Hydrogen Ion Secretion by the Collecting Duct as a Determinant of the Urine to Blood P_{CO_2} Gradient in Alkaline Urine

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ABSTRACT Several theories have been advanced to explain the elevation in urinary P_{CO_2} during bicarbonate loading and include: (*a*) H^+ secretion, (*b*) countercurrent system for CO_2, (*c*) the "ampholyte" properties of bicarbonate, and (*d*) mixing of urine of disparate bicarbonate and buffer concentrations. In this study microelectrodes were used to measure *in situ* and equilibrium pH (pH_{is} and pH_{eq}) and P_{CO_2} in control and bicarbonate loaded rats before and after infusion of carbonic anhydrase. The disequilibrium pH method ($pH_{dq} = pH_{is} - pH_{eq}$) was used to demonstrate H^+ secretion. Control rats excreting an acid urine (pH = 6.04±0.06) failed to display a significant disequilibrium pH at the base (BCD), or tip (TCD) of the papillary collecting duct. Urine pH (7.54±0.12), and urine to blood (U–B) P_{CO_2} increased significantly during $NaHCO_3$ loading while P_{CO_2} at the BCD and TCD also increased (95±4 and 122±4). Furthermore, an acid disequilibrium pH was present at both the BCD and TCD (−0.42±0.04 and −0.36±0.03) and was obliterated by carbonic anhydrase. Comparison of the P_{CO_2} in the BCD or TCD with the adjacent vasa recta revealed similar values ($r = 0.97$). It is concluded that H^+ secretion by the collecting duct into bicarbonate containing fluid with delayed dehydration of H_2CO_3, is the most likely determinant of the U–B P_{CO_2} in alkaline urine. Similar values for P_{CO_2} in the collecting duct and the adjacent vasa recta suggests trapping of CO_2 in the medullary countercurrent system. The rise in P_{CO_2} occurs both along the collecting duct and after exit from the papilla.

INTRODUCTION

There has been general agreement since the early observations of Mainzer and Bruhn (1), Pitts and Lotspeich (2), and Ryberg (3), that the CO_2 tension of alkaline urine may exceed that of systemic arterial blood by two- to fourfold. Considerable controversy has existed regarding the mechanism by which this elevation in urinary P_{CO_2} occurs (4–7). Despite this controversy the urine to blood P_{CO_2} gradient (U–B P_{CO_2})[1] during bicarbonate administration has been widely used an an index of hydrogen ion secretion by the "distal nephron" in studies involving whole kidney clearance techniques in experimental animals and in man (7–11). In fact, the failure of patients with classical distal renal tubular acidosis to generate an elevated urinary P_{CO_2} during bicarbonate loading has been proposed as a means of categorizing the type of urinary acidification defect in this disorder ("secretory defect") (9).

Pitts and Lotspeich (2) first proposed that H^+ secretion into bicarbonate-containing fluid in the more distal nephron segments resulted in the formation and subsequent delayed dehydration of H_2CO_3. Thus, as envisioned by these investigators, CO_2 would be formed in areas of the collecting system where surface-volume relationships would be unfavorable for CO_2 diffusion, resulting in elevated urinary CO_2 tensions.

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[1] *Abbreviations used in this paper*: pH_{dq}, disequilibrium pH: pH_{eq}, equilibrium pH; pH_{is}, *in situ*.

Additional support for this hypothesis was derived from the studies of Ochwadt and Pitts (12) in which it was observed that systemic administration of carbonic anhydrase, which presumably appeared in the urine, obliterated the U–B P_{CO_2} gradient. In contrast, Kennedy, Orloff, and Berliner (13) emphasized that the mixing of urine of low bicarbonate and high nonbicarbonate buffer concentration with urine of high bicarbonate, low nonbicarbonate buffer concentration (at different pH) could also serve to elevate the urinary P_{CO_2} ("mixing hypothesis") (13).

Pak Poy and Wrong (14) noted that high urinary P_{CO_2} could also be achieved by a medullary countercurrent system but these authors suggested that the CO_2 would be derived primarily from metabolic sources. Uhlich, Baldamus, and Ullrich (15) suggested by indirect techniques, that the CO_2 tension in the collecting tubule exceeded that of the vasa recta by ~30 mm Hg. Furthermore, this sizable gradient was abolished by carbonic anhydrase infusion. This study cast doubt on the medullary countercurrent hypothesis and further underscored the importance of delayed dehydration. Although of an indirect nature, this study has served as the foundation of the U–B P_{CO_2} gradient method used in whole kidney clearance studies. The suggestion of a diffusion barrier for CO_2 across the collecting duct (5, 6, 15) differs markedly from recent findings across the superficial proximal convoluted tubule reported by our laboratory (16, 17).

Recently, the H^+ secretory hypothesis has been called into question by physicochemical considerations. Arruda and associates (5) and Maren (6) have emphasized the "ampholyte" properties of bicarbonate as an explanation for the expected increase in CO_2 tension in highly alkaline aqueous solution or urine when the concentration of carbonate ($CO_3^=$) and CO_2 are equal. The linear relationship between the U–B P_{CO_2} gradient and urinary bicarbonate concentration observed by Arruda et al. (8) in a variety of animal models and man have led to the hypothesis that a large component of the U–B P_{CO_2} gradient is a result of the ampholyte effect initiated by water abstraction in the distal nephron and a subsequent increase in bicarbonate concentration (5). Thus, these investigators suggested that the low U–B P_{CO_2} gradient observed in distal renal tubular acidosis (9) was critically dependent on urinary concentrating ability, which is often defective in this disease (5). These findings were further supported by the theoretical considerations of Maren (6). In contrast, however, Stinebaugh and associates (7) have recently reevaluated the linear relationship between U–B P_{CO_2} and urinary bicarbonate concentration in alkaline urine in several species. These studies led these authors to the conclusion that the most plausible explanation for this linear relationship was a H^+ secretory process that increased as a function of the increase in urine bicarbonate concentration (7). It was suggested that a disequilibrium pH could occur as a result of H^+ secretion or alternatively by concentration of bicarbonate in the terminal nephron with simultaneous back diffusion of carbon dioxide (7). These studies were necessarily of an indirect nature, however, since a disequilibrium pH has not been demonstrated previously in the collecting tubule.

