Direct Determination of P_{CO_2} in the Rat Renal Cortex

THOMAS D. DUBOSE, JR., LEO R. PUCACCO, DONALD W. SELDIN, NORMAN W. CARTER, and JUHA P. KOKKO, *Department of Internal Medicine, The University of Texas, Health Science Center at Dallas, Southwestern Medical School, Dallas, Texas 75235*

ABSTRACT The mechanism by which the kidney reabsorbs sodium bicarbonate could be a result of: (*a*) H^+ secretion, (*b*) direct HCO_3^- reabsorption, or (*c*) a combination of both processes. Most of the studies which have supported the H^+ secretory theory have involved the assumption that tubular fluid and arterial P_{CO_2} were equal. We have utilized a new P_{CO_2} microelectrode to directly determine *in situ* P_{CO_2} of tubular fluid and stellate vessel blood in the cortex of the rat kidney during control conditions and after alterations in acid-base status. In 21 control rats, proximal tubular fluid P_{CO_2} exceeded systemic arterial P_{CO_2} (ΔP_{CO_2}) by 25.9±0.92 mm Hg ($P < 0.001$). The values obtained for both distal tubular fluid and stellate vessel blood were not significantly different from proximal tubular P_{CO_2}. Evaluation of P_{CO_2} in the proximal tubules of Munich-Wistar rats did not reveal evidence for a declining profile for P_{CO_2} along the length of the nephron. When proximal bicarbonate reabsorption was increased or decreased acutely by alterations in acid-base status, ΔP_{CO_2} changed in parallel. Furthermore, benzolamide administration significantly reduced ΔP_{CO_2}. We conclude: (*a*) that the P_{CO_2} in tubular fluid is significantly greater than systemic arterial P_{CO_2}, (*b*) that there is no tendency for the observed P_{CO_2} to fall along the proximal tubule, (*c*) the mean P_{CO_2} in the proximal and distal tubules as well as the stellate vessel is not significantly different, thereby rendering the concept of a "diffusion barrier" for CO_2 in the proximal tubule unlikely, and (*d*) the level of renal cortical P_{CO_2} appears to vary directly with the magnitude of bicarbonate reabsorption.

INTRODUCTION

The reabsorption of bicarbonate by the proximal tubule has been assumed to be a result of either: (*a*) hydrogen secretion into bicarbonate-containing tubular fluid, thereby generating carbonic acid (1–6), (*b*) direct bicarbonate reabsorption (7), or (*c*) a combination of both mechanisms (8).

Most of the studies supporting hydrogen secretion as the mechanism mediating bicarbonate reabsorption have involved the necessary assumption that systemic arterial and tubular fluid P_{CO_2}[1] are equal. In fact, this assumption was inherent in the calculation of a disequilibrium pH because tubular and arterial P_{CO_2} were simply assumed to be the same (2, 3, 9). The direct measurement of proximal tubular P_{CO_2} and an assessment of its determinants, therefore, has recently assumed a central role in the evaluation of the respective mechanisms mediating bicarbonate reabsorption (10, 11). Karlmark and Danielson (12) were the first to suggest that tubular fluid might not be in equilibrium with arterial blood. Despite the wide range of CO_2 tensions reported by these investigators, the inference was made that a declining profile for P_{CO_2} existed along the length of the proximal tubule (12). In this study, however, the P_{CO_2} of tubular fluid was not determined directly, but was calculated by indirect techniques from measurements of *in situ* pH. More recently, Sohtell and Karlmark (13) have reported preliminary direct measurements of *in situ* P_{CO_2}, the magnitude of which exceeded arterial blood by 16.6±4.5 mm Hg in 10 proximal tubules. Based on these indirect and direct findings, Karlmark (14), Malnic and Steinmetz (10), and Giebisch and Malnic (11) have suggested that the CO_2 generated in the tubular lumen as a result of hydrogen secretion encounters a "diffusion barrier" for CO_2 in the early proximal tubule.

The present study was designed to elucidate the magnitude and determinants of P_{CO_2} in proximal tubular fluid. We have utilized a new P_{CO_2} microelectrode modified from that originally described by Caflisch and Carter (15). The specific purposes of the present study were threefold. First, proximal tubular P_{CO_2}

Portions of this study were presented at the 10th Annual Meeting of the American Society of Nephrology, 20–22 November 1977.

Received for publication 14 November 1977 and in revised form 24 March 1978.

[1] *Abbreviations used in this paper*: ΔP_{CO_2}, difference of P_{CO_2} between cortical puncture site and systemic arterial blood.

 J. Clin. Invest. © *The American Society for Clinical Investigation, Inc.*, 0021-9738/78/0801-338 $1.00

was compared with that of other portions of the nephron as well as the surrounding vascular structures to determine whether a diffusion barrier for CO_2 was present across the proximal tubular epithelium. Second, P_{CO_2} was measured sequentially, from early to late proximal tubule, to evaluate the possibility of a declining profile for CO_2. Third, the relationship between the magnitude of bicarbonate reabsorption and CO_2 generation was evaluated by determining the effect of variations in bicarbonate reabsorption on the level of P_{CO_2} in the cortex. The results of these in vivo and additional in vitro studies are then utilized to attempt to define the possible mechanisms of CO_2 generation in the renal cortex.

METHODS

Adult Sprague-Dawley or mutant Munich-Wistar rats (mean wt = 202 g) were allowed free access to food and water up to the time of intraperitoneal injection of Inactin (Promonta, Hamburg, West Germany) 100 mg/kg body wt. After tracheostomy, the left jugular vein was doubly cannulated with two PE 50 catheters. The left femoral artery was cannulated for blood pressure monitoring (Statham Transducer, model P23 Db, Statham Instruments, Division, Gould, Inc., Oxnard, Calif.) and for collection of arterial blood gases and electrolytes. The animal's body temperature was maintained at 37°C on a thermostatically controlled heating table. The left kidney was prepared for micropuncture in the usual manner (16), and bathed continuously with mineral oil equilibrated with CO_2 gas in a standard bubbling chamber and then maintained at 37°C. The P_{CO_2} of the bathing solution was adjusted as needed to closely approximate (at the cortical surface) the arterial P_{CO_2} of the animal. To assure that the P_{CO_2} of the bathing solution, per se, could not serve to falsely elevate the level of P_{CO_2} within the structures of the renal cortex, the P_{CO_2} of the oil bath was measured directly with the P_{CO_2} microelectrode before and after each individual puncture. The P_{CO_2} of the oil bath was always maintained at a level below that observed *in situ*. Furthermore, experiments were performed with mineral oil not equilibrated with CO_2 gas to assure that this solution could not serve to elevate the P_{CO_2} *in situ*. In addition, equilibrated and nonequilibrated Ringer's bicarbonate baths were tested in a few rats, and the results obtained were not significantly different from the findings with a mineral oil bath. Urine was collected with a PE 90 suprapubic bladder catheter for the right, nonexperimental kidney and a PE 50 ureteral catheter for the left kidney, both under water-equilibrated mineral oil. After completion of the initial surgery, each animal received an intravenous infusion of Ringer's bicarbonate (Na = 140, Cl = 115, HCO_3 = 30, and K = 5 meq/liter) at a rate equivalent to 1% body wt/h (control period). Identification of the various superficial segments selected for micropuncture was accomplished by observing the passage of Lissamine green dye (30 μl of 10% solution) as previously described (16). Proximal transit times > 13 s or arterial blood pressure < 110 mm Hg were causes for rejection at this point.

