Renal bicarbonate reabsorption in the rat. I. Effects of hypokalemia and carbonic anhydrase.

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Research Article

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Renal Bicarbonate Reabsorption in the Rat

I. Effects of Hypokalemia and Carbonic Anhydrase

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Abstract

Free-flow micropuncture studies were carried out on superficial rat proximal and distal tubules to assess the participation of different nephron segments in bicarbonate transport. Particular emphasis was placed on the role of the distal tubule, and microcalorimetric methods used to quantitate bicarbonate reabsorption. Experiments were carried out in control conditions, during dietary potassium withdrawal, and after acute intravenous infusions of carbonic anhydrase. We observed highly significant net bicarbonate reabsorption in normal acid-base conditions as evidenced by the maintenance of significant bicarbonate concentration gradients in the presence of vigorous fluid absorption. Distal bicarbonate reabsorption persisted in hypokalemic alkalosis and even steeper transepithelial concentration gradients of bicarbonate were maintained. Enhancement of net bicarbonate reabsorption followed the acute intravenous administration of carbonic anhydrase but was limited to the nephron segments between the late proximal and early distal tubule. The latter observation is consistent with a disequilibrium pH along the proximal straight tubule (S_3 segment), the thick ascending limb of Henle, and/or the early distal tubule.

Introduction

Two mechanisms have been identified to account for acidification of tubular fluid. Na/H exchange in the apical brush border membrane takes place along the proximal convoluted tubule and is also present in the thick ascending limb of Henle's loop of the rat and mouse, but not the rabbit (1–5). In addition, a directly electrogenic, ATP-driven H-ion pump has been detected in the cortical and medullary collecting duct (6–8). Such a primary active mechanism of hydrogen ion (H) secretion has also been firmly established in the turtle bladder (9). In the distal nephron, the situation is complicated by observations of net bicarbonate secretion into the collecting duct. Thus, both H^+ and bicarbonate secretion occur and may coexist in separate and specialized cell types (10, 11).

Early micropuncture studies have identified the distal tubule as a site where transepithelial pH and bicarbonate gradients similar to those across the proximal tubule are observed (12–14).[1] In view of the progressive increase of inulin concentrations, net bicarbonate reabsorption of the order of 5–10% of the filtered load has been attributed to this segment (13). However, more recent studies in which single distal tubules were pump-perfused with artificial solutions in conditions of normal acid-base balance did not detect significant bicarbonate reabsorption. Net bicarbonate reabsorption was only detected in rats in metabolic acidosis (16, 17).

The present study attempts to reconcile these divergent views, in particular to ascertain whether the results obtained in perfused tubule segments apply to free-flow conditions. To this end, we have applied, in a first series of experiments, the picapnotherm technique to measure bicarbonate transport along the distal tubule under free-flow and normal acid-base conditions.

In additional experiments, we have extended our segmental analysis of tubule bicarbonate transport to dietary potassium (K) deprivation. K depletion in the rat may be associated with increased proximal tubular bicarbonate reabsorption and lead to metabolic alkalosis (18–20). The role of the distal tubule in this disorder has not yet been defined.

Finally, there are also unresolved problems concerning the role of carbonic anhydrase in distal tubule acidification. From the presence of an acid disequilibrium pH, Vieira and Malnic (14) and Rector et al. (21) have drawn the conclusion that the distal tubule fluid had no access to the catalytic action of carbonic anhydrase. However, the presence of a disequilibrium pH in the distal tubule has been questioned (22). We addressed this issue in our present study in the following manner. If carbonic anhydrase were functionally absent in either the cells lining the straight part of the proximal tubule, Henle's loop, or in distal tubule cells, administration of carbonic anhydrase might dissipate a disequilibrium pH and lower the gradient against which H secretion has to proceed. As a consequence, bicarbonate reabsorption along these segments could be stimulated.

Our results confirm the earlier free-flow observations by (*a*) demonstrating significant bicarbonate reabsorption along the distal tubule, even in nonacidotic conditions. (*b*) In K-depleted rats we observed the establishment of steeper transepithelial bicarbonate gradients and greater fractional bicarbonate reabsorption along the distal tubule than under control conditions. (*c*) Carbonic anhydrase infusion induced increased bicarbonate reabsorption along the S_3 segment, the loop of Henle, and/or

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1. The term "distal tubule" in this paper refers to the segment of the nephron between the macula densa region and the first confluence with another distal tubule to form a collecting duct. This nephron segment comprises at least three different segments: the distal convoluted tubule, the connecting tubule, and the initial collecting duct (15). In our experiments the early distal puncture site includes the first or first two of these segments, and the late distal site, also the initial collecting duct.

the early distal tubule, but no further transport stimulation was observed beyond the first third of the superficial distal tubule.

