Total Acid-Labile CO_2 Content in Dog Renal Cortex and Medulla[1] (34455)

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Information concerning the renal corticomedullary distribution of bicarbonate (HCO_3^-) and CO_2 is of importance since either may affect intra- and extracellular pH (1), H^+ secretion rate by renal collecting duct cells (2), and in addition, certain metabolic processes (3) which may occur in the renal medulla.

We have attempted to determine whether there is a corticomedullary gradient for total CO_2 ($CO_2 + HCO_3^-$) by measuring the acid-labile CO_2 (4) content of renal cortex and medulla. The acid-labile CO_2 content of tissue includes gaseous or dissolved CO_2, HCO_3^-, and carbamino-bound CO_2. Thus, a corticomedullary gradient for any one of these moieties could account for any difference observed in the measured acid-labile CO_2 content between renal cortex and medulla. Due to the countercurrent arrangement of the tubules and blood vessels in the renal medulla, it seemed likely that trapping of the gaseous CO_2, which is produced locally, could raise the medullary acid-labile CO_2 above that in the cortex. The following experiments were done to test this hypothesis.

Methods. Female mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg iv); additional doses of 10 mg/kg iv pentobarbital were given as needed. Duplicate 5-ml arterial (5) samples were obtained in oiled syringes for anaerobic pH determination using the Cambridge Research Model pH meter thermostated at 38°. Samples (5-ml) for determination of urine pH were obtained through a self-retaining bladder catheter. The left kidney and its pedicle were exposed through a flank incision, the pedicle was then clamped and the kidney excised. The kidney was cut transversely into six sections (the poles were discarded) and as much medulla as possible (the position of the arcuate vessels was used to determine the corticomedullary junction) was rapidly separated from the cortex with stainless-steel cork borers of appropriate size. The kidney sections were imxediately placed in liquid nitrogen, in a CO_2-free, nitrogen atmosphere (6). Less than 30 sec elapsed from the time of clamping of the pedicle to the time of freezing the tissue in liquid nitrogen. Aliquots of frozen cortex and medulla were pulverized while immersed in liquid N_2, in a stainless-steel mortar which was immersed in liquid nitrogen. Approximately 1-g aliquots of frozen cortical or medullary tissue were then transferred to tared flasks containing 0.1 *N* NaOH and 0.5% ferric fluoride (as enzyme inhibitor) (4). The flasks were immediately closed, the tissue allowed to thaw, and the wet weight of the tissue samples was taken. Determination of the total tissue CO_2 content was done in triplicate using the manometric Van Slyke apparatus according to the method of Danielson and Hastings (4). All handling of the frozen tissue samples was done in a nitrogen atmosphere free of CO_2 (6).

Results. The results of all five experiments done are summarized in Table I. The total acid-labile CO_2 content of renal medullary tissue is significantly greater than that of renal cortical tissue. The variability in the determination of total CO_2 content was similar in tissue samples from the same animal and in tissue samples from different animals. In the one experiment (Expt. 3) where only one

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TABLE I. Acid-Labile CO_2 Content in Dog Kidney.

Expt.	pH		Acid-labile CO_2 content (μmoles/g wet wt)[a]		
	Blood	Urine	Cortex	Medulla	Corticomedullary difference[b]
1	—	—	10.50 ± .46 (3)	13.25 ± .19 (3)	—2.73
2	7.25	6.98	13.36 ±1.55 (3)	16.50 ±1.39 (3)	—3.17
3	7.32	7.01	16.25 (1)	16.22 ±1.22 (4)	+0.03
4	7.24	7.00	14.58 ± .50 (4)	16.77 ± .31 (3)	—2.19
5	7.28	7.30	10.68 ± .60 (3)	16.17 ±1.14 (2)	—5.49
Means ± 1 SE			13.07 ± .99	15.78 ± .57	—2.71 ± .79
No. of experiments			(5)	(5)	(5)
					$p < .05$

[a] Means ± 1 SE are presented. The number in parentheses indicates the number of separate CO_2 determinations done on a single tissue sample.

[b] Negative figures indicate that the CO_2 content is higher in medulla.

cortical sample was analyzed, the renal cortical CO_2 content exceeded that in the renal medulla. Comparison of the CO_2 content observed in this experiment with those of the other experiments, indicated that this difference is due to a relatively high cortical CO_2 content rather than to a decrease in medullary CO_2 content.

