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

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

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

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

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

INTRODUCTION

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

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

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

METHODS

Experiments were performed using male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 156–325 g. The rats were prepared for microperfusion as previously described (9). Briefly, rats were anesthetized with an intraperitoneal injection of Inactin (100–120 mg/kg), and placed on a heated table that maintained body temperature at 37°C. The right femoral artery was catheterized for monitoring blood pressure and obtaining blood samples. The left kidney was exposed using a flank incision, and immobilized in a Lucite cup. The ureter was cannulated (PE-50) to ensure the free drainage of urine. Throughout surgery, rats were infused intravenously with a bicarbonate Ringer’s solution (NaCl 105 mM, NaHCO3 25 mM, Na2HPO4 4 mM, KCl 5 mM, MgSO4 1 mM, CaCl2 1.8 mM) at 1.2 ml/h. Proximal tubular transit time was measured after injection of 0.02 ml of 10% lissamine green dye intravenously, and only those kidneys in which transit time was < 13 s were accepted for study. After completion of surgery, rats were divided into four experimental groups.

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

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

Alkalotic volume expansion. The rats were infused with a bicarbonate solution (NaHCO3 180 mM, KHCO3 30 mM) at the same rates as in group 2. This infusion was chosen so as to avoid changes in plasma K concentration. Plasma K concentration was 5.0±0.1 (n = 20) in this group as compared with 5.0±0.2 (n = 7) in isohydric expansion.

Metabolic acidosis. The rats were infused with 0.5 M NH4Cl, 2% body wt at 70 μl/min, followed by a maintenance infusion of normal saline at 1.2 ml/h.

Prior to the infusions, plasma samples were obtained for determination of protein concentration (refractometry) and hematocrit. 30 min after maintenance infusions were started, a blood sample was obtained for determination of pH and Pco2 (Corning model 165 blood gas analyzer, Corning Glass Works, Medfield, MA). Subsequent plasma samples were then obtained for determination of protein concentration and hematocrit throughout the experiment.

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

The perfusion solutions used are listed in Table I. In the permeability studies, perfusate 1 was used, which was designed to minimize volume flux and was not gassed. In the absorption studies, perfusates 2 and 3 were used and were gassed with 90% O2/10% CO2, in order to achieve a CO2 tension of ~ 60–70 mmHg (9). The bicarbonate concentration of perfusate 2 was varied by replacing NaCl with NaHCO3. All perfusion solutions contained 0.05% FD&C green dye No. 3 and exhaustively dialyzed [methoxy-3H] inulin.

Analysis. The collected samples were kept in contact with Hepes equilibrated paraffin oil, bubbled with 10% CO2 (9). The samples were transferred into constant bore tubing for measurement of collected volume. A 25–35-nl aliquot for the absorptive studies, and a 45-nl aliquot for the permeability studies, was then removed for determination of total CO2 concentration and the remaining fluid was transferred to a vial for liquid scintillation counting. Total CO2 concen-

<table>
<thead>
<tr>
<th>TABLE I Perfusion Solutions</th>
<th>Solutions*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mM</td>
</tr>
<tr>
<td>NaCl</td>
<td>120</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>24</td>
</tr>
<tr>
<td>KCl</td>
<td>5</td>
</tr>
<tr>
<td>MgSO4</td>
<td>1</td>
</tr>
<tr>
<td>CaCl2</td>
<td>1.5</td>
</tr>
<tr>
<td>Na2HPO4</td>
<td>1</td>
</tr>
<tr>
<td>Glucose</td>
<td>5</td>
</tr>
<tr>
<td>Alanine</td>
<td>5</td>
</tr>
<tr>
<td>Urea</td>
<td>5</td>
</tr>
<tr>
<td>Raffinose</td>
<td>55</td>
</tr>
<tr>
<td>Acetazolamide</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* All perfusion fluids contained 0.05% FD & C green dye No. 3 and [methoxy-3H] inulin. Perfusion 1 was not gassed while perfusates 2 and 3 were gassed with 90% O2/10% CO2. The bicarbonate concentration of perfusate 2 was varied by replacing NaCl with NaHCO3.
tration was measured using microcalorimetry (picano-
therm) (13).
Calculations. The perfusion rate \( (V_o) \) was calculated as
\[
V_o = (I_e/I_o) V_L,
\]
where \( I_e \) and \( I_o \) represent the inulin concentration in the
collected and perfused fluids, respectively, and \( V_L \) is the
collection rate. Volume flux \( (J_v) \) was calculated as:
\[
J_v = (V_o - V_l)/L,
\]
where \( L \) equals the perfused length.