To insure stability with respect to acid-base balance during the control or experimental periods, arterial blood gases were frequently monitored (30- to 45-min intervals) in each animal by withdrawal of 250 μl of femoral arterial blood into a heparinized glass syringe. Arterial blood gases, urine pH, and urine P_{CO_2} were determined on a Corning blood gas analyzer (model 165, Corning Medical, Corning Glass Works, Medfield, Mass.) which was carefully calibrated with pH standard buffers, and analytically balanced gas mixtures/Biomed Instruments, Inc., Chicago, Ill.) the accuracy of which were counterchecked on an IL Duo-Matic blood gas machine (model 123, Instrumentation Laboratory, Inc., Lexington, Mass.).

We have noted that animals prepared for micropuncture in the standard manner are frequently mildly hypoxic ($P_{CO_2} \cong 75$ mm Hg) and may be unstable with respect to respiratory rates which can vary from hypoventilation (P_{CO_2} = 48–51 mm Hg) to hyperventilation (P_{CO_2} = 25–30 mm Hg). Although the explanation for this problem is not totally apparent, we have found that it can be alleviated in part by: (*a*) careful maintenance of the anesthetized state with 100 mg/kg intraperitoneal Inactin (Promonta, Hamburg, West Germany) plus very small supplementary intravenous injections of Inactin (0.05 mg in 0.2 ml 0.9% NaCl) as often as needed, and (*b*) utilization of either a volume-regulated rodent ventilator (Harvard Apparatus Co., Inc., Millis, Mass.) to which 40% oxygen (balance air) is added and the respiratory rate adjusted to maintain P_{CO_2} between 35 and 40 mm Hg and P_{O_2} between 90 and 100 mm Hg; or by utilization of a tracheal T tube through which the same inspired oxygen content is delivered. Both procedures result in very stable arterial blood gases over a prolonged period (2–3 h). In any case, animals were rejected in which stable blood gases could not be maintained. Furthermore, *in situ* P_{CO_2} measurements (as described below) were always bracketed before and after determination (not greater than 30 min) by obtaining arterial blood gases to insure stability. Data were not accepted if arterial P_{CO_2} before and after data collection varied > ±5.0 mm Hg.

The *in situ* P_{CO_2} of proximal and distal tubular fluid was determined with a new P_{CO_2} microelectrode of a 4–6-μm tip diameter. This microelectrode is a modified and significantly improved version of the original CO_2 microelectrode described by Caflisch and Carter (15). The P_{CO_2} microelectrode described here also has advantages when compared with the microelectrode recently utilized by Sohtell and Karlmark (13). Our P_{CO_2} microelectrode has the characteristics of obtaining near theoretic slope (maximal sensitivity predicted by the Nernst equation) (57–61 mV/$\log_{10}$ P_{CO_2}), and tip diameters sufficiently small (4–6 μm) to allow access to all superficial structures. The electrodes employed in our experiments (Fig. 1) have utilized the new glass-membrane pH microelectrode as the pH sensor. This pH sensor, as described by Pucacco and Carter (17), obtains near theoretic slope (59 mV/pH U at 22°C and 61 mV/pH U at 37°C). As can be seen in Fig. 1, the pH electrode is placed in a weak bicarbonate solution (1 mM $NaHCO_3$ plus 100 mM NaCl) behind a 5–15-μm thick silicon rubber membrane (gas permeable, liquid impermeable). The circuit is completed with a silver: silver chloride reference wire and the entire electrode is sealed at the butt end with epoxy cement. The silver wire from the pH electrode is connected to the input terminal of a Keithley model 602 solid state electrometer (Keithley Instruments, Inc., Cleveland, Ohio) using a shielded cable. The silver reference wire from the bicarbonate solution is connected to the low impedance input terminal of the same electrometer. The potential sensed by the electrometer and recorded on a Rikadenki multi-pen recorder (Soltec Corp., Sun Valley, Calif.) is proportional to the pH of the bicarbonate solution with a sensitivity of 61 mV/pH U (37°C). The bicarbonate concentration in the electrode is 1.0 mM. It is obvious from the Henderson-Hasselbalch relationship that the pH of the bicarbonate solution will be determined by and directly proportional to the partial pressure of the CO_2 gas in solution. The pH of the bicarbonate solution will

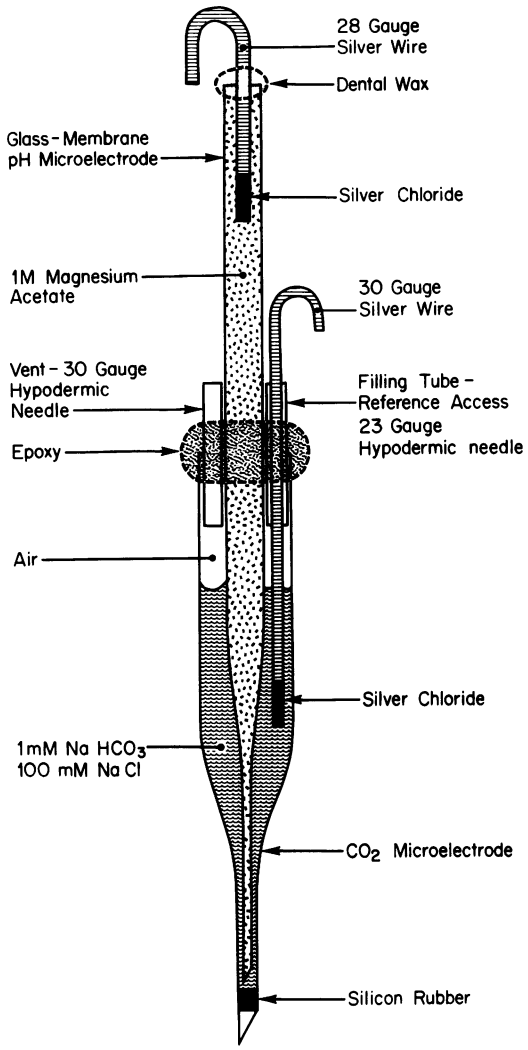

FIGURE 1 Schematic of P_{CO_2} microelectrode. Electrode consists of an outer shell into which a glass-membrane pH electrode is placed in a weak bicarbonate solution behind a gas-permeable membrane.

therefore vary in a predictable manner as the P_{CO_2} of the solution is varied. Because the pH electrode obtains near theoretic slope, the difference in potential recorded between two analytically determined gases, 5% (35 mm Hg) and 20% (140 mm Hg) will be ≅ 0.61 pH U or 36 mV. This represents the means by which the P_{CO_2} microelectrode is calibrated in actual use.

The manufacturing procedure for the present electrode differs from those previously described (13, 15) in that the steps are reversed. That is, the gas-selective membrane (silicon rubber) is installed first (not last) so that a "dry seal" (silicon rubber to glass) is made, thus avoiding the problem of a liquid pathway between the unknown solution and the reference bicarbonate solution, and is so maintained throughout the actual operation of the electrode. The outer shell of the P_{CO_2} electrode is then filled with the bicarbonate solution and finally, the pH electrode is inserted and advanced to a position as near the CO_2 membrane as possible.

