The Critical Importance of Urinary Concentrating Ability in the Generation of Urinary Carbon Dioxide Tension

JOSE A. L. ARRUDA, LUÍZ NASCIMENTO, PRADEEP K. MEHTA, DONALD R. RADEMACHER, JOHN T. SEHY, CHRISTOF WESTENFELDER, and NEIL A. KURTZMAN, The Sections of Nephrology, University of Illinois Abraham Lincoln School of Medicine and the Veterans Administration West Side Hospital, Chicago, Illinois, 60612

ABSTRACT Measurement of urine to blood (U-B) carbon dioxide tension (Pco2) gradient during alkalinization of the urine has been suggested to assess distal H+ secretion. A fact that has not been considered in previous studies dealing with urinary Pco2 is that dissolution of HCO3 in water results in elevation of Pco2 which is directly proportional to the HCO3 concentration. To investigate the interrelationship of urinary HCO3 and urinary acidification, we measured U-B Pco2 in (a) the presence of enhanced H+ secretion and decreased concentrating ability i.e., chronic renal failure (CRF), (b) animals with normal H+ secretion and decreased concentrating ability, Brattleboro (BB) rats, and (c) the presence of both impaired H+ secretion and concentrating ability (LiCl treatment and after release of unilateral ureteral obstruction). At moderately elevated plasma HCO3 levels (30-40 meq/liter), normal rats achieved a highly alkaline urine (urine pH > 7.8) and raised urine HCO3 concentration and U-B Pco2. At similar plasma HCO3 levels, BB rats had a much higher fractional water excretion and failed to raise urine pH, urine HCO3 concentration, and U-B Pco2 normally. At a very high plasma HCO3 (>50 meq/liter), BB rats raised urine pH, urine HCO3 concentration, and U-B Pco2 to the same levels seen in normals. CRF rats failed to raise urine pH, urine HCO3, and U-B Pco2 to the same levels seen in normals. At moderately elevated plasma HCO3 levels; at very high plasma HCO3 levels, CRF rats achieved a highly alkaline urine but failed to raise U-B Pco2. Dogs and patients with CRF were also unable to raise urine pH, urine HCO3 concentration, and U-B Pco2 normally at moderately elevated plasma HCO3 levels.

In rats, dogs, and man, U-B Pco2 was directly related to urine HCO3 concentration and inversely related to fractional water excretion. At moderately elevated plasma HCO3 levels, animals with a distal acidification defect failed to raise U-B Pco2; increasing the plasma HCO3 to very high levels resulted in a significant increase in urine HCO3 concentration and U-B Pco2. The observed urinary Pco2 was very close to the Pco2 which would be expected by simple dissolution of a comparable amount of HCO3 in water. These data demonstrate that, in highly alkaline urine, urinary Pco2 is largely determined by concentration of urinary HCO3 and cannot be used as solely indicating distal H+ secretion.

INTRODUCTION

It has been well established that alkalinization of the urine is associated with elevation of urinary carbon dioxide tension (Pco2) in excess of blood Pco2 (1). More recently, it has been suggested that measurement of the urine to blood (U-B) Pco2 gradient in alkaline urine can be used to assess distal hydrogen ion secretion (2-4). We have recently demonstrated that in highly alkaline urine (urine pH > 7.8), U-B Pco2 is not influenced by acute decreases in glomerular filtration rate (GFR), urine flow, bicarbonate excretion, phosphate concentration and excretion, and parathyroid hormone administration (4, 5). The failure of U-B Pco2 to rise with alkalinization of the urine has been taken as evidence of a distal acidification defect (2, 4). Steinmetz et al. (6) indicated, however, that, to

1 Abbreviations used in this paper: BB, Brattleboro (rats); CK, contralateral kidney; CRF, chronic renal failure; GFR, glomerular filtration rate; Pco2, carbon dioxide tension; POK, postobstructed kidney; TF/P, tubular fluid concentration/plasma concentration; Tn/GFR, maximal HCO3 reabsorption/GFR; U-B, urine to blood; V, urine flow.
interpret the meaning of low urinary PCO₂ in distal renal tubular acidosis, one must take into account urinary bicarbonate concentration. Inasmuch as urinary PCO₂ is related to urinary bicarbonate concentration, it is possible that the concentrating defect of patients with distal renal tubular acidosis may account at least in part for the low urinary PCO₂ (6). One observation that remains unexplained is the failure of patients with chronic renal failure (CRF) to raise U-B PCO₂ with alkalinization of urine (7–9). This phenomenon is somewhat surprising in that studies of acid excretion in CRF have demonstrated that net acid excretion per nephron is enhanced in renal failure (10, 11). In the present study we evaluated the factors controlling the formation of urinary PCO₂ in CRF in animals with concentrating defects and(or) acidification defects.