Methods

Preparation of animals. Free-flow micropuncture experiments were carried out in male Wistar rats (Charles River-Kingston, Stone Ridge, NY) kept in group cages at 21°C and at controlled daylight (7 a.m. to 7 p.m.). Rats were fasted overnight but had free access to water up to the time of the experiment. Animals were anesthetized with Inactin (Promonta, West Germany) using a dose of 120 mg/kg body wt, intraperitoneally, tracheostomized, and placed on a thermoregulated table designed to hold body temperature at 37°C. The right carotid artery was catheterized for blood pressure monitoring and periodic blood sampling for measurements of hematocrit, radioactive inulin, pH and total CO_2 concentration. The left jugular vein was cannulated with PE-50 tubing and used for infusion via a syringe pump (Harvard Apparatus Co., Inc., S. Natick, MA).

The left kidney was exposed through a flank incision, made free of perirenal fat, decapsulated, and immobilized in a lucite chamber. The kidney was bathed with prewarmed (37°C) paraffin oil. The left ureter was catheterized for collection of urine.

Three groups of rats were studied: (A) control rats, maintained on a diet of standard Purina laboratory Chow (Ralston Purina Co., St. Louis, MO); (B) low potassium rats, maintained on a low potassium diet (no. 170550, Teklad, Madison, WI) for 5–7 wk prior to the experiment; (C) rats kept on standard diet but receiving, after a control period, an i.v. bolus of 10 mg of bovine erythrocyte carbonic anhydrase (no. C7500, Sigma Chemical Co., St. Louis, MO). This carbonic anhydrase is largely composed of carbonic anhydrase type C, and physiologically active (21). This priming injection was then followed by a sustaining infusion of 0.5 mg/min of the enzyme.

The experimental design was the same in all three groups of rats. After completion of surgery, they received [*methoxy*-^{3}H]inulin (New England Nuclear, Boston, MA) at 110 μCi/h after a priming injection of 90 μCi. Rats of groups A and B were infused at a rate of 6.6 ml/h with a solution containing: for animals in group A, 121 mM NaCl, 25 mM $NaHCO_3$, and 4 mM KCl; and for animals in group B, 125 mM NaCl, and 25 mM $NaHCO_3$. Both solutions were equilibrated with 5% CO_2 and 95% O_2 to achieve a final pH of 7.4. Rats of group C were infused at a rate of 4.6 ml/h with a solution containing 90 mM NaCl, 4 mM KCl, and 60 mM $NaHCO_3$.

Micropuncture. Collections of tubule fluid samples were begun in groups A and B after a 1-h equilibration period. In group C, a 1-h control period was followed by an experimental period in which carbonic anhydrase was administered. Free-flow micropuncture techniques were those published in previous work from this laboratory (13, 18). Superficial late proximal and early and late distal segments were identified by the i.v. injection of 20 μl FDC solution (5% FD & C green dye no. 1, pH 7.4). Puncture sites were identified by filling the tubule with a microfil silicone rubber compound (Canton Biomedical Products, Boulder, CO). The kidneys were then macerated overnight in 25% NaOH and the puncture sites determined by microdissection. Puncture sites were grouped as early distal (between 20% and 40% distal tubule length) and late distal (60–95% distal tubule length).

Analytic methods. Tubule fluid total CO_2 concentration was determined by microcalorimetry (Picapnotherm, W-P Instruments, Inc., New Haven, CT) immediately after collections (23). In order to avoid loss of CO_2, all the mineral oil used, i.e., that to cover the kidney surface, to fill either the picapnotherm vials or the collecting micropipettes, was equilibrated to cortical carbon dioxide tension (P_{CO_2}) values (22) with a solution containing 100 mM Hepes, 48 mM $NaHCO_3$, and equilibrated with 6.7% CO_2 (15). Each analysis was bracketed by running standards of $NaHCO_3$ solutions. The accuracy of the method was ±5%. The blood acid-base status was assessed using a gas analyzer (model 213-329, Instrumentation Laboratory, Inc., Lexington, MA). Total plasma CO_2 was determined using a carbon dioxide analyzer (model 960, Corning Medical, Medfield, MA). [^{3}H]inulin radioactivity was measured by a liquid scintillation spectrometer (model 92, Searle, Chicago, IL) in a 77% solution of Aquasol (New England Nuclear). Urine volumes were estimated gravimetrically. The volumes of collected tubular fluid samples were measured in a calibrated constant bore capillary. Plasma potassium was determined by standard flame photometry.