The concentration of total \( \text{CO}_2 \) was measured in the
perfused and collected fluids as well as in plasma samples
obtained during the experiment. Total \( \text{CO}_2 \) includes dissolved
\( \text{CO}_2 \), bicarbonate, and carbonate. At the pH, \( \text{PCO}_2 \), and
bicarbonate concentrations encountered in the absorption
experiments, total \( \text{CO}_2 \) can be considered a reasonable estimate
of bicarbonate concentration. In the permeability experi-
ments, however, because dissolved \( \text{CO}_2 \) concentration was a
significant component of total \( \text{CO}_2 \), bicarbonateconcen-
tration was calculated by subtracting the dissolved \( \text{CO}_2 \)(0.03
\times \( \text{PCO}_2 \)) from the measured total \( \text{CO}_2 \) concentration in the
collected fluid (9). For groups 1–3, arterial \( \text{PCO}_2 \) averaged
\(~36 \text{ mmHg} \) in the permeability studies and the plasma-dis-
solved \( \text{CO}_2 \) was therefore assumed equal to 1.1 mM. In these
groups, the dissolved \( \text{CO}_2 \) content of the collected fluid
was assumed equal to 1.8 mM. This was based on the arterial
\( \text{PCO}_2 \) of 36 mmHg, and a \( \Delta \text{PCO}_2 \) (renal cortical \( \text{PCO}_2 \) –
arterial \( \text{PCO}_2 \)) of \(~25 \text{ mmHg} \) which has been measured in
hydroperfusion (14), and expansion alkalinosis. In groups 1–3 the
exact magnitude of the correction for dissolved \( \text{CO}_2 \) was not
quantitatively important. However, in group 4 (acidosis),
because the bicarbonate concentrations are small, this
correction becomes more important. We therefore used the
measured arterial \( \text{PCO}_2 \) from each experiment to calculate the
plasma-dissolved \( \text{CO}_2 \). As the exact \( \Delta \text{PCO}_2 \) has not been
measured in this condition, we chose the lowest value ob-
tained by DuBose et al. (14) of 8 mmHg. Our bicarbonate
permeability in acidosis therefore represents a maximum
estimate of the true value. The perfused bicarbonate concen-
tration in the permeability studies was assumed equal to
zero as there was no bicarbonate added and the solutions
were not gassed. 25 measurements of this perfusion fluid
revealed [total \( \text{CO}_2 \)] = \(-0.1\pm0.1 \text{ mM} \). Net bicarbonate flux
\( (J_{\text{bic}}) \) was calculated as:
\[
J_{\text{bic}} = (C_O V_O - C_L V_L)/L,
\]
where \( C_O \) and \( C_L \) represent the perfused and collected bi-
carbonate concentrations, respectively.

In the permeability studies the log mean driving force was
used to calculate the permeability \( (P_{\text{bic}}) \).

\[ P_{\text{bic}} = -J_{\text{bic}} / \ln(C_O/(C_F - C_L)), \]

where \( C_F \) equals the concentration of bicarbonate in the
plasma.5

In the absorptive studies, net flux \( (J_{\text{net}}) \) was divided into
two components: (a) passive bicarbonate diffusion \( (J_{\text{bic}}) \)
and (b) proton secretion \( (J_{\text{pro}}) \),6 using the equations:
\[
J_{\text{net}} = J_{\text{bic}} + J_{\text{pro}}.
\]
\[
J_{\text{bic}} = P_{\text{bic}} \times (C_L - C_F),
\]

where \( C_L \) equals the arithmetic mean luminal total \( \text{CO}_2 \)
concentration.7

Data are presented as mean\pmSEM and groups are com-
pared by the unpaired two-tailed \( t \) test, unless otherwise
stated.