METHODS

Studies in rats

The rats were allowed a normal food and water intake before the day of study. They were anesthetized with 10 mg/100 g Inactin (Promonta, Hamburg, West Germany) given intraperitoneally. Tracheostomy was performed, and one carotid artery and jugular vein were cannulated. The bladder was catheterized through an abdominal incision. Blood pressure was monitored throughout the experiment. At the start of the experiment, 131I-iothalamate diluted in saline 0.75 μCi/ml was infused by an infusion pump at a rate of 0.024 ml/min throughout the course of the experiment as a marker of GFR. A 60-min equilibration period was allowed before any collection was started. Urine samples were collected under mineral oil in preweighed glass vials, and the urine volume was determined gravimetrically. Blood samples were collected from the carotid artery during the midportion of each clearance collection; collections were of a 20-min duration. The following groups of rats were studied:

Group I—normal rats. 10 normal rats, weighing between 200 and 300 g, were infused with 0.9 M NaHCO₃ at a rate of 6 ml/h to achieve a stable plasma HCO₃ level between 30 and 40 meq/liter. Once the plasma concentration had reached the desired level, three to four collections were obtained.

Group II—CRF rats. 10 normal rats, weighing between 200 and 300 g, had the secondary branches of the renal artery dissected and ligated to produce infarction of approximately 80% of the kidney. 1 wk later the contralateral kidney was removed. These rats were studied =10 days later. 0.9 M NaHCO₃ was infused at a rate of 6 ml/h to increase plasma HCO₃ between 30 and 40 meq/liter; three clearance collections were then obtained. The infusion was then increased to 8 ml/h; when the urine pH reached a value >7.8, two to three additional collections were obtained.

Group III—Brattleboro rats. Six Brattleboro (BB) rats, weighing between 125 and 175 g, were infused with 0.9 M NaHCO₃ at a rate of 6 ml/h; when plasma HCO₃ reached a stable level between 30 and 40 meq/liter, two collections were obtained. NaHCO₃ infusion was then increased to 8 ml/h; when the urine pH was >7.8, two to three additional collections were obtained.

Studies in dogs

The dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.); subsequent small doses were administered as needed to maintain light anesthesia. An arterial catheter was used to sample blood. 131I-iothalamate in 0.9% saline (115 μCi/liter) was infused at a rate of 0.6 ml/min as a marker of GFR. An endotracheal tube was inserted and connected to a Bird respirator (Bird Corp., Palm Springs, Calif.); arterial PCO₂ was maintained between 40 and 50 mm Hg by appropriate manipulation of the respirator. An equilibration period of at least 40 min was allowed before starting collections. The following groups of dogs were studied:

Group I—chronic renal failure dogs. Six normal dogs were infused with 0.9 M NaHCO₃ to achieve a stable plasma HCO₃ level between 30 and 40 meq/liter. When this level was achieved, three to four collections were obtained. 1 wk later these dogs were operated on under sterile conditions. The secondary and tertiary branches were dissected and ligated to produce infarction of 80% of the kidney as described by Schultz et al. (12). The contralateral kidney was removed 1 wk later. 7–10 days later these animals underwent the second part of the study using a protocol identical to that used in the first part of the study.

Three additional dogs were also studied. The first part of the study was identical to that described above. In the second part of the study the diseased kidney was studied in the presence of the contralateral kidney.

Group II—lithium-treated dogs. Five normal dogs were treated with LiCl, 3 meq/kg intraperitoneally for 3 days including the day of the study. Urine was collected from bilateral ureteral catheters. NaHCO₃ (0.9 M was infused at a bolus dose of 100 meq followed by 1 ml/min in order to achieve a urine pH >7.8. After a 30-min equilibration period, two to three collections were obtained. NaHCO₃ infusion was then increased to 2–3 ml/min in order to achieve a plasma HCO₃ value >50 meq/liter. The ureteral pressure of one kidney was elevated to 40–60 mm of H₂O to prevent an increase in urine flow. Two to three additional urine and blood samples were then obtained. A previous study from this laboratory has shown that elevation of ureteral pressure does not influence urinary PCO₂ (4).

Group III—unilateral ureteral obstruction. Four normal dogs had one ureter ligated completely under anesthesia. 18–24 h later both ureters were identified and cannulated. After urine flow had become stable, urine collections were started. NaHCO₃ (0.9 M) was infused at a rate of 1 ml/min in order to achieve a urine pH >7.8. Two to three clearance collections were obtained. The infusion of NaHCO₃ was increased to 2–3 ml/min until the plasma HCO₃ levels were >50 meq/liter. Additional urine and blood samples were then obtained.

Studies in humans

Eight patients with CRF were included in this study. This research protocol was approved by the Institutional Review Committee at the University of Illinois and West Side Veterans Administration Hospitals. All patients gave informed consent to participate in the study. The cause of CRF was chronic glomerulonephritis (three patients), nephrosclerosis (four patients), and systemic lupus erythematosus (one patient). Patients with the nephrotic syndrome, obstructive uropathy, chronic pyelonephritis, congestive heart failure, and diabetes were excluded from the study. All patients included were in stable condition at the time of the study. They were ingesting a normal diet containing Urinary Carbon Dioxide Tension 923
were achieved their hospital admission for evaluation for chronic hemodialysis. The patients who were on diuretics had these drugs discontinued for at least 3 days before the study.