The P_{CO_2} microelectrodes utilized in these experiments were calibrated by immersing the tip into 10-mM $NaHCO_3$ solutions equilibrated with gases of known (analyzed) content, 2, 5, 10, and 20% CO_2 (balance O_2), maintained at 37°C in a Lucite (E. I. du Pont de Nemours Co., Wilmington, Del.) equilibration chamber. This chamber is designed so that the solution to be equilibrated with CO_2 is placed in a long, narrow cylinder so that the gas enters the cylinder from below through a fritted disk allowing small bubble dispersion of gas constantly flowing throughout calibration. The chamber enclosing the cylinders is maintained at 37°C by a recirculating water bath. Because the bicarbonate solution placed in the cylinder is of known content (10 mM) the effectiveness of CO_2 equilibration can be monitored by a commercial Beckman glass pH electrode (Beckman Instruments, Inc., Fullerton, Calif.). This provides a valuable check point for the calibration system. After obtaining values for the electrical potential thus observed in the 35- and 140-mm Hg P_{CO_2} standard solutions, the electrode tip is placed into standard pH 4 buffer. There are two reasons for this step: (*a*) to allow the P_{CO_2} within the microelectrode to drop well below 35 mm Hg very rapidly (P_{CO_2} of pH 4 buffer = 0); and (*b*) to verify the integrity of the CO_2 membrane, i.e. if a liquid pathway exists, pH 4 buffer will rapidly titrate the alkaline $NaHCO_3$ solution and result in measurement of an acid pH. If no liquid pathway exists, the pH 4 buffer will simply reduce the CO_2 content within the electrode toward zero and thus, the pH will increase. The gas selectivity of the CO_2 membrane was assured in this manner immediately before and after obtaining data in vivo. A P_{CO_2} microelectrode assembled in this manner will have the following characteristics: tip diameter of 4–6 μm, slope (sensitivity) of 33–36 mV between 35 and 140 mm Hg P_{CO_2} (57–61 mV/$\log_{10}$ P_{CO_2}), response time of 1–4 min, stability of intercept (drift) of < 1–3 mV/30 min, and a 1- to 2-day lifetime. In Fig. 2, the electrical response of seven typical microelectrodes (4–7 μm OD) to standard solutions

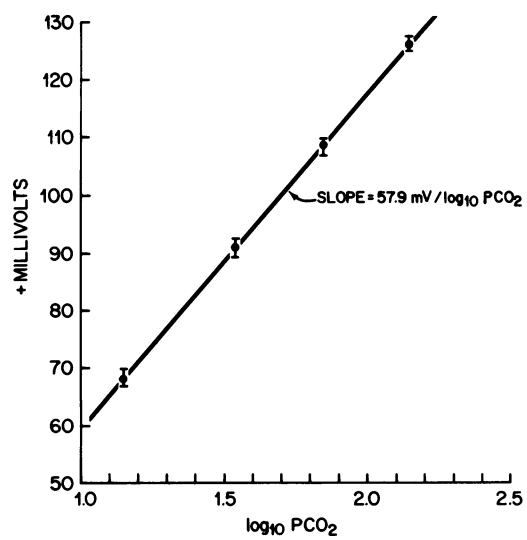

FIGURE 2 Electrical response of seven typical microelectrodes. The microelectrodes were placed into bicarbonate solutions equilibrated with known CO_2 gases at 22°C and the electrical voltage plotted as a function of $\log_{10}$ P_{CO_2}. Each point represents the mean response of seven electrodes and the vertical lines represent the range. Theoretic slope at 22°C equals 59.4 mV/$\log_{10}$ P_{CO_2}.

equilibrated in vitro with various known CO_2 tensions at 22°C is displayed. Each point represents the mean value obtained at the respective CO_2 tension. The vertical lines represent the range of the measured voltage. The slope obtained in this case ranged from 56 to 60 mV/$\log_{10}$ P_{CO_2} with an average value of 57.9 mV/$\log_{10}$ P_{CO_2}. Theoretic slope at this temperature is 59.4 mV/$\log_{10}$ P_{CO_2}. After demonstrating logarithmic linearity in this manner, calibrations were performed using only the 5% (35.0 mm Hg) and 20% (140.0 mm Hg) CO_2 equilibrated solutions.

A comparison of the P_{CO_2} of rat blood determined by both a 7.0-μm P_{CO_2} microelectrode and a standard P_{CO_2} macroelectrode (Corning model 165, Corning Medical, Glass Works) is depicted in Table I. There was no significant difference in the determined values with these two techniques (r = .998). To further validate the accuracy of the P_{CO_2} microelectrode for determination of blood P_{CO_2} in vivo, direct punctures of the renal vein were compared with the values for P_{CO_2} in the structures of the renal cortex in four rats. Furthermore, the values for renal vein P_{CO_2} were compared to systemic arterial P_{CO_2} as determined with the standard P_{CO_2} macroelectrode. This comparison was utilized to (*a*) verify appropriate variation in the level of P_{CO_2} sensed in vivo, and (*b*) to compare directly the values obtained for blood P_{CO_2} with macro- and microelectrodes. Therefore, by obtaining data in this manner, validation of measurements of P_{CO_2} for blood was thus assured.

All calibrations and determinations of *in situ* P_{CO_2} were carried out on a standard micropuncture table totally enclosed in a Faraday cage that was connected to earth ground. All electrical equipment remained outside the cage. This was necessary because of the high resistance of the microelectrode (10^9–10^{10} Ω). In addition, the micropuncturist was required to remain quite motionless and in contact with the floor of the cage. With these precautions, tracings virtually free of interference could be obtained.

After completion of the surgery, and a 1-h equilibration period while receiving the Ringer's infusion at 1% body wt/h, arterial blood gases were obtained to establish acceptability of the rat during the control period. The P_{CO_2} microelectrode was then calibrated as described above. Three to five data points were obtained from the previously mapped, superficial nephrons including early and late proximal convoluted tubules, early and late distal convoluted tubules, and star vessels. The postpuncture calibration was then immediately performed (pH 4 buffer was also utilized to rule out liquid pathways) and arterial blood gases were obtained. The electrode was almost always stable in terms of minimal drift and reproducibility of intercept and slope. However, instances in which electrode drift > 3 mV occurred, resulted in rejection of the data. After obtaining adequate control data, the experimental period was instituted as determined by the respective groups.

TABLE I

Comparison of Blood P_{CO_2} as Determined by P_{CO_2} Microelectrode and a Standard P_{CO_2} Macroelectrode‡ In Vitro§*

	P_{CO_2} microelectrode	P_{CO_2} macroelectrode	ΔP_{CO_2}
	mm Hg	*mm Hg*	*±mm Hg*
1	37.8	37.1	0.7
2	41.5	40.6	0.9
3	38.5	38.8	0.3
4	101.0	97.5	3.5
5	65.5	67.7	2.2
6	31.5	32.0	0.5
7	82.0	80.8	1.2
8	43.0	42.3	0.7
9	24.9	27.4	2.5
10	4.8	6.2	1.4
Mean±SEM			1.4±0.31
r			0.998

* 7 μm P_{CO_2} electrode.

‡ Corning model 615 (Corning Medical, Glass Works).

§ Blood obtained from four rats maintained at 37°C under oil.

Group I (control Sprague-Dawley rats) (n = 21). Data were obtained from these rats, as described above, over a 1.0- to 1.5-h control period in which the precautions just mentioned were utilized to insure stability of both the animal and the electrode. 21 male Sprague-Dawley rats (mean = 205 g) served as controls.