The disequilibrium pH method ($pH_{dq} = pH_{is} - pH_{eq}$) has been used by several investigators to demonstrate delayed dehydration, and thus H^+ secretion in other nephron segments (18, 19). Recently, we have used newly developed microelectrode techniques to measure P_{CO_2}, *in situ* pH (pH_{is}), and equilibrium pH (pH_{eq}).

The purpose of the present study was to examine the role of delayed dehydration in the generation of the U–B P_{CO_2} gradient by micropuncture of the surgically exposed papilla of the rat in the presence and absence of carbonic anhydrase.

METHODS

Preparation of rats for micropuncture. Studies were performed after 100 mg/kg, i.p. Inactin anesthesia (Promonta, Hamburg, West Germany) on young mutant Munich-Wistar rats weighing 60–150 g. All rats were allowed free access to tap water and standard rat chow until the time of the experiment. The rat was placed on a thermostatically controlled heating table and maintained at 37.5°C. After tracheostomy, polyethylene catheters (PE 50) were inserted into the left jugular vein for infusion and into the left femoral artery for constant blood pressure monitoring and blood collection, and into the bladder for urine collection from the right nonexperimental kidney (under oil). The left kidney was then gently separated from the adrenal gland and peritoneal attachment. The renal papilla was exposed by temporarily displacing the papilla into the renal pelvis and carefully excising the ureter. The kidney was then placed in a lucite cap stabilized by 3% agar and continuously bathed with mineral oil equilibrated with 5% CO_2–95% O_2, maintained at 37°C and illuminated with a small fiber optic light source. This technique has been described previously in detail (20). After jugular vein cannulation, rats were infused with Ringer's bicarbonate ($Na^+ = 140$, $Cl^- = 110$, $HCO_3^- = 25$, $K^+ = 5$ meq/liter) at 1% of body wt/h.

Microelectrode techniques

P_{CO_2} microelectrode. The *in situ* P_{CO_2} of tubule fluid at the base of the collecting duct (defined as the earliest accessible portion of the papillary collecting duct), tip of the collecting duct (opening of duct or direct puncture at tip) (distance between tubule puncture sites of 2.1±0.5 mm), and an adjacent vasa recta were obtained by puncture at each site with a P_{CO_2} microelectrode of 6–9 μm tip Diam. The construction, testing, electrical characteristics, and calibration of these electrodes were exactly as described previously (16). Electrodes having a sensitivity of <57 mV/$\log_{10}$ P_{CO_2} were discarded.

In situ pH. The *in situ* pH (pH_{is}) at the base and tip

collecting duct was determined with single or double barrelled glass membrane pH microelectrodes of 7–10 μm tip Diam as reported previously (18). Electrodes having a sensitivity of <57 mV/pH unit were not used. Initially all collecting duct punctures were with double-barrelled electrodes, however, no difference in pH was noted with single or double-barrelled electrodes during any of the physiological conditions examined. Subsequently, in five rats transepithelial potential difference was determined with 2–3-μm beveled pipettes filled with 2.5 M KCl and 0.5 M KNO_3. In 15 tubules the range of potential was −1.0 to +2.0 mV in controls and −3.0 to 0.0 mV during bicarbonate loading. These findings indicate that the transepithelial potential difference in the papillary collecting duct of the exposed papilla (length ~2.0 mm) is not of sufficient magnitude to affect adversely the accuracy of a single-barrelled pH electrode having near theoretical slope. Therefore, most of the *in situ* pH data were obtained with single-barrelled electrodes because of ease of construction. Calibration before and after in vivo use was as described previously (16, 18).

Equilibrium pH. The equilibrium pH (pH_{eq}) was measured in vivo with a composite probe consisting of an aspiration pipette into which a single-barrelled pH electrode was inserted. This electrode was designed and constructed in our laboratory and has been described in detail (18). This electrode consists of an outer shell (aspiration or collection pipette) of borosilicate glass capillary (2 mm) that was pulled to a taper length of 6–7 mm and beveled at a 50–60° angle to an outer tip Diam of 4–9 μm. A pH electrode was constructed as previously described (18) except that the geometry of the outer shell and pH electrode were taken into account to allow insertion of the pH electrode up to a distance of some 200–400 μm from the tip of the outer shell. After sealing the butt end with epoxy cement and allowing a suitable cure, the pipette was filled with mercury and attached to a hydraulic device via PE-50 tubing. Both the tubing and hydraulics were filled with mercury. Therefore, standard pH buffer or tubule fluid was aspirated behind a mercury column to avoid CO_2 loss. Before use this electrode was calibrated by aspirating standard pH buffer at 37°C (pH = 6.84 and 7.384, respectively) into the pipette under microscopic control to assure that the pH electrode was in contact with the buffer. An attempt was made to withdraw a volume of buffer similar to that expected in vivo. Only electrodes with a sensitivity of at least 57 mV/pH unit were accepted. The lucite chamber used for calibration at 37°C was as reported previously (16). In actual use in a micropuncture experiment, tubule fluid was aspirated and allowed to reach chemical equilibrium while the aspiration pipette tip remained within the tubule lumen. The theoretical advantages of an equilibrium pH electrode constructed in this manner were: (*a*) it was not necessary to remove tubule fluid to an equilibration chamber in vitro, and (*b*) no assumption regarding the level of papillary P_{CO_2} was required. This electrode was tested extensively in vitro to: (*a*) assure isolation of the sample with respect to ionic diffusion of H^+ into or out of the tubule sample, (*b*) assure that CO_2 gas was not lost over the period of time required to make the pH measurement, and (*c*) to assure that the CO_2 produced from the dehydration of the H_2CO_3 contained in the tubule sample from an "off-equilibrium" source (i.e., where a disequilibrium pH was present) would not erroneously lower the true equilibrium pH. The results of this testing have been reported previously (18). With regard to the third problem however, nonbicarbonate buffers could also contribute as a potential source of CO_2 by this mechanism. For example if the concentration of nonbicarbonate buffers was 5.0 mM (7), and the disequilibrium pH equal to −0.50 pH U, then 2.5 mM of CO_2 could be produced as the H_2CO_3 was titrated by the nonbicarbonate buffers. This means that if this CO_2 were trapped in the equilibrium pH electrode, the equilibrium pH observed would be falsely acid. Such a problem would be highly unlikely since the tip of the equilibrium electrode through which collection is made is open and remains within the tubule lumen during operation. However, to assure that such a problem did not exist in our system, the determined equilibrium pH was compared with the equilibrium pH calculated from determined P_{CO_2} and total CO_2 concentration (Henderson-Hasselbach equation, α = 0.0309, pK'_1 = 6.13). In these experiments care was taken to assure that the loss of CO_2 gas from the collected sample was negligible by aspirating tubule fluid directly into a beveled volumetric constriction pipette between oil layers equilibrated with CO_2. The sample was then transferred immediately to the microcalorimeter (21). The results of the comparison of determined and calculated equilibrium pH (pH_{eq}) from five rats are displayed in Fig. 1. Each point represents a paired determination. Thus, since there is a close correlation between the equilibrium pH determined by these two techniques, the type of consideration noted above could not be adversely affecting the results and, in this system, nonbicarbonate buffers could not contribute significantly to the equilibrium pH measured in the papillary collecting duct.