The normal subjects included in this study were six healthy subjects (four of the six are authors of this paper) who volunteered for the study. All patients and normal subjects were encouraged to ingest substantial amounts of water 2 h before the study to achieve a good urine flow. 1 h before the study, 10 μCi 1251-iothalamate with 0.1 ml epinephrine was injected subcutaneously. One intravenous catheter was used for infusion of NaHCO₃ in the opposite arm and another intravenous catheter was used to sample blood. Blood for determination of base-line plasma electrolytes and blood gases were collected before starting infusion of NaHCO₃. NaHCO₃ (0.9 M) was given at a bolus dose of 100–150 meq followed by infusion at a rate of 2–3 ml/min. When plasma HCO₃ achieved a stable level above 30 meq/liter, the bladder was emptied completely, and clearance collections were started. Collections were of a 10–30-min duration. Blood and urine were sampled at the midpoint of each collection. No Foley catheter was used in the study, because neither patients nor the normal subjects had any difficulty in emptying the bladder. Three to four collections were obtained in each subject.

Studies in vitro

Four different urine samples from bicarbonate-loaded dogs who had a highly alkaline urine were studied in vitro. After measurement of pH and Pco₂, NaHCO₃ was added to each sample to yield a final concentration of 100 or 200 meq/liter. The solution was stirred thoroughly, and after complete dissolution of the added NaHCO₃, pH and Pco₂ were measured again.

GFR, blood and urinary electrolytes, and statistical analyses were performed as previously described (13). Li levels were measured with a flame photometer. The values shown in the tables are the average of two to three collections. Data are presented as mean±SEM.

RESULTS

Studies in rats

The mean GFR in normal and CRF rats was 3.17 ±0.18 and 0.33±0.06 ml/min, respectively (P < 0.001). BB rats had a mean GFR of 1.45±0.18 ml/min. Urinary Pco₂ in normal, BB, and CRF rats is shown in Table I and Figs. 1–3. At a plasma HCO₃ of 34.8 meq/liter,
normal rats had a highly alkaline urine and significantly elevated urine PCO$_2$ and U-B PCO$_2$ (Table I, Figs. 1 and 2). At comparable plasma HCO$_3$ levels, CRF rats failed to raise urine pH, U-B PCO$_2$, and urine HCO$_3$ concentration to the same level seen in normals.

![Figure 2](image)

**Figure 2** U-B PCO$_2$ is plotted against fractional water excretion, (V/GFR) x 100, for normal, BB, and CRF rats.

Urine flow (V) was significantly lower in CRF than in normal rats; the fraction of filtered water that was excreted, (V/GFR) x 100, was significantly higher in CRF than in normals. At a plasma HCO$_3$ concentration of 56.4 meq/liter, CRF rats raised urine pH to the same level as normal rats; urine HCO$_3$ concentration increased significantly but still remained significantly lower than that of normal rats. U-B PCO$_2$ failed to rise, and (V/GFR) x 100 increased significantly.

At a plasma HCO$_3$ level of 37.2 meq/liter, BB rats also failed to achieve a highly alkaline urine and to

![Figure 3](image)

**Figure 3** U-B PCO$_2$ (left panel) and urine PCO$_2$ (right panel) are plotted against urine HCO$_3$ concentration for normal, CRF, and BB rats.

### Table I

**Urinary rCO$_2$ in Normal, BB and CRF Rats**

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Urine</th>
<th>Ormolality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCO$_3$</td>
<td>rCO$_2$</td>
<td>pH</td>
</tr>
<tr>
<td></td>
<td>meq/liter</td>
<td>mm Hg</td>
<td>mm Hg</td>
</tr>
<tr>
<td>Normal (n = 10)</td>
<td>34.8±1.42</td>
<td>35.0±1.75</td>
<td>7.92±0.02</td>
</tr>
<tr>
<td>CRF (n = 10)</td>
<td>35.5±1.19</td>
<td>41.0±1.17</td>
<td>7.40±0.16</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>NS</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>56.4±2.00</td>
<td>44.0±1.00</td>
<td>7.89±0.03</td>
</tr>
<tr>
<td>BB (n = 6)</td>
<td>37.0±3.36</td>
<td>46.0±1.46</td>
<td>7.11±0.35</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Normal vs. CRF</td>
<td>(A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>(B)</td>
<td></td>
<td></td>
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<tr>
<td>Normal vs. BB</td>
<td>(A)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>NS</td>
<td>0.001</td>
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<tr>
<td></td>
<td>(B)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
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<td></td>
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</tbody>
</table>

A and B refer to values obtained at plasma HCO$_3$ levels (A) between 30 and 40 meq/liter and (B) higher than 50 meq/liter.

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increase U-B Pco₂. At a plasma HCO₃ of 55.8 meq/liter, BB rats increased urine pH, urine HCO₃ concentration, and U-B Pco₂ to the same level seen in normal rats (Figs. 1–3). Observe that urine osmolality increased significantly, but this increase in urine osmolality is achieved at a very high plasma osmolality.

In Figs. 2 and 3, U-B Pco₂ is plotted against (V/GFR) × 100 and urine HCO₃ concentration, respectively. It is clear that U-B Pco₂ is inversely related to V/GFR (y = 37.8 − 0.82x, r = 0.82, P < 0.005), and directly to urine HCO₃ concentration (y = −8.5 + 0.24x, r = 0.88, P < 0.005) (Fig. 3).

**Studies in dogs**

**Normal and CRF dogs.** In CRF urine pH, urine HCO₃ concentration and U-B Pco₂ were significantly lower than in control despite identical plasma HCO₃ levels and similar V (Table II, Fig. 4). Observe, however, that (V/GFR) × 100 was significantly higher in CRF than in normal dogs. U-B Pco₂ was inversely related to (V/GFR) × 100 (Fig. 5) (y = 20.8 − 0.55x, r = 0.72, P < 0.025).