Group II (control Munich-Wistar rats) (n = 5). The mutant Munich-Wistar rat was utilized because of the presence of surface glomeruli and thus, the accessibility of the early proximal convoluted tubule. These rats were selected for micropuncture in exactly the same manner as group I. The P_{CO_2} microelectrode was inserted at intervals along the entire length of the proximal tubule from later to earlier segment to determine the presence or absence of a profile for CO_2.

Group III (stimulation of proximal bicarbonate reabsorption) (n = 7). After collection of control data, as in the previous groups, seven Sprague-Dawley rats were subjected to acute respiratory acidosis (10% CO_2 via respirator), and acute volume depletion by administration of intravenous furosemide (5.0 mg/kg body wt). Urinary electrolyte and volume losses were not replaced and 1 h after initiation of respiratory acidosis and furosemide administration, micropuncture was again performed as described above. Thus, proximal bicarbonate reabsorption was maximally increased by a combination of: volume depletion, hypokalemia, and respiratory acidosis.

Group IV (inhibition of proximal tubular bicarbonate reabsorption) (n = 7). After a suitable control period, seven male Sprague-Dawley rats were simultaneously volume expanded with 0.9% NaCl to equal 10% body wt in 1 h, acutely loaded with KCl (0.25-mM solution at 3.0 ml/h) and hyperventilated with the respirator. After a 1.0-h equilibration period, the saline diuresis was maintained by infusion of 0.9% NaCl, and 0.25 mM KCl at a rate designed to replace urinary losses. This resulted in a volume-expanded, hyperkalemic (K^+ = 4.1 meq/liter controls vs. 6.8 meq/liter experimental) rat with acute respiratory alkalosis. This condition was employed to maximally decrease proximal bicarbonate reabsorption. Data were then obtained in exactly the same manner as in controls. These animals remained stable throughout the remaining 45- to 60-min period of observation.

Group V (carbonic anhydrase inhibition) (n = 5). After obtaining control data in exactly the same manner as group I, five Sprague-Dawley rats received a bolus injection of benzolamide 2.0 mg/kg body wt followed by a maintenance infusion of 2.0 mg/kg per h in a 300-mM/liter $NaHCO_3$ solution infused at 1.2 ml/h. 5–10 min after benzolamide administration, an alkaline diuresis was observed. $NaHCO_3$ was administered to replace urinary losses and prevent metabolic acidosis (18). 10–15 min after benzolamide administration, micropuncture was again performed in exactly the same manner during a subsequent 30-min period.

The results are expressed as the mean±SEM for each period in each of the five groups. Statistical significance between mean values was calculated using the Student's t test for paired or unpaired data as appropriate.

TABLE II

Summary of Systemic and In Situ P_{CO_2} in 21 Control Rats

	Systemic blood		Urine		Proximal tubule		Distal tubule		Star vessel	
	pH	P_{CO_2}	pH	P_{CO_2}	P_{CO_2}	ΔP_{CO_2}*	P_{CO_2}	ΔP_{CO_2}*	P_{CO_2}	ΔP_{CO_2}*
	U	*mm Hg*	*U*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*
Mean	7.34	39.2	6.15	43.4	65.1	25.9	67.1	26.7	64.8	24.9
SEM	±0.01	±1.09	±0.07	±2.73	±1.21	±0.92	±1.43	±1.11	±1.74	±1.27
(*n*)‡	(42)	(42)	(21)	(21)	(68)	(68)	(54)	(54)	(28)	(28)

* ΔP_{CO_2} = *in situ* P_{CO_2} minus systemic P_{CO_2} ($P < 0.001$).

‡ Number of determinations or punctures.

Proximal vs. distal vs. star vessel P_{CO_2} not significantly different.

RESULTS

Group I (Sprague-Dawley controls) (n = 21). A summary of the findings in the 21 control Sprague-Dawley rats is presented in Table II. Systemic arterial pH was 7.34±0.01 U and arterial P_{CO_2} was 39.2±1.09 mm Hg. Urinary pH was 6.15±0.07 U and P_{CO_2} was 43.4±2.73 mm Hg. In the proximal tubule, the *in situ* P_{CO_2} was 65.1±1.21 mm Hg ($n = 68$) which indicates a significant difference in P_{CO_2} between tubular fluid and systemic arterial blood (ΔP_{CO_2}) of 25.9±0.92 mm Hg ($P < 0.001$). The *in situ* P_{CO_2} in the distal tubule and star vessel was similarly elevated (67.1±1.43 and 64.8±1.74 mm Hg, respectively). This resulted in a ΔP_{CO_2} of 26.7±1.11 mm Hg in the distal tubule and 24.9±1.27 mm Hg in the star vessel, respectively. Furthermore, the values obtained in the distal tubule and star vessel were not significantly different from that value obtained in the proximal segment ($P > 0.05$).

Group II (Munich-Wistar control) (n = 5). The findings of arterial blood and urine pH and P_{CO_2}, and the micropuncture findings for this group are summarized in Table III. The systemic arterial blood, urinary pH, and P_{CO_2} values were not significantly different from those values observed in the Sprague-Dawley controls (Table II). This mutant strain of rat, because of the presence of surface glomeruli, allowed the examination of the early proximal tubule at the glomerulus (Table III). Note that the P_{CO_2} in the early proximal convoluted tubule (74.5±3.31 mm Hg) was not significantly different from the P_{CO_2} in the last accessible superficial proximal segment (71.9±1.72 mm Hg). In addition, single proximal tubules punctured sequentially in retrograde fashion did not exhibit values for P_{CO_2} which were different along the nephron. As in the Group I rats, the P_{CO_2} obtained in the early and late distal tubule was not significantly different from the value in the early and late proximal tubule ($P < 0.05$). In addition, the star vessel P_{CO_2} was the same (74.6 ±2.87 mm Hg) as in the tubular fluid ($P > 0.05$). The P_{CO_2} and ΔP_{CO_2} obtained in the Munich-Wistar rats was not significantly greater than the values in the Sprague-Dawley rats.

Group III (stimulation) (n = 7) and group IV (suppression of proximal bicarbonate reabsorption (n = 7). To examine the response of the renal *in situ* P_{CO_2} to variations in bicarbonate reabsorption, these two groups were evaluated in the manner described in Methods. The findings are summarized in Table IV and Fig. 3. The values for arterial and urine pH and P_{CO_2} as well as the *in situ* P_{CO_2} in the segments examined during the control period were not different for groups III and IV, and compare favorably to the controls in group I. When bicarbonate reabsorption was stimulated (group III) by acute respiratory acidosis and volume depletion, arterial pH decreased to

TABLE III

P_{CO_2} Profile along the Superficial Nephron in Five Control Munich-Wistar Rats

	Systemic blood		Urine		EPCT*		LPCT		EDCT		LDCT		Star vessel	
	pH	P_{CO_2}	pH	P_{CO_2}	P_{CO_2}	ΔP_{CO_2}	P_{CO_2}	ΔP_{CO_2}	P_{CO_2}	ΔP_{CO_2}	P_{CO_2}	ΔP_{CO_2}	P_{CO_2}	ΔP_{CO_2}
	U	*mm Hg*	*U*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*
Mean	7.33	42.8	6.17	47.2	74.5	29.2	71.9	31.6	75.9	31.3	71.5	29.4	74.6	31.4
SEM	±0.02	±2.06	±0.07	±6.81	±3.31	±2.93	±1.72	±1.89	±3.71	±3.77	±1.89	±1.62	±2.87	±2.10
(*n*)	(10)	(10)	(5)	(5)	(5)	(5)	(16)	(16)	(6)	(6)	(14)	(14)	(6)	(6)

* EPCT earliest accessible proximal tubule at surface glomerulus.