Physiological conditions

Surgical exposure of the renal papilla in the mutant Munich-Wistar rat would be expected to disrupt the normal anatomical relationship whereby the papilla is bathed in pelvic urine. In addition, exposure would result in the loss of CO_2 gas from the exposed papilla. Therefore, preliminary

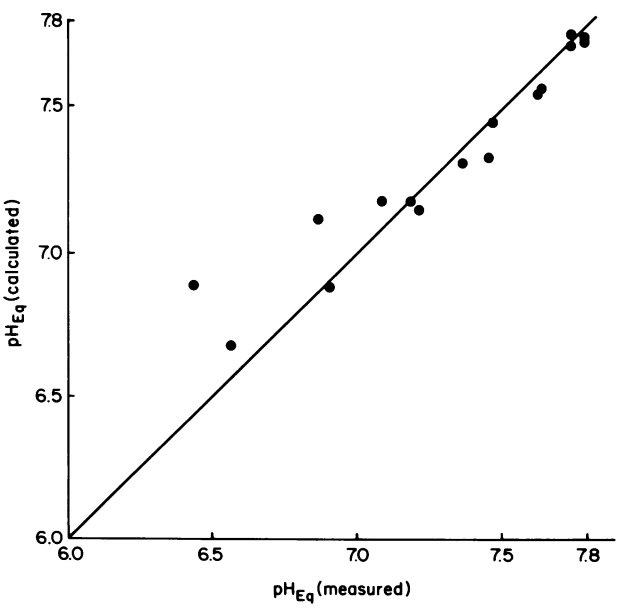

FIGURE 1 Comparison of pH_{eq} calculated from [tCO_2] and P_{CO_2} determinations (ordinate) vs. pH_{eq} measured by aspiration equilibrium pH microelectrode. Two techniques are not significantly different (r = 0.956) (pH_{eq}^{cal} = 0.74 pH_{eq}^{meas} + 1.9).

experiments were designed to allow direct determination of the P_{CO_2} of urine in the pelvic space. P_{CO_2} microelectrodes of 15–20 μm tip Diam were assembled. Six hydropenic rats in normal acid-base balance, and excreting an acid urine equal to 6.10 pH U were prepared for micropuncture and the renal pelvis was left intact. The mean P_{CO_2} of urine in the pelvic space surrounding the renal papilla was 36.5±1.2 mm Hg. When the P_{CO_2} electrode was then advanced into the papillary interstitium, equal values were obtained. In subsequent experiments (below) in which the renal pelvis was surgically excised, the renal papilla was bathed in heated mineral oil equilibrated with 5% CO_2. To assure that the mineral oil surrounding the papilla was maintained at 35 mm Hg, the P_{CO_2} of the oil was frequently measured with a P_{CO_2} microelectrode of 6–9 μm tip Diam.

Group I (controls) (n = 7)

Seven mutant Munich-Wistar rats served as controls after surgical exposure of the papilla while receiving a maintenance infusion of Ringer's bicarbonate at 1% body wt/h. Care was taken to maintain acid-base balance as described previously (16).

Group II (bicarbonate loading) (n = 25)

25 animals received 300 mM $NaHCO_3$ at 1.8% body wt/h in an attempt to minimize volume overexpansion. Micropuncture was initiated after a 1.5-h equilibration period.

Group III (bicarbonate load plus carbonic anhydrase) (n = 14)

14 of the same rats in group II then received carbonic anhydrase as an intravenous bolus (10 mg) and maintenance infusion (20 mg/h). Micropuncture was initiated 30 min after the bolus injection. Carbonic anhydrase was prepared from bovine erythrocytes (C-7500, Sigma Chemical Co., St. Louis, Mo.) and assayed for activity in vitro before use. The presence of carbonic anhydrase activity in urine from the right kidney was verified by the micromethod of Maren (22).

Arterial blood pH and P_{CO_2} and urine pH and P_{CO_2} were determined on a blood gas analyzer (model 165, Corning Medical, Medfield, Mass.). The blood $[HCO_3^-]$ was calculated with the Henderson-Hasselbalch equation ($\alpha = 0.0301$, $pK'_a = 6.10$), whereas for urine $[HCO_3^-]$ the α was 0.0309, and the pK'_a was corrected for ionic strength by the method of Hastings and Sendroy (23).

The results are expressed as mean±SE in each group. Statistical significance was calculated using the Student's *t* test for paired or unpaired data as appropriate.

RESULTS

The systemic arterial and urinary pH, P_{CO_2} and $[HCO_3^-]$ values and the U–B P_{CO_2} for all three grups are displayed in Table I. The control rats (group 1) were in normal acid-base balance and excreted an acid urine. These findings are consistent with previous controls in our laboratory (16, 18, 21). The U–B P_{CO_2} gradient was less than zero in this condition ($P < 0.01$). The animals receiving 300 mM $NaHCO_3$ (groups II and III) developed an acute metabolic alkalosis and excreted an alkaline urine (untouched kidney). In group II rats the P_{CO_2} in urine from the right kidney was 136.3±11.8 mm Hg, so that the U–B P_{CO_2} gradient was 97.4±10.6 mm Hg. After administration of carbonic anhydrase the urine pH increased significantly (7.54±0.12 to 8.17±0.05), the urine P_{CO_2} decreased significantly (136.3±11.8 to 59.5±2.3 mm Hg) and the U–B P_{CO_2} decreased, as expected (97.4±10.6 to 17.7±4.0) ($P < 0.001$). The U–B P_{CO_2} remained significantly greater than zero after carbonic anhydrase ($P < 0.01$), however.