In the presence of a normal contralateral kidney, the diseased kidney also failed to raise U-B Pco₂ (Fig. 6). Observe, however, that the diseased kidney achieved a urine pH of the same magnitude as that of the normal kidney. Urine HCO₃ concentration was also decreased in the diseased kidney.

**Li-treated dogs.** Li-treated dogs developed hyperchloremic metabolic acidosis with an alkaline urine (Table III). At a plasma HCO₃ of 37.5 meq/liter, Li-treated dogs had a highly alkaline urine, but U-B Pco₂ was only 14.0 ± 2.80 mm Hg, which is significantly lower than that of normal dogs (Tables II and IV). Increasing the plasma HCO₃ levels to 62.8 meq/liter, with simultaneous elevation of the ureteral pressure in one kidney, resulted in a significant increase in urine osmolality, urine HCO₃ concentration, and in U-B Pco₂. In the contralateral kidney, urine HCO₃ concentration, urine osmolality, and U-B Pco₂ failed to rise (Table IV).

**Unilateral ureteral obstruction.** After the release of unilateral obstruction, the post-obstructed kidney (POK) had a significantly higher baseline urine pH than the contralateral kidney (CK) (Table V). GFR and urine osmolality were also lower in the POK than in the CK. At a plasma level of 42.8 meq/liter,
U-B Pco2 and urine HCO3 concentration were significantly lower in the POK than in the CK. Elevation of plasma HCO3 to 61 meq/liter resulted in a significant increase in urine HCO3 concentration in the POK with concomitant elevation of U-B Pco2. In the CK, urine HCO3 concentration and U-B Pco2 failed to rise further. Observe that despite a significant rise in U-B Pco2 and urine HCO3 concentration in the POK, these values are still significantly lower than in the CK.

In Fig. 7 U-B Pco2 is plotted against urine HCO3 concentration for normal, CRF, and Li-treated dogs and for dogs with unilateral ureteral obstruction. It can be seen that, at comparable levels of urine HCO3 concentration, the values of U-B Pco2 for normal dogs and dogs with a distal acidification defect overlap. Observe that U-B Pco2 was linearly related to urine HCO3 concentration \( y = 1.5 + 0.14x, \ r = 0.88, \ P < 0.005 \).

Studies in humans

The mean GFR in normal subjects was 140.7±9.58 ml/min and that of CRF patients was 18.7±9.54 ml/min \( (P < 0.001) \). U-B Pco2 in the normal subjects and in patients with CRF are shown in Table VI and Fig. 8. Observe that subjects L. N., J. A., and P. C. failed to achieve a highly alkaline urine and to raise U-B Pco2 despite high plasma bicarbonate levels; these subjects were excreting a high fraction of the filtered water and, therefore, had a low urinary HCO3 concentration. Subjects J. S., G. A., and M. R. excreted a much lower fraction of filtered water and were able to raise urine HCO3 concentration. All patients with CRF excreted a high fraction of the filtered water and thus failed to raise urine HCO3 concentration and U-B Pco2. U-B Pco2 was inversely related to (V/GFR) \( \times 100 \) \( (y = 26.7 - 0.54x, \ r = 0.56, \ P < 0.05) \) (Fig. 8).

Studies in vitro

Table VII shows that the addition of NaHCO3 to highly alkaline dog urine in vitro uniformly results in a marked elevation of Pco2 while the pH remains constant.

DISCUSSION

The demonstration that the urinary Pco2 is considerably greater than that of blood during bicarbonate loading has been felt to signify the presence of an intact distal acidification mechanism \( (2-4) \). Almost totally ignored has been the fact that increasing bicarbonate concentration in any aqueous fluid results in a concomitant increase in Pco2 \( (14, 15) \). This phenomenon relates solely to the bicarbonate concentration and does not require the addition of hydrogen ion.

To study the role of urinary concentration in the formation of urinary Pco2, we measured U-B Pco2 in animals with enhanced H+ secretion and decreased concentrating ability (renal failure), normal H+ secretion and decreased concentrating ability (BB rats) and in animals with both impaired H+ secretion and impaired concentrating ability (Li-treated dogs and POK). Dissolving HCO3 in water results in elevation of Pco2; this increase in Pco2 is proportionate to HCO3 concentration and is secondary to the equilibrium of HCO3 according to the following reaction: \( \text{HCO}_3^- + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{CO}_3^- \) \( (14, 15) \). We have previously demonstrated that urinary Pco2 is linearly related to urinary HCO3 concentration \( (4) \). The failure of urinary HCO3 to rise may, thus, be the critical factor responsible for the low urinary Pco2 in renal tubular acidosis. Steinmetz et al. \( (6) \) have contended that, to prove that urinary Pco2 is diminished in renal

