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

JOSE A. L. ARRUDA, LUIZ 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 (P_{CO_2}) 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 P_{CO_2} is that dissolution of HCO_3 in water results in elevation of P_{CO_2} which is directly proportional to the HCO_3 concentration. To investigate the interrelationship of urinary HCO_3 and urinary acidification, we measured U-B P_{CO_2} 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 HCO_3 levels (30–40 meq/liter), normal rats achieved a highly alkaline urine (urine pH > 7.8) and raised urine HCO_3 concentration and U-B P_{CO_2}. At similar plasma HCO_3 levels, BB rats had a much higher fractional water excretion and failed to raise urine pH, urine HCO_3 concentration, and U-B P_{CO_2} normally. At a very high plasma HCO_3 (>50 meq/liter), BB rats raised urine pH, urine HCO_3 concentration, and U-B P_{CO_2} to the same levels seen in normals. CRF rats failed to raise urine pH, urine HCO_3, and U-B P_{CO_2} normally at moderately elevated plasma HCO_3 levels; at very high plasma HCO_3 levels, CRF rats achieved a highly alkaline urine but failed to raise U-B P_{CO_2}. Dogs and patients with CRF were also unable to raise urine pH, urine HCO_3 concentration, and U-B P_{CO_2} normally at moderately elevated plasma HCO_3 levels. In rats, dogs, and man, U-B P_{CO_2} was directly related to urine HCO_3 concentration and inversely related to fractional water excretion. At moderately elevated plasma HCO_3 levels, animals with a distal acidification defect failed to raise U-B P_{CO_2}; increasing the plasma HCO_3 to very high levels resulted in a significant increase in urine HCO_3 concentration and U-B P_{CO_2}. The observed urinary P_{CO_2} was very close to the P_{CO_2} which would be expected by simple dissolution of a comparable amount of HCO_3 in water. These data demonstrate that, in highly alkaline urine, urinary P_{CO_2} is largely determined by concentration of urinary HCO_3 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 (P_{CO_2})[1] in excess of blood P_{CO_2} (1). More recently, it has been suggested that measurement of the urine to blood (U-B) P_{CO_2} 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 P_{CO_2} 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 P_{CO_2} 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

Dr. Arruda is a Research Associate of the Veterans Administration West Side Hospital in Chicago, Ill.

Received for publication 7 February 1977 and in revised form 25 May 1977.

[1] *Abbreviations used in this paper*: BB, Brattleboro (rats); CK, contralateral kidney; CRF, chronic renal failure; GFR, glomerular filtration rate; P_{CO_2}, carbon dioxide tension; POK, postobstructed kidney; TF/P, tubular fluid concentration/plasma concentration; Tm/GFR, maximal HCO_3 reabsorption/GFR; U-B, urine to blood; V, urine flow.

interpret the meaning of low urinary P_{CO_2} in distal renal tubular acidosis, one must take into account urinary bicarbonate concentration. Inasmuch as urinary P_{CO_2} 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 P_{CO_2} (6). One observation that remains unexplained is the failure of patients with chronic renal failure (CRF) to raise U-B P_{CO_2} 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 P_{CO_2} 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, ^{125}I-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 $NaHCO_3$ at a rate of ≅6 ml/h to achieve a stable plasma HCO_3 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 $NaHCO_3$ was infused at a rate of 6 ml/h to increase plasma HCO_3 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 $NaHCO_3$ at a rate of ≅6 ml/h; when plasma HCO_3 reached a stable level between 30 and 40 meq/liter, two collections were obtained. $NaHCO_3$ 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. ^{125}I-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 P_{CO_2} 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 $NaHCO_3$ to achieve a stable plasma HCO_3 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 Schultze 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. $NaHCO_3$ (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. $NaHCO_3$ infusion was then increased to 2–3 ml/min in order to achieve a plasma HCO_3 value >50 meq/liter. The ureteral pressure of one kidney was elevated to 40–60 mm of H_2O 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 P_{CO_2} (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. $NaHCO_3$ (0.9 M) was infused at a rate of 1 ml/min to achieve a urine pH > 7.8. Two to three clearance collections were obtained. The infusion of $NaHCO_3$ was increased to 2–3 ml/min until the plasma HCO_3 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

≅60–100 meq of NaCl/day and were studied at the time of 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 ^{125}I-iothalamate with 0.1 ml epinephrine was injected subcutaneously. One intravenous catheter was used for infusion of $NaHCO_3$; in the opposite arm 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 $NaHCO_3$. $NaHCO_3$ (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 HCO_3 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 P_{CO_2}, $NaHCO_3$, 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 $NaHCO_3$, pH and P_{CO_2} 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 P_{CO_2} in normal, BB, and CRF rats is shown in Table I and Figs. 1–3. At a plasma HCO_3 of 34.8 meq/liter,

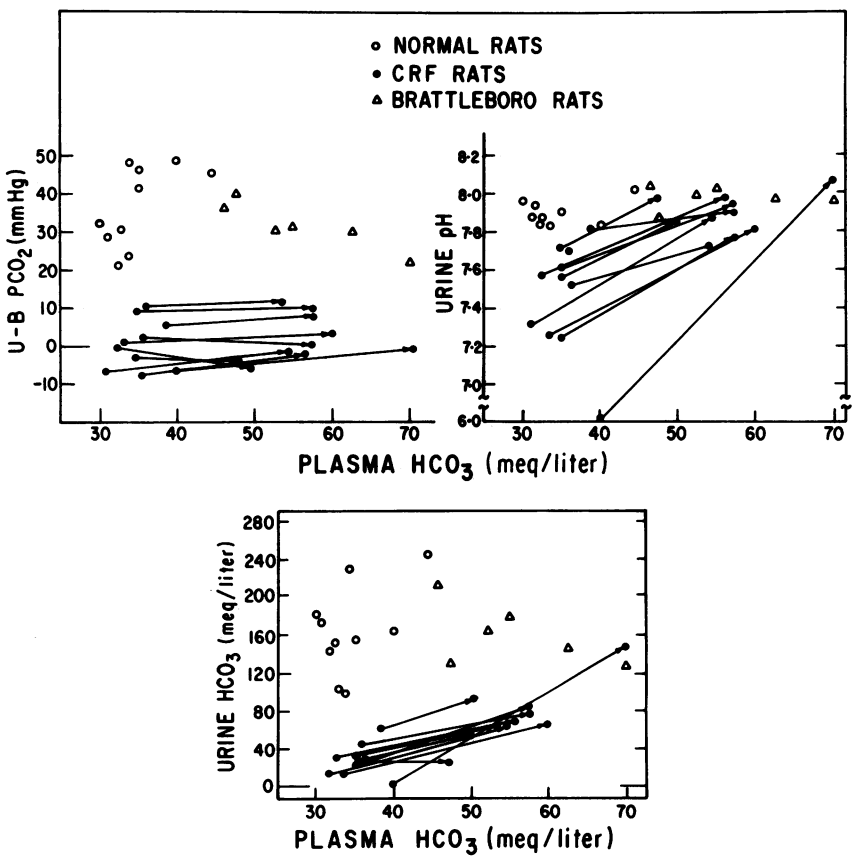

FIGURE 1 Left upper panel: U-B P_{CO_2} is plotted against plasma HCO_3 in normal rats, (○), BB rats (△), and CRF rats (●). The values of CRF rats are shown at moderately elevated plasma HCO_3 levels (30–40 meq/liter) and at very high plasma HCO_3 levels (>60 meq/liter). Right upper panel: urine pH is plotted against plasma HCO_3. Lower panel: urine HCO_3 concentration is plotted against plasma HCO_3.

