Kidney International, Vol. 21 (1982), pp. 795–799

Elevated urinary Pco_2 in the rat: An intrarenal event

MARK L. GRABER, HOWARD H. BENGELE, and EDWARD A. ALEXANDER

Thorndike Memorial Laboratory, Renal Section, Boston City Hospital, and Departments of Medicine and Physiology, Boston University School of Medicine, Boston, Massachusetts

Elevated urinary Pco_2 in the rat: An intrarenal event. During a bicarbonate diuresis, final urine Pco_2 considerably exceeds systemic Pco_2, an effect thought to reflect the postpapillary delayed dehydration of carbonic acid. To test this explanation, Pco_2 tensions along the inner medullary collecting duct (IMCD) of bicarbonate-loaded rats were measured directly using Pco_2 microelectrodes. With systemic Pco_2 held at 40 mm Hg, IMCD Pco_2 exceeded systemic Pco_2 in every measurement by an average of 20 to 30 mm Hg. A significant increment in Pco_2 was seen between 50% IMCD length and the papilla tip. During the infusion of carbonic anhydrase, IMCD Pco_2 was reduced but not to systemic levels. Finding elevated Pco_2 along the terminal IMCD deemphasizes the importance of postpapillary delayed dehydration and suggests the possibility that bicarbonaturia is associated with papillary accumulation of carbon dioxide.

Pco_2 urinaire élevée chez le rat: Un phénomène intra-rénal. Au cours d'une diurèse induite par la perfusion de bicarbonate, la Pco_2 de l'urine définitive est considérablement supérieure à la Pco_2 systémique, fait que l'on attribue à la déshydratation postpapillaire retardée d'acide carbonique. Pour vérifier cette explication, la Pco_2 le long du tube collecteur de la médullaire interne (TCMI) chez des rats recevant du bicarbonate étaient mesuré directement en utilisant des microélectrodes à Pco_2. Lorsque la Pco_2 systémique était maintenue à 40 mm Hg, la Pco_2 dans le TCMI dépassait à chaque mesure la Pco_2 de 20 à 30 mm Hg en moyenne. Une augmentation significative de la Pco_2 était notée entre la moitié de la longueur du TCMI et la pointe de la papille. Pendant la perfusion d'anhydrase carbonique la Pco_2 du TCMI diminuait mais n'atteignait pas les valeurs systémiques. Le fait que la Pco_2 soit élevée le long du TCMI terminal fait perdre son importance à l'hydrolyse postpapillaire retardée et suggère la possibilité que la bicarbonaturie soit associée à une accumulation papillaire de carbon dioxide.

When a bicarbonate diuresis occurs in humans, dogs, or rats, the Pco_2 of final urine considerably exceeds the arterial blood Pco_2 [1, 2]. This urine to blood Pco_2 gradient (U-B Pco_2) is thought to reflect acidification events in the distal nephron, a hypothesis which was considerably strengthened by the studies of Halperin et al showing that patients with documented distal acidification defects were unable to generate a normal U-B Pco_2 [3].

Ochwadt and Pitts proposed that the genesis of the U-B Pco_2 involved the postpapillary delayed dehydration of carbonic acid [4], and this formulation has been accepted widely. In this scheme, Ochwadt and Pitts assumed: *(1)* that distal acidification involves secretion of hydrogen ion, which reacts with the plentiful luminal bicarbonate ion to form carbonic acid, *(2)* that all papillary structures have the same carbon dioxide tension, namely, that set by the systemic arterial Pco_2, and *(3)* that there is no access of the luminal fluid to carbonic anhydrase. With these constraints, Ochwadt and Pitts envisioned that the dehydration of carbonic acid to carbon dioxide and water could be delayed until urine left the papilla and entered the lower urinary tract where the relatively low ratio of surface area to urine volume would tend to maintain a urine to blood Pco_2 gradient. Consistent with this formulation, the Pco_2 gradient was abolished when the dehydration reaction was accelerated by infused (and filtered) carbonic anhydrase [4].