Table I. Functional Parameters in Control and Hypokalemic Rats

	Control rats (n = 9)	Low-K rats (n = 6)	P
Body weight, *g*	270±9.5	260±11	NS
Plasma K, *meq/liter*	4.8±0.1	2.5±0.1	<0.02
Plasma (HCO_3^-), *mM*	27.1±1.1	36.0±1.3	<0.001
Blood pH	7.42±0.04	7.53±0.01	<0.02
Blood P_{CO_2}, *mmHg*	44.9±2.1	44.3±1.5	NS
Urine pH	6.5±0.2	6.3±0.2	NS
Hct	46.1±1.4	46.9±1.7	NS
GFR, *ml/min/kidney*	1.13±0.11	1.00±0.10	NS
(U/P)In	89.9±10.9	131±16	NS
$FE_{HCO_3^-}$, %	0.99±0.11	0.41±0.10	<0.05

Values are mean±SEM. Abbreviations: (U/P)In, urine to plasma inulin ratio; $FE_{HCO_3^-}$, fractional bicarbonate excretion, percent of filtered load; Hct, hematocrit; n, number of rats.

Calculations. Glomerular filtration rate (GFR)[2] and fractional bicarbonate excretion rate were determined using standard formulas, as were single-nephron GFR (SNGFR) and fractional bicarbonate delivery in free-flow micropuncture experiments. Because SNGFR values based on late proximal fluid collections tend to overestimate the true SNGFR values by interfering with tubuloglomerular feedback (24, 25), we have chosen to use distal SNGFR values to estimate the mean glomerular, late proximal, and distal bicarbonate loads.

All data are expressed as mean±SEM. Student's t test for paired or unpaired data as appropriate was used to evaluate the significance of differences. Regression lines were obtained by the least squares method, and the significance of slopes was calculated by analysis of variance. Correlation coefficients (r) were also calculated for the variation of data with tubular length.

Results

HCO_3 reabsorption in proximal and distal tubules of control and low-K rats. Table I provides a summary of data on body weights, blood and urine electrolytes, systemic acid-base parameters, and renal clearances (GFR) in control and potassium-depleted animals. As expected, plasma potassium was markedly depressed in group B, whereas blood pH and bicarbonate were significantly increased. Fractional bicarbonate excretion was significantly decreased in K-depleted rats. No difference between hematocrit values was found between control and K-depleted rats, whereas GFR, although not significantly different, showed a tendency to be lower in the latter groups of animals.

Figs. 1 and 2 summarize data on the progress of inulin and total CO_2 concentrations along the superficial distal tubule of the control group. It is evident that significant fluid reabsorption took place along the distal tubule of control rats. No significant change in the tubule bicarbonate concentration was observed

2. *Abbreviations used in this paper:* CA, carbonic anhydrase; GFR, glomerular filtration rate; SNGFR, single-nephron GFR; TF/P, tubular fluid/plasma (concentration).

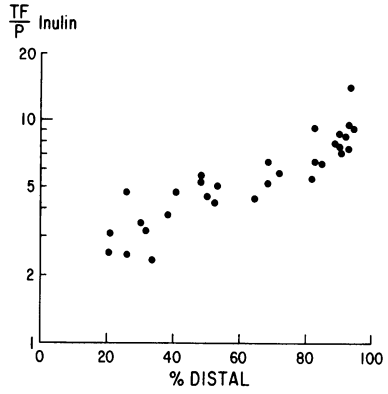

Figure 1. Summary of inulin tubular fluid/plasma concentration ratios as function of distal tubule length of control rats. The equation of the regression line is $\ln y = 0.752 + 0.01475x$, where y = TF/P In and x = localization (tubule length, percent). (Exponential regression). Slope $P < 0.01$. $r = 0.892$.