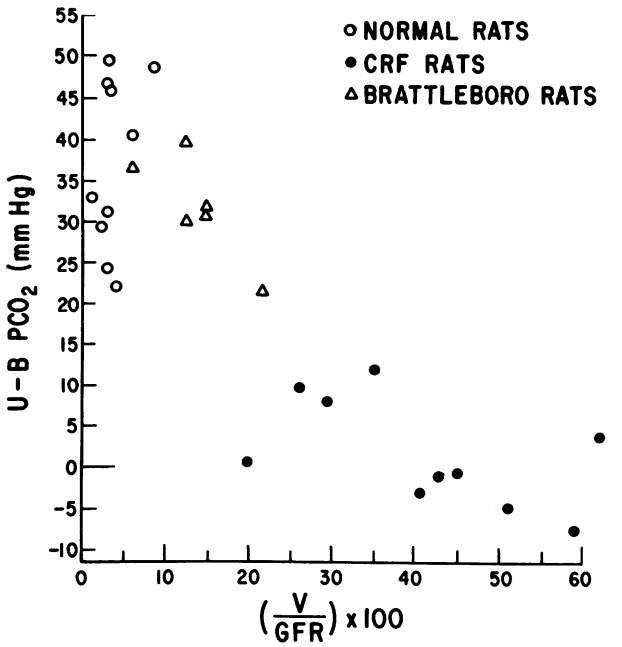

FIGURE 2 U-B P_{CO_2} is plotted against fractional water excretion, (V/GFR) × 100, for normal, BB, and CRF rats.

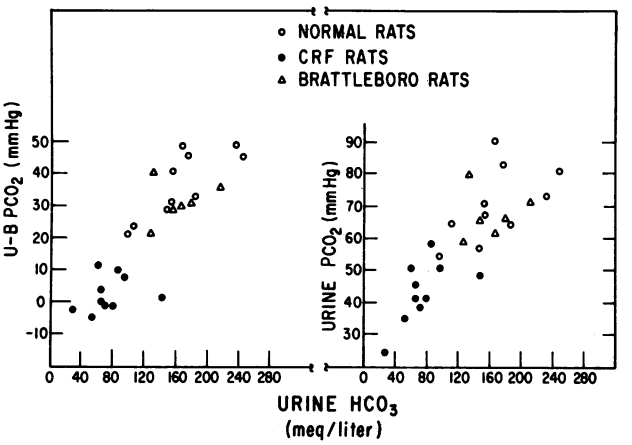

FIGURE 3 U-B P_{CO_2} (left panel) and urine P_{CO_2} (right panel) are plotted against urine HCO_3 concentration for normal, CRF, and BB rats.

normal rats had a highly alkaline urine and significantly elevated urine P_{CO_2} and U-B P_{CO_2} (Table I, Figs. 1 and 2). At comparable plasma HCO_3 levels, CRF rats failed to raise urine pH, U-B P_{CO_2}, and urine HCO_3 concentration to the same level seen in normals. Urine flow (V) was significantly lower in CRF than in normal rats; the fraction of filtered water that was excreted, (V/GFR) × 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 P_{CO_2} failed to rise, and (V/GFR) × 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

TABLE I
Urine PCO_2 in Normal, BB and CRF Rats

	Plasma		Urine						Osmolality	
	HCO_3	PCO_2	pH	PCO_2	U-B PCO_2	HCO_3	V	(V/GFR) × 100	Plasma	Urine
	meq/liter	*mm Hg*		*mm Hg*		*meq/liter*	*μl/min*	*%*	*mosmol/kg H_2O*	
Normal (n = 10)	34.8±1.42	35.0±1.75	7.92±0.02	73.0±3.69	37.3±3.31	168.3±15.12	126.0±17.80	4.0±0.67		
CRF (n = 10)										
A	35.5±1.19	41.0±1.17	7.40±0.16	41.5±2.54	0.5±2.09	27.8±5.60	62.4±16.56	20.2±3.31		
P <	0.001	NS	0.05	NS	NS	0.01	0.01	0.01		
B	56.4±2.00	44.0±1.00	7.89±0.03	44.5±2.85	0.5±1.82	74.0±10.85	133.2±29.10	41.0±4.47		
BB (n = 6)										
A	37.0±3.36	46.0±1.46	7.11±0.35	35.9±6.31	−10.1±7.39	27.0±11.07	160.0±47.32	9.3±2.21	288.0±4.15	177.0±12.64
P <	0.001	0.05	0.05	0.001	0.001	0.001	NS	NS	0.01	0.01
B	55.8±3.79	37.2±2.06	7.98±0.03	68.0±3.21	31.8±2.49	163.0±13.79	189.0±26.50	13.8±2.15	399.0±9.88	390.0±24.20
Normal vs. CRF										
(A)										
P <	NS	0.01	0.001	0.001	0.001	0.001	0.02	0.001		
(B)										
P <	0.001	0.01	NS	0.001	0.001	0.001	NS	0.001		
Normal vs. BB										
(A)										
P <	NS	0.001	0.01	0.001	0.001	0.001	NS	0.02		
(B)										
P <	NS	NS	NS	NS	NS	NS	NS	0.001		

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.

TABLE II
Urine PCO_2 in Dogs before and after Induction of CRF

	GFR	V	(V/GFR) × 100	Plasma		Urine				
				HCO_3	PCO_2	pH	PCO_2	U-B PCO_2	HCO_3	PO_4
	ml/min		*%*	*meq/liter*	*mm Hg*		*mm Hg*		*meq/liter*	*mmol/liter*
Control (n = 6)	46.40±3.42	1.50±0.71	1.70±0.29	31.30±0.30	43.30±1.09	8.02±0.02	67.30±1.30	24.00±1.29	205.80±7.91	26.70±4.87
P <	0.001	NS	0.001	NS	NS	0.001	0.001	0.001	0.001	0.02
CRF (n = 6)	7.00±1.93	0.90±0.12	20.20±6.53	31.30±0.44	41.20±1.60	6.85±0.09	44.70±2.32	3.50±1.84	14.20±12.36	14.20±4.06

increase U-B P_{CO_2}. At a plasma HCO_3 of 55.8 meq/liter, BB rats increased urine pH, urine HCO_3 concentration, and U-B P_{CO_2} 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 P_{CO_2} is plotted against (V/GFR) × 100 and urine HCO_3 concentration, respectively. It is clear that U-B P_{CO_2} is inversely related to V/GFR ($y = 37.8 - 0.82x$, $r = 0.82$, $P < 0.005$), and directly to urine HCO_3 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 HCO_3 concentration and U-B P_{CO_2} were significantly lower than in control despite identical plasma HCO_3 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 P_{CO_2} 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 P_{CO_2} (Fig. 6). Observe, however, that the diseased kidney achieved a urine pH of the same magnitude as that of the normal kidney. Urine HCO_3 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 HCO_3 of 37.5 meq/liter, Li-treated dogs had a highly alkaline urine, but U-B P_{CO_2} was only 14.0±2.80 mm Hg, which is significantly lower than that of normal dogs (Tables II and IV). Increasing the plasma HCO_3 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 HCO_3 concentration, and in U-B P_{CO_2}. In the contralateral kidney, urine HCO_3 concentration, urine osmolality, and U-B P_{CO_2} 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,

