Micropuncture Determination of pH, P_{CO_2}, and Total CO_2 Concentration in Accessible Structures of the Rat Renal Cortex

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ABSTRACT Previous studies evaluating the mechanism of renal HCO_3^- reabsorption have assumed equilibrium between systemic arterial blood and tubular fluid P_{CO_2}. We have recently reported that the P_{CO_2} in proximal and distal tubular fluid as well as the stellate vessel significantly exceeded arterial P_{CO_2} by 25.9 ±0.92 mm Hg. The purpose of this study was to determine directly, for the first time, pH, P_{CO_2}, and total CO_2 concentration in the accessible structures of the rat renal cortex with both microelectrodes and microcalorimetry. In addition, the concentrations of chloride and total CO_2 were compared in the stellate vessel. The data demonstrate that: (*a*) values for total [CO_2] in both the proximal tubule and stellate vessel calculated from *in situ* determination of pH and P_{CO_2} closely agree with the measured values for total [CO_2]; (*b*) values for chloride concentration in the stellate vessel are significantly less than the corresponding values in systemic plasma ($\Delta[Cl^-]$ = 5.6 meq/liter); and (*c*) the rise in [HCO_3^-] from systemic to stellate vessel plasma closely approximates the observed reciprocal fall in [Cl^-] in this structure.

INTRODUCTION

Recent studies in which the P_{CO_2} of proximal tubule fluid has been directly measured with a microelectrode have indicated that the P_{CO_2} significantly exceeds systemic arterial blood P_{CO_2} (1, 2). Furthermore, we have demonstrated that the P_{CO_2} along the length of the accessible proximal convoluted tubule, the distal tubule, and the stellate vessel was the same, indicating that CO_2 gas was in, or near, diffusion equilibrium through the renal cortex (1). One of the methods previously employed to examine the mechanism of renal bicarbonate reabsorption has been the disequilibrium pH calculation (3–5). However, one assumption inherent in this calculation, as it has been applied previously, is that the P_{CO_2} of tubular fluid and systemic arterial blood are equal (3–5). However, the demonstration of an elevated P_{CO_2} throughout the renal cortex by microelectrode techniques raises important questions regarding both methodology and, more importantly, the mechanism by which this level of CO_2 tension is achieved. Therefore, before the mechanism of bicarbonate reabsorption can be more clearly defined, the value for P_{CO_2} in the accessible structures of the nephron must be firmly established.

The present studies were designed to validate the magnitude of the P_{CO_2} observed in the accessible structures of the rat renal cortex by comparison of directly determined values of pH and P_{CO_2} and thus the calculated total CO_2 concentration with the total CO_2 concentration measured by the microcalorimetric method (picapnotherm). Furthermore, we have attempted to correlate stellate vessel total CO_2 concentration with chloride concentration.

METHODS

Adult Sprague-Dawley rats (mean wt, 225 g) were anesthetized by intraperitoneal injection with Inactin (ByK Golden-Lomberg Chemische Fabrik GmbH, Konstanz, West Germany) 100 mg/kg body wt and prepared for micropuncture exactly as reported (6).

The *in situ* pH of proximal tubules and stellate vessels was determined with glass membrane pH microelectrodes of 4- to 7-μm tip diameter as recently developed in this laboratory (7). *In situ* P_{CO_2} was determined with P_{CO_2} microelectrodes as previously described (1). Total CO_2 concen-

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tration ($[CO_2]_T$)[1] was measured by the microcalorimetric method of Vurek et al. (8). The $[CO_2]_T$ of plasma as determined by the microcalorimetric method was compared to the standard macrotechnique (Natelson Microgasometer, model 650, Scientific Industries, Inc., Bohemia, N. Y.). The values obtained with these two techniques were not statistically different.