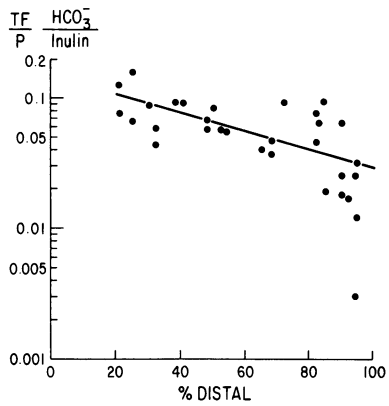

Figure 3. Summary of HCO_3/inulin tubular fluid/plasma concentration ratios as function of distal tubule length of control rats. The equation of the regression line is $\ln y = -1.816 - 0.0197x$ ($r = -0.645$) (slope $P < 0.01$).

(see Fig. 2). The observation of constant bicarbonate concentrations along the distal tubule was confirmed in a subset of micropuncture experiments in which early and late fluid collections were done in identical tubules. The mean of early and late bicarbonate concentrations was 9.8±0.98 and 9.2±1.56 mM, respectively ($n = 4$).

In Fig. 3 fractional bicarbonate delivery of the control group is plotted as a function of distal tubular length. There is a significant negative correlation ($r = 0.65$, $P < 0.01$) between total CO_2/inulin tubular fluid/plasma concentration (TF/P) ratios and distal tubular length. Between 20% and 95% distal tubular length, a mean of some 6% of the filtered bicarbonate is reabsorbed. Accordingly, significant bicarbonate reabsorption takes place along the superficial distal tubule.

In Fig. 4 we present data on the fractional reabsorption of bicarbonate along the distal tubule during hypokalemia. These data are based on measurements of individual TF/P inulin and TF/P bicarbonate (total CO_2) ratios. The regression line for TF/P inulin ratios was $\ln(\text{TF/P In}) = 1.019 + 0.01746x$ ($r = 0.926$, slope $P < 0.01$), that for TF/P bicarbonate $\ln(\text{TF/P } HCO_3^-) = -1.2873 - 0.00943x$, $r = -0.461$, slope $P < 0.05$). Inspection of Fig. 4 indicates that significant net bicarbonate reabsorption was also uniformly observed along the distal tubule in hypokalemic rats.

In Fig. 5 and Table II, TF/P inulin, TF/P bicarbonate, and TF/P bicarbonate/inulin concentration ratios are summarized for control and for K-depleted rats. SNGFR values are given in Table II. In Fig. 5, values are compared at late proximal (*upper panel*), early distal (*middle panel*), and late distal (*lower panel*) punctures sites. It should be noted that, at all three nephron sites, inulin TF/P ratios are slightly higher in low-K than in control animals. A comparison of bicarbonate TF/P ratios is shown in the middle column. In low-K rats, this ratio is significantly decreased in the late proximal tubule, but not different from control values in the early and the late distal tubule. On the other hand, it is noteworthy that the hypokalemic state is characterized by steeper absolute transepithelial bicarbonate concentration differences particularly across the late distal tubule epithelium. This is partly due to the higher absolute bicarbonate level in arterial blood in hypokalemia. Whereas under control conditions, the mean late distal bicarbonate concentration is 7.7±1.2 mM and the transepithelial concentration difference is 19.4±1.2 mM, corresponding values in low-K rats are, 4.9±1.0 and 31.1±1.0 mM, respectively.

A summary of fractional bicarbonate deliveries to various tubule sites is given in the last column of Fig. 5. We note that in hypokalemic rats, compared with control animals, smaller fractions of total bicarbonate are present in the late proximal, early and late distal tubule. We found both in control and K-depleted rats a significant difference between early and late distal delivery of bicarbonate.

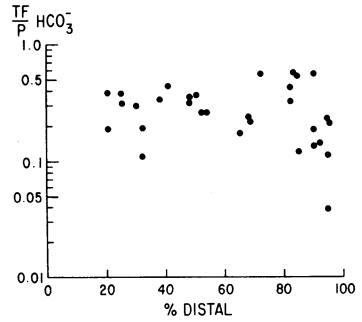

Figure 2. Summary of HCO_3 tubular fluid/plasma concentration ratios as function of distal tubule length of control rats. The equation of the regression line is $\ln y = -1.0496 - 0.00517x$ with $r = -0.227$, slope $P > 0.10$.