The hypothesis of postpapillary delayed dehydration has been testable recently using advances in microelectrode technology to assess directly the distal acidification process in the collecting duct. Two of the key assumptions have been verified in our laboratory [5] and by others [6, 7]: First, the medullary collecting duct is capable of acidification by hydrogen secretion [5]. Second, as evidenced by an acid disequilibrium pH, this process takes place in the absence of luminal carbonic anhydrase [5, 6]. The purpose of this study was to test directly the last key argument of the Ochwadt-Pitts hypothesis by determining whether or not the papillary Pco_2 is set to systemic arterial Pco_2. The results contradict the postpapillary dehydration scheme and indicate that carbon dioxide tensions are elevated already along the medullary collecting duct. In addition, carbonic anhydrase infusion did not lower collecting duct Pco_2 to systemic levels suggesting that at least part of the U-B Pco_2 gradient may be generated independent of delayed carbonic acid dehydration.

Methods

Male Charles River rats, weighing 280 to 330 g each, were maintained on Purina 5001 rat chow and water until the morning of each experiment. Anesthesia was induced by intraperitoneal Inactin (Promonta, Hamburg, West Germany), 120 mg/kg body weight. After a tracheostomy was performed, PE-50 polyethylene catheters were placed in the jugular vein for infusion and in the femoral artery for blood sampling and monitoring of blood pressure. The left kidney was isolated, and the papilla exposed as described previously [5]. Final urine bathed the papilla and was then collected via a PE-50 catheter fed through the left ureter. All animals were maintained at 37° C, and ventilated with 40% oxygen to maintain arterial Pco_2 near 40 mm Hg. Arterial blood, 400 μl, was sampled every 45 min, and the volume was replaced by blood from a donor litter-mate.

Received for publication August 11, 1981
and in revised form November 23, 1981

0085–2538/82/0021–0795 $01.00

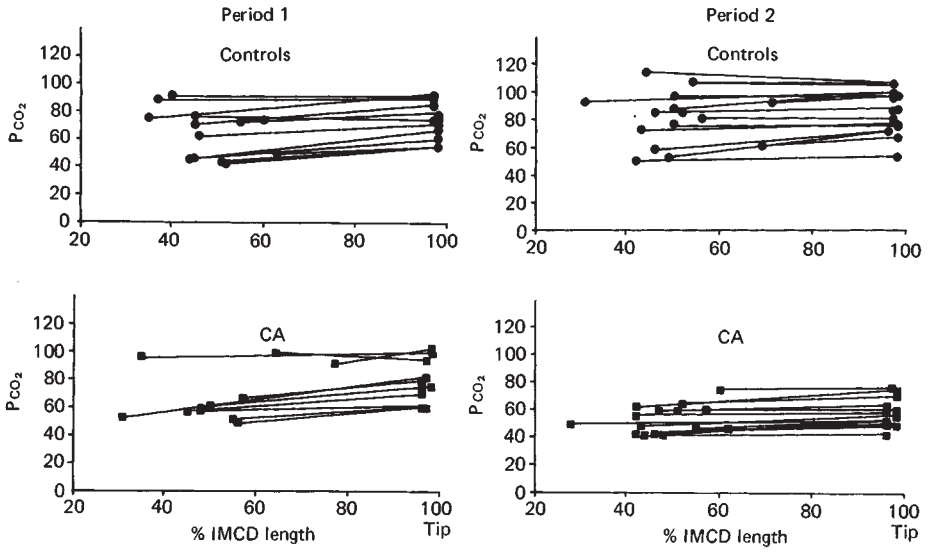

Fig. 1. *Paired (deep and shallow) measurements of P_{CO_2} tension (mm Hg) as a function of length along the inner medullary collecting duct* (IMCD). Two to four pairs per rat were obtained in bicarbonaturic control rats (*upper panels*) and animals given carbonic anhydrase during period 2 (*lower panels*).