RESULTS

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

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

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5 DuBose, T. D., Jr., and M. S. Lucci. Personal
communication.

6 Since this manuscript was first prepared, measured values
for \( \Delta \text{PCO}_2 \) have been published (15) that disagree somewhat
with those of DuBose et al. (14). However, because the cor-
rection for \( \text{PCO}_2 \) is small, there is not a large effect on the
results. Bicarbonate permeability would increase by 0.3
\times 10^{-7} \text{ in hydroperfusion, } 0.3 \times 10^{-7} \text{ in isohydronic expansion, and
0.2 \times 10^{-7} \text{ in alkalotic expansion if the } \Delta \text{PCO}_2 \text{ of Gennari
et al. is used.}

7 In calculating the driving force for passive bicarbonate
diffusion, only the chemical driving force is considered, be-
cause the electrical driving force is small. Using a \( \text{PHCO}_3/\text{PCl} \) of 0.44 and a \( \text{PNa}/\text{PCl} \) of 1.22 (17), one can calculate
that the effect of chloride-bicarbonate gradients on PD is
small. In addition, the effect of any PD on bicarbonate flux
would be small because of the low mean bicarbonate con-
centration.
TABLE II
Plasma Values

<table>
<thead>
<tr>
<th></th>
<th>Protein</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>Pco₂</td>
</tr>
<tr>
<td>Hydropenia</td>
<td>7.44±0.01</td>
<td>36.3±0.9</td>
</tr>
<tr>
<td>Isohydric expansion</td>
<td>7.46±0.01</td>
<td>35.0±0.6</td>
</tr>
<tr>
<td>Alkalotic expansion</td>
<td>7.65±0.01</td>
<td>35.3±0.6</td>
</tr>
<tr>
<td>Acidosis</td>
<td>7.18±0.02</td>
<td>28.5±0.7</td>
</tr>
</tbody>
</table>

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

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

Bicarbonate permeability was measured by perfusing tubules with a bicarbonate-free perfusate (perfusate 1 in Table I) and measuring bicarbonate appearance. Bicarbonate permeability was 2.6±0.3×10⁻⁷ cm²/s in hydropenia and was increased by 50% to 3.9±0.4×10⁻⁷ cm²/s in volume-expanded rats (P < 0.05) (Table IV). From these permeability coefficients and the measured blood to lumen bicarbonate concentration gradients, a rate of passive bicarbonate diffusion can be calculated for the above absorption studies (Eq. 6). The rate of proton secretion can then be calculated as the difference between net bicarbonate absorption and passive bicarbonate diffusion (Eq. 5). Proton secretion was not significantly inhibited by ECF volume expansion: 114±5 pmol/mm·min in hydropenia vs. 126±5 pmol/mm·min in isohydric ex-

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

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Length, mm</th>
<th>Perfusion rate, nL/min</th>
<th>Perfused</th>
<th>Collected</th>
<th>Mean luminal</th>
<th>Volume flux, nL/mm·min</th>
<th>Net</th>
<th>Passive</th>
<th>Proton secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydropenia</td>
<td>8</td>
<td>2.08±0.26</td>
<td>14.88±0.24</td>
<td>24.5±0.3</td>
<td>12.7±2.1</td>
<td>18.6±1.1</td>
<td>1.92±0.11</td>
<td>105±4</td>
<td>−9±1</td>
<td>114±5</td>
</tr>
<tr>
<td>Isohydric expansion</td>
<td>9</td>
<td>1.77±0.25</td>
<td>14.29±0.20</td>
<td>23.6±0.7</td>
<td>14.1±1.4</td>
<td>19.2±1.1</td>
<td>2.24±0.16</td>
<td>107±5</td>
<td>−18±3</td>
<td>126±5</td>
</tr>
<tr>
<td>Alkalotic expansion</td>
<td>8</td>
<td>1.92±0.22</td>
<td>14.12±0.36</td>
<td>24.1±0.3</td>
<td>27.6±1.1</td>
<td>25.9±0.5</td>
<td>2.07±0.09</td>
<td>28±8§</td>
<td>−34±3</td>
<td>62±10§</td>
</tr>
<tr>
<td>Acidosis</td>
<td>8</td>
<td>1.76±0.14</td>
<td>14.50±0.28</td>
<td>23.6±0.2</td>
<td>7.4±1.4</td>
<td>15.5±0.8</td>
<td>1.87±0.09</td>
<td>149±11¶</td>
<td>7±1</td>
<td>142±11¶</td>
</tr>
</tbody>
</table>

