these circumstances. Similar results have been reported in the rat proximal convoluted tubule (1, 20, 25) and the turtle urinary bladder (35).

In Figs. 1 and 2 approximately half the tubules were perfused with solutions containing glucose, alanine, citrate, and lactate (IV and V, Table 1) while the rest were perfused with solutions that did not contain them (I and II, Table 1). As shown in Figs. 1 and 2, there was no effect of these organic solutes on either the reabsorption or back-leak of total CO₂. Schafer et al. (34) previously found that the removal of glucose and alanine from the luminal perfusate did not influence the rate of fluid absorption in the superficial rabbit proximal straight tubule.

Two types of straight tubules. We did not prospectively identify the anatomic origin of the proximal straight tubules which are depicted in Figs. 1 and 2. Following the report of Kawamura et al. (18) that there were functional differences between proximal straight tubules from superficial and juxtamedullary nephrons, we identified and studied the two types of tubules. By definition, the glomeruli of superficial nephrons lie near surface of cortex and those of juxtamedullary nephrons near the corticomedullary border (13). Although the glomeruli of individual proximal straight tubules were not localized in our dissections, we distinguished between the tubules from superficial and juxtamedullary nephrons by the morphologic and anatomic features which are illustrated in Fig. 3 (modified from Peter (26)). The tubules from juxtamedullary nephrons are attached to longer thin limbs of loops of Henle (36) and are larger in diameter. The external diameters of tubules from juxtamedullary nephrons ranged from 50 to 70 μm compared with 40-50 for superficial nephrons. The internal diameters were 30 and 20 μm, respectively. The tubules from superficial nephrons were relatively straight with a few undulations towards their cortical end, while those from juxtamedullary nephrons on the other hand were "kinky." The origin of the tubules was identified prior to studying their function.

Each tubule was perfused both with solution I (initial total CO₂ of 0 mM) and solution II (initial CO₂ averaged 26.8 ± 0.3 mM) while bathed in the control bath. The
perfusion rates were adjusted so that the collection rate (Vf) was the same for both sets of collections in each tubule. The results are presented in Table 2.

The net transport of total CO₂ from bath to lumen (J₁) was significantly greater in the tubules from superficial nephrons (−2.2 ± 0.3 pmol/cm·s) than in the tubules from juxtamedullary nephrons (−1.4 ± 0.2 pmol/cm·s).

Tubules of both types absorbed total CO₂ when perfused with solution II. The mean concentration difference between collected and perfused fluids (collected minus perfused; Table 2) was −4.4 ± 0.8 mM in tubules from juxtamedullary nephrons and −2.9 ± 0.6 mM in tubules from superficial nephrons perfused at similar rates. Assuming that the rate of fluid absorption in each type of tubule was equal to that reported by Kawamura et al. (18), lumen-to-bath transport of total CO₂ (J₁b; equation 3) was significantly greater in the juxtamedullary than in the superficial nephrons.

Ionic permeability ratios. Kawamura et al. (18) reported that the proximal straight tubules of juxtamedullary nephrons are less permeable to chloride than to sodium while those from superficial nephrons are more permeable to chloride than sodium.

In the tubules from superficial nephrons the mean sodium chloride dilution potential was 6.9 mV (positive in the lumen, which contained the higher concentration of sodium chloride) and the calculated sodium/chloride permeability ratio was 0.43 ± 0.05 (Table 3) confirming the earlier conclusions (18, 34) that this nephron segment is more permeable to chloride than to sodium. The mean bicarbonate/chloride bi-ionic potential was 2.1 mV (positive in the lumen which contained the higher concentration of chloride) and the calculated bicarbonate/chloride permeability ratio was 0.35 ± 0.03.

### Table 2. Total CO₂ transport rates of proximal straight tubules

<table>
<thead>
<tr>
<th>Origin of Tubules</th>
<th>Superficial</th>
<th>Juxtamedullary</th>
<th>Diff*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, mm</td>
<td>2.0</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Collection rate,</td>
<td>2.9</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>0 mM total CO₂ in perfuse (solution I)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CO₂ collected conc, mM</td>
<td>5.1</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>CO₂ transport, bath to lumen, J₁b; pmol/cm·s</td>
<td>−2.2±0.3</td>
<td>−1.4±0.2</td>
<td>−0.8±0.4 P&lt;.05</td>
</tr>
<tr>
<td>26.8 mM total CO₂ in perfuse (solution II)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total CO₂ collected minus perfused conc, mM</td>
<td>−2.9</td>
<td>−4.4</td>
<td></td>
</tr>
<tr>
<td>Rate of fluid absorption†,</td>
<td>0.47</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>n/ml/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO₂ transport, lumen to bath, J₁b; pmol/cm·s</td>
<td>3.2±0.2</td>
<td>4.9±0.5</td>
<td>−1.7±0.5 P&lt;.005</td>
</tr>
</tbody>
</table>

Transport values are means ± SE; n, number of tubules. * Difference in transport rates between superficial and juxtamedullary tubules; unpaired two-tailed t statistics. † Taken from Kawamura et al. (18).