Micropuncture data

The micropuncture findings are displayed in Tables II, III, IV, and Figs. 2–5.

P_{CO_2}. Control rats (group I) excreting an acid urine (6.04±0.06) had papillary collecting duct P_{CO_2} values that were very similar to systemic arterial values (35.2±1.2 at the base, and 36.5±1.5 mm Hg at the tip of the collecting duct) (Table II.). Thus the U–B P_{CO_2}, and the papillary-blood P_{CO_2} values were similar in control animals.

Bicarbonate-loaded rats (group II) excreting an alkaline urine displayed markedly elevated values for P_{CO_2} at the base (95.4±4.1) and tip collecting duct (122.2±4.3) (Table II, Fig. 2). The increase in P_{CO_2} from base to tip was significant ($P < 0.01$). Thus, the directly measured papillary collecting duct tip minus systemic blood P_{CO_2} (PCD-B P_{CO_2}) was 54.1±4.1 and 79.6±4.3, respectively (Tables II and III). As displayed in Table III and Fig. 3 the U–B P_{CO_2} from the untouched right kidney was slightly, but not significantly, greater than the PCD-B P_{CO_2} at the tip collecting duct. When 10 rats in this group having a urine pH nearer 8.0 pH units were analyzed separately, similar results were obtained. Specifically, values for arterial blood ($pH_a = 7.53±0.05$, $Paco_2 = 44±2.1$, $[HCO_3^-] = 36.8±1.1$) and urine (pH = 7.93±0.06 and $P_{CO_2} = 92±4.2$) were more alkaline and the urine P_{CO_2} slightly lower. The micropuncture findings for P_{CO_2} in these 10 rats were as follows: BCD = 90±4.7 and TCD = 115±6.8. These findings do not differ from the results obtained in all 25 rats in group II. Therefore, these results were combined.

In the 14 rats receiving $NaHCO_3$ and carbonic anhydrase infusion (group III) the P_{CO_2} at the base and tip collecting duct decreased markedly after infusion of the enzyme (95.4 to 68.1 and 122.2 to 78.3, respectively) (Table II). As shown in Fig. 3, carbonic anhydrase markedly reduced the U–B and PCD-B P_{CO_2} but did not obliterate this difference so that values of P_{CO_2} in both collected urine (right kidney) and the microelectrode determined value at the papillary tip (left kidney) remained significantly greater than sys-

TABLE I
Systemic Arterial and Urine Values

Group	Arterial blood			Urine (right-untouched)			
	pH	Pa_{CO_2}	$[HCO_3^-]$	pH	P_{CO_2}	$[HCO_3^-]$	U-B P_{CO_2}
I. Controls							
Mean	7.36	37	20.1	6.04	32	0.9	−5
SEM	±0.01	±1	±0.37	±0.06	±2.5	—	±1.3
(*n*)	(7)			(7)			(7)
II. 300 mM $NaHCO_3$							
Mean	7.50	44	33.0	7.54	136	108.3	97
SEM	±0.02	±1.7	±0.9	±0.12	±11.8	±9.3	±10.6
(*n*)	(25)			(25)			(25)
III. 300 mM $NaHCO_3$ + Carbonic anhydrase							
Mean	7.55	41	34.1	8.17	59	162.5	18
SEM	±0.02	±1.9	±1.1	±0.05	±2.3	±10.2	±4.0
(*n*)	(14)			(14)			(14)
P (II vs. III)	NS	NS	NS	<0.001	<0.001	<0.01	<0.001

temic arterial blood. (17.7±4.0 and 39.8±3.0, respectively) (Table III and Fig. 3).

Comparison of collecting duct and vasa recta P_{CO_2}. In five control and five bicarbonate-loaded rats comparison of P_{CO_2} at either the base or tip of the collecting duct with the P_{CO_2} in the immediately adjacent vasa recta was made (Fig. 4). Each point represents a paired tubule-vasa recta determination. Note that a highly significant correlation ($r = 0.97$) was obtained.

Disequilibrium pH. The values of the microelectrode determined *in situ* pH (pH_{is}), equilibrium pH (pH_{eq}) and the difference, or the disequilibrium pH (pH_{dq}) for controls, bicarbonate-loaded, and bicarbonate-loaded and carbonic anhydrase infused rats are displayed in Table IV for each micropuncture site. In control rats values for pH_{is} and pH_{eq} were similar at both the base (6.51±0.06, 6.44±0.06) and tip (6.47±0.07, 6.45±0.06) of the collecting duct so that a disequilibrium pH was not observed (i.e., pH_{dq} not

TABLE II
Micropuncture Data: P_{CO_2} Papillary Collecting Duct

Condition	P_{CO_2}		PCD-B P_{CO_2}*	
	Base	Tip	Base	Tip
I. Control				
Mean	35	36	−3	−3
SEM	±1.2	±1.5	±1.3	±1.4
(*n*)	(15)	(20)		
II. $NaHCO_3$				
Mean	95	122	54	80
SEM	±4.1	±4.3	±4.1	±4.3
(*n*)	(38)	(52)		
III. $NaHCO_3$ + Carbonic anhydrase				
Mean	68	78	29	40
SEM	±2.4	±2.8	±3.2	±3.0
(*n*)	(44)	(50)		
P II vs. III	<0.001	<0.001	<0.001	<0.001

* Papillary collecting duct minus systemic blood P_{CO_2}.