EPCT vs. LPCT vs. EDCT vs. LDCT not significantly different.

TABLE IV

Effect of Acute Alterations in Acid-Base Status on In Situ P_{CO_2} and the Cortical to Systemic P_{CO_2} Gradient

	Systemic blood		Urine	PCT		DCT		Star vessel	
	pH	P_{CO_2}	pH	P_{CO_2}	ΔP_{CO_2}	P_{CO_2}	ΔP_{CO_2}	P_{CO_2}	ΔP_{CO_2}
	U	*mm Hg*	*U*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*
Group III (n = 7)									
Control	7.39±0.01 (14)	35.8±1.4 (14)	6.39±0.05 (7)	60.3±0.69 (23)	26.2±1.1 (23)	59.5±0.72 (17)	24.3±1.2 (17)	60.9±1.4 (10)	25.1±2.1 (10)
Stimulation*	7.13±0.03 (14)	73.6±3.1 (14)	5.89±0.30 (7)	113.1±4.3 (32)	43.0±2.6 (32)	115.7±4.6 (20)	44.3±3.0 (20)	120.8±5.6 (14)	49.1±3.3 (14)
P	<0.001	<0.001	NS	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Group IV (n = 7)									
Control	7.32±0.02 (14)	37.3±1.5 (14)	6.00±0.13 (7)	59.7±2.6 (23)	22.8±2.0 (23)	64.7±4.3 (12)	26.8±3.3 (12)	60.2±3.7 (9)	23.4±2.6 (9)
Suppression‡	7.49±0.02 (14)	15.5±1.4 (14)	7.24±0.04 (7)	27.2±1.2 (33)	8.5±0.6 (33)	25.2±1.8 (17)	7.7±0.67 (17)	26.1±2.0 (10)	7.5±0.83 (10)
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

* Hypercapnia, volume depletion, and hypokalemia.

‡ Hypocapnia, volume expansion, and hyperkalemia.

Data expressed as mean±SEM. Number of determinations or punctures are in the parentheses.

7.13±0.03 U and P_{CO_2} increased to 73.6±3.1 mm Hg (Table IV). The *in situ* P_{CO_2} and ΔP_{CO_2} was significantly increased in the proximal tubule (113.1±4.3 and 43.0±2.6), distal tubule (115.7±4.6 and 44.3±3.0), and star vessel (120.8±5.6 and 49.1±3.3) mm Hg, respectively ($P < 0.001$ stimulation vs. control for all

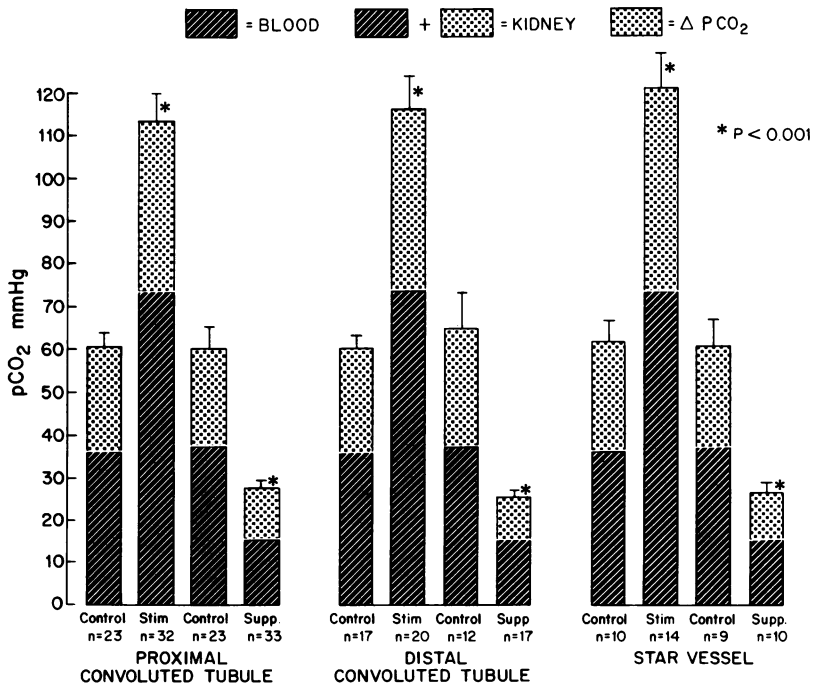

FIGURE 3 Response of *in situ* P_{CO_2} to variations in proximal tubular bicarbonate reabsorption. The highest point of the bar represents the *in situ* P_{CO_2} at each micropuncture site. Systemic arterial P_{CO_2} is represented by the hatched portion of the bars and ΔP_{CO_2} by the stippled area. Stimulation (stim) of bicarbonate reabsorption resulted in significant increases in P_{CO_2} and ΔP_{CO_2}. The converse was observed with suppression (supp) of bicarbonate reabsorption ($P < 0.001$). n = number of determinations.

TABLE V
Effect of Benzolamide on In Situ P_{CO_2} of the Rat Renal Cortex

	Systemic blood			Urine		Proximal tubule		Distal tubule		Star vessel	
	pH	P_{CO_2}	HCO_3^-	pH	P_{CO_2}	P_{CO_2}	ΔP_{CO_2}	P_{CO_2}	ΔP_{CO_2}	P_{CO_2}	ΔP_{CO_2}
	U	*mm Hg*	*mM*	*U*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*	*mm Hg*
Control (n = 5)	7.36	40.9	22.1	6.13	39.5	73.0	32.6	73.0	32.1	71.1	32.4
	±0.01	±2.6	±1.0	±0.10	±2.4	±3.2	±2.6	±3.8	±2.9	±4.4	±3.4
	(10)	(10)	(10)	(5)	(5)	(23)	(23)	(21)	(21)	(11)	(11)
Benzolamide	7.35	37.4	19.4	7.92	98.9	59.5	22.9	58.7	22.7	60.8	25.7
(2.0 mg/kg)	±0.01	±1.6	±0.28	±0.06	±6.2	±1.8	±1.3	±2.2	±1.2	±2.7	±2.8
	(10)	(10)	(10)	(5)	(5)	(27)	(27)	(22)	(22)	(10)	(10)
P	NS	NS	<0.025	<0.001	<0.001	<0.001	<0.001	<0.025	<0.005	<0.01	<0.05
Percent change						−16.4	−26.3	−14.3	−24.6	−10.0	−18.8
						±1.8	±2.8	±2.5	±3.2	±3.1	±6.7

segments, Table IV and Fig. 3). Conversely, in the group IV rats, when bicarbonate reabsorption was suppressed, arterial pH increased and arterial P_{CO_2} decreased significantly (7.49±0.02 U and 15.5±1.4 mm Hg). After suppression of proximal bicarbonate reabsorption, an alkaline urine was elaborated (7.24 ±0.04 U). The *in situ* P_{CO_2} and ΔP_{CO_2} obtained in this group was significantly less than during the control period: proximal tubule 27.2±1.2 and 8.5±0.6 mm Hg, distal tubule 25.2±1.8 and 7.7±0.67 mm Hg, and star vessel 26.1±2.0 and 7.5±0.83 mm Hg, respectively (P value for all segments < 0.001). Note that despite a marked decrease in the P_{CO_2} difference between tubular fluid and arterial P_{CO_2}, at no time did these values reach equality. Furthermore, for both groups, the values for P_{CO_2} obtained in each of the three segments punctured were not significantly different from each other. Thus, suppression or stimulation of bicarbonate reabsorption resulted in parallel changes in the observed *in situ* P_{CO_2} and ΔP_{CO_2}.