All rats were infused with 0.3M sodium bicarbonate, containing 8 μCi/ml carboxy-^{14}C inulin, at 6.6 ml/hr, and received a separate infusion of normal saline at 0.3 ml/hr. A 1-hr equilibration time separated the completion of the surgical preparation from the two 45-min experimental periods. Two groups of five rats were studied; both groups were prepared and treated identically through the end of the first period. During the second period, one group of rats (CA) received carbonic anhydrase (Bovine erythrocyte carbonic anhydrase B, Sigma Chemical Co., St. Louis, Missouri) at 3.3 mg/hr in the saline infusion after a 4 mg bolus dissolved in donor blood. Control rats received the same volume of donor blood and the same saline infusion but without carbonic anhydrase. The second period was started 20 min after the CA bolus. In preliminary experiments, the carbonic anhydrase activity in final urine was measured using a semiquantitative assay with a lower detection limit of 2 ng/μl [8].

No activity was detectable before enzyme infusion, and activity was present in 200-fold dilutions of final urine during enzyme infusion.

Collecting duct P_{CO_2} was measured with the P_{CO_2} microelectrode of Pucacco and Carter [9]. The electrodes were modified for inner medullary collecting duct (IMCD) catheterization by elongating the neck portion to a uniform diameter of 20 to 30 μ for a distance of 1 cm. The electrode was inserted gently retrograde into the IMCD using a Leitz micromanipulator; the depth of insertion was measured as the electrode was withdrawn from the papilla. Distances are expressed as a percentage of the total IMCD length determined on a sagital section of kidney at the end of each experiment. IMCD P_{CO_2} was measured in deep (~3 mm into papilla) and shallow (~1 mm into papilla) pairs; the pairing order was varied randomly. The electrode was brought to less than 10 mm Hg P_{CO_2} in room air before each measurement. Before and after two to three pairs of readings, the electrode was calibrated at 37° C in thermoregulated wells of 10 mM sodium bicarbonate bubbled from analyzed tanks of 4, 8, and 15% carbon dioxide. Integrity of the microelectrode's silicone membrane was confirmed during each calibration by insuring that the P_{CO_2} reading approached zero with the electrode immersed in pH 4 buffer. The slope of the P_{CO_2} response was 55 to 60 mV/log P_{CO_2} for all electrodes used. Measurements were discarded if red cells were seen in the duct effluent.

Arterial blood gases were measured on a Radiometer BMS-Mk2 microsystem. Glomerular filtration rate was calculated as the clearance of radioactive inulin. Bicarbonate concentration in blood was calculated, assuming a pK of 6.10 and a solubility of 0.0306. Statistical comparisons were performed by use of the paired or unpaired *t* test, or by analysis of variance.

Results

Arterial blood gases were not significantly different among the two periods of controls and the first period of the CA rats. Mean pH was 7.61 ± 0.01, P_{CO_2} 39 ± 1 mm Hg, and bicarbonate 39 ± 1 mM. During CA infusion, pH was 7.69 ± 0.02, P_{CO_2} 36 ± 1, and bicarbonate 42 ± 1. Mean pH of urine from the unexposed kidney was 7.93 ± 0.02 in controls and period 1 of the CA animals, increasing to 8.18 ± 0.02 during CA infusion.

Two major findings are seen in the individual measurements of collecting duct P_{CO_2} shown in Figure 1. First, in every instance the P_{CO_2} tension in the collecting duct exceeded the systemic arterial P_{CO_2} of that animal. Second, there was a significant increment of P_{CO_2} tension between the deep and tip measurement sites, and this increment was seen in both periods of both groups.

In the first period of the control animals, mean P_{CO_2} at the deeper measurement site averaged 64 ± 4.8 mm Hg, increasing to 71.4 ± 3.6 near the papilla tip (P = 0.006). Comparable findings were seen in the first period of the CA animals, P_{CO_2} increased from 67.9 ± 5.7 to 78.0 ± 4.7, (P = 0.006). Neither the mean values of P_{CO_2} nor the increment in P_{CO_2} along the IMCD differed significantly between the two groups during the first period.