TABLE III
Comparison of Right (Untouched) and Left (Experimental) Kidney

Condition	Urine		Tip PCD	
	P_{CO_2}	U-B P_{CO_2}*	P_{CO_2}	P-B P_{CO_2}‡
II. $NaHCO_3$	136	97	122	80
	±11.8	±10.6	±4.3	±4.3
	(25)	(25)	(52)	(52)
III. $NaHCO_3$ + Carbonic anhydrase	59	18	78	40
	±2.3	±4.0	±2.8	±3.0
	(14)	(14)	(50)	(50)
P	<0.001	<0.001	<0.001	<0.001

* Urine minus blood P_{CO_2}.
‡ Papillary minus blood P_{CO_2}.

different than zero; +0.07±0.04 and +0.02±0.04, respectively). In bicarbonate-loaded rats (group II) *in situ* pH was significantly more acid than equilibrium pH at both sites (7.24±0.08 vs. 7.66±0.06 at the base $P < 0.001$, and 7.35±0.09 vs. 7.71±0.05 at tip $P < 0.01$) (Table IV and Fig. 5). Thus, a significant acid disequilibrium pH was observed at both base (−0.42±0.04) and tip collecting duct (−0.36±0.03). In the 10 rats in group II analyzed separately and having a mean urine pH of 7.93±0.03 U, the values for disequilibrium pH were not different ($P > 0.05$), i.e., base collecting duct = −0.40±0.04 and tip collecting duct = −0.35±0.03 U, (significantly greater than zero, $P < 0.01$). Carbonic anhydrase infusion (group III) completely obliterated the disequilibrium pH, at both sites, and did so by increasing *in situ* pH to values not different from equilibrium pH (pH_{dq} = +0.06 and +0.03, respectively) (Fig. 5). Note that equilibrium pH in groups II and III did not differ significantly in either the presence or absence of carbonic anhydrase (Fig. 5).

DISCUSSION

Of the several theories advanced to explain the observed increase in urinary P_{CO_2} above systemic arterial levels during bicarbonate loading, H^+ secretion into bicarbonate-containing fluid has been the most widely accepted mechanism (2, 4, 7, 10, 12). Recent studies have emphasized the "ampholyte" properties of bicarbonate and the critical importance of urinary concentrating ability (5, 6), thus seriously questioning the contribution of H^+ secretion to the generation of the elevated U-B P_{CO_2} difference. The present study was

TABLE IV
Disequilibrium pH Papillary Collecting Duct

Condition	Base CD			Tip CD		
	pH_{is}	pH_{eq}	pH_{dq}	pH_{is}	pH_{eq}	pH_{dq}
I. Controls	6.51	6.44	+0.07	6.47	6.45	+0.02
	±0.06	±0.06	±0.04	±0.07	±0.06	±0.04
			(28)			(33)
P vs. 0			NS			NS
II. $NaHCO_3$	7.24	7.66	−0.42	7.35	7.71	−0.36
	±0.08	±0.06	±0.04	±0.09	±0.05	±0.03
			(48)			(26)
P vs. 0			<0.001			<0.01
III. $NaHCO_3$	7.74	7.68	+0.06	7.73	7.70	+0.03
+ Carbonic anhydrase	+0.06	±0.06	±0.05	±0.08	±0.05	±0.03
			(18)	(14)		(14)
P vs. 0			NS			NS
II vs. III			<0.001			<0.001

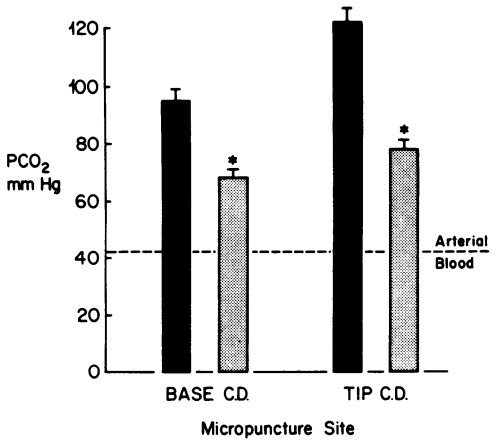

FIGURE 2 Microelectrode P_{CO_2} values in $NaHCO_3$ loaded (black bars) and $NaHCO_3$ loaded and carbonic anhydrase infused rats (stippled bars) at both micropuncture sites, the base and tip of the papillary collecting duct. The dashed line represents mean systemic arterial P_{CO_2}. The reduction in P_{CO_2} after carbonic anhydrase is highly significant. $^*P < 0.001$.

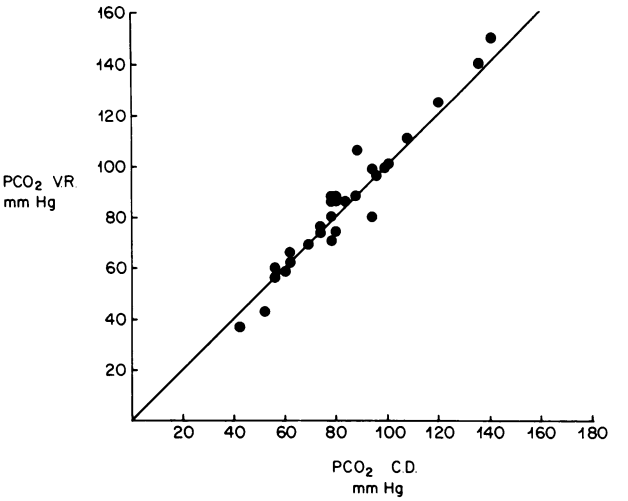

FIGURE 4 Comparison of P_{CO_2} in the papillary collecting duct (abcissa) and the immediately adjacent vasa recta (ordinate). Each point represents a paired determination and includes controls and bicarbonate loaded rats. The P_{CO_2} is similar in both structures ($r = 0.967$, $y = 1.097x - 6.19$).

designed to evaluate P_{CO_2} and the presence, or absence, of H^+ secretion by recently developed microelectrode techniques employed in a papillary micropuncture setting. This study represents the first report of direct determination of these parameters in the rat and thus adds insight to the process by which elevated urinary CO_2 tensions are achieved.