<table>
<thead>
<tr>
<th>Na</th>
<th>K</th>
<th>Cl</th>
<th>HCO3</th>
<th>pH</th>
<th>Pco2</th>
<th>Li</th>
<th>Urine pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>meq/liter</td>
<td>meq/liter</td>
<td>meq/liter</td>
<td>meq/liter</td>
<td>mm Hg</td>
<td>mm Hg</td>
<td>meq/liter</td>
<td>meq/liter</td>
</tr>
<tr>
<td>139.1±2.26</td>
<td>4.0±0.27</td>
<td>113.0±2.39</td>
<td>13.8±1.32</td>
<td>7.24±0.03</td>
<td>32.9±2.30</td>
<td>4.6±0.25</td>
<td>7.40±0.17</td>
</tr>
</tbody>
</table>

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TABLE IV
Urinary Pco2 in Li-Treated Dogs

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GFR</td>
<td>pH</td>
</tr>
<tr>
<td></td>
<td>ml/min</td>
<td>V</td>
</tr>
<tr>
<td>EK*</td>
<td>21.5±2.05</td>
<td>6.6±1.46</td>
</tr>
<tr>
<td>C1</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>UF</td>
<td>11.5±2.13</td>
<td>2.0±0.48</td>
</tr>
</tbody>
</table>

* Experimental kidney subjected to elevation of ureteral pressure (UP).
† First control period.
‡ Second control period.
§ Measurement of U-B Pco2 in the CK available only in four dogs.

Tubular acidosis as compared to normal subjects, one must determine urinary Pco2 in patients with distal renal tubular acidosis at high urinary HCO3 levels. They pointed out that, in the study of Halperin et al. (2), urinary HCO3 concentrations were lower in the patients with distal renal tubular acidosis than in the controls. This criticism seems to be applicable to all papers dealing with urinary Pco2 in the presence of a distal acidification defect (2, 4, 16–18).

In Figs. 9–11 the interrelationship of urinary HCO3 concentration, urinary Pco2, and fractional water excretion is examined in normal, BB, and CRF rats. Fig. 9 shows that, at a plasma HCO3 of 35 meq/liter, normal rats have a HCO3 concentration of 17 meq/liter in the end of the proximal tubule; these calculations are made on the basis of the observed GFR, plasma HCO3, and Tm/GFR in vivo and on the assumption that there is 60% fluid reabsorption in the proximal tubule (19). Further water reabsorption between the end of the proximal tubule and late distal tubule will result in an increase in HCO3 concentration to 70 meq/liter in the distal tubule, a value very close to that observed by Vieira and Malnic (20). Additional removal of water in the collecting duct to yield a fractional water excretion similar to that seen in vivo will result in a HCO3 concentration very similar to that seen in vivo. This HCO3 concentration yields 74.5 mm Hg Pco2 in vitro (see below), a value very close to that observed in vivo (Table I). Fig. 10 shows similar calculations for BB rats. At a plasma HCO3 of 35 meq/liter, BB rats had a fractional water excretion of 10% and based on Tm/GFR of 33 meq/liter, BB rats were able to raise urine HCO3 only to 20 meq/liter, a value close to that observed in vivo (Table I). The theoretical calculations presented in Fig. 10 show that all that is required to raise urinary HCO3 concentration

TABLE V
Urinary Pco2 in the POK

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GFR</td>
<td>pH</td>
</tr>
<tr>
<td></td>
<td>ml/min</td>
<td>(V/GFR) x 100</td>
</tr>
<tr>
<td>EK*</td>
<td>21.5±2.05</td>
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</tr>
<tr>
<td>UF</td>
<td>11.5±2.13</td>
<td>2.0±0.48</td>
</tr>
</tbody>
</table>

A and B refers to values obtained at two different plasma HCO3 levels.
treated dogs, concentration in FIGURE 7 U-B

However, an failed levels
dicted from meq/liter.
to the levels observed is a plasma concentration of 55 meq/liter.
In BB rats, at a highly alkaline urinary pH, the observed urinary PCO₂ is also very close to that predicted from the concentration of HCO₃. In CRF, however, an increase in plasma HCO₃ to very high levels failed to increase urinary HCO₃, because the fraction of the excreted filtered water also increased twofold. It is obvious from the calculations in Fig. 11 that, in CRF, a plasma HCO₃ in excess of 200 meq/liter would be necessary to attain a urine HCO₃ similar to that seen in normals. In CRF and in BB rats, at a lower urinary pH, the observed urinary PCO₂ is higher than the calculated PCO₂. Two factors may account for this phenomenon. First, these calculations may be valid only for highly alkaline urine, and second, the fact that the observed PCO₂ was higher than the calculated PCO₂ may indicate the presence of H⁺ secretion.

These observations demonstrate convincingly that urinary PCO₂ is critically dependent on urinary HCO₃ concentration. The present data also demonstrate that, in the presence of a distal acidification defect, elevation of urinary HCO₃ concentration accomplished either by an increase of plasma HCO₃ in the POK or by an increase in plasma HCO₃ and elevation of ureteral pressure in Li-treated dogs results in a significant increase in urinary pCO₂. Indeed, in the Li-treated dogs, the urinary PCO₂ reached the same level seen in normal dogs despite the fact that these animals had unequivocal distal renal tubular acidosis. These observations are in total agreement with the suggestion of Steinmetz et al. (6) that, in distal renal tubular acidosis, urinary PCO₂ may rise normally if urinary HCO₃ concentration is high enough.

These observations suggest that the ability to remove water in the collecting duct and therefore raise urinary HCO₃ concentration is essential for the formation of a high urinary PCO₂ in a highly alkaline urine.