The observed greater total CO_2 content of renal medullary tissue might arise from a difference in water content in the two areas of the kidney (7). However, in dog, the medullary water content exceeds that of the cortex (tissue water is 81% ± 0.3% of wet weight in the medulla vs 78% ± 0.3% in the cortex (7)). In Table II are summarized the calculated total acid-labile CO_2 contents of dog medullary and cortical tissues expressed per gram of wet or dry weight, and also per gram of tissue water. The medullary CO_2 content per gram wet weight is higher by 17% than that of the cortex and higher by 39% when expressed per gram dry weight. Thus, the acid-labile CO_2 concentration in medullary tissue H_2O is greater than in cortical tissue water.

Discussion. Our observations do not permit us to identify whether it is the carbamino-CO_2, the gaseous CO_2, or the $[HCO_3^-]$ which accounts for the high medullary acid-labile CO_2 content.

Any difference in carbamino-CO_2 content between renal cortex and medulla would be due to a difference in the concentration of terminal amino groups of proteins between the two regions (1). Although there is a corticomedullary gradient for albumin (8), it is doubtful whether the increased albumin content could form enough carbamino-CO_2 to account for the observed difference in CO_2 content. Further, the concentration of hemoprotein in medulla is lower than in cortex

TABLE II. Renal Acid-Labile CO_2 Content on Wet- and Dry-Weight Basis.

	Total acid-labile CO_2 content[a]		
	Cortex	Medulla	Cortex—medulla
μmoles/g wet wt	13.07 ± 0.99	15.78 ± 0.57	—2.71 ± 0.79
μmoles/g dry wt	59.40 ± 4.50	83.10 ± 3.0	—23.70 ± 5.44
μmoles/g tissue H_2O	16.75 ± 1.27	19.45 ± 0.71	—2.70 ± 1.48

[a] Means ± 1 standard error of the mean are presented. The standard errors for the derived data (i.e., per gram dry weight and per gram of tissue water) were calculated as the geometric mean of the standard error for the CO_2 content (per gram wet weight) and for the renal tissue water content (9).

(9). Thus, it is unlikely that carbamino-CO_2 is the basis for the high CO_2 content of medulla.

During alkalosis the P_{CO_2} of the urine and presumably of the medulla may be as high as 200 mm Hg. (10). Assuming a solubility coefficient for CO_2 in renal medullary tissue water of 0.2834 μmoles/ml per mm Hg (which is similar to that in whole blood (11)), and assuming a tissue water content of 81% of wet tissue weight (7), the concentration of dissolved CO_2 at P_{CO_2} of 200 mm Hg is 4.58 μmoles/g wet weight. Thus, in alkalosis, if the P_{CO_2} in the medulla and in the urine were the same, the medullary P_{CO_2} alone could account for acid-labile CO_2 content of renal medulla which is higher than in cortical tissue.

If we now assume that the higher medullary acid-labile CO_2 content observed in our experiments is due entirely to a difference in P_{CO_2} this would require that the medullary P_{CO_2} be 95 mm Hg greater than the cortical P_{CO_2}. If cortical P_{CO_2} is 45 mm Hg (similar to venous P_{CO_2}) a medullary CO_2 tension of 140 mm Hg would be necessary to account for our experimental observations as being due entirely to a high medullary P_{CO_2}. However, the reported urine P_{CO_2} under experimental circumstances similar to ours is not above 100 mm Hg (12). Further, Ulich *et al.* (13) have recently reported that the P_{CO_2} in vasa recta blood of normal rats (during NaCl infusion) is only ~ 10 mm Hg greater than that of arterial blood. If the P_{CO_2} of vasa recta blood is of similar magnitude in dog, the contribution of the gaseous CO_2 moiety to the total CO_2 of medulla is minimal. Thus, the high medullary total CO_2 content reported here is best accounted for by an accumulation of HCO_3^- in the medulla. Further, since Ulich *et al.* (13) observed no increase in HCO_3^- of vasa recta blood above that present in arterial blood, most of the increased HCO_3^- content is located in the extravascular compartment of the medullary tissue, and most probably in the intracellular compartment.

Summary. Total acid-labile CO_2 was measured in aliquots of dog renal cortex and medulla frozen in liquid nitrogen. A higher (15.87 μmoles/g wet weight vs 13.55 μmoles/g wet weight, $p<.05$) acid-labile CO_2 content was found in the renal medulla. It is suggested that the higher medullary total acid-labile CO_2 content is primarily due to a high $[HCO_3^-]$ in the medullary intracellular fluid rather than to a high medullary P_{CO_2}.

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