Several new findings emerge from these studies: (*a*) The demonstration of a significant acid disequilibrium pH in conjunction with a significantly elevated CO_2 tension in the papillary collecting duct during bicarbonate loading supports the view that hydrogen ion secretion is a determinant of the U-B P_{CO_2} gradient. (*b*) The observed increase in P_{CO_2} above systemic levels occurred both along the papillary collecting duct and after exit from the papilla. (*c*) Comparison of CO_2

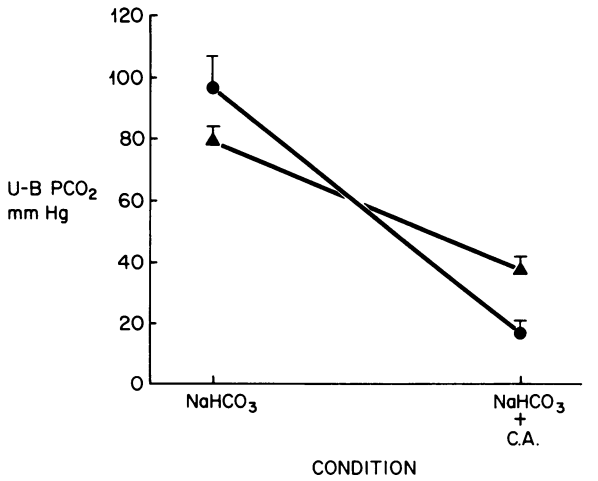

FIGURE 3 Comparison of right urinary P_{CO_2} (dots) and left papillary tip P_{CO_2} (triangles) minus blood P_{CO_2} (U-B P_{CO_2}) in $NaHCO_3$ loaded animals before and after carbonic anhydrase (CA).

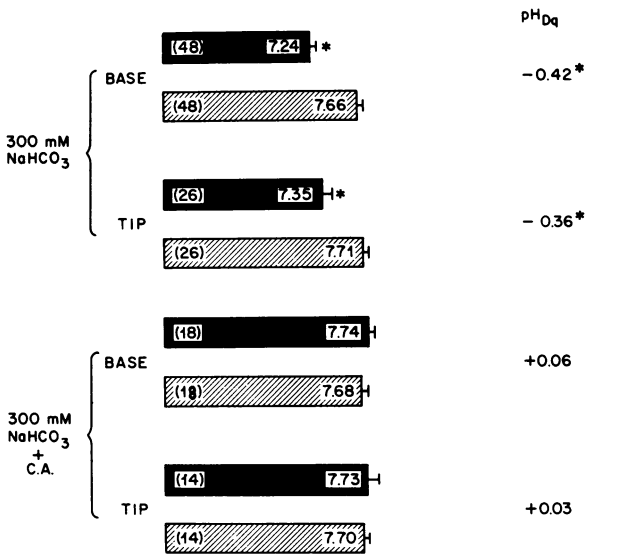

FIGURE 5 Summary of pH determinations in $NaHCO_3$ loaded rats before and after carbonic anhydrase (CA) infusion (left column) at both micropuncture sites (base and tip). The top (black) bar in both conditions at each site represents *in situ* pH while the hatched bars represent equilibrium pH. The values for the resulting pH_{dq} are displayed in the right column. The starred values for pH_{dq} are significantly greater than zero. ($P < 0.001$).

tension in both collecting duct fluid and the adjacent vasa recta revealed similar values, suggesting trapping of CO_2 in the medullary countercurrent system.

It has been widely appreciated that the P_{CO_2} of urine in man and experimental animals is near or below systemic arterial levels during normal or control conditions (4). In the present study it is demonstrated that the P_{CO_2} measured with a microelectrode by micropuncture of the papillary collecting duct of control rats is similar to the P_{CO_2} of systemic arterial blood (34.5 vs. 36.6 mm Hg). Furthermore, direct puncture of the pelvic space prior to exposure of the renal papilla revealed similar values for papillary collecting duct and pelvic space P_{CO_2}. Previous explanations for urinary CO_2 tensions, at or below plasma values during the excretion of an acid urine have included (*a*) reabsorption of bicarbonate in the terminal nephron decreasing the concentration of HCO_3^- generating a disequilibrium between CO_2 and H_2CO_3 (24), (*b*) a mixture of urine with differing concentrations of bicarbonate and nonbicarbonate buffers (13), and (*c*) passive back diffusion of undissociated organic acids (25). As pointed out by Rector (4) none of these explanations serve to support either H^+ secretion or HCO_3^- reabsorption in the collecting duct as a determinate of urinary CO_2 tensions. The failure to demonstrate a significant disequilibrium pH in the papillary collecting duct of control rats in the present study indicates that bicarbonate reabsorption in this segment, during this condition, is exceedingly low. This is not surprising in view of the very low luminal concentrations of bicarbonate and high concentrations of non-bicarbonate buffer in the collecting duct during excretion of an acid urine. Although the value for collecting duct P_{CO_2} agrees closely with the recent findings of Graber and associates (26), we have not, in contrast, demonstrated an acid disequilibrium pH in the collecting duct of control rats. Graber and associates, demonstrated an acid disequilibrium pH in control (−0.26 pH units) and acidotic rats (−0.13 pH U). The microcatherization technique used by Graber et al. (27) allows examination of a larger portion of the medullary collecting duct than in the present study. Different electrode techniques were used in the former study, as well, since aspiration into the microcatheter was required for the fluid to make contact with the pH electrode while equilibrium pH was measured in vitro. Based on the dehydration rate constant of 49.6/s (28), the equilibrium concentration of H_2CO_3 would be approached with a half-time of 1.4×10^{-2} s. Although nonbicarbonate buffers would reduce the disequilibrium pH by consumption of H_2CO_3, this effect might be offset by prolongation of the time to reach equilibrium. These differences are not easily resolved, therefore. These investigators have interpreted their findings as consistent with acidification by a H^+ secretory process along the medullary collecting duct in these conditions (27).

The fact that the P_{CO_2} of highly alkaline urine exceeds systemic arterial P_{CO_2} by several fold has been widely appreciated for several decades (1–3). Both the mechanism and clinical relevance of this observation have been a source of debate for many years, however. The major areas of disagreement have been reviewed in detail recently (4, 6, 7). The classical studies of Pitts and Lotspeich (2), and Ochwadt and Pitts (12) supported a proton secretion mechanism which, in urine containing bicarbonate, would generate H_2CO_3 that would dehydrate forming CO_2 at the uncatalyzed (slow) rate allowing trapping of CO_2 in the collecting system where surface-volume relationships would be unfavorable for diffusion. Thus, these investigators suggested an important role for delayed dehydration of H_2CO_3, as well as the postpapillary nature of this process in the generation of a high U–B P_{CO_2} difference (12).