**TABLE VI**

*Urine pCO₂ in CRF Patients and Normal Subjects*

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Urine</th>
<th>HCO₃</th>
<th>pCO₂</th>
<th>pH</th>
<th>pCO₂</th>
<th>U-B pCO₂</th>
<th>HCO₂⁻</th>
<th>V</th>
<th>(V/GFR) × 100</th>
<th>PO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HCO₃</td>
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<td>mm Hg</td>
<td>meq/liter</td>
<td>mm Hg</td>
<td>mm Hg</td>
<td>mm Hg</td>
<td>meq/liter</td>
<td>m/min</td>
<td>%</td>
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<tr>
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</table>

*Urinary Carbon Dioxide Tension*
It must be emphasized that our study, as do all previous such studies, measures urinary pCO₂ in urine that has left the kidney. The PCO₂ measured in these urine samples may or may not reflect the PCO₂ in the collecting duct. Two possibilities concerning the PCO₂ in the collecting duct immediately present themselves. First, as water is abstracted from the collecting duct, bicarbonate concentration rises, and PCO₂ likewise increases. Assuming that the collecting duct is highly permeable to CO₂ (an assumption that has been made by almost all workers in the field), then CO₂ will diffuse across the collecting duct. This may result in an equilibration of PCO₂ across the collecting duct. Thus, the PCO₂ at the end of the collecting duct will be 40 mm Hg in the normal animal. However, as soon as the urine leaves the kidney, the high bicarbonate concentration in postpapillary urine will establish a new equilibrium resulting in an elevation of PCO₂. The second possibility is that, although CO₂ freely diffuses across the collecting duct, it is immediately regenerated by the decomposition of bicarbonate.

The relatively brief exposure of urine to the collecting duct, <20 s (21), combined with the continuing elevation of bicarbonate concentration, will make it impossible to dissipate the CO₂ gradient in the collecting duct owing to the continuous generation of H₂CO₃ from bicarbonate. This hypothetical series of events results in a situation in which the PCO₂ in the collecting duct will be considerably greater than that of vasa recta blood. The only measurement of PCO₂ in the collecting duct and vasa recta supports this view (22). The difficulty with the interpretation of this observation is that the PCO₂ is calculated from pH measurements in the collecting duct and vasa recta. The issue will be resolved only when direct measurements of collecting duct PCO₂ are made using a carbon dioxide electrode.

Observe, however, that regardless of which of these two hypotheses is correct, indeed regardless of whether both are incorrect, urinary PCO₂, measured in the final

![Figure 8](image_url)

**FIGURE 8** U-B Pco₂ (left upper panel), urine pH (right upper panel), and urine HCO₃ concentration (lower panel) are plotted against fractional water excretion, (V/GRF) x 100, in normal subjects, and in CRF patients.

### Table VII

**Addition of NaHCO₃ to Highly Alkaline Dog Urine in Vitro**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial pH</th>
<th>Initial pCO₂</th>
<th>Addition of NaHCO₃ (meq/liter)</th>
<th>Final pH</th>
<th>Final pCO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.02</td>
<td>60</td>
<td>100</td>
<td>8.03</td>
<td>86</td>
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<tr>
<td>2</td>
<td>8.08</td>
<td>58</td>
<td>100</td>
<td>8.08</td>
<td>80</td>
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<td></td>
<td></td>
<td></td>
<td>200</td>
<td>8.08</td>
<td>115</td>
</tr>
<tr>
<td>3</td>
<td>8.03</td>
<td>78</td>
<td>200</td>
<td>8.00</td>
<td>112</td>
</tr>
<tr>
<td>4</td>
<td>7.98</td>
<td>71</td>
<td>200</td>
<td>8.08</td>
<td>114</td>
</tr>
</tbody>
</table>

The measurement of PCO₂ was done at 1 min after addition of NaHCO₃ to the urine samples.
NORMAL RATS

FILTERED - REABSORBED
105 - 84 = 21 μeq/liter
60% H2O REABSORPTION
3.0 - 1.8 = 1.2 ml/min
GFR : 3.0 ml/min
PHCO3 : 35 meq/liter
Tm/GFR : 28 meq/liter GFR
H2O REABSORPTION (0.9 ml/min)
HCO3 CONCN LEAVING PT
17.5 meq/liter
HCO3 REACHING DN
21 μeq/min
H2O : 0.3 ml/min
HCO3 CONCENTRATION
70 meq/liter
H2O REABSORPTION (0.18 ml/min)
URINE HCO3 : 175 meq/liter
V : 0.12 ml/min
(V/VGFR) x 100 = 4%
THEORETICAL PCO2
74.5 mmHg

FIGURE 9  Bicarbonate concentration in the proximal tubule (PT), distal nephron (DN), and in the final urine of normal rats. Calculations were based on the GFR, plasma HCO3, Tm/GFR, and (V/GFR) x 100 observed in vivo. Fluid reabsorption in the PT was based on TF/P inulin ratio of reference 19. For simplicity of calculation all the reabsorption of HCO3 was assumed to occur in the PT. 30% of fluid filtered is reabsorbed between the level of the PT and the end of DN. Further water removal in the collecting duct to yield a fractional water excretion similar to that seen in vivo results in a HCO3 concentration very close to that observed in vivo. The theoretical PCO2 is the PCO2 calculated from the HCO3 concentration (see text).

urine, must be influenced to a major degree by the bicarbonate concentration. This point is emphasized by the in vitro addition of sodium bicarbonate to highly alkaline dog urine. The calculation of PCO2 resulting from the reaction of bicarbonate in aqueous solution to form carbonic acid (described below) cannot be used to calculate with great accuracy the expected PCO2 in the in vitro studies. This is so because the addition of sodium bicarbonate to these urines renders them so concentrated that their behavior varies markedly from that of an ideal solution. The information necessary to calculate theoretical PCO2 that will accurately predict that observed in the presence of such highly concentrated urinary sodium bicarbonate solution is not available.