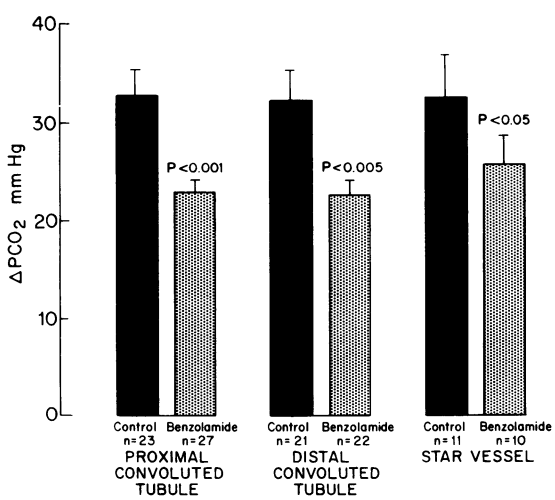

FIGURE 4 Response of ΔP_{CO_2} to benzolamide administration (five rats). The solid bars represent ΔP_{CO_2} values obtained in the control period and the stippled bars represent values obtained after benzolamide. n = number of determinations at each of the three micropuncture sites.

Group V (carbonic anhydrase inhibition) (n = 5). The effect of 2.0 mg/kg body wt benzolamide on the renal cortical *in situ* P_{CO_2} is summarized in Table V and Fig. 4. There was no significant change in systemic arterial pH or P_{CO_2} after benzolamide although a small but significant decrease in arterial bicarbonate concentration was noted (22.1±1.0 to 19.4±0.28 mM/liter). Carbonic anhydrase inhibition resulted in the elaboration of an alkaline urine (7.92±0.06 U) (P < 0.001) which is evidence for drug effect. No significant alterations in blood pressure were noted and all rats diuresed promptly after the bolus injection. The micropuncture findings in Table V and Fig. 4 demonstrate a significant decrease in the *in situ* P_{CO_2} after benzolamide: 73.0±3.2 to 59.5±1.8 in the proximal convoluted tubule, 73.0±3.8 to 58.7±2.2 in the distal convoluted tubule, and 71.1±4.4 to 60.8±2.7 in the star vessel. The respective values for ΔP_{CO_2} were: 32.6±2.6 to 22.9±1.3 in the proximal, 32.1±2.9 to 22.7±1.2 in the distal, and 32.4±3.4 to 25.7±2.8 in the star vessel. Therefore, after benzolamide, a 26.2% decrease in ΔP_{CO_2} in the proximal tubule was observed (P < 0.001). Again, the values for *in situ* P_{CO_2} were not significantly different in all segments of the renal cortex.

P_{CO_2} of renal vein. In 4 of 21 rats in group I (controls), measurement of P_{CO_2} in the renal vein was determined directly with a microelectrode as described in Methods. The mean P_{CO_2} in renal vein was 41.1 ±1.60 mm Hg (n = 5) which was not significantly different from the systemic arterial P_{CO_2} of 39.2±1.09 mm Hg (P > 0.05) as determined with a standard macro-

electrode. The mean renal vein P_{CO_2} was, however, significantly lower than the P_{CO_2} of the renal cortical structures ($P < 0.001$).

DISCUSSION

A critical feature in the analysis of the mechanism mediating proximal bicarbonate reabsorption is the direct measurement of tubular fluid P_{CO_2} and an assessment of its determinants. Most studies have necessarily assumed identical values for arterial blood and tubular fluid P_{CO_2} (2, 3, 9). In the few studies in which an attempt has been made to estimate P_{CO_2} either indirectly or directly, the calculated (12, 19), or determined (13) CO_2 tension in proximal tubular fluid exceeded that of arterial blood. Moreover, a profile was described in which the P_{CO_2} fell along the length of the proximal tubule, reaching equilibrium with arterial blood in the later segments (12).

The present investigation was designed to provide direct data concerning the determination of P_{CO_2} in the kidney. Four key findings emerge: (*a*) utilizing a new technique for direct determination of *in situ* P_{CO_2}, it is demonstrated that under normal conditions proximal tubular fluid P_{CO_2} exceeds arterial P_{CO_2} (Tables II,III). (*b*) Tubular fluid P_{CO_2} is uniformly elevated along the entire length of the proximal tubule with no tendency to fall in the later segments (Table III). (*c*) All structures examined in the renal cortex (the entire proximal and distal tubule, and star vessel) display the same P_{CO_2} (Tables II,III). (*d*) The level of cortical P_{CO_2} varies directly with the magnitude of bicarbonate reabsorption (Figs. 3,4).

Karlmark and Danielson (12) have previously presented evidence that proximal tubular P_{CO_2} exceeds arterial P_{CO_2}, but this study suffers from the disadvantage of relying on indirect methods, i.e., the Astrup technique. This technique involves the measurement of *in situ* pH after which tubular fluid is collected and equilibrated under oil at various CO_2 gas tensions. The P_{CO_2} required to return the sample to the previously measured *in situ* pH is assumed to represent luminal P_{CO_2}. This technique, therefore, represents a calculation of P_{CO_2}, not a determination. Utilizing this indirect approach the authors calculated a wide variation in luminal P_{CO_2} from as high as 90 mm Hg in the early segments of the proximal tubule to as low as 26.6 mm Hg in the late proximal tubule during a period when arterial P_{CO_2} was 33.2 mm Hg.

In a subsequent publication, Sohtell and Karlmark (13) have reported direct measurements of P_{CO_2} in the proximal tubule, with values 16–17 mm Hg higher than systemic arterial blood, but without information regarding localization within the proximal tubule, or of a profile for CO_2 along the nephron. The P_{CO_2} microelectrode utilized by Sohtell and Karlmark (13) differs from that employed in our study in several respects, the most important of which is their use of an antimony pH electrode as the primary pH sensor. The problems inherent in the measurement of pH of biological solutions with the antimony electrode have been well documented (20–22). The necessity to compensate for the effects of variations in ionic composition, temperature, and dissolved gases when this electrode is employed as a pH sensor, has long been appreciated (20–22). When employed as a component of a P_{CO_2} microelectrode it is not necessary to compensate for the deleterious anion effect (13). However, the most serious defects of the antimony electrode involve the influence of both temperature and the partial pressure of all gases in solution (CO_2, O_2, and N_2, as well as inert gases) (21, 22). Therefore, appropriate precautions must be taken to minimize these errors when antimony is used in a P_{CO_2} electrode. Furthermore, the P_{CO_2} microelectrode utilized by Sohtell and Karlmark is characterized by a sensitivity considerably less than the theoretic value; i.e. they reported a slope of 37–47 $mV/\log_{10} P_{CO_2}$ instead of a theoretical value of 61 $mV/\log_{10} P_{CO_2}$. In contrast, our electrode has the following characteristics: sensitivity of 57–61 $mV/\log_{10} P_{CO_2}$ (near theoretic or Nernstian response), stability of intercept, a tip diameter sufficiently small to allow direct measurement in all segments of the nephron, and does not exhibit sensitivity to O_2 or N_2. The present study, therefore, circumvents the disadvantages inherent in the indirect and direct methodology employed in the studies cited above (12, 13). Repeated determinations (68 proximal tubules) in 21 control rats clearly establish that proximal tubular P_{CO_2} exceeds arterial P_{CO_2} under normal acid-base conditions (Table II).