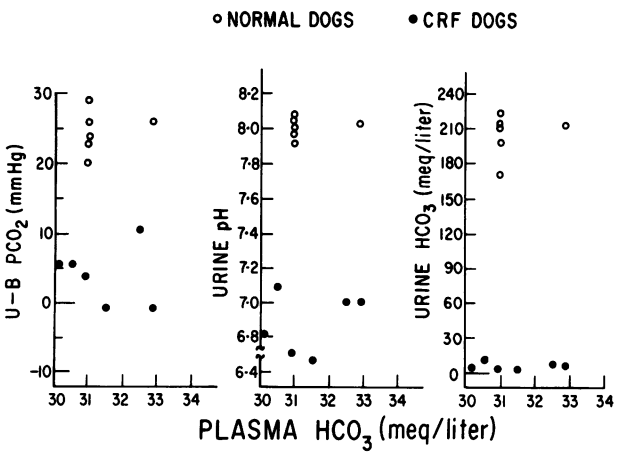

FIGURE 4 U-B P_{CO_2} (left panel), urine pH (middle panel), and urine HCO_3 concentration (right panel) are plotted against plasma HCO_3 before and after induction of CRF in dogs.

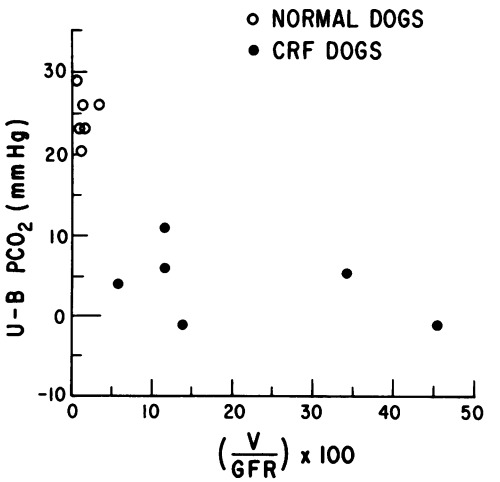

FIGURE 5 U-B P_{CO_2} is plotted against fractional water excretion, (V/GFR) × 100, in dogs before and after induction of CRF.

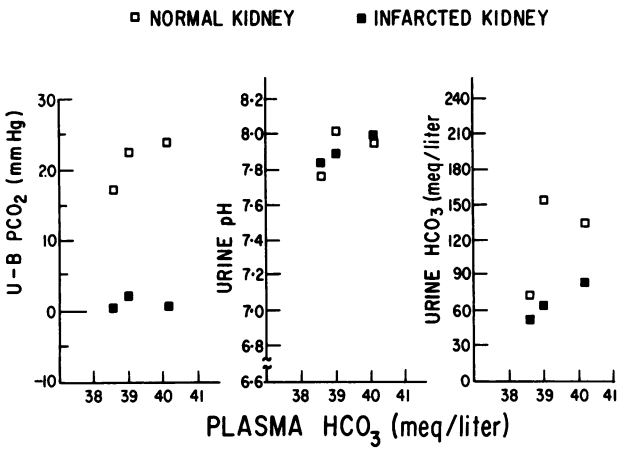

FIGURE 6 U-B P_{CO_2} (left panel), urine pH (middle panel), and urine HCO_3 concentration are plotted against plasma HCO_3 in dogs with a normal kidney and an infarcted kidney.

U-B P_{CO_2} and urine HCO_3 concentration were significantly lower in the POK than in the CK. Elevation of plasma HCO_3 to 61 meq/liter resulted in a significant increase in urine HCO_3 concentration in the POK with concomitant elevation of U-B P_{CO_2}. In the CK, urine HCO_3 concentration and U-B P_{CO_2} failed to rise further. Observe that despite a significant rise in U-B P_{CO_2} and urine HCO_3 concentration in the POK, these values are still significantly lower than in the CK.

In Fig. 7 U-B P_{CO_2} is plotted against urine HCO_3 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 HCO_3 concentration, the values of U-B P_{CO_2} for normal dogs and dogs with a distal acidification defect overlap. Observe that U-B P_{CO_2} was linearly related to urine HCO_3 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 P_{CO_2} 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 P_{CO_2} despite high plasma bicarbonate levels; these subjects were excreting a high fraction of the filtered water and, therefore, had a low urinary HCO_3 concentration. Subjects J. S., G. A., and M. R. excreted a much lower fraction of filtered water and were able to raise urine HCO_2 concentration. All patients with CRF excreted a high fraction of the filtered water and thus failed to raise urine HCO_3 concentration and U-B P_{CO_2}. U-B P_{CO_2} was inversely related to (V/GFR) × 100 ($y = 26.7 - 0.54x$, $r = 0.56$, $P < 0.05$) (Fig. 8).

Studies in vitro

Table VII shows that the addition of $NaHCO_3$ to highly alkaline dog urine in vitro uniformly results in a marked elevation of P_{CO_2} while the pH remains constant.

DISCUSSION

The demonstration that the urinary P_{CO_2} 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 P_{CO_2} (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 P_{CO_2}, we measured U-B P_{CO_2} 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 HCO_3 in water results in elevation of P_{CO_2}; this increase in P_{CO_2} is proportionate to HCO_3 concentration and is secondary to the equilibrium of HCO_3 according to the following reaction: $HCO_3^- + HCO_3^- \leftrightharpoons H_2CO_3 + CO_3^-$ (14, 15). We have previously demonstrated that urinary P_{CO_2} is linearly related to urinary HCO_3 concentration (4). The failure of urinary HCO_3 to rise may, thus, be the critical factor responsible for the low urinary P_{CO_2} in renal tubular acidosis. Steinmetz et al. (6) have contended that, to prove that urinary P_{CO_2} is diminished in renal

TABLE III
Base Line Plasma Electrolytes in Li-Treated Dogs

Na	K	Cl	HCO_3	pH	PCO_2	Li	Urine pH
		meq/liter			*mm Hg*	*meq/liter*	
139.1±2.26	4.0±0.27	113.0±2.39	13.8±1.32	7.24±0.03	32.9±2.30	4.6±0.25	7.40±0.17