38 mM perfusate

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Length, mm</th>
<th>Perfusion rate, nL/min</th>
<th>Perfused</th>
<th>Collected</th>
<th>Mean luminal</th>
<th>Volume flux, nL/mm·min</th>
<th>Net</th>
<th>Passive</th>
<th>Proton secretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isohydric expansion</td>
<td>6</td>
<td>2.28±0.21</td>
<td>14.25±0.60</td>
<td>36.6±0.2</td>
<td>15.5±3.7</td>
<td>26.1±2.0</td>
<td>2.08±0.12</td>
<td>159±5</td>
<td>4±6</td>
<td>155±6</td>
</tr>
<tr>
<td>Alkalotic expansion</td>
<td>9</td>
<td>1.51±0.16</td>
<td>14.15±0.31</td>
<td>39.4±0.5</td>
<td>39.1±1.2</td>
<td>39.3±0.7</td>
<td>1.36±0.30</td>
<td>60±5§</td>
<td>−5±2</td>
<td>64±5§</td>
</tr>
</tbody>
</table>

Net bicarbonate absorption was measured with either the 24 or 38 mM bicarbonate perfusate in the various animal groups. Using the bicarbonate permeabilities of Table IV, net absorption was divided into fluxes related to passive bicarbonate diffusion and proton secretion.

* Previously reported data (9).
¶ NS, vs. hydropenia.
§ P < 0.001 vs. isohydric expansion.
* P < 0.002 vs. hydropenia.
† P < 0.05 vs. hydropenia.
bicarbonate concentrations slow proton secretion and provide a large gradient for bicarbonate back-diffusion (9). To examine this question tubules were perfused with solution 3 (Table I) in hydropenic and volume-expanded animals. This perfusate is similar to that found normally in the late proximal tubule of hydropenic rats that bicarbonate concentration is 5 mM and organics are absent. The results are shown in Fig. 1. Net bicarbonate absorption was inhibited in volume expanded rats as compared with hydropenic rats: 7±3 vs. 19±3 pmol/mm·min, respectively (P < 0.01). This inhibition of net bicarbonate absorption was totally due to the increased rate of bicarbonate back-diffusion in the volume expanded animals (−45±2 pmol/mm·min) as compared to hydropenic animals (−33±1 pmol/mm·min) (P < 0.001). The calculated rates of proton secretion were the same in hydropenia (52±3 pmol/mm·min) and isohydric expansion (52±4 pmol/mm·min) (NS). Collected bicarbonate concentrations were higher in isohydric expansion (6.1±0.4 mM) as compared to hydropenia (4.9±0.5 mM), but this was of border line significance (0.05 < P < 0.1). Thus, when luminal bicarbonate concentration is low, volume expansion leads to an inhibition of net bicarbonate absorption, which is due totally to the expansion-induced increase in bicarbonate permeability. Volume status does not affect the rate of proton secretion.