$[CO_2]_T$ was also calculated to afford comparison with the determined value. The concentration of CO_2 was calculated from the directly measured pH and P_{CO_2} in the same proximal tubule from the Henderson-Hasselbalch expression with the following apparent pK_a's: systemic arterial blood, 6.10; stellate vessel blood, 6.12; and solubility coefficients (α) of 0.0301 for all of the above. Determination of pH, P_{CO_2} and $[CO_2]_T$ from the same stellate vessel was not technically possible. In the stellate vessel; P_{CO_2} *in situ* was determined first, after which pH *in situ* was measured. Stellate vessel blood was then collected in siliclad-coated glass micropipettes with a rapid taper and a tip diameter of 10- to 13-μm as described by Weinstein and Szyjewiez (9). Collection of stellate vessel blood was initiated with gentle suction after injection of a small droplet of oil to confirm that the selected vessel received its blood flow from below the cortex. The sample was then capped with CO_2-equilibrated mineral oil (P_{CO_2}, 35–40 mm Hg) and both ends of the pipette were heat sealed in a Water-Welder (Henes Mfg. Co., Phoenix, Ariz.). The P_{CO_2} of the mineral oil was maintained at a lower level than that observed in the renal cortex (1) to avoid the possibility of falsely elevating the $[CO_2]_T$. The sample was then centrifuged at 15,000 rpm for 5.0 min and a 14-nl aliquot was immediately transferred directly to the picapnotherm. Proximal tubular fluid samples were obtained from the same segments of the proximal tubule as those in which pH and P_{CO_2} had been measured. Early and late proximal segments were identified with 10% lissamine green (50 μl) as previously described (6). Tubular fluid samples were transferred directly into the picapnotherm chamber.

In an additional group of control rats, maintained as described above, the chloride concentration in stellate vessel plasma was determined. Chloride concentration in stellate vessel plasma, and in systemic arterial plasma was measured with the micro-coulometric technique of Ramsay et al. (10). The interference of plasma protein with this technique has been long-appreciated (9, 10). Therefore, to negate this error the following modifications were employed. First, the diluent was changed from 1 N H_2SO_4 to a 6:1 mixture of chloride reagent (including gel as commonly employed in the Buchler-Cotlove macrochloride technique (Buchler Instruments Div., Searle Diagnostics Inc., Fort Lee, N. J.). Second, the silver wire was frequently cleaned and(or) cut to assure adequate electrical contact because plasma proteins tend to build up on the wire increasing the electrical resistance and preventing repeated determinations. Third, a thorough mixing of the modified diluent and plasma was assured. Fourth, the reference electrode (agar bridge) was frequently changed. When modified in this manner, the comparison between determination of chloride concentration in plasma by the standard macrotechnique and the microcoulometric technique was linear.

Calculations. Measured stellate vessel plasma $[CO_2]_T$ and chloride concentrations were corrected for plasma water by estimating the protein concentration in stellate vessel plasma according to the expression of Bresler (11).

The filtration fraction was estimated by determination of stellate vessel hematocrit as described by Brenner and Galla (12). Systemic plasma chloride was corrected by using the factor 1.06 (13).

The results are presented as mean±SE. Statistical significance between mean values was calculated by the Student's *t* test for paired or unpaired data, as appropriate.

RESULTS

The hematocrit (Hct) in systemic blood was 45±0.3% and in stellate vessel blood was 53±0.7%. The determined values for pH and P_{CO_2} in systemic arterial blood for 16 control rats are displayed in Table I. Systemic arterial pH was 7.34±0.01 U and P_{CO_2} was 39.2±1.09 mm Hg, whereas the calculated $[HCO_3^-]$ was 22.1 meq/liter. The values for P_{CO_2} *in situ* in the early proximal tubule, late proximal tubule, early distal tubule, and stellate vessel were significantly greater than the value for systemic arterial P_{CO_2} ($P < 0.001$) and are in close agreement with values recently reported from our laboratory (1). Values for pH *in situ* (Table I) obtained with glass membrane pH microelectrodes are in agreement with values obtained by other investigators with other microelectrode techniques for segments of the proximal and distal tubules (3,–5, 14). Thus, paired determinations of pH and P_{CO_2} in the same nephron resulted in a calculated $[HCO_3^-]$ of 18.0 meq/liter in the early proximal tubule and 7.4 meq/liter in the late proximal tubule. Calculated values for $[HCO_3^-]$ are not displayed in segments of the distal tubule because reaction equilibrium cannot be assumed in this structure. The calculated value for $[HCO_3^-]$ in the late proximal tubule (7.4 meq/liter) is similar to values reported by others (5, 14, 15) and lends indirect support to our determined value for P_{CO_2} in this structure. In the stellate vessel the values for pH (7.27±0.01) and P_{CO_2} (65.2±1.29) resulted in a calculated value for $[HCO_3^-]$ of 28.2 meq/liter.