During the second period in the control animals, P_{CO_2} increased from 80.1 ± 4.9 to 83.5 ± 3.9 (P = 0.02) along the IMCD. Both levels were significantly greater than during the

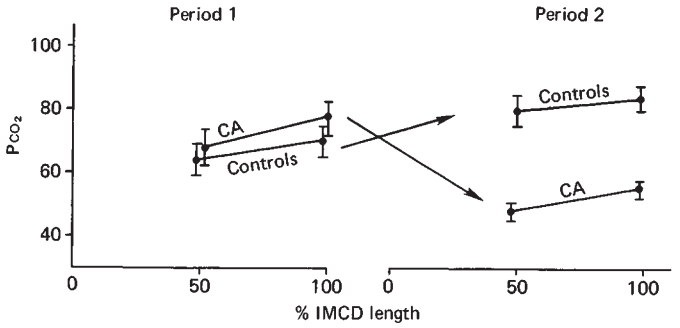

Fig. 2. *Mean P_{CO_2} values from Figure 1, ±SEM, plotted at the mean measurement site.*

first period in the same animal. During CA infusion (second period of the CA group), P_{CO_2} increased from the proximal value of 47.8 ± 2.6 to 56.7 ± 2.9 near the papilla tip ($P = 0.003$).

The effects of carbonic anhydrase infusion are seen more clearly in Figure 2, where the mean values of the IMCD P_{CO_2} tensions are plotted. The luminal P_{CO_2} tensions were reduced significantly and substantially as compared to mean values of the time controls (period 2 of control animals) or to the pre-infusion values of the first period in the same animals (all $P <$ 0.005). Despite the large reduction in the absolute value of luminal P_{CO_2}, there was still a significant increment along the duct, and each measurement exceeded the systemic P_{CO_2}.

Discussion

The central tenet of the postpapillary delayed dehydration hypothesis is that urinary P_{CO_2} is set to systemic P_{CO_2} within the papilla and does not rise above systemic values until urine leaves the papilla. Despite the virtually unanimous acceptance of this scheme [1, 2, 10, 11], a variety of theoretical arguments and experimental data are in direct conflict with this central assumption.

First, the hypothesis predicts that there would be no U-B P_{CO_2} gradient if the dehydration reaction was instantaneous. Several laboratories, however, have shown that the infusion of carbonic anhydrase does not necessarily obliterate the U-B P_{CO_2} gradient although detectable enzyme levels are present in the final urine [12–14]. Second, the P_{CO_2} of the urine in the renal pelvis is already elevated, with little further change in the lower urinary passages [15, 16]. Third, the proposed scheme is difficult to reconcile with the kinetics of carbonic acid dehydration [17]. Even when an uncatalyzed rate and the effect of nonbicarbonate buffers to delay the reaction are accepted, the dehydration is expected to reach equilibrium within a few seconds [17, 18]. In contrast, the transit time from late distal tubule to final urine is probably longer; estimates range anywhere from 15 sec to several minutes [19–21]. Accepting these kinetic data would indicate that the only acidification site capable of contributing to postpapillary elevation of carbon dioxide would be some small fraction of the terminal IMCD. Knowing how small this fraction might be would require more precise estimates of the relevant kinetics than are presently available. Finally, Uhlich, Baldamus, and Ullrich [22] have estimated P_{CO_2} tensions in the papillary structures indirectly, using an Astrup type of analysis in which the pH of an aspirated sample is measured immediately and again at various known tensions of carbon dioxide. These authors concluded that during a bicarbonate diuresis, papillary P_{CO_2} tensions were elevated above systemic levels.

Our results confirm that elevated tensions of carbon dioxide exist within the papillary collecting duct with measurements of collecting duct P_{CO_2} made directly with a P_{CO_2} microelectrode. DuBose and Pucacco have reported similar results recently [6, 7]. We conclude that the elevated P_{CO_2} seen in final urine during bicarbonate loading is, at least in part, an intrarenal event.