TABLE IV
Urinary PCO_2 in Li-Treated Dogs

	GFR	V	Plasma HCO_3	Plasma PCO_2	Urine pH	Urine PCO_2	Urine U-B PCO_2	Urine HCO_3	Urine Osmolality	Urine PO_4
	ml/min		*meq/liter*	*mm Hg*		*mm Hg*		*meq/liter*	*mosmol/kg H_2O*	*mmol/liter*
EK* (n = 5)										
C_1‡	21.5±2.25	6.4±1.46	37.5±4.45	41.8±1.18	7.97±0.02	55.9±1.50	14.1±3.80	125.8±5.53	370.0±4.10	2.8±1.79
$P <$	0.01	0.05	0.02	NS	NS	0.01	0.02	0.01	0.01	NS
UP	11.5±2.13	2.0±0.48	62.8±5.64	45.8±3.08	8.01±0.01	73.5±3.04	27.7±2.43	183.0±10.35	430.0±11.75	1.0±0.87
CK (n = 4)										
C_1	23.7±2.80	6.0±0.34	37.5±4.40	39.0±1.18	7.98±0.02	55.0±2.03	15.9±2.50‖	122.7±7.50	379.0±10.40	1.0±0.61
$P <$	NS	NS	0.05	0.05	NS	NS	NS	NS	NS	NS
C_2§	20.0±3.50	7.2±1.50	66.6±4.91	47.8±3.42	7.97±0.04	63.0±4.00	15.1±4.20‖	139.1±8.07	370.0±4.32	0.3±0.18

* Experimental kidney subjected to elevation of ureteral pressure (UP).
‡ First control period.
§ Second control period.
‖ Measurement of U-B PCO_2 in the CK available only in four dogs.

tubular acidosis as compared to normal subjects, one must determine urinary PCO_2 in patients with distal renal tubular acidosis at high urinary HCO_3 levels. They pointed out that, in the study of Halperin et al. (2), urinary HCO_3 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 PCO_2 in the presence of a distal acidification defect (2, 4, 16–18).

In Figs. 9–11 the interrelationship of urinary HCO_3 concentration, urinary PCO_2, and fractional water excretion is examined in normal, BB, and CRF rats. Fig. 9 shows that, at a plasma HCO_3 of 35 meq/liter, normal rats have a HCO_3 concentration of 17 meq/liter in the end of the proximal tubule; these calculations are made on the basis of the observed GFR, plasma HCO_3, 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 HCO_3 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 HCO_3 concentration very similar to that seen in vivo. This HCO_3 concentration yields 74.5 mm Hg PCO_2 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 HCO_3 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 HCO_3 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 HCO_3 concentration

TABLE V
Urinary PCO_2 in the POK

	GFR	V	(V/GFR) × 100	Plasma HCO_3	Plasma PCO_2	Urine pH	Urine PCO_2	Urine U-B PCO_2	Urine HCO_3	Urine Osmolality	Urine PO_4	Base line pH
	ml/min		*%*	*meq/liter*	*mm Hg*		*mm Hg*		*meq/liter*	*mosmol/kg H_2O*	*mmol/liter*	
POK (n = 4)												
A	20.0±2.50	1.8±0.46	9.0±1.50	42.8±1.95	44.0±4.58	7.87±0.05	51.0±3.78	7.0±3.51	86.4±15.52	294.0±27.50	3.0±1.35	6.69±0.39
$P <$	NS	0.01	0.02	0.001	NS	NS	0.05	0.02	0.05	NS	NS	
B	16.6±3.03	3.7±0.23	23.0±5.31	61.0±1.27	47.0±2.00	7.92±0.04	67.0±3.58	20.0±1.65	139.3±11.00	303.0±26.70	2.4±1.40	
CK (n = 4)												
A	35.2±1.50	2.7±0.35	7.6±1.35			8.00±0.03	78.8±2.50	34.8±5.15	199.0±22.00	478.0±45.00	9.3±1.85	5.59±0.28
$P <$	0.05	NS	0.01			NS	NS	NS	NS	NS	NS	
B	25.2±3.15	3.5±0.11	13.5±3.07			7.99±0.03	84.0±2.31	36.8±3.60	205.0±21.00	447.0±35.75	4.8±1.15	
Comparison between POK and CK												
A	0.02	NS	NS			NS	0.001	0.01	0.01	0.02	0.05	0.05
B	0.02	NS	NS			NS	0.01	0.01	0.05	0.02	0.05	

A and B refer to values obtained at two different plasma HCO_3 levels.

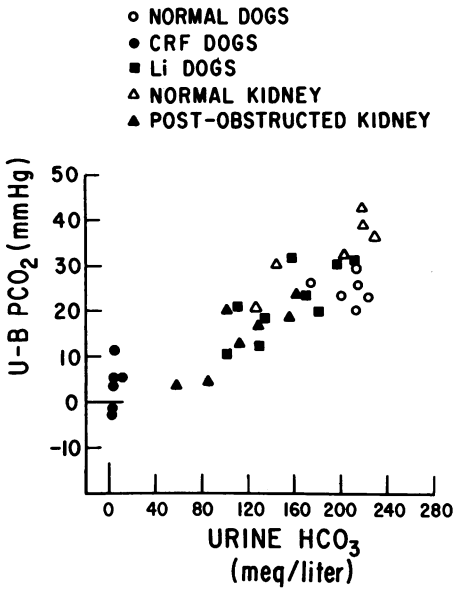

FIGURE 7 U-B P_{CO_2} is plotted against urine HCO_3 concentration in dogs before and after induction of CRF, in Li-treated dogs, and in dogs with unilateral ureteral obstruction.

to the levels observed is a plasma concentration of 55 meq/liter.

In BB rats, at a highly alkaline urinary pH, the observed urinary P_{CO_2} is also very close to that predicted from the concentration of HCO_3. In CRF, however, an increase in plasma HCO_3 to very high levels failed to increase urinary HCO_3, 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 HCO_3 in excess of 200 meq/liter would be necessary to attain a urine HCO_3 similar to that seen in normals. In CRF and in BB rats, at a lower urinary pH, the observed urinary P_{CO_2} is higher than the calculated P_{CO_2}. 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 P_{CO_2} was higher than the calculated P_{CO_2} may indicate the presence of H^+ secretion.

These observations demonstrate convincingly that urinary P_{CO_2} is critically dependent on urinary HCO_3 concentration. The present data also demonstrate that. in the presence of a distal acidification defect, elevation of urinary HCO_3 concentration accomplished either by an increase of plasma HCO_3 in the POK or by an increase in plasma HCO_3 and elevation of ureteral pressure in Li-treated dogs results in a significant increase in urinary pCO_2. Indeed, in the Li-treated dogs, the urinary P_{CO_2} 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 P_{CO_2} may rise normally if urinary HCO_3 concentration is high enough.

These observations suggest that the ability to remove water in the collecting duct and therefore raise urinary HCO_3 concentration is essential for the formation of a high urinary P_{CO_2} in a highly alkaline urine.