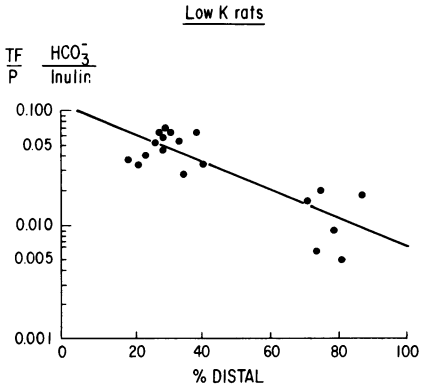

Figure 4. Summary of HCO_3/inulin tubular fluid/plasma concentration ratios in hypokalemic rats as function of distal tubule length. The equation of the regression line is $\ln y = -2.2802 - 0.0275x$ ($r = -0.827$, $P < 0.01$).

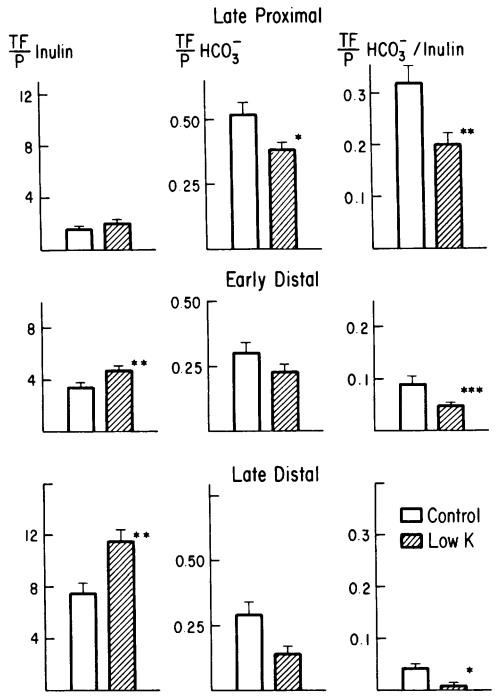

Figure 5. Upper panel: Inulin, HCO_3, and HCO_3–inulin tubule fluid/plasma concentration ratios in late proximal tubule of control and low-K rats. *Middle panel:* Inulin, HCO_3, and HCO_3–inulin tubule fluid plasma concentration ratios in early distal tubules of control and low-K rats. The localization (mean±SEM) of the puncture site, expressed as percentage of distal tubular length, was 29.3±2.7 in control rats and 30.0±1.9 in low-K rats. *Lower panel:* Inulin, HCO_3, and HCO_3–inulin tubule fluid/plasma concentration ratios in late distal tubule of control and low-K rats. The localization (mean±SEM) of the puncture sites, expressed as percentage of the distal tubular length, was 82±2.3% in control rats and 79.8±2.5% in low-K rats. $*P < 0.02$; $**P < 0.01$; $***P < 0.001$ control rats vs. low-K rats.

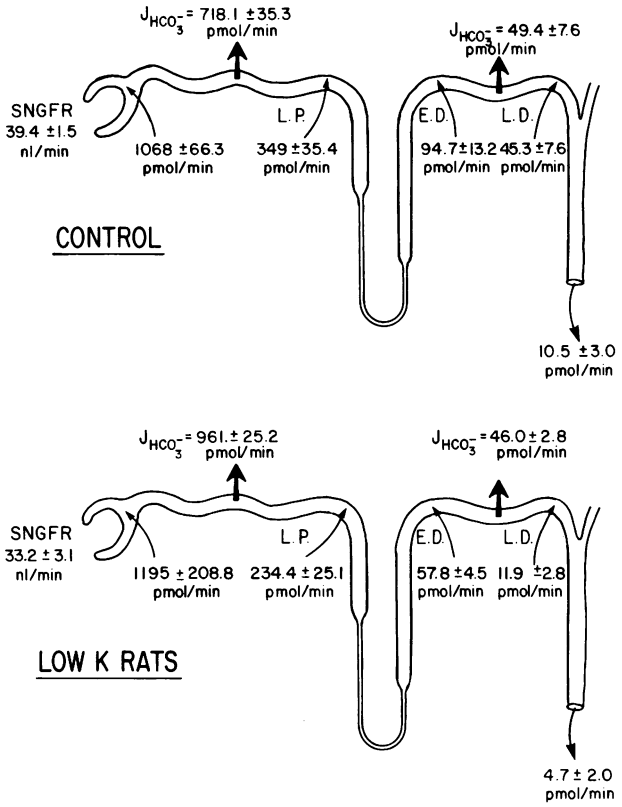

Figure 6. Schematic summary of HCO_3 reabsorption along the nephron of control rats (*upper panel*) and low-K rats (*lower panel*). Filtered load (J) of bicarbonate and delivery of bicarbonate to various tubule sites and into the final urine expressed in pmol · min^{-1}.