To determine if a declining profile for P_{CO_2} exists along the length of the accessible proximal tubule, we measured P_{CO_2} from early to late proximal convoluted tubule in the mutant Munich-Wistar rat (Table III). The data demonstrate equal values for P_{CO_2} from near the glomerulus to the last accessible proximal segment. This finding is in opposition to the previous indirect studies of Karlmark and Danielson (12).

On the basis of a presumed fall of P_{CO_2} to arterial levels along the proximal tubule, Malnic and Steinmetz (10), Giebisch and Malnic (11), and Karlmark (14), have recently proposed that a "diffusion barrier" for CO_2 exists across the proximal tubular epithelium. The demonstration in our study that P_{CO_2} is not significantly different throughout the entire proximal tubule mitigates against this hypothesis. To examine this issue further, we have determined the P_{CO_2} in all the accessible structures of the superficial cortex in a variety of conditions of acid-base balance. The data demonstrate similar values for P_{CO_2} in the proximal and distal nephron as well as in the stellate vessel (Tables II–V and Figs. 3, 4). Our findings are therefore

compatible with the recent observations of Warnock and Rector (23) in which an exceedingly high CO_2 permeability was demonstrated in isolated perfused rabbit proximal straight tubules.

The present study also demonstrates that variations in the magnitude of bicarbonate reabsorption results in parallel changes in the P_{CO_2} gradient between arterial blood and the superficial cortex (ΔP_{CO_2}) (Tables IV, V, and Figs. 4, 5). When bicarbonate reabsorption is increased and large amounts of bicarbonate are added to peritubular capillary blood, ΔP_{CO_2} increases significantly; conversely, when bicarbonate reabsorption is decreased, ΔP_{CO_2} decreases significantly. It should be noted that two different methods were utilized to decrease bicarbonate reabsorption: (*a*) a combination of acute hypocapnia, volume expansion, and hyperkalemia (Table IV and Fig. 3); and (*b*) benzolamide administration (Table V and Fig. 4). Benzolamide administration results in partial inhibition of bicarbonate reabsorption in vivo, despite near total inhibition of renal carbonic anhydrase activity in vitro (24). In both instances, however, ΔP_{CO_2} fell significantly. The magnitude of the reduction in ΔP_{CO_2} during combined hypocapnia and volume depletion greatly exceeded the reduction observed after carbonic anhydrase inhibition. It is interesting to speculate that this difference might be due to the direct effect of hypocapnia on the pH of the renal tubular cell. That is, the cell becomes alkalotic and cannot elaborate hydrogen ions even from the uncatalyzed hydration reaction. After carbonic anhydrase inhibition, however, the uncatalyzed hydration reaction might continue to provide hydrogen ions for secretion. The fact, however, that two entirely different methods, each depressing bicarbonate reabsorption by separate mechanisms, resulted in a reduction of ΔP_{CO_2} suggests that this reduction was not a function of the specific manner by which bicarbonate transport was depressed, but rather of the extent of residual bicarbonate reabsorption.

In this regard, it is attractive to assume that the observed association between the magnitude of proximal bicarbonate reabsorption (or of H^+ ion secretion) and the generation of P_{CO_2} is attributable to the addition of reabsorbed bicarbonate to peritubular blood. Because blood contains nonbicarbonate buffers of strong buffering capacity, the addition of reabsorbed bicarbonate to peritubular capillary blood would generate CO_2 (as dictated by the Henderson-Hasselbach relationship) from the reaction between the added bicarbonate and H^+ ions contributed by blood buffers.

Two additional sources of CO_2 generation may be operative either in addition to, or instead of, the mechanism proposed above. Luminal production of CO_2 would be an obvious consequence of dehydration of carbonic acid formed by the reaction between secreted H^+ and filtered bicarbonate. Certainly, abundant evidence supports the notion that the P_{CO_2} of urine is a function of the concentration of bicarbonate and other buffers (25, 26). The CO_2 formed in the proximal tubule could, as a result of a high proximal tubular permeability to CO_2, approach equilibrium in the other structures of the renal cortex. A second potential source of CO_2 generation might be metabolic CO_2 production. Schwartz et al. (27) and Schwartz and Steinmetz (28) have estimated, however, that <2.0 mm Hg P_{CO_2} could be contributed from this process in the turtle urinary bladder in the absence of exogenous CO_2. In this species, metabolic CO_2 production as a source for the gradients observed in the mammalian kidney, seems far too small. It must be cautioned, however, that similar measurements in rat kidney have not been performed, and this possibility remains a consideration. It is conceivable then, that each of these three possible mechanisms of renal CO_2 generation may be operative in the cortex. However, if the reported value for pH in the star vessel of 7.51 is correct (29), then the latter two mechanisms are less likely.

The finding of an elevated P_{CO_2} throughout the renal cortex, although somewhat surprising initially, is quite compatible with previously reported values for *in situ* pH (2, 3, 9) and bicarbonate concentration (9, 12, 18). In fact, if one assumes reaction equilibrium in the proximal tubule, a pH of 6.71, and a bicarbonate concentration of 8 mM, the calculated P_{CO_2} for these values would be 65 mm Hg. This type of calculation was performed by Brodsky and Schilb (7) in their review to emphasize that proximal tubular P_{CO_2} might exceed that of systemic arterial blood. These values are in agreement with our directly determined value for P_{CO_2} in the proximal tubule. Utilizing the measured values in the distal tubule for the bicarbonate concentration and pH in normal rats equal to 6.3 mM and 6.45 U, respectively (3), the calculated P_{CO_2} is 93.7 mm Hg. This value is significantly greater, not less than, our actual measurement.

Despite the high CO_2 tensions observed throughout the cortex, the P_{CO_2} in the renal artery and vein are the same and equal to systemic arterial blood. We have confirmed this finding by direct measurement of P_{CO_2} in these structures in vivo. How then might a P_{CO_2} of 65 mm Hg in the renal cortex return to 40 mm Hg in the renal vein? There are at least three explanations for this observation. It is possible that the addition of water and NaCl, in excess of bicarbonate, to the capillary bed might be responsible for reducing venous P_{CO_2} by diluting the bicarbonate concentration without significantly altering pH. The medullary collecting tubule is the most likely site for such a process. Tubular bicarbonate reabsorption might be low, both because the capacity for bicarbonate transport is small and because the tubular fluid concentration at this site is

usually reduced. As a result of the buffering properties of blood, the dilution of bicarbonate would shift the equilibrium in favor of CO_2 hydration, thereby lowering CO_2 tension. A second mechanism that could contribute to the reduction of CO_2 tension from cortex to renal vein is the addition of a nonbicarbonate buffer to peritubular blood. A nonbicarbonate buffer, such as ammonia, could act as a proton acceptor and thereby shift the equilibrium reaction toward bicarbonate as described above, consuming CO_2. The third process that aids to dissipate cortical P_{CO_2} gradients is the countercurrent arrangement of the renal circulation. The close proximity of the arterial and venous circulation throughout the kidney (30) allows these vessels to serve as countercurrent exchangers so that equilibrium would occur before or near the renal hilus. All of these processes might be acting in concert to reduce the P_{CO_2} in the renal vein to that in the renal artery. At present, until studies evaluating P_{CO_2} in the deeper structures are available, the highly speculative nature of these proposals must be emphasized.