TABLE VI
Urine pCO_2 in CRF Patients and Normal Subjects

	Plasma		Urine						
	HCO_3	pCO_2	pH	pCO_2	U-B pCO_2	HCO_3^-	V	(V/GFR) × 100	PO_4
	meq/liter	*mm Hg*		*mm Hg*		*meq/liter*	*ml/min*	*%*	*mmol/liter*
CRF patients									
J. M.	34.2	39.7	6.88	52.3	12.6	8.4	1.5	14.2	3.8
I. S.	35.1	45.0	7.54	47.0	2.0	34.9	2.4	23.1	5.0
R. M.	31.3	43.0	7.22	47.0	4.0	16.2	4.6	20.4	3.5
F. H.	30.9	44.0	7.62	51.2	7.2	47.1	7.8	16.6	2.9
A. S.	34.5	42.5	7.57	54.5	12.0	41.5	7.6	20.5	2.3
L. G.	32.5	43.0	7.05	42.5	−0.5	14.8	2.3	41.1	2.1
F. O.	33.3	44.0	7.15	61.3	17.3	19.1	2.3	17.7	6.2
R. C.	30.6	42.5	7.08	47.4	4.9	11.7	2.2	73.3	1.5
Normal subjects									
L. N.	33.9	42.1	7.41	52.1	10.0	26.6	17.1	9.6	1.2
J. A.	33.4	48.6	7.41	48.6	0.0	23.4	23.7	18.9	0.3
P. C.	32.6	38.0	7.47	53.5	15.5	30.9	17.6	11.7	1.4
J. S.	32.5	45.8	7.92	96.5	50.7	221.5	2.5	2.1	18.9
G. A.	32.2	48.0	7.79	78.0	30.0	119.5	6.8	5.6	2.1
M. R.	33.1	46.0	7.85	103.0	57.0	196.3	4.2	2.8	4.3
CRF	32.8±0.26	43.0±0.56	7.26±0.09	50.4±2.04	7.4±2.08	24.2±5.21	3.8±1.04	28.4±7.05	3.4±0.20
$P <$	NS	NS	0.05	0.05	0.05	0.05	0.05	0.05	NS
Normal	33.0±0.61	44.5±1.66	7.64±0.09	72.0±9.80	27.5±9.34	103.0±36.69	12.0±3.52	8.5±2.59	4.7±1.18

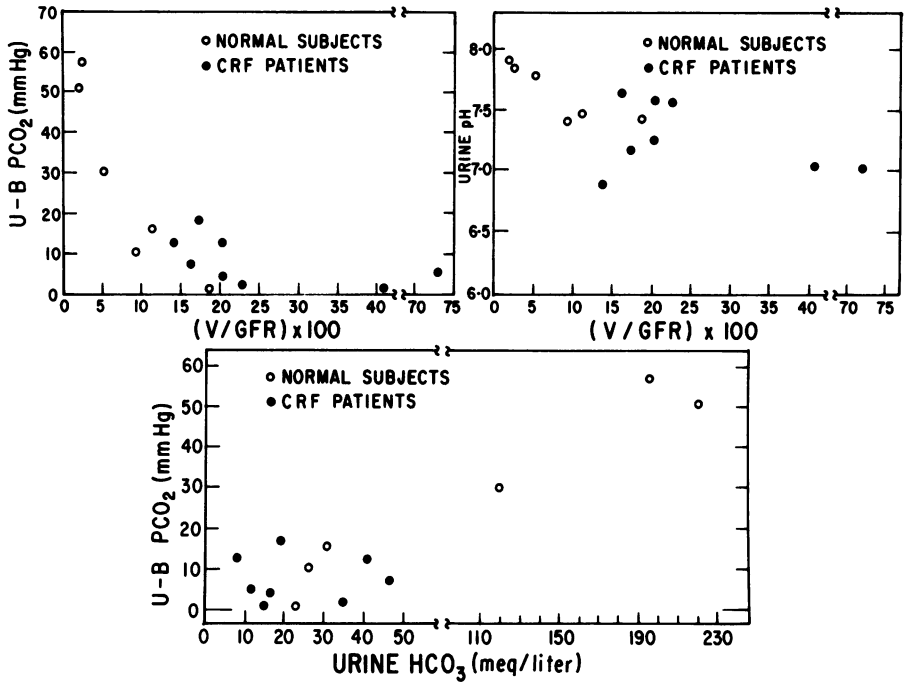

FIGURE 8 U-B P_{CO_2} (left upper panel), urine pH (right upper panel), and urine HCO_3 concentration (lower panel) are plotted against fractional water excretion, (V/GRF) × 100, in normal subjects, and in CRF patients.

It must be emphasized that our study, as do all previous such studies, measures urinary pCO_2 in urine that has left the kidney. The P_{CO_2} measured in these urine samples may or may not reflect the P_{CO_2} in the collecting duct. Two possibilities concerning the P_{CO_2} in the collecting duct immediately present themselves. First, as water is abstracted from the collecting duct, bicarbonate concentration rises, and P_{CO_2} likewise increases. Assuming that the collecting duct is highly permeable to CO_2 (an assumption that has been made by almost all workers in the field), then CO_2 will diffuse across the collecting duct. This may result in an equilibration of P_{CO_2} across the collecting duct. Thus, the P_{CO_2} 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 P_{CO_2}. The second possibility is that, although CO_2 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 CO_2 gradient in the collecting duct owing to the continuous generation of H_2CO_3 from bicarbonate. This hypothetical series of events results in a situation in which the P_{CO_2} in the collecting duct will be considerably greater than that of vasa recta blood. The only measurement of P_{CO_2} in the collecting duct and vasa recta supports this view (22). The difficulty with the interpretation of this observation is that the P_{CO_2} is calculated from pH measurements in the collecting duct and vasa recta. The issue will be resolved only when direct measurements of collecting duct P_{CO_2} 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 P_{CO_2}, measured in the final

TABLE VII
Addition of $NaHCO_3$ to Highly Alkaline Dog Urine in Vitro

Sample	Initial pH	Initial P_{CO_2}	Addition of $NaHCO_3$	Final pH	Final P_{CO_2}
			meq/liter		
1	8.02	60	100	8.03	86
2	8.08	58	100	8.08	80
			200	8.08	115
3	8.03	78	200	8.00	112
4	7.98	71	200	8.08	114

The measurement of P_{CO_2} was done at 1 min after addition of $NaHCO_3$ to the urine samples.