The failure of urinary PCO2 to rise in renal tubular acidosis may thus be due, at least in part, to the concentrating defect present in this condition. Renal tubular acidosis, either primary or secondary, is usually associated with a defect in urinary concentration. In the case of primary distal renal tubular acidosis, the concentrating defect has been attributed to potassium depletion and nephrocalcinosis (23, 24). Virtually every cause of clinical or experimental distal renal tubular acidosis is associated with a concentrating defect.

The high urinary PCO2 of alkaline urine has been attributed to several mechanisms which include delayed dehydration of carbonic acid (6), mixing of acid and alkaline urine, trapping of medullary CO2, and restriction to diffusion of CO2 across the collecting duct (1, 4, 25, 26). The present observations suggest another mechanism whereby the urinary PCO2 can be raised. When NaHCO3 is dissolved in water, the following reactions take place (14):

\[ 2\text{H}_2\text{O} = \text{H}_2\text{O}^+ + \text{OH}^- \]

\[ K_w = 1.01 \times 10^{-14} = [\text{H}_2\text{O}'][\text{OH}^-] \] (1)

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FIGURE 10  Bicarbonate concentration in the proximal tubule, distal nephron, and final urine of BB rats at a plasma HCO₃ of 35 meq/liter (labeled A) and at a plasma HCO₃ of 55 meq/liter (labeled B). Calculations are based on the GFR, plasma levels of HCO₃, Tm/GFR, and (V/GFR) x 100 observed in vivo. Fluid reabsorption in the proximal tubule in BB rats was assumed to be 80% of the filtered fluid. Observe that HCO₃ concentrations in the final urine are very close to the observed values.

\[
\begin{align*}
    \text{HCO}_3^- + \text{H}_2\text{O} & \rightleftharpoons \text{H}_3\text{O}^+ + \text{CO}_3^- \\
    K_2 &= 6.0 \times 10^{-11} = \frac{[\text{H}_3\text{O}^+][\text{CO}_3^-]}{[\text{HCO}_3^-]} \quad (2)
\end{align*}
\]

\[
\begin{align*}
    \text{HCO}_3^- + \text{H}_2\text{O} & \rightleftharpoons \text{H}_2\text{CO}_3 + \text{OH}^- \\
    K_b &= 3.1 \times 10^{-8} = \frac{[\text{H}_2\text{CO}_3][\text{OH}^-]}{[\text{HCO}_3^-]} \quad (3)
\end{align*}
\]

\[
\begin{align*}
    \text{CO}_2 + \text{H}_2\text{O} & \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{H}_3\text{O}^+ + \text{HCO}_3^- \\
    K_1 &= 3.5 \times 10^{-7} = \frac{[\text{H}_3\text{O}^+][\text{HCO}_3^-]}{[\text{CO}_2]} \quad (4)
\end{align*}
\]

\[
\begin{align*}
    \text{HCO}_3^- + \text{H}_2\text{O} & \rightleftharpoons \text{H}_2\text{CO}_3 + \text{CO}_3^- \\
    \text{CO}_2 + \text{H}_2\text{O} \\
    K &= \frac{K_2}{K_1} = \frac{[\text{CO}_2][\text{CO}_3^-]}{[\text{HCO}_3^-]^2} \quad (5)
\end{align*}
\]

The [Pco₂] of an ideal solution at 25°C and zero ionic strength containing 0.3 M NaHCO₃ can be calculated in the following way from the fifth equation above:

\[
K = \frac{K_2}{K_1} = 1.71 \times 10^{-4} = \frac{[\text{CO}_2][\text{CO}_3^-]}{[\text{HCO}_3^-]^2};
\]

let \( x = [\text{CO}_3^-] = [\text{CO}_3^+] \); therefore,

\[
1.71 \times 10^{-4} = \frac{x^2}{[0.3 - 2x]^2} \quad (or) \quad 1.31 \times 10^{-2} = \frac{x}{0.3 - 2x};
\]

therefore, \( x = 0.00383 \) M \( \text{CO}_2 = \text{CO}_3^+ \); therefore, \( \text{Pco}_2 = 3.83 \text{ mM of CO}_2 / 0.03 = 127.7 \text{ mm Hg} \).