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

### Table IV

**Effects of Systemic pH and Volume Status on Bicarbonate Permeability**

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Length, mm</th>
<th>Collected Mean</th>
<th>Luminal</th>
<th>Plasma</th>
<th>Perfusion rate, pmol/mm·min</th>
<th>Bicarbonate secretory flux, pmol/mm·min</th>
<th>Bicarbonate permeability, X10⁷ cm²/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydropenia*</td>
<td>12</td>
<td>1.96±0.17</td>
<td>3.9±0.4</td>
<td>2.0±0.2</td>
<td>21.5±1.0</td>
<td>14.62±0.37</td>
<td>31±4</td>
<td>2.6±0.3</td>
</tr>
<tr>
<td>Isohydric</td>
<td>18</td>
<td>1.85±0.15</td>
<td>5.4±0.5</td>
<td>2.8±0.3</td>
<td>23.1±0.5</td>
<td>14.41±0.22</td>
<td>46±5</td>
<td>3.9±0.41</td>
</tr>
<tr>
<td>Expanslon</td>
<td>9</td>
<td>1.66±0.16</td>
<td>8.1±1.0</td>
<td>4.2±0.6</td>
<td>37.9±1.0</td>
<td>15.00±0.33</td>
<td>79±6</td>
<td>3.9±0.34</td>
</tr>
<tr>
<td>Acidosis</td>
<td>8</td>
<td>1.91±0.23</td>
<td>1.7±0.3</td>
<td>0.9±0.2</td>
<td>8.7±0.4</td>
<td>14.04±0.44</td>
<td>14±3</td>
<td>3.0±0.5†</td>
</tr>
</tbody>
</table>

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

* Previously reported data (9).
† P < .05 vs. hydropenia.
§ NS vs. isohydric expansion.
‖ NS vs. hydropenia.
isohydric expansion: 2.59±0.11 nl/mm·min in hydro-
penia vs. 1.93±0.11 nl/mm·min in isohydric expan-
sion (P < 0.001). Cogan et al. and Berry and Cogan
found that reduction in peritubular protein proto-
centration in vivo and in vitro specifically inhibited NaCl
absorption but did not inhibit absorption of bicarbo-
nate or organic solutes (8, 18). In the tubules perfused
with solution 2, it could be calculated that ~50% of
the volume absorption was related to sodium bicar-
bonate, glucose, and alanine absorption, thus making
it difficult to detect an effect on volume absorption.
In the tubules perfused with solution 3, >95% of the
volume absorption was related to NaCl, and thus the
effect of volume expansion was able to be detected.
Previously, Morgan and Berliner perfused proximal
tubules with normal saline and found a similar inhi-
bition of volume absorption with ECF volume expa-

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

The inhibition of bicarbonate absorption induced by
peritubular alkalosis could be related to either an
effect on proton secretion or an effect on passive bi-
carbonate diffusion. An effect on passive bicarbonate
diffusion could in turn be related to an increase in
bicarbonate permeability or to an alteration in the
driving force for bicarbonate diffusion. To resolve
these alternatives we used two approaches, both of
which led to similar conclusions. First, we measured
bicarbonate permeability (Table IV). This value was
3.9±0.3 × 10⁻⁷ cm²/s in alkalotic expansion, which
was not different from the value in isohydric expansion
(3.9±0.4 × 10⁻⁷ cm²/s). Thus, peritubular pH does not
independently affect bicarbonate permeability. In ad-
dition, bicarbonate permeability is small, so that pas-
sive bicarbonate diffusion accounts for only a small
part of the difference in net bicarbonate absorption
between alkalotic and isohydric expansion (20 and 9%)
with the 24 and 38 mM perfusates, respectively, Table
III). Thus, the major effect in the depression of net
bicarbonate absorption relates to a >50% inhibition in
the rate of proton secretion during alkalosis (Table III).