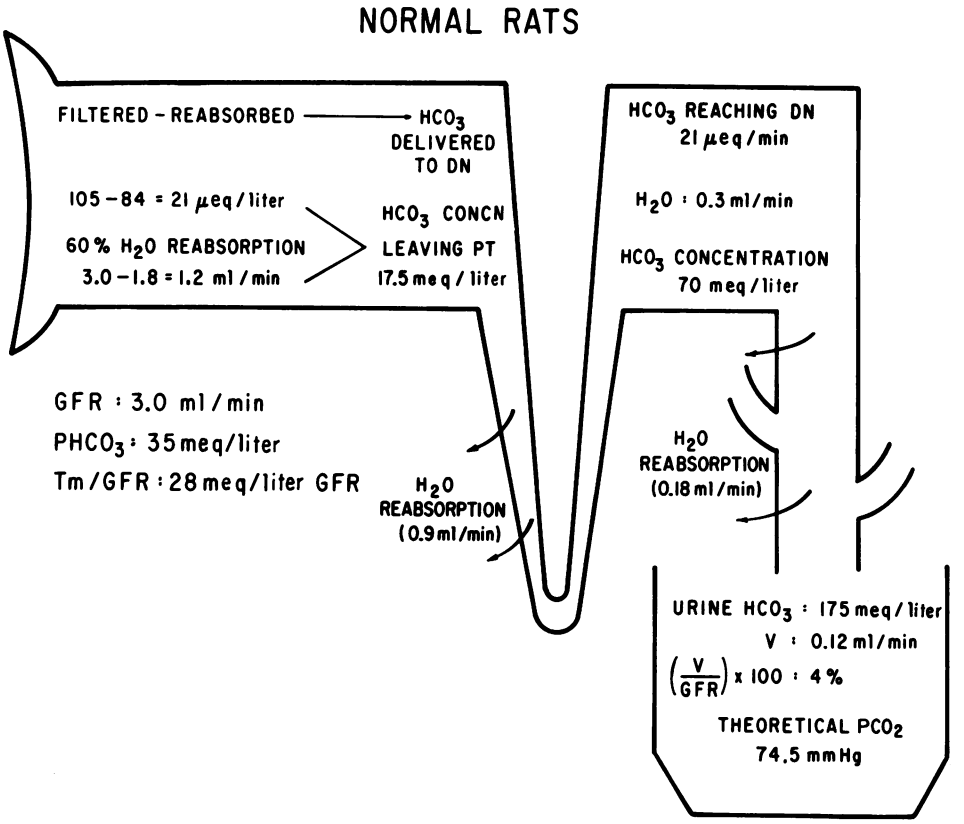

FIGURE 9 Bicarbonate concentration in the proximal tubule (PT), distal nephron (DN), and in the final urine of normal rats. Calculations were based on the GFR, plasma HCO_3, Tm/GFR, and (V/GFR) × 100 observed in vivo. Fluid reabsorption in the PT was based on TF/P inulin ratio of reference 19. For simplicity of calculation all the reabsorption of HCO_3 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 HCO_3 concentration very close to that observed in vivo. The theoretical P_{CO_2} is the P_{CO_2} calculated from the HCO_3 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 P_{CO_2} 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 P_{CO_2} 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 P_{CO_2} that will accurately predict that observed in the presence of such highly concentrated urinary sodium bicarbonate solution is not available.

The failure of urinary P_{CO_2} 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 P_{CO_2} 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 CO_2, and restriction to diffusion of CO_2 across the collecting duct (1, 4, 25, 26). The present observations suggest another mechanism whereby the urinary P_{CO_2} can be raised. When $NaHCO_3$ is dissolved in water, the following reactions take place (14):

$$2H_2O \rightleftharpoons H_3O^+ + OH^-$$

$$K_w = 1.01 \times 10^{-14} = [H_3O][OH^-] \quad (1)$$

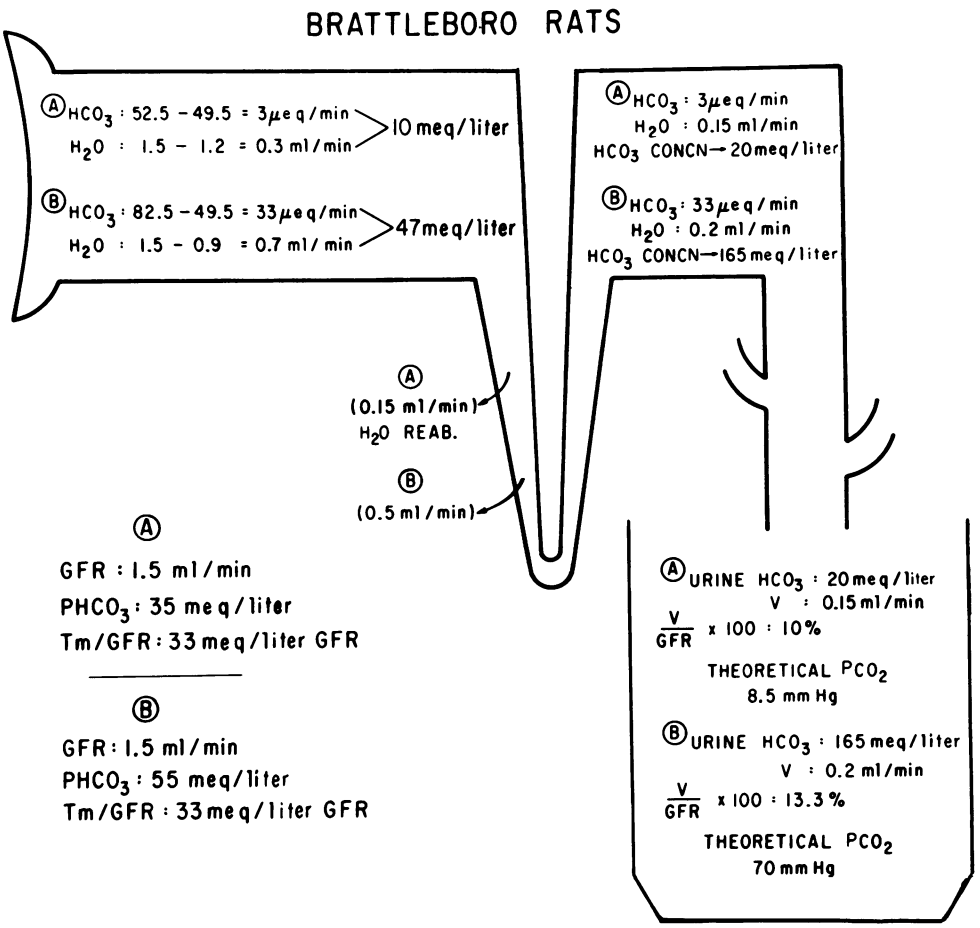

FIGURE 10 Bicarbonate concentration in the proximal tubule, distal nephron, and final urine of BB rats at a plasma HCO_3 of 35 meq/liter (labeled A) and at a plasma HCO_3 of 55 meq/liter (labeled B). Calculations are based on the GFR, plasma levels of HCO_3, Tm/GFR, and (V/GFR) × 100 observed in vivo. Fluid reabsorption in the proximal tubule in BB rats was assumed to be 80% of the filtered fluid. Observe that HCO_3 concentrations in the final urine are very close to the observed values.

$$HCO_3^- + H_2O \rightleftharpoons H_3O^+ + CO_3^=$$

$$K_2 = 6.0 \times 10^{-11} = \frac{[H_3O^+][CO_3^=]}{HCO_3^-} \quad (2)$$

$$HCO_3^- + H_2O \rightleftharpoons H_2CO_3 + OH^-$$

$$K_b = 3.1 \times 10^{-8} = \frac{[H_2CO_3][OH^-]}{HCO_3} \quad (3)$$

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H_3O^+ + HCO_3^-$$

$$K_1 = 3.5 \times 10^{-7} = \frac{[H_3O^+][HCO_3^-]}{CO_2} \quad (4)$$

$$HCO_3^- + HCO_3^- \rightleftharpoons H_2CO_3 + CO_3^=$$
$$\Downarrow\Uparrow$$
$$CO_2 + H_2O$$

$$K = \frac{K_2}{K_1} = \frac{[CO_2][CO_3^=]}{[HCO_3^-]^2}. \quad (5)$$

The $[P_{CO_2}]$ of an ideal solution at 25°C and zero ionic strength containing 0.3 M $NaHCO_3$ can be calculated in the following way from the fifth equation above:

$$K = \frac{K_2}{K_1} = 1.71 \times 10^{-4} = \frac{[CO_2][CO_3^=]}{[HCO_3^-]^2};$$

let $x = [CO_2] = [CO_3^=]$; therefore,

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

therefore, $x = 0.00383$ M $CO_2 = CO_3^=$; therefore, P_{CO_2} = 3.83 mM of $CO_2 \div 0.03 = 127.7$ 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 P_{CO_2} resulting from a solution of HCO_3 in urine. If one takes into account the effects of temperature (37°C) and the ionic strength of blood,