Fig. 1 represents a comparison between measured (microcalorimetric) values for $[CO_2]_T$ (abcissa) and

TABLE I

Determined pH, pCO_2 and Calculated $[HCO_3^-]$ in the Structures of the Renal Cortex (Controls)

	$pH_{in\ situ}$	$P_{CO_2\ in\ situ}$	$[HCO_3^-]_{cal}$
	U	*mm Hg*	*meq/liter*
SAB	7.34±0.01	39.2±0.09	22.1
EPCT	7.06±0.15	65.6±1.32	18.0
LPCT	6.70±0.50	65.1±1.21	7.4
EDCT	6.69±0.15	67.2±1.52	—
LDCT	6.39±0.04	67.1±1.43	—
SV	7.27±0.01	65.2±1.29	28.2

Abbreviations used in this table: EDCT, early distal tubule; EPCT, early proximal tubule; $[HCO_3^-]_{cal}$, bicarbonate concentration calculated from determined pH and P_{CO_2}. LDCT, late distal tubule; LPCT, late proximal tubule; SAB, systemic arterial blood.

[1] *Abbreviations used in this paper*: $[CO_2]_T$, total CO_2 concentration; Hct, hematocrit; SV (subscript), stellate vessel.

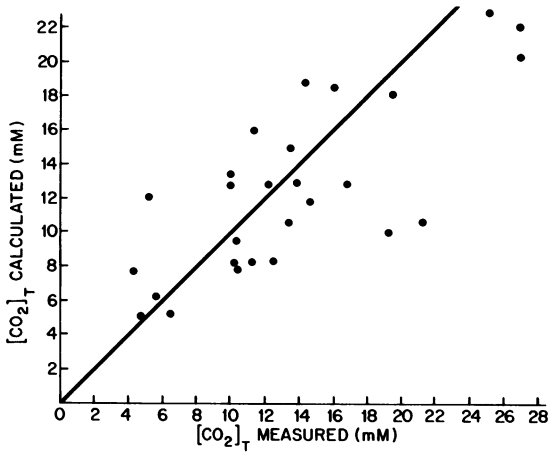


FIGURE 1 Comparison of calculated and measured $[CO_2]_T$ in the superficial proximal convoluted tubule (control rats). Each point represents a paired determination in a single tubule and the line is the line of identity. The ratio of calculated to measured $[CO_2]_T$ does not differ from 1.00 ($P > 0.05$). The slope of the regression line ($y = 0.6X + 4.7$) which describes the data is 0.6 and the correlation coefficient is 0.76. The slope of the regression line and the line of identity are not significantly different ($P > 0.05$).

values for $[CO_2]_T$ calculated from directly determined pH and P_{CO_2} *in situ* (ordinate). Each point represents a paired determination in a single tubule and the line is the line of identity. The slope of the regression line that describes the data is 0.60 and the correlation coefficient (r) is 0.76. The data scatter about the line of identity and the mean ratio of calculated to determined $[CO_2]_T$ does not differ statistically from 1.00 ($P > 0.05$). A similar relationship exists when the measured P_{CO_2} is compared with the P_{CO_2} calculated from the observed pH and $[CO_2]_T$. Thus, the two techniques are not significantly different.

In Table II a similar comparison between calculated and measured $[CO_2]_T$ in the stellate vessel is displayed. 48 calculated and 19 measured values are compared in seven control rats. Again, there is no significant difference in the calculated and determined values with either a nonpaired Student's t test or a two-way analysis of variance. Paired comparisons were not possible in this structure. Values for systemic arterial pH, P_{CO_2}, and $[HCO_3^-]$ were 7.39±0.01 U, 39.0±1.32 mm Hg, and 21.0±1.12 meq/liter, respectively, and thus, did not differ significantly from the control animals mentioned above.