Based on several lines of evidence, we speculate that the elevated P_{CO_2} in the IMCD reflects elevated carbon dioxide tensions throughout the papilla during bicarbonate loading. The indirect P_{CO_2} measurements of Uhlich, Baldamus, and Ullrich, showing elevated P_{CO_2} in the vasa rectae, would be one point in favor of this view. More recently, vasa recta P_{CO_2} was measured directly by DuBose and was found to be elevated to levels comparable to those in adjacent collecting ducts [7]. These elevated levels of P_{CO_2} in the vasa recta could in theory reflect carbon dioxide generated outside the collecting duct. Alternatively, to the extent that the IMCD epithelium permits free diffusion of carbon dioxide, the elevated vasa recta tensions simply might reflect diffusional loss from the collecting duct lumen. If diffusion of collecting duct P_{CO_2} does comprise the major source of papillary P_{CO_2}, the P_{CO_2} increment seen along the IMCD must reflect a similar gradient of carbon dioxide through the papilla, with P_{CO_2} tension progressively increasing between the papillary base and tip. A carbon dioxide countercurrent exchange system, as proposed by Uhlich, Baldamus, and Ullrich [22], and also Ullrich, Kramer, and Boylan [23] presumably would maintain this gradient.

The possibility of carbon dioxide accumulation in the papillary microenvironment carries potentially important implications for collecting duct physiology. Because other acidifying epithelia display carbon dioxide-dependent acidification rates [10], it is conceivable that the papillary accumulation of carbon dioxide during bicarbonaturia may serve to regulate the local epithelial acidification rates. A variety of carbon dioxide-regulated metabolic processes besides those related to acidification could be affected as well.

Elevation of carbon dioxide tension within the papilla would require some reexamination of the proposal that the U-B P_{CO_2} gradient reflects the distal acidification process. If it does, our results suggest that segments proximal to the IMCD contribute to this gradient, because P_{CO_2} already was elevated in the more proximal parts of this segment. The concept of papillary P_{CO_2} accumulation also suggests an alternative explanation for the subnormal U-B P_{CO_2} gradient in subjects with distal renal tubular acidosis [2, 3, 24]. Currently, it is thought that part of the subnormal U-B P_{CO_2} reflects the reduced urinary bicarbonate concentrations which result from the concentrating defect usually found in this condition [24]. If the concentrating defect were associated also with increased papillary blood flow, medullary washout could reduce papillary carbon dioxide accumulation and thus contribute to the subnormal P_{CO_2} in final urine.

In these studies we did not investigate the mechanisms responsible for elevating the collecting duct P_{CO_2} tension. Likely possibilities have been suggested by others (1, 2, 10, 11) and include the formation of carbon dioxide from *(1)* the titration of luminal bicarbonate by secreted hydrogen, and/or

(2) the ampholyte effect, from rising concentrations of luminal bicarbonate, due to bicarbonate secretion or water reabsorption. It is also conceivable that some part of the IMCD P_{CO_2} could derive from carbon dioxide generated outside of the lumen. The elevated carbon dioxide tension probably cannot be accounted for by carbon dioxide produced from metabolism because collecting duct P_{CO_2} is subsystemic when final urine is acid [5].

The effect of infused carbonic anhydrase (CA) was to depress collecting duct P_{CO_2}, although not to systemic levels. We derive two conclusions from this finding: First, opposite to the postpapillary delayed dehydration scheme, it is clear that elevation of urinary P_{CO_2} can be accomplished independent of delayed carbonic acid dehydration. Second, because P_{CO_2} increased along the IMCD even in the presence of enzyme, at least this portion of the final urinary P_{CO_2} reflects some process performed by the IMCD, as opposed to representing the dehydration of carbonic acid formed by some earlier segment. Both of these conclusions depend on the assumption that there is no possibility of a disequilibrium state when carbonic anhydrase is present in the tubular fluid. The extremely high turnover number of this enzyme provides compelling evidence that the assumption is valid. Moreover, we have shown previously during aciduria that even a lower total CA dose (5 mg bolus + 2 mg/hr) prevents the demonstration of the IMCD disequilibrium pH present before enzyme infusion [5].