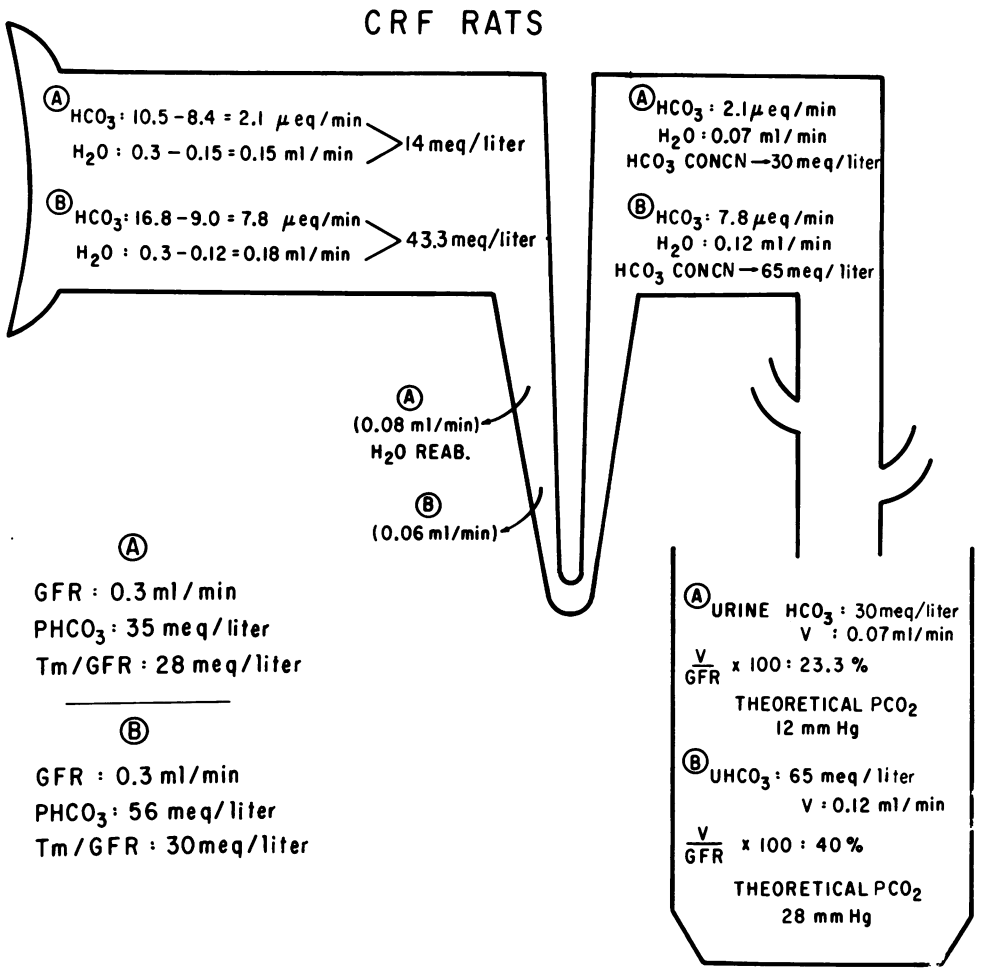

FIGURE 11 Bicarbonate concentration in the proximal tubule, distal nephron, and final urine of CRF rats at two different levels of plasma HCO_3 (labeled A and B). Calculations based on GFR, plasma HCO_3 levels, Tm/GFR, and (V/GFR) × 100 observed in vivo. Fluid reabsorption in PT of CRF was assumed to be 50%, calculation based on TF/P inulin ratio of reference 19. Observe that urine HCO_3 concentrations are very similar to those observed in vivo. Theoretical P_{CO_2} 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 P_{CO_2} of 0.3 M solution of $NaHCO_3$ is 141 mm Hg.

These observations demonstrate that the high urinary P_{CO_2} of alkaline urine as measured in vitro is critically dependent on the presence of a high urinary HCO_3 concentration which in equilibrium with water will result in a high urinary P_{CO_2}. As can be seen from Figs. 10 and 11, the calculated P_{CO_2} 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 pCO_2 of highly alkaline urine can be largely explained as consequence of a rise in urinary HCO_3 concentration. From the equation $H + HCO_3 \rightleftharpoons H_2CO_3 \rightleftharpoons CO_2 + H_2O$, 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 P_{CO_2}. It is likely that in highly alkaline urine, urinary HCO_3 concentration plays the more important role in the elevation of urinary P_{CO_2}. It is impossible at present to determine precisely how much of each of these factors contribute to the elevation of urinary P_{CO_2}. It is very likely, however, that the difference between the observed P_{CO_2} and the expected P_{CO_2} is determined by H+ secretion.

In moderately alkaline urine, however, the achievement of a high urinary P_{CO_2} seems to be due mainly to hydrogen ion secretion because the amount of bicarbonate present is clearly not sufficient to raise urinary P_{CO_2} (4). We have previously demonstrated

that, at comparable urinary bicarbonate concentrations, urinary P_{CO_2} was significantly lower in dogs with renal tubular acidosis than in normal dogs. In moderately alkaline urine, urinary P_{CO_2} 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: $H^+ + HPO_4^= \rightleftharpoons H_2PO_4^-$ As the tubular fluid proceeds towards equilibrium, the pH will rise, and $H_2PO_4^-$ will react with HCO_3, thus increasing the P_{CO_2}. Previous studies have suggested that it is possible to increase urinary P_{CO_2} 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 P_{CO_2}.

The observation that carbonic anhydrase administration lowers urinary P_{CO_2} to the level of blood P_{CO_2} 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 P_{CO_2} in highly alkaline urine. The dissipation of the U-B P_{CO_2} 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 P_{CO_2} (in vitro) lowers the P_{CO_2} of these solutions to very low levels (31). When the solutions are kept under oil, carbonic anhydrase fails to lower the P_{CO_2}. Obviously there cannot be a disequilibrium pH in a solution of bicarbonate, and the mechanism whereby carbonic anhydrase lowers the P_{CO_2} of this solution must lie elsewhere. It is possible that carbonic anhydrase, by accelerating the dehydration of carbonic acid, raises the P_{CO_2} at the interface of the liquid and the air and therefore favors diffusion of CO_2 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 CO_2. This explanation is supported by the fact that carbonic anhydrase fails to lower P_{CO_2} 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 P_{CO_2} 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 P_{CO_2} is critically dependent on urinary bicarbonate concentration. The failure of urinary bicarbonate concentration to rise impairs the increase in urinary P_{CO_2}, even though hydrogen ion secretion is either normal or enhanced, e.g., diabetes insipidus and CRF. The urinary P_{CO_2} 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 P_{CO_2} largely reflects urinary concentration.

ACKNOWLEDGMENTS

We wish to thank Roderich Walter, Ph.D., Head of the Department of Physiology in the University of Illinois School of Basic Medical Sciences for providing us with BB rats.

This research was supported by Central Office Grant 7083-04; Basic Institutional Support Grant 3324-01, and Research Associate Grant 052-01, all from the Veterans Administration.