### Table 3. Permeability ratios and coefficients in proximal straight tubules

<table>
<thead>
<tr>
<th>Origin of Tubules</th>
<th>Superficial</th>
<th>Juxtamedullary</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl dilution potential, mV*</td>
<td>6.9</td>
<td>−3.5</td>
</tr>
<tr>
<td>Na/Cl permeability ratio</td>
<td>0.43</td>
<td>2.0</td>
</tr>
<tr>
<td>HCO₃⁻/Cl bi-ionic potential, mV*</td>
<td>2.1</td>
<td>0.8</td>
</tr>
<tr>
<td>HCO₃⁻/Cl permeability ratio</td>
<td>0.35</td>
<td>0.53</td>
</tr>
<tr>
<td>Permeability coeff., 10⁻⁵ cm/s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloride</td>
<td>5.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>2.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Corrected for liquid junction potentials. † Isotopic chloride permeability coefficient, taken from Kawamura et al. (18).

### Discussion

Total CO₂, which was measured in the present experiments, includes bicarbonate and carbonic acid in addition to dissolved carbon dioxide. Therefore, it is important to distinguish which form was transported under the different conditions. In the experiments measuring absorption of total CO₂, decrease in the concentration of bicarbonate must account for most of the change in total CO₂ concentration since approximately 95% of the total CO₂ of the initial perfusates (pH 7.4) is in the form of bicarbonate. As reviewed elsewhere, there is uncertainty whether bicarbonate is directly reabsorbed by renal tubules, or whether, as seems more likely, it reacts with secreted hydrogen ions to form carbon dioxide (7, 28, 29, 37).

Proximal convoluted tubules are believed to be...
permeable to both bicarbonate and molecular carbon dioxide, based on studies by micropuncture in rats (24). Substantial permeability to bicarbonate was inferred by Bank and Aynedjian (1) who perfused with bicarbonate-free solutions and found a mean concentration of 15 mM bicarbonate in the collected fluid. The concentration of bicarbonate that accumulated in the tubule was deemed too high to be accounted for by permeation of molecular carbon dioxide alone. Malnic and de Mello-Aires (20), on the other hand, directly demonstrated rapid permeation of molecular carbon dioxide. They perfused with a solution containing 100 mM bicarbonate. When the solution was initially equilibrated with air, the pH fell rapidly, due to entry of molecular carbon dioxide from the blood. When the solution was initially equilibrated with carbon dioxide, as a control, there was no immediate rapid change in pH. We presume that in the present studies the appearance of total CO₂ in the collected fluid when the perfusate was free of CO₂ was due to both processes. Note in Fig. 2 that even at the fastest rates of perfusion there was 2.5 to 4 mM of total CO₂ in the collected fluid. We infer that this principally represents rapid entry of dissolved molecular carbon dioxide from the bath. At slow rates of perfusion the concentration of total CO₂ in the collected fluid was even higher. We infer that this additional total CO₂ represents permeation of bicarbonate per se. The electrophysiological studies (Fig. 4 and Table 3) afford additional evidence that bicarbonate permeates the epithelium.

Following the report of Kawamura et al. (18), that proximal straight tubules from superficial and juxtamedullary nephrons have different permeabilities to sodium and chloride, it was of interest to consider whether there are also differences in the transepithelial movement of CO₂. As noted in Table 2, proximal straight tubules from juxtamedullary nephrons have a smaller rate of net total CO₂ movement from bath to lumen ($J_{\text{bl}}$) than do tubules from superficial nephrons, and reabsorb total CO₂ more rapidly as well. This finding may have implications for total renal function since acidification by the proximal straight tubule presumably is a factor determining the pH and bicarbonate content of the tubule fluid which is delivered distally. Considering the difference in handling of total CO₂ between the proximal tubule segments from superficial and juxtamedullary nephrons, redistribution of glomerular filtration between the nephron populations might conceivably alter urinary acidification. Examples of such redistribution include the selective increase in glomerular filtration rate in superficial nephrons during acute volume expansion, and in the juxtamedullary nephrons during contraction of the extracellular fluid volume (2, 12, 16, 17, 38). The resulting redistribution of the filtered load of bicarbonate between nephrons containing proximal straight tubules which handle total CO₂ differently could affect urinary acidification. Along this line, Garrella et al. (14) suggested that redistribution of glomerular filtration rate in volume-expanded animals resulted in changes in the relative rates of reabsorption of anions.

These experiments were made possible by the technical developments of Dr. Gerald G. Vurek. His continuing support and encouragement are deeply appreciated. It is a pleasure to acknowledge the superb technical assistance of Nordica Green. Dr. Clifford S. Patlak and Dr. Jack Orloff provided stimulating discussions throughout the course of this work. Dr. Floyd C. Rector, Jr., provided many fertile suggestions following the completion of the experiments reported herein.

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