The above calculations are applicable only to an ideal solution at 25°C. Inasmuch as urine is not an ideal solution these calculations would be at best an approximation of the Pco₂ resulting from a solution of HCO₃ in urine. If one takes into account the effects of temperature (37°C) and the ionic strength of blood,
Figure 11 Bicarbonate concentration in the proximal tubule, distal nephron, and final urine of CRF rats at two different levels of plasma HCO₃ (labeled A and B). Calculations based on GFR, plasma HCO₃ levels, Tm/GFR, and (V/GFR) × 100 observed in vivo. Fluid reabsorption in PT of CRF was assumed to be 50%, calculation based on TF/P inulin ratio of reference 19. Observe that urine HCO₃ concentrations are very similar to those observed in vivo. Theoretical PCO₂ values are lower than those observed in vivo.

$K_1 = 7.9 \times 10^{-7}$, and $K_2 = 1.66 \times 10^{-10}$, and therefore, $K = 2.1 \times 10^{-4}$ (15). Using this new value for $K$, the PCO₂ of 0.3 M solution of NaHCO₃ is 141 mm Hg.

These observations demonstrate that the high urinary PCO₂ of alkaline urine as measured in vitro is critically dependent on the presence of a high urinary HCO₃ concentration which in equilibrium with water will result in a high urinary PCO₂. As can be seen from Figs. 10 and 11, the calculated PCO₂ values of normal rats and of BB rats with a high urinary pH are very close to those found in vivo. These observations suggest that urinary pCO₂ of highly alkaline urine can be largely explained as consequence of a rise in urinary HCO₃ concentration. From the equation $H^+ + HCO₃ ⇌ H₂CO₃ ⇌ CO₂ + H₂O$, it can be seen that either an increase in hydrogen ion concentration or an increase in bicarbonate concentration will shift the equilibrium of the reaction to the right and result in an elevation of urinary PCO₂. It is likely that in highly alkaline urine, urinary HCO₃ concentration plays the more important role in the elevation of urinary PCO₂. It is impossible at present to determine precisely how much of each of these factors contribute to the elevation of urinary PCO₂. It is very likely, however, that the difference between the observed PCO₂ and the expected PCO₂ is determined by H⁺ secretion.

In moderately alkaline urine, however, the achievement of a high urinary PCO₂ seems to be due mainly to hydrogen ion secretion because the amount of bicarbonate present is clearly not sufficient to raise urinary PCO₂ (4). We have previously demonstrated

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that, at comparable urinary bicarbonate concentrations, urinary PCO₂ was significantly lower in dogs with renal tubular acidosis than in normal dogs. In moderately alkaline urine, urinary PCO₂ is mainly determined by urinary phosphate concentration (4). Hydrogen ion secretion in the collecting duct will result in a disequilibrium pH and titration of phosphate according to the following reaction: \( H^+ + HPO_4^{2-} \rightleftharpoons H_2PO_4^- \). As the tubular fluid proceeds towards equilibrium, the pH will rise, and H₂PO₄ will react with HCO₃⁻ thus increasing the Pco₂. Previous studies have suggested that it is possible to increase urinary PCO₂ in man undergoing water diuresis (27, 28). In these experiments urine bicarbonate and phosphate concentrations were very low, and it is difficult to explain the mechanism responsible for the elevation of urinary PCO₂.

The observation that carbonic anhydrase administration lowers urinary PCO₂ to the level of blood PCO₂ whereas urinary bicarbonate concentration remains unchanged (29) could be used as an argument against the thesis that urinary bicarbonate concentration is the main determinant of urinary PCO₂ in highly alkaline urine. The dissipation of the U-B PCO₂ gradient after carbonic anhydrase administration has been interpreted as indicating the existence of carbonic acid off of equilibrium (acid disequilibrium pH) in the distal nephron (29, 30). The addition of carbonic anhydrase either to a solution of bicarbonate or to urine with a high PCO₂ (in vitro) lowers the PCO₂ of these solutions to very low levels (31). When the solutions are kept under oil, carbonic anhydrase fails to lower the PCO₂. Obviously there cannot be a disequilibrium pH in a solution of bicarbonate, and the mechanism whereby carbonic anhydrase lowers the PCO₂ of this solution must lie elsewhere. It is possible that carbonic anhydrase, by accelerating the dehydration of carbonic acid, raises the PCO₂ at the interface of the liquid and the air and therefore favors diffusion of CO₂ into air; there is then a shift of the equilibrium of the following reaction: \( H^+ + HCO_3^- \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 \) to the right with a consequent elevation of the pH of the solution because of the continuing loss of CO₂. This explanation is supported by the fact that carbonic anhydrase fails to lower PCO₂ appreciably when the sample was kept under oil. Thus, the previous experiments with infusion carbonic anhydrase can be used neither to support the existence of a disequilibrium pH in the collecting duct nor to indicate that the mechanism of elevation of the urinary PCO₂ is due to delayed dehydration of carbonic acid. The observations do not exclude the existence of a disequilibrium pH in the distal nephron; the demonstration of such a phenomenon must use evidence other than that derived from carbonic anhydrase administration.

In conclusion, in highly alkaline urine, urinary PCO₂ is critically dependent on urinary bicarbonate concentration. The failure of urinary bicarbonate concentration to rise impairs the increase in urinary PCO₂, even though hydrogen ion secretion is either normal or enhanced, e.g., diabetes insipidus and CRF. The urinary PCO₂ found in highly alkaline urine is very close to that expected from the behavior of a similar solution of bicarbonate in vitro. These data suggest that, in highly alkaline urine, urinary PCO₂ largely reflects urinary concentration.

ACKNOWLEDGMENTS

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