Before the present investigation, no studies have directly determined the disequilibrium pH or P_{CO_2} in the papillary collecting duct. One micropuncture study by Uhlich, Baldamus, and Ullrich (15) used indirect techniques to estimate P_{CO_2}. These workers calculated P_{CO_2} from pH and bicarbonate determinations and demonstrated that collecting duct P_{CO_2} exceeded vasa recta blood P_{CO_2} by 40 mm during bicarbonate loading. Furthermore, this difference between tubule lumen and vasa recta was increased by carbonic anhydrase inhibition and obliterated by infusion of excess carbonic anhydrase. Thus, they concluded that the trapping of CO_2 by a medullary countercurrent system could not account for the high P_{CO_2} of alkaline urine (15).

In contrast, the findings in the present study demonstrate that the P_{CO_2} in the papillary collecting duct during bicarbonate loading was markedly elevated with respect to systemic arterial blood but was not significantly different than the P_{CO_2} in the adjacent vasa recta. These findings strongly support capture of CO_2 in the medullary counter-current system. Furthermore, a significant rise in P_{CO_2} was noted along the length of the papillary collecting duct available for micropuncture. Moreover, a significant acid disequilibrium pH was demonstrated at both the base and tip of the collecting duct in the same condition (−0.42 and −0.36) (Table V). When carbonic anhydrase was administered in amounts which achieved assayable activity in final urine, the disequilibrium pH was completely obliterated while P_{CO_2} fell dramatically indicating clearly that delayed dehydration of H_2CO_3 was an important factor in the generation of the elevated CO_2 tension in the collecting tubule. The fact that the

TABLE V
Summary of Micropuncture Findings

	BCD		TCD	
	P_{CO_2}	pH_{dq}	P_{CO_2}	pH_{dq}
Controls	35	+0.07	36	+0.02
$NaHCO_3$	95	−0.42	122	−0.36
$NaHCO_3$ + Carbonic anhydrase	68	+0.06	78	+0.03

P_{CO_2} of urine from the right untouched kidney remained at a level significantly greater than systemic arterial blood (59.5±2.3 vs. 41.0±1.9) after carbonic anhydrase is in agreement with previous studies (6, 29, 30). Very early studies demonstrating a complete return of urinary P_{CO_2} to systemic arterial levels after carbonic anhydrase infusion were based on calculated, not determined CO_2 tensions (12).

The CO_2 tension measured by the microelectrode at the tip of the collecting duct in the left papilla compared favorably, before and after carbonic anhydrase with that observed in the right final urine (standard macro technique). The observation of a slightly, but significantly higher CO_2 tension in the experimental kidney after carbonic anhydrase deserves further comment. The magnitude of the decrease in CO_2 after carbonic anhydrase depends on the magnitude of the prevailing CO_2 in the medullary interstitium, not systemic blood. When carbonic anhydrase is added to an open beaker of a $NaHCO_3$ solution in vitro, the P_{CO_2} falls as a result of CO_2 formation throughout the solution and acceleration of CO_2 loss at the liquid-gas interface (5, 6). The papillary interstitium may not be an entirely open system and the dissipation of CO_2 could proceed at a slower rate. Furthermore, the demonstration of similar values for P_{CO_2} in both collecting duct and vasa recta all along the length of exposed papilla, suggests trapping of CO_2 by a medullary countercurrent system. Since a disequilibrium pH of −0.36 U was observed in the tip of the papillary collecting duct it would be predicted that as chemical equilibrium is achieved, or as the acidic portion of nonbicarbonate buffers react with HCO_3^- at the higher pH (7), CO_2 would be generated and final urine should have a CO_2 tension ~25 mm Hg greater than the P_{CO_2} at the tip of the collecting duct. Comparison of these values in the present study (Table III) indicates that the observed increase in P_{CO_2} from left papillary tip (122±4.3 mm Hg) to right bladder urine (136±11.8) was not significant. The explanation for this observation is not entirely clear. It may not be appropriate to compare left and right renal findings in the papillary preparation since obvious differences in blood flow, concentrating ability, and electrolyte handing have been well described (20). Furthermore, it is generally appreciated that urine issuing out of the papillary collecting duct courses back over the papilla, which may allow equilibration of CO_2 between urine and papillary interstitium. Finally, CO_2 could diffuse out of the collecting system (albeit slight) or lost during handling of the urine sample.

In addition to hydrogen ion secretion, other possible mechanisms could result in a relative excess of H_2CO_3. These possibilities as recently outlined by Stinebaugh et al. (7), and Warnock and Rector (31) include bicarbonate secretion into buffer containing fluid (32), mixture of acid and alkaline tubule fluid from different nephron populations (13), water abstraction from tubule fluid resulting in concentration of H_2CO_3 and HCO_3^- in conjunction with CO_2 loss (24), and finally the dissociation of HCO_3^- to $CO_3^=$ and H_2CO_3 ("ampholyte" effect) (5, 6). Bicarbonate secretion has been observed in various types of acidifying epithelia including the rabbit cortical collecting tubule (32). The contribution of secretion to the final concentration of bicarbonate in urine has not been established, however. Previous micropuncture studies have demonstrated that the concentration of bicarbonate in the superficial distal tubule is quite high during acute metabolic alkalosis (18, 33). The bicarbonate concentration in the distal tubule during bicarbonate loading can be calculated from the equilibrium pH (7.47) and P_{CO_2} (65 mm Hg) to be 43.9 mM (18). These same studies failed to demonstrate a significant acid disequilibrium pH in the distal nephron during bicarbonate loading (18). Furthermore the anticipated increase in bicarbonate concentration from distal tubule to papillary collecting duct is modest, when water abstraction is considered, and casts doubt on a high capacity secretory process in the collecting tubule. Recently, Lombard and associates (34) have demonstrated that the net secretion of bicarbonate in the cortical collecting tubule of the rabbit is quite low. These same investigators demonstrated bicarbonate reabsorption, not secretion, in the medullary collecting tubule (34). Moreover, the capacity for bicarbonate reabsorption in the medullary collecting tubule exceeded that in the cortical collecting tubule by several fold (34). Compatible with this latter observation are recent micropuncture studies that have demonstrated bicarbonate reabsorption in the papillary collecting duct of the surgically exposed rat papilla (35). It seems highly unlikely, therefore, that bicarbonate secretion could be playing a significant role in the generation of an acid disequilibrium pH in the papillary collecting duct.