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

Effect of metabolic acidosis. Since peritubular
alkalosis inhibited proximal tubular bicarbonate ab-
sorption, we studied rats made acutely acidotic by am-
monium chloride infusion (Table II) to examine

Effects of ECF Volume and Plasma pH on Proximal Acidification
whether bicarbonate absorption was stimulated when compared to normal hydropenic rats. Net bicarbonate absorption from a 24-mM bicarbonate perfusate (solution 2) was increased (149±11 pmol/mm · min) compared to normal hydropenic rats (105±4 pmol/mm · min) (P < 0.002) (Table III). Again, this increase could be related to a stimulation of proton secretion or to a more favorable gradient for bicarbonate diffusion. To resolve these possibilities, bicarbonate permeability was measured in metabolic acidosis. Due to the lack of knowledge of the exact renal PCO2 in this condition, we were only able to estimate a maximum bicarbonate permeability (see Methods). This was 3.0±0.5 × 10−7 cm2/s, and was not statistically different from that measured in hydropenia (Table IV). Using this maximal permeability, there was an increase in passive bicarbonate efflux in metabolic acidosis, but a stimulation of proton secretion was also observed. Proton secretion increased by 25% from 114±5 pmol/mm · min in hydropenia to 142±11 pmol/mm · min in metabolic acidosis (P < 0.05) (Table III).

**DISCUSSION**

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

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

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

<table>
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<tr>
<th>Table V: Effect of Alkalosis on Bicarbonate Absorption in the Presence of High Luminal Bicarbonate Concentration</th>
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<tbody>
<tr>
<td>Isohydric expansion</td>
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<td>Perfusion rate, nl/min</td>
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<td>Mean luminal [tCO3], mM</td>
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<td>Plasma [tCO3], mM</td>
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<tr>
<td>Gradient, mM</td>
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<tr>
<td>Net tCO3 flux, pmol/mm · min</td>
</tr>
<tr>
<td>Passive HCO3 flux, pmol/mm · min</td>
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<td>Proton secretion, pmol/mm · min</td>
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Tubules were perfused in isohydrically and alkalotically expanded animals with solutions of high bicarbonate concentration. The perfusate bicarbonate concentrations were chosen such that the concentration gradients would be reversed (lumen greater than plasma) and of equal magnitude.

**TABLE V**

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

Although we found that bicarbonate permeability was 50% larger in volume expansion as compared to hydropenia, it was small in both conditions. As such its effects on net bicarbonate absorption were small. When tubules were perfused with 24 mM bicarbonate we were unable to detect an effect on net bicarbonate absorption. When tubules were perfused with 5 mM bicarbonate, there was an easily detectable inhibition of net bicarbonate absorption that was totally attributable to the effect of volume expansion on bicarbonate permeability and was small (12 pmol/mm·min). In both series, the calculated rates of proton secretion were not affected by volume status. The physiologic significance of the volume expansion-induced increase in bicarbonate permeability remains unclear at this time. The effect on net bicarbonate absorption is small when compared with the usual delivery out of the PCT in the plasma replete rat (300 pmol/min) (8). It is therefore unclear whether the small increases in delivery that will result will lead to bicarbonaturia in the absence of an inhibitory effect of volume expansion distal to the PCT or in juxta-medullary nephrons. A change in bicarbonate permeability in the pars recta may also be present and if so could be a more important determinant of bicarbonate excretion.

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

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

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

The mechanism of the effect of peritubular bicarbonate concentration or pH on active proton secretion can be further elucidated from our results. In a previous study, bicarbonate absorption was measured as a function of mean luminal bicarbonate concentration in hydropenic rats (9). The closed circles in Fig. 2 represent the calculated rates of proton secretion in these previous studies. Proton secretion was a saturable function of luminal bicarbonate concentration with a \( K_{1/2} \) of \( \sim 16 \text{ mM} \) and a maximal rate \((J_{\text{max}}')\) of 200 pmol/mm·min. In the present study, when tubules in alkalotic rats were perfused with 24, 38, and 94 mM bicarbonate, the rates of proton secretion were 62, 64, and 80 pmol/mm·min, respectively. These results are plotted in Fig. 2 as a function of mean luminal bicarbonate concentration (open circles). The inhibitory effect on the rate of proton secretion of a 15-mM in-
increase in plasma bicarbonate concentration was not counterbalanced by a similar or greater increase in luminal bicarbonate concentration. Cohen and Steinmetz also found that proton secretion in the turtle bladder was more sensitive to serosal pH than to luminal pH (29).