The IMCD luminal fluid is thought to be without access to carbonic anhydrase in the absence of enzyme infusion [5]. In this case, delayed dehydration probably does contribute to elevated P_{CO_2} in the collecting duct. Although it might be thought that the magnitude of this component would be estimated by the difference in IMCD P_{CO_2} before and during CA infusion, this is not necessarily valid. As emphasized by Arruda et al [14] and Maren [17], CA will tend to dissipate a carbon dioxide gradient regardless of the mechanism of its generation, a process which may involve CA-facilitated diffusion of carbon dioxide through aqueous as well as lipid compartments [25]. It is also possible that the reduction in IMCD P_{CO_2} during CA infusion reflects a change in the segmental acidification/alkalinization rates. We found, as have others [4, 12, 13], that whole kidney bicarbonate clearance was unchanged by CA infusion, but direct measurements of IMCD acidification/alkalinization rates before and during CA infusion are needed to clarify the question of whether CA does or does not affect these rates.

The question remains whether or not any of the U-B P_{CO_2} gradient reflects postpapillary events. In theory, some contribution of postpapillary delayed dehydration must be present, given a finite dehydration time. This contribution cannot be quantitated easily, because measuring the intrarenal component involves exposing the papilla, a procedure which contaminates final urine with blood from the cut ureter. Nor can it be assumed that the final urinary P_{CO_2} is that from the unexposed kidney, because acidification by the exposed and nonexposed papilla is probably not identical (unpublished observations).

In summary, we have presented evidence that the elevation of urinary P_{CO_2} above systemic levels is an intrarenal event. This finding deemphasizes the importance of postpapillary delayed dehydration and is consistent with the formulation that bicarbonate diuresis is associated with the papillary accumulation of carbon dioxide.

Acknowledgments

A preliminary report of these studies was presented at the 1980 meetings of the American Society of Nephrology. These studies were supported by National Institutes of Health grants HL-17458 and AM-7053. Part of this work was done during the tenure of research fellowships to Dr. Graber from the American Heart Association, Central Massachusetts Division and American Heart Association, Massachusetts Affiliate, Inc., 13-410-798, and from the Charles H. King Trust, Boston, Massachusetts. We appreciate the technical assistance of Dr. C. Caflisch and E. R. McNamara and the secretarial assistance of M. Shea.

Reprint requests to Dr. E. A. Alexander, Renal Unit, Thorndike 416, Boston City Hospital, 818 Harrison Avenue, Boston, Massachusetts 02118, USA