Although the admixture of urine of varying bicarbonate and nonbicarbonate buffer concentration could serve to elevate the urinary P_{CO_2} in certain conditions

(13), the demonstration by Rector and associates of high urinary CO_2 tensions during a combined water and bicarbonate diuresis in phosphate-depleted subjects (36) casts considerable doubt on this possibility as a sole determinant of the U-B P_{CO_2}. In the present study the excretion of nonbicarbonate buffers would be expected to be low (7) and "mixing" would be minimal from the base to tip of papilla since the majority of branch points occur proximal to this site. Furthermore, a significant acid disequilibrium pH was observed along the length of the papillary collecting duct examined, i.e., did not dissipate. Nonbicarbonate buffers may serve to further increase CO_2 tension in conditions in which the concentration of H_2CO_3 is slightly displaced from equilibrium, as in the present study, since, as pH rises, the acidic portion of the buffer (HA) would react with HCO_3^- to produce H_2CO_3 and thus CO_2 (13, 37). For example, at an equilibrium pH of 7.71 (papillary tip) 2 mM of buffer would produce ~0.22 mM of CO_2 and 5 mM of buffer would produce 0.55 mM of CO_2. Since nonbicarbonate buffer content was not measured in the present study, a correlation between the magnitude of the acid disequilibrium pH, the P_{CO_2}, and the amount of nonbicarbonate buffer present in the papillary collecting duct is not possible. The extent to which urinary P_{CO_2} is dependent on the combined effects of proton secretion and nonbicarbonate buffer content cannot be stated with certainty, therefore. A role for nonbicarbonate buffer as an additional determinant of the high CO_2 tension observed in the present study seems likely, however.

Another possible cause for the disequilibrium pH observed in the papillary collecting duct in this study would be CO_2 loss in a segment capable of significant concentration of bicarbonate and carbonic acid as originally proposed by Reid and Hills (24). Loss of CO_2 from collecting duct lumen to vasa recta is suggested by the demonstration of similar values for P_{CO_2} in these structures (Fig. 4). That there was not adequate concentration in this segment to generate a disequilibrium pH of the magnitude reported in this study is evidenced by the findings in Tables IV and V. The bicarbonate concentration increased from base to tip (104–140 meq/liter) (calculated from the measured pH_{eq} and P_{CO_2}). The tubular fluid to plasma inulin ratio increased slightly (15–22.5) from base to tip in animals similarly prepared for papillary micropuncture in this laboratory. Therefore, ~2% of the filtered load of bicarbonate was reabsorbed between base and tip. To generate a disequilibrium pH of −0.3 U (less than that observed at the base), the concentration of H_2CO_3 would be required to double. For water abstraction to account alone for such an increase in H_2CO_3 concentration, a similar increase in bicarbonate concentration would be expected (i.e., 104–208 meq/liter). To explain these findings on this basis alone seems untenable. Therefore, proton secretion seems the most likely determinant of the disequilibrium pH and elevated P_{CO_2} observed in the papillary collecting duct. Finally, in previous studies from this laboratory a disequilibrium pH was not observed in the superficial distal tubule (18). From the calculated concentration of bicarbonate (43.9 mM) in this segment in identically prepared rats, the transit time from distal tubule to base collecting duct of ~2 min, and the failure for water abstraction alone to account for the observation of a disequilibrium pH of −0.42 U at the base, it seems unlikely that a mechanism similar to that proposed by Reid and Hills (24), or mixing (13) could account for the observed changes between the distal tubule and base collecting duct as well. Because of the inaccessibility of the nephron segments involved, neither of these mechanisms can be totally eliminated, however.

Recent emphasis has been placed on the physicochemical properties of bicarbonate and the linear relationship between the urinary bicarbonate concentration and the U-B P_{CO_2} difference (5). The demonstration by Stinebaugh and associates (7) that this relationship can be altered by various physiological maneuvers and pathophysiological conditions as well as the theoretical considerations and calculations of these investigators, make a primary role for the "ampholyte" effect unlikely. The demonstration in the present study of an acid disequilibrium pH in association with elevated papillary CO_2 tensions both of which were markedly reduced by carbonic anhydrase is further evidence against CO_2 generation of this magnitude from a purely physicochemical process.

The recent demonstration that a spontaneous disequilibrium pH did not exist in the superficial distal tubule during bicarbonate loading (18), was interpreted as evidence for a low capacity proton secretory system in this segment. This finding has been strengthened by the recent in vivo microperfusion studies of Lucci and associates (38) which demonstrated that significant bicarbonate reabsorption was not present despite the existence of sodium reabsorption and potassium secretion in the superficial distal tubule (38). In this regard, and in view of the demonstration in the present study of a significant acid disequilibrium pH at the base of the papillary collecting duct, it is interesting to speculate that the medullary collecting duct, proximal to the first accessible micropuncture site, participates importantly in the acidification process by proton secretion. It is conceivable therefore, that the disequilibrium pH observed at the base papillary collecting duct (−0.42) pH U, underestimates the maximum disequilibrium pH achievable in the terminal

nephron. Furthermore, since carbonic anhydrase accelerates the conversion of carbonic acid to CO_2, and CO_2 loss from the collecting duct, the observation of a marked decrease in P_{CO_2} at the papillary base after carbonic anhydrase administration (Table II) suggests that an effect of the enzyme occurs in nephron segments proximal to the papillary collecting duct prior to accessibility to, and trapping in, the medullary countercurrent system.

The demonstration of an acid disequilibrium pH in the papillary collecting duct, only in association with elevated CO_2 tensions in equilibrium in the collecting duct and vasa recta, suggests that hydrogen ion secretion is a major determinant of the increase in the U–B P_{CO_2} gradient during an alkaline diuresis. An important role for a medullary countercurrent system, trapping CO_2 in the renal medulla, is also likely. Because of the technical limitations of this study, a role for mixing of dissimilar urines (13), "concentration" with CO_2 loss (24), and the effect of nonbicarbonate buffers cannot be totally eliminated as potential contributors to this process with certainty. In view of these findings, and the recent extensive clearance studies by Stinebaugh and associates (7, 10), Halperin et al. (9), and Arruda et al. (8), its is concluded that when appropriate consideration for urinary concentrating ability, nonbicarbonate buffer content, and urinary bicarbonate concentration is made, the U–B P_{CO_2} difference can be considered a reliable qualitative index of hydrogen ion secretion by the collecting tubule.

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