Values for chloride concentration in systemic and stellate vessel plasma are displayed in Table III. When corrected for plasma water (see Methods) there was a significant decrease in $[Cl^-]$ in the stellate vessel ($P < 0.005$). Thus, the fall in chloride concentration of 5.6 meq/liter was compatible with the observed rise in $[HCO_3^-]$ of 6.1 meq/liter (Table I).

TABLE II
Comparison of Calculated and Measured $[CO_2]_T$ in the Stellate Vessel

Rat	Calculated $[CO_2]_T$	n	Measured $[CO_2]_T$	n
1	31.0	(5)	28.8	(3)
2	24.2	(5)	27.1	(2)
3	37.6	(8)	31.2	(1)
4	32.5	(7)	30.0	(4)
5	35.2	(11)	27.5	(3)
6	26.7	(6)	29.4	(4)
7	22.9	(6)	20.0	(2)
Mean±SEM	30.0±2.11	(48)	27.7±1.39	(19)
	P		NS	

DISCUSSION

The present investigation was designed to provide direct determinations of all of the participants in the Henderson-Hasselbalch expression, namely pH, P_{CO_2}, and $[CO_2]_T$ (or HCO_3^- concentration) in the same animal. Three key findings emerge: (*a*) values for $[CO_2]_T$ in both the proximal tubule and stellate vessel of the rat renal cortex calculated from *in situ* pH and P_{CO_2} closely agree with the values for $[CO_2]_T$ measured by the microcalorimetric method (Fig. 1); (*b*) values for chloride concentration in the stellate vessel are significantly less than the corresponding values in systemic plasma ($\Delta = 5.6$ meq/liter) (Table III); and (*c*) the rise in $[HCO_3^-]$ from systemic to stellate vessel plasma closely approximates the observed reciprocal fall in $[Cl^-]$ in this structure ($\Delta = 6.1$ meq/liter).

The demonstration and subsequent validation of an elevated CO_2 tension in these structures assumes importance because a critical feature in previous analyses of the mechanism mediating bicarbonate reabsorption have relied on the disequilibrium pH calculation (3–5), a calculation that involves the necessary assumption that tubular fluid and systemic arterial P_{CO_2} are equal.

Brodsky and Schilb (16) have emphasized that the validity of this assumption requires the direct determination of *in situ* P_{CO_2}. Karlmark and Danielson (14), using indirect techniques, suggested that tubular fluid might not be in equilibrium with systemic arterial blood. In addition, Sohtell and Karlmark (2) have reported direct measurements of proximal tubular P_{CO_2} (with an antimony P_{CO_2} microelectrode) the magnitude of which exceeded arterial blood by 16.6 mm Hg. More recently, Sohtell (17) has reported a mean proximal tubular P_{CO_2} of 60.6 mm Hg. This value closely agrees with our previously reported value of 65.1±1.21 mm Hg in this segment, a value which significantly exceeded systemic arterial P_{CO_2} by 25.9±0.92 mm Hg (1). However, Sohtell (17) reports values for P_{CO_2} in the

TABLE III
Comparison of Systemic and Stellate Vessel [Cl^-] Control Rats (n = 7)

Uncorrected			Corrected-plasma water		
$*[Cl^-]_p$	$[Cl^-]_{sv}$	ΔCl	$*[Cl^-]_p$	$[Cl^-]_{sv}$	ΔCl
110.0±1.34 (14)	102.4±1.21 (33) $P < 0.001$	7.91±0.74	116.7±1.42 (14)	111.2±1.3 (33) $P < 0.005$	5.6±0.86

$*_p$, plasma.

stellate vessel that do not differ from systemic arterial values. Therefore, these results (17) are in sharp distinction to our findings of an elevated P_{CO_2} in the stellate vessel that does not differ from the value in the proximal tubule (1). Although the problems encountered when an antimony electrode is used as a pH sensor have been long appreciated (18, 19) and recently emphasized (20), when employed as a component of a P_{CO_2} microelectrode it is not necessary to compensate for the deleterious affects of anions on the antimony response (2). However, the electrode employed by Sohtell (2, 17) was calibrated in a gas stream, thus raising the possibility of an error caused by the sensitivity of the antimony electrode, to temperature variations (19), or as a result of other gases in solution (20). We have demonstrated close agreement between our P_{CO_2} microelectrode and a macroelectrode for measurement of blood P_{CO_2} (1). Although this is an unlikely source of error, Sohtell has not published a similar validation. These possibilities must remain conjectural because we are unable at present to explain these differences.