Although previous models of the mechanism of bicarbonate reabsorption assuming equilibrium for CO_2 between systemic blood and tubular fluid are clearly in error, our findings cannot differentiate between H^+ ion secretion or primary bicarbonate reabsorption. Before this process can be more clearly defined, measurement of *in situ* pH together with an accurate assessment of equilibrium pH must be performed to correctly calculate disequilibrium pH.

ACKNOWLEDGMENTS

The authors wish to acknowledge the expert technical assistance of Mr. John Green and Ms. Jane Cutrer as well as the secretarial assistance of Miss Peggy McConnell. Mr. Pucacco contributed to this study while a graduate student in the Biomedical Engineering Program at Southern Methodist University.

This work was supported in part by U. S. Public Health Service program project grant 5 PO1 HL11662, National Institute of Arthritis, Metabolism and Digestive Diseases, grant 1 RO1 AM14677, U. S. Public Health Service training grant 5 TO1 HL05469, and by Biomedical research support grant 5 SO7 RR05426.

REFERENCES

1. Pitts, R. F., and R. S. Alexander. 1945. The nature of the renal tubular mechanism for acidifying the urine. *Am. J. Physiol.* **144:** 239–254.
2. 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.
3. Vieira, F. L., and G. Malnic. 1968. Hydrogen ion secretion by rat renal cortical tubules as studied by an antimony electrode. *Am. J. Physiol.* **214:** 710–718.
4. Rector, F. C., Jr. 1976. Renal acidification and ammonia production; chemistry of weak acids and bases; buffer mechanisms. *In* The Kidney. B. M. Brenner and F. C. Rector, Jr., editors. W. B. Saunders Co., Philadelphia. 1st edition. **1:** 318–343.
5. Malnic, G., and G. Giebisch. 1972. Mechanism of renal hydrogen ion secretion. *Kidney Int.* **1:** 280–296.
6. Murer, H., U. Hopfer, and R. Kinne. 1976. Sodium/proton antiport in brush-border membrane vesicles isolated from rat small intestine and kidney. *Biochem. J.* **154:** 597–604.
7. Brodsky, W. A., and T. P. Schilb. 1974. The means of distinguishing between hydrogen secretion and bicarbonate reabsorption: theory and applications to the reptilian bladder and mammalian kidney. *Curr. Top. Membranes Transp.* **5:** 161–224.
8. Maren, T. H. 1974. Chemistry of the renal reabsorption of bicarbonate. *Can. J. Physiol. Pharmacol.* **52:** 1041–1050.
9. Malnic, G., M. Mello Aires, and G. Giebisch. 1972. Micropuncture study of renal tubular hydrogen ion transport in the rat. *Am. J. Physiol.* **222:** 147–158.
10. Malnic, G., and P. R. Steinmetz. 1976. Transport processes in urinary acidification. *Kidney Int.* **9:** 172–188.
11. Giebisch, G., and G. Malnic. 1976. Studies on the mechanism of tubular acidification. *Physiologist.* **19:** 511–524.
12. Karlmark, B., and B. G. Danielson. 1974. Titratable acid, pCO_2 bicarbonate, and ammonium ions along the rat proximal tubule. *Acta Physiol. Scand.* **91:** 243–258.
13. Sohtell, M., and B. Karlmark. 1976. In vivo micropuncture pCO_2 measurements. *Pflugers Arch. Eur. J. Physiol.* **363:** 179–180.
14. Karlmark, B. 1971. Renal tubular pCO_2. *Proc. Int. Union Physiol. Sci.* **12:** 206. (Abstr.)
15. Caflisch, C. R., and N. W. Carter. 1974. A micro pCO_2 electrode. *Anal. Biochem.* **60:** 252–257.
16. DuBose, T. D., D. W. Seldin, and J. P. Kokko. 1978. Segmental chloride reabsorption in the rat nephron as a function of load. *Am. J. Physiol.* **3:** F97–F105.
17. Pucacco, L. R., and N. W. Carter. 1976. A glass-membrane pH microelectrode. *Anal. Biochem.* **73:** 501–512.
18. Kunau, R. T. 1972. The influence of the carbonic anhydrase inhibitor, benzolamide (CL-11,366) on the reabsorption of chloride, sodium, and bicarbonate in the proximal tubule of the rat. *J. Clin. Invest.* **51:** 294–306.
19. Malnic, G., M. Mello Aires, and A. C. Cassola. 1974. Kinetic analysis of renal tubular acidification by antimony microelectrodes. *In* Ion Selective Microelectrodes. H. J. Berman and N. C. Hebert, editors. Plenum Publishing Corporation, New York. 1st edition. 89–107.
20. Puschett, J. B., and P. E. Zurbach. 1974. Re-evaluation of microelectrode methodology for the in vivo determination of pH and bicarbonate concentration. *Kidney Int.* **6:** 81–91.
21. Perley, G. A. 1939. Characteristics of the antimony electrode. *Industrial and Engineering Chemistry (Analytical Edition).* **11:** 319–322.
22. Caflisch, C., L. R. Pucacco, and N. W. Carter. 1978. The manufacture and utilization of antimony pH electrodes. *Kidney Int.* **14:** 12–27.
23. Warnock, D. G., and F. E. Rector, Jr. 1977 CO_2 permeability of the rabbit proximal straight tubule. *Abstracts of the American Society of Nephrology.* **10:** 124A (Abstr.)
24. Maren, T. H. 1967. Carbonic anhydrase: chemistry, physiology, and inhibition. *Physiol. Rev.* **47:** 595–781.
25. Arruda, J. A. L., L. Nascimento, S. K. Kumar, and N. A. Kurtzman. 1976. Factors influencing the formation of urinary carbon dioxide tension. *Kidney Int.* **11:** 307–317.

26. Arruda, J. A. L., L. Nascimento, P. K. Mehta, D. R. Rademacher, J. T. Sehy, C. Westenfelder, and N. A. Kurtzman. 1977. The critical importance of urinary concentrating ability in the generation of urinary carbon dioxide tension. *J. Clin. Invest.* **60:** 922–935.
27. Schwartz, J. H., J. T. Finn, G. Vaughn, and P. R. Steinmetz. 1974. Distribution of metabolic CO_2 and the transported ion species in acidification by turtle bladder. *Am. J. Physiol.* **226:** 283–289.
28. Schwartz, J. H., and P. R. Steinmetz. 1971. CO_2 requirements for H^+ secretion by the isolated turtle bladder. *Am. J. Physiol.* **220:** 2051–2057.
29. Garcia-Filho, E. M., and G. Malnic. 1976. pH in cortical peritubular capillaries of rat kidney. *Pflugers Arch. Eur. J. Physiol.* **363:** 211–217.
30. Kriz, W., J. M. Barrett, and S. Petter. 1976. The renal vasculature: anatomical-functional aspects. *In* International Review of Physiology, Kidney and Urinary Tract Physiology. K. Thurau, editor. University Park Press, Baltimore. 2nd edition. **11:** 1–21.