References

1. KURTZMAN NA, ARRUDA JAL: Physiologic significance of urinary carbon dioxide tension. *Miner Electrolyte Metabol* 1:241–246, 1978 1978
2. STINEBAUGH BJ, ESQUENAZI R, SCHLOEDER FX, SUKI WN, GOLDSTEIN MB, HALPERIN ML: Control of the urine-blood P_{CO_2} gradient in alkaline urine. *Kidney Int* 17:31–39, 1980
3. HALPERIN ML, GOLDSTEIN MB, HAIG A, JOHNSON MD, STINEBAUGH BJ: Studies on the pathogenesis of type I (distal) renal tubular acidosis as revealed by the urinary P_{CO_2} tensions. *J Clin Invest* 53:669–677, 1974
4. OCHWADT BK, PITTS RF: Effects of intravenous infusion of carbonic anhydrase on carbon dioxide tension of alkaline urine. *Am J Physiol* 185:426–429, 1950
5. GRABER ML, BENGELE HH, SCHWARTZ JH, ALEXANDER EA: pH and P_{CO_2} profiles of the rat inner medullary collecting duct. *Am J Physiol* 241 (10):F669–F676, 1981
6. DUBOSE TD JR, PUCACCO LR: Evidence that an acid disequilibrium pH is a determinant of papillary collecting duct P_{CO_2} (*abstract*), *Kidney Int* 19:238, 1981
7. DUBOSE TD JR: Urine to blood P_{CO_2} gradient is an index of hydrogen ion secretion by collecting duct (*abstract*). *Clin Res* 29:460A, 1981
8. SCHWARTZ JH, ROSEN S, STEINMETZ PR: Carbonic anhydrase function and the epithelial organization of H^+ secretion in turtle urinary bladder. *J Clin Invest* 51:2653–2662, 1972
9. PUCACCO LR, CARTER NW: An improved P_{CO_2} microelectrode. *Anal Biochem* 90:427–434, 1978
10. WARNOCK DG, RECTOR FC JR: Renal acidification mechanisms, chap 10, in *The Kidney* (2nd ed.), edited by BRENNER BM and RECTOR FC JR, Philadelphia, W. B. Saunders Co., 1981, pp. 440–494
11. PEREZ GO, OSTER JR, SONNEBORN R, CASSADY J, VAAMONDE CA: Analysis of the variables influencing urinary P_{CO_2} during $NaHCO_3$ and water loading in normal man. *Proc Soc Exp Biol Med* 157:97–102, 1978
12. STINEBAUGH BJ, GHARAFRY E, GOLDSTEIN MB, HALPERIN ML, SCHLOEDER FX, SUKI WN: Disequilibrium pH and bicarbonate reabsorption. *Nephron* 20:141–146, 1978
13. GUIGNARD JP: Mecanisme de la reabsorption renale des bicarbonates chez le rat. *Helv Physiol Acta* 24:193–226, 1966
14. ARRUDA JAL, ROSEMAN MK, SEHY JT, MEHTA PK, KURTZMAN NA: In vivo and in vitro studies of urinary acidification. *Miner Electrolyte Metabol* 3:122–135, 1980
15. KENNEDY TJ JR, ORLOFF J, BERLINER RW: Significance of carbon dioxide tension in urine. *Am J Physiol* 169:596–608, 1952
16. HILLS AG, REID EL: P_{CO_2} and P_{NH_3} in mammalian kidney and urinary tract related to urine pH and flow. *Am J Physiol* 219:423–434, 1970
17. MAREN TH: Carbon dioxide equilibria in the kidney: The problems of elevated carbon dioxide tension, delayed dehydration and disequilibrium pH. *Kidney Int* 14:395–405, 1978
18. BRODSKY WA, MILEY JF, KAIM JJ, SHAH NP: Characteristics of acidic urine after loading with weak organic acids in dogs. *Am J Physiol* 193:108–122, 1958

19. GOTTSCHALK CW, MOREL F, MYLLE M: Tracer microinjection studies of renal tubular permeability. *Am J Physiol* 209:173–178, 1965
20. FOWLER N, GIEBISCH G, WHITTEMBURY G: Distal tubular tracer microinjection study of renal tubular potassium transport. *Am J Physiol* 229:1227–1233, 1975
21. DANIELSON RA, SCHMIDT-NIELSEN B: Recirculation of urea analogs from renal collecting ducts of high- and low-protein-fed rats. *Am J Physiol* 223:130–137, 1972
22. UHLICH E, BALDAMUS CA, ULLRICH KJ: Verhalten von CO_2-Druck und bicarbonat im Gegenstromsystem des Nierenmarks. *Pfluegers Arch* 303:31–48, 1968
23. ULLRICH KJ, KRAMER K, BOYLAN JW: Present knowledge of the counter-current system in the mammalian kidney, in *Heart Kidney and Electrolytes*, edited by FREIDBERG CK, New York, Grune and Stratton, 1962, pp. 1–37
24. ARRUDA JAL, NASCIMENTO L, MEHTA PK, RADEMACHER DR, SEHY JT, WESTENFELDER C, KURTZMAN NA: The critical importance of urinary concentrating ability in the generation of urinary carbon dioxide tension. *J Clin Invest* 60:922–935, 1977
25. GUTKNECHT J, BISSON MA, TOSTESON FC: Diffusion of carbon dioxide through lipid bilayer membranes. *J Gen Physiol* 69:779–794, 1977