The demonstration of close agreement for the calculated and measured values for $[CO_2]_T$ strongly supports our previous demonstration of an elevated CO_2 tension in or near equilibrium throughout the superficial cortex of the rat kidney (1). It is therefore interesting to note that when $[CO_2]_T$ is calculated using the animals' systemic arterial P_{CO_2} instead of the observed *in situ* P_{CO_2}, a line is obtained that is shifted down and lies below the actual points depicted in Fig. 1. This comparison assumes importance because it demonstrates clearly that our observation (1) was not a technical artifact and that the previous assumption of equality for systemic arterial and tubular fluid P_{CO_2} was not entirely correct (3–7). Furthermore, this study represents the first report of paired determinations of these values (pH, P_{CO_2}, and $[CO_2]_T$) using two independent techniques; that is, microelectrodes and microcalorimetry.

Our findings may also be taken as evidence against the recently advanced view that the proximal tubular epithelium might serve as a "diffusion barrier" for CO_2 (21–23). Additional evidence against the concept of a diffusion barrier for CO_2 has been advanced by Warnock and Rector (24), by the demonstration of an exceedingly high permeability for CO_2 gas in the isolated, perfused rabbit proximal straight tubule.

The finding of a pH of 7.27±0.01 U in the stellate vessel differs from the finding of Garcia-Filho and Malnic (7.51 U) (25). These investigators used an antimony pH microelectrode to measure stellate vessel blood pH (pH_{sv}) during free flow and during perfusion of the peritubular capillaries with artificial solutions. However, their protocol differed from ours in that their animals received an infusion of 12 mM $NaHCO_3$ plus 3% mannitol at a faster rate (6.0 ml/h vs. 2.0 ml/h). The arterial P_{CO_2} of their control rats (34.3 mm Hg) was significantly lower than in our animals (39.2 mm Hg). In addition, because the Sb electrode was calibrated in a solution of 20 mM phosphate and no bicarbonate, an alkaline error equivalent to 0.16 pH U is apparent (20). This would result in a corrected pH_{sv} in their study equal to 7.35 pH U. Despite this correction, their reported value for pH_{sv} remains significantly higher than our value and we are unable to totally explain this discrepancy.

The demonstration of a significantly lower value for chloride in the stellate vessel ($\Delta Cl^- = 5.6$ meq/liter; Table III), is compatible with the findings of both Weinstein and Szyjewiez (9) and Atherton (26). Furthermore the observed fall in $[Cl^-]$ is compatible with the observed rise in $[HCO_3^-]$ in this structure. Although we do not report values for sodium concentration in the stellate vessel, Weinstein and Szyjewiez (9) reported values that were not different than systemic plasma. Our findings, in addition to the findings cited above (9, 26), support the concept of addition of early proximal reabsorbate to the efferent arteriole before its rapid and direct ascent to the capsular surface, an anatomical finding emphasized by Weinstein and Szyjewiez (27).

We previously offered three possible mechanisms to explain the generation of CO_2 to the level observed in the renal cortex (1). These mechanisms include: (*a*) the direct addition of reabsorbed bicarbonate to peritubular capillary blood, (*b*) metabolic CO_2 production, and (*c*) luminal CO_2 production as a consequence of the dehydration of carbonic acid formed from secreted