The failure of increasing mean luminal bicarbonate concentration to stimulate proton secretion in alkalosis (Fig. 2) suggests that \( J_{\text{proton}} \) has been reached and is decreased from that in isohydric rats. Clearance studies by Monclair et al. (33) and Langberg et al. (34) defined a \( J_{\text{max}} \) for bicarbonate absorption by varying the filtered load of bicarbonate, and also found that peritubular alkalolemia caused a decrease in \( J_{\text{max}} \). In the appendix, we present a kinetic model for the Na:H exchanger which is used to explain the mechanistic significance of \( J_{\text{proton}} \). Using the model, it is demonstrated that an alkalosis-induced increase in cell pH would be expected to decrease \( J_{\text{max}} \). However, the expected magnitude of the change in cell pH is not sufficient to explain the entire observed decrease in \( J_{\text{proton}} \) and thus an alteration in the intrinsic properties of the Na:H exchanger is postulated. This intrinsic change in the Na:H exchanger in alkalosis, could be a decreased number of carriers, or a pH-dependent alteration in the structure and function of the carrier. Acidosis, which stimulated proton secretion (open triangle, Fig. 2), would have the opposite effect. Recent studies in the turtle bladder have suggested that increased CO₂ tension stimulates acidification by increasing the number of proton pumps in the luminal membrane (35).

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

In metabolic acidosis the distal nephron must increase ammonium and titratable acid excretion in order to increase bicarbonate regeneration. It is therefore important to minimize bicarbonate delivery from the proximal tubule such that required distal bicarbonate reclamation is small. Cogen et al. (8) have measured end-proximal total CO₂ concentrations in metabolic acidosis of 1.6 mM (equivalent to bicarbonate concentrations <0.5 mM). The ability of the proximal tubule to achieve these low bicarbonate concentrations and pH is determined by a balance between proton secretion and bicarbonate back leak. Because our bicarbonate permeability in acidosis is a maximum number, we cannot rule out a decrease in bicarbonate permeability in this condition. However, the stimulation of proton secretion that we observed will also contribute to the ability of the proximal tubule to maintain low luminal bicarbonate concentrations and thus limit distal bicarbonate delivery.

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

APPENDIX

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

\[ J_{\text{proton}} = k[H^+]_{l} - k_{-1}[Na^+]_{l}[H^+], \]

where \([Na^+]_{l}\) and \([Na^+]_{c}\) are the sodium concentrations in the lumen and cell, respectively, \([H^+]_{l}\) and \([H^+]_{c}\) are the hydrogen ion concentrations in the lumen and cell, respectively, and \(k_{1}\) and \(k_{-1}\) are rate constants. If one assumes that \([Na^+]_{l}\) and \([Na^+]_{c}\) are constant in the present experiments, new rate constants, \(k_{1}\) and \(k_{-1}\) can be defined such that:

\[ J_{\text{proton}} = k[H^+]_{c} - k^{-1}[H^+]_{l}. \]

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

The model would also predict that the change in \([J_{\text{proton}}]\) would be proportional to the change in \([H^+]_{c}\); if \(k_{1}\) remained constant. Using the measured plasma bicarbonate concentrations (Table II), it can be calculated that the peritubular hydrogen ion concentration in the aliphatic animals is ~35% lower than in the isohydric animals. In this study, \([H^+]_{c}\) was not measured, but the data of Struyvenberg et al. (31) suggest that a 35% reduction in peritubular hydrogen ion concentration would only lead to an 11% reduction in \([H^+]_{c}\). As this 11% reduction in \([H^+]_{c}\) cannot account for the 50% reduction in \([J_{\text{proton}}]\) (Fig. 2), \(k_{1}\) must be decreased in alkalosis. These results would therefore suggest that increased intracellular pH affects the rate of proton secretion by two mechanisms: (a) changing the chemical driving force for protons ([\(H^+]_{c}\)], and (b) altering the intrinsic properties of the Na:H exchanger \([k_{1}].\)

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