REFERENCES

1. Pitts, R. F., and W. D. Lotspeich. 1946. Bicarbonate and the renal regulation of acid-base balance. *Am. J. Physiol.* **147:** 138–154.
2. Halperin, M. L., M. B. Goldstein, A. Haig, M. D. Johnson, and B. J. Stinebaugh. 1974. Studies on the pathogenesis of type I (distal) renal tubular acidosis as revealed by the urinary PCO_2 tensions. *J. Clin. Invest.* **53:** 669–677.
3. Sebastian, A., E. McSherry, and R. C. Morris, Jr. 1974. On the mechanism of inappropriately low urinary carbon dioxide tension ($U\text{-}P_{CO_2}$) in classic (type 1) renal tubular acidosis (RTA). *Clin. Res.* **22:** 544A. (Abstr.)
4. Arruda, J. A. L., L. Nascimento, S. K. Kumar, and N. A. Kurtzman. 1977. Factors influencing the formation of urinary carbon dioxide tension. *Kidney Int.* **11:** 307–317.
5. Arruda, J. A. L., L. Nascimento, C. Westenfelder, and N. A. Kurtzman. 1977. Effect of parathyroid hormone on urinary acidification. *Am. J. Physiol.* **232:** F429–433.
6. Steinmetz, P. R., Q. Al-Awqati, and W. J. Lawson. 1976. Renal tubular acidosis. *Am. J. Med. Sci.* **271:** 41–51.
7. Pak-Poy, R. K., and O. Wrong. 1960. The urinary pCO_2 in renal disease. *Clin. Sci. (Oxf.).* **19:** 631–639.
8. Fillastre, J. P., R. Ardaillou, and G. Richet. 1969. pH et pCO_2 urinaires en réponse à une surcharge alcaline au cours de l'insuffisance rénale chronique. *Nephron.* **6:** 91–101.
9. Schloeder, F. X., W. N. Suki, B. J. Stinebaugh, M. B. Goldstein, and M. L. Halperin. 1976. Urinary pCO_2 as an index of distal nephron H^+ secretion in chronic renal failure. Proceedings of the American Society for Nephrology, Washington. 112. (Abstr.)
10. Dorhout Mees, E., J. Machado, E. Slatopolsky, S. Klahr, and N. S. Bricker. 1966. The functional adaptation of the diseased kidney to ammonium excretion. *J. Clin. Invest.* **45:** 289–296.
11. Seldin, D. W., A. J. Coleman, N. W. Carter, and F. C. Rector, Jr. 1967. The effect of Na_2SO_4 on urinary acidification in chronic renal disease. *J. Lab. Clin. Med.* **69:** 893–903.
12. Schultze, R. G., D. D. Taggart, H. Shapiro, J. P. Pernell, S. Caglar, and N. S. Bricker. 1971. On the adaptation in potassium excretion associated with nephron reduction in the dog. *J. Clin. Invest.* **50:** 1061–1068.
13. Kurtzman, N. A. 1970. Regulation of renal bicarbonate

reabsorption by extracellular volume. *J. Clin. Invest.* **49**: 586–595.
14. Blaedel, W. J., and V. W. Meloche. 1963. Acid base equilibrium. *In* Elementary Quantitative Analysis. Harper and Row, Publishers, New York. 2nd edition. 327–335.
15. Edsall, J. T., and J. Wyman. 1958. Carbon dioxide and carbonic acid. *In* Biophysical Chemistry. Academic Press, Inc., New York. 1st edition. 550–589.
16. Thirakomen, K., N. Kozlov, J. A. L. Arruda, and N. A. Kurtzman. 1976. Renal hydrogen ion secretion following the release of unilateral ureteral obstruction. *Am. J. Physiol.* **231**: 1233–1239.
17. Nascimento, L., D. Rademacher, R. Hamburger, J. A. L. Arruda, and N. A. Kurtzman. 1977. On the mechanism of lithium-induced renal tubular acidosis. *J. Lab. Clin. Med.* **89**: 455–462.
18. Roscoe, J. M., M. B. Goldstein, J. L. Halperin, D. R. Wilson, and B. J. Stinebaugh. 1976. Lithium-induced impairment of urine acidification. *Kidney Int.* **9**: 344–350.
19. Su, W. S., H. S. Aynedjian, and N. Bank. 1976. A micropuncture study of HCO_3 reabsorption by the remnant kidney. Proceedings American Society for Nephrology, Washington. 80. (Abstr.)
20. Vieira, F. C., and G. Malnic. 1968. Hydrogen ion secretion by rat renal cortical tubules as studied by an antimony electrode. *Am. J. Physiol.* **214**: 710–718.
21. Morel, F., M. Mylle, and C. W. Gottschalk. 1965. Tracer microinjection studies of effect of ADH on renal tubular diffusion of water. *Am. J. Physiol.* **209**: 179–187.
22. Uhlich, E., C. A. Baldamus, and K. J. Ullrich. 1968. Verhalten von CO_2-druck und bicarbonat im gegenstromsystem des nierenmarks. *Pflugers Arch.* **303**: 31–48.
23. Seldin, D. W., and J. D. Wilson. 1966. Renal tubular acidosis. *In* The Metabolic Basis of Inherited Diseases. J. B. Stanbury, J. Wyngaarden, and D. S. Frederickson, editors. McGraw-Hill Book Co., New York. 2nd edition. 1230–1246.
24. Sebastian, A., E. McSherry, and R. C. Morris. 1976. Metabolic acidosis with special reference to the renal acidosis. *In* The Kidney. B. Brenner and F. C. Rector, Jr., editors. W. B. Saunders, Philadelphia. 1st edition. **1**: 615–660.
25. Kennedy, T. J., Jr., J. Orloff, and R. W. Berliner. 1952. Significance of carbon dioxide in urine. *Am. J. Physiol.* **169**: 596–608.
26. Reid, E. L., and A. G. Hills. 1965. Diffusion of carbon dioxide out of the distal nephron in man during antidiuresis. *Clin. Sci. (Oxf.).* **28**: 15–28.
27. Portwood, R. M., D. W. Seldin, F. C. Rector, Jr., and R. Cade. 1959. The relationship of urinary CO_2 tension to bicarbonate excretion. *J. Clin. Invest.* **38**: 770–776.
28. Rector, F. C., Jr., R. M. Portwood, and D. W. Seldin. 1959. Examination of the mixing hypothesis as an explanation for elevated urinary CO_2 tensions. *Am. J. Physiol.* **197**: 861–864.
29. Ochwadt, B. K., and R. F. Pitts. 1956. Effect of intravenous infusion of carbonic anhydrase on carbon dioxide tension of alkaline urine. *Am. J. Physiol.* **185**: 426–429.
30. Rector, F. C., Jr., N. W. Carter, and D. W. Seldin. 1965. The mechanism of bicarbonate reabsorption in the proximal and distal tubules of the kidney. *J. Clin. Invest.* **44**: 278–290.
31. Arruda, J. A. L., M. K. Roseman, J. T. Sehy, P. K. Mehta, and N. A. Kurtzman. 1977. On the mechanism of the formation of urine (U) PCO_2: Effect of carbonic anhydrase (CA) and Tris (T) on urine-blood (U-B) PCO_2 gradient. *Clin. Res.* (Abstr.) In press.