H^+ and filtered HCO_3^-. The demonstration in the present study of a pH in the stellate vessel of 7.27 would obviously make the first mechanism less likely because the addition of HCO_3^- to blood should result in a pH more alkaline than systemic arterial blood and, in addition, the magnitude of bicarbonate addition required to achieve a P_{CO_2} of 65 mm Hg in the efferent arteriole by this mechanism alone exceeds the amount of bicarbonate filtered. The second mechanism, although clearly possible, seems unlikely in view of the findings of Schwartz et al. (28, 29) in which a contribution of less than 2.0 mm Hg could be attributed to this source in the turtle urinary bladder. Furthermore, we have previously demonstrated that the level of renal cortical P_{CO_2} varied directly with the magnitude of bicarbonate reabsorption (1). Variations of this magnitude would not be expected if metabolic sources served as the sole source of renal cortical P_{CO_2}. If the third proposal, luminal CO_2 production, is responsible for the generation of an elevated cortical P_{CO_2}, it is apparent that the classical view of H^+ secretion could not account for our observation of an elevated P_{CO_2} and reduced pH in the stellate vessel because luminal CO_2 would be consumed in the cellular hydration reaction. As an alternative to the classical view, hydrogen ions could be generated by the dissociation of water into OH^- and H^+ (30). The CO_2 generated in the tubule lumen would rapidly diffuse into the peritubular capillary. The explanation of a "respiratory acidosis" in stellate vessel plasma as observed in our study requires, therefore, the functional separation of that OH^- liberated from water and the CO_2 ultimately added to this structure. Separation of OH^- and CO_2 could be achieved by either countercurrent tubular-vascular flow or sequestration of OH^- at or near the peritubular membrane, or in the peritubular plasma in an environment devoid of carbonic anhydrase. The expected changes in pH, P_{CO_2}, and $[CO_2]_T$ would be based on the sequence of addition of CO_2 and OH^- to the peritubular capillary. Such an analysis is considered in detail in the Appendix. The subsequent consumption of CO_2 in the stellate vessel by reabsorbed OH^- would serve to raise the pH and lower the P_{CO_2} of peritubular blood as equilibrium is approached downstream of this structure. The time required to achieve complete equilibrium with respect to pH might be quite slow, as predicted in several recent studies (31, 32).

The further characterization of the generation and maintenance of an elevated renal cortical P_{CO_2}, and the relationship of this process to the mechanism of bicarbonate reabsorption remains speculative and is the subject of future investigation in our laboratory.

APPENDIX

The changes observed in pH, P_{CO_2}, and $[HCO_3^-]$ or $[CO_2]_T$ from systemic arterial to stellate vessel blood requires consideration of: (*a*) the amount of filtered bicarbonate available for reabsorption and thus addition to the peritubular capillary, and (*b*) the source of CO_2 responsible for the rise in P_{CO_2} and fall in pH in this structure. These two problems will be considered in detail.

(*a*) *Comparison of the amount of filtered bicarbonate and amount of bicarbonate required to raise stellate vessel bicarbonate concentration to observed level.* Since arterial pH = 7.40 U, P_{CO_2} =40 mm Hg, Hct = 50%, $[HCO_3^-]$ = 24.5 meq/liter, whole blood CO_2 = 20.5 mM/liter, and filtration fraction (FF) = 0.30, the glomerular filtration rate (GFR) per liter of renal blood flow (RBF) would equal to:

$$\text{GFR/liter of RBF} = (\text{Hct})(\text{FF}) = 0.15 \text{ liters}$$

and

$$\text{efferent RBF} = (1 - \text{GFR}) = 0.85 \text{ liters.}$$

Therefore, the filtered amount of HCO_3^- would be equal to:

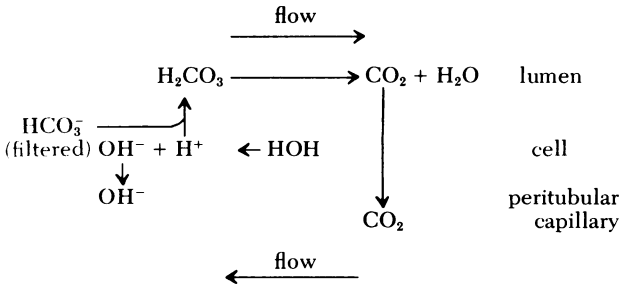
$$0.15 \text{ liters GFR} \times 24.5 \text{ meq/liter} = 3.68 \text{ meq.}$$

This should be compared to the values measured in this study in the stellate vessel: pH_{sv} = 7.27, $P_{CO_{2sv}}$ = 65.2 mm Hg, Hct_{sv} = 58%, and $[HCO_3^-]_{sv}$ = 28.2 meq/liter or whole blood CO_{2sv} = 24 mmol/liter. The amount of filtered HCO_3^- required to raise stellate vessel whole blood CO_2 from 20 to 24 would be equal to:

$$(24 - 20)(0.85) = 3.40 \text{ meq.}$$

Thus, the amount of filtered bicarbonate available (3.68 meq) compares favorably with the amount required to raise the stellate vessel bicarbonate to the observed level (3.40 meq).

(*b*) *Explanation of observations in stellate vessel.* Assume (*a*) Filtered HCO_3^- = 3.68 meq (see above), (*b*) all HCO_3^- is reabsorbed by conversion to CO_2 in proximal tubule lumen, and (*c*) P_{CO_2} of renal arterial blood is 40 mm Hg. For each 0.85 liters of efferent renal blood, 3.68 meq of H^+ ion must be removed from hemoglobin and added to proximal tubule fluid as a result of H^+ secretion. In a dynamic system containing erythrocytes in one compartment, it is assumed that all H^+ must be ultimately derived from the hemoglobin of the erythrocytes in the peritubular capillary. This would produce 3.68 meq of CO_2 in the tubule lumen. Based on the assumption of a high permeability coefficient for CO_2 (24), this CO_2 would flow down its concentration gradient (lumen to blood) and rapidly achieve diffusion equilibrium so that 3.68 meq/0.85 liters = 4.32 meq/liter of CO_2 would be added to stellate vessel plasma. The resulting alterations in pH, P_{CO_2}, and bicarbonate in the stellate vessel would depend on the sequence of addition of OH^- and CO_2 to reabsorbate. One means by which CO_2 could be added before OH^- could be by separation of CO_2 and OH^- in an environment devoid of carbonic anhydrase (peritubular membrane or interstitium) so that the reaction between these two entities would occur at the uncatalyzed rate. This would indicate that a slight disequilibrium should exist, with respect to pH, in the stellate vessel. This may not be incompatible with the demonstration of similar values for calculated and measured $[CO_2]_T$ as demonstrated in this study (Table II), because minor differences in the two techniques might not allow the detection of a slight disequilibrium. If there were countercurrent blood flow with respect to tubule fluid flow, as indicated by the arrows below, and as has been reported between stellate vessel and late proximal tubule (33, 34), such as sequence could be easily explained, i.e. CO_2 produced in the lumen would be added before OH^-.



Based on the calculation above, 4.32 mM of CO_2 would be added to each liter of efferent arteriolar blood. Thus, when added to arterial blood, initially consisting of the values in (*a*) above, would then yield: $pH_{sv} = 7.29$, $P_{CO_{2sv}} = 61$, whole blood $CO_2 = 24.0$ mM/liter, buffer base = 50 meq/liter, and $[HCO_3^-]_{sv} = 28.5$ meq/liter.

Note that these calculated values approximate those values observed in vivo (Table II). Subsequently, as OH^- is added downstream (4.32 meq) the following changes would be expected: pH = 7.41, $P_{CO_2} = 48$, whole blood $CO_2 = 24.0$ mmol/liter, buffer base$^+$ = 54.3 meq/liter, and $[HCO_3^-]_p = 30$ meq/liter.

If, conversely, OH^- were added first, the pH_{sv} would be 7.54 and $P_{CO_{2sv}}$ would be 31, a possibility that does not agree with the observed values. With countercurrent flow, a respiratory acidosis would be observed in the early efferent arteriole (stellate vessel) before return toward neutrality. It should be emphasized, however, that we do not consider the data either evidence for, or against a functional countercurrent system for CO_2 in the renal cortex. The calculations, however, indicate that addition of CO_2 before OH^- or simply a less than instantaneous consumption of CO_2 by OH^- would be compatible with our findings. The half-times required to obtain equilibrium in blood for both CO_2 and pH have been demonstrated to be quite slow (31, 32), and thus would be compatible with this hypothesis.

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