Fig. 6 provides an overview of absolute bicarbonate reabsorption along the nephron in control and hypokalemic conditions. First, a comparison of the amount of bicarbonate filtered in the two conditions indicates no significant differences. Although the mean SNGFR fell in hypokalemia ($P < 0.05$), plasma bicarbonate rose enough to maintain similar bicarbonate loads. Second, we note that in hypokalemia the absolute rate of proximal tubule bicarbonate reabsorption exceeds that in control rats.[3] This difference is significant ($P < 0.001$) and of similar magnitude as that observed in a series of in vivo perfusion studies under similar experimental conditions (18). Third, a comparison between distal reabsorption rates in control and hypokalemic rats demonstrates no significant difference in absolute reabsorption rates along this nephron segment. Fourth, fractional reab-

3. It should be noted that these mean transport rates of bicarbonate are estimates based on measurements of distal SNGFR (see Methods). If end-proximal collection rates are used (see Table II), there still remains a significant difference between control (789±36.7 pmol · min^{-1}) and hypokalemic rats (998±24.6 pmol · min^{-1}) ($P < 0.01$).

Table II. Micropuncture Data for Animals Placed on Either Control or Potassium-deficient Diet

	SNGFR		[TF/P]inulin		[TF/P]HCO_3		[TF/P]HCO_3/inulin	
	Control	Low K	Control	Low K	Control	Low K	Control	Low K
	nl · min⁻¹							
Late proximal	43.3±2.7 (9)	34.5±2.8 (9)	1.60±.05 (9)	1.99±.13 (9)	0.519±.05 (9)	0.382±.03 (9)	0.327±.03 (9)	0.196±.021 (9)
Early distal	39.2±2.9 (9)	33.5±1.9 (14)	3.36±.32 (9)	4.67±.21 (14)	0.295±.04 (9)	0.228±.023 (14)	0.089±.012 (9)	0.048±.004 (14)
Late distal	35.5±2.3 (17)	33.3±4.3 (6)	7.43±.57 (17)	11.45±.84 (6)	0.285±.045 (17)	0.137±.029 (6)	0.042±.007 (17)	0.012±.003 (6)

(*n*), number of tubules.

sorption of the early distal bicarbonate load is higher in hypokalemia than in control conditions (80±4% vs. 52±7%, $P < 0.05$). The early distal delivery of bicarbonate was significantly reduced in hypokalemia ($P < 0.01$), reflecting a higher reabsorption rate of bicarbonate along more proximal tubule segments. Finally, inspection of Fig. 6 shows that urinary bicarbonate reabsorption, compared with control rats, is also more complete in K-depleted animals ($P < 0.05$).

Effect of intravenous carbonic anhydrase infusion on bicarbonate reabsorption. Table III provides a summary of functional parameters, blood acid-base, clearance, and urinary excretion data obtained in rats before and after the i.v. infusion of carbonic anhydrase (CA). With the exception of a modest increast in blood pH, the administration of CA did not change blood or urine composition. The fractional excretion of bicarbonate was significantly reduced. This reduction of bicarbonate excretion after CA probably underestimates the true stimulating effect of CA on bicarbonate reabsorption. This is due to the further increase of bicarbonate excretion with time that would have occurred in the absence of CA. Thus, when bicarbonate excretion was measured in a separate group of control animals ($n = 4$) in two 1-h clearance periods, there was a significant further increase ($P < 0.05$) in urinary fractional bicarbonate excretion (1.14±0.354% second hour) as compared to the first hour (0.676±0.342%).

Fig. 7 and Table IV summarize mean SNGFR, inulin, bicarbonate, and bicarbonate/inulin TF/P concentration ratios at the late proximal (*upper panel*), early distal (*middle panel*), and late distal (*lower panel*) puncture sites.

These data are based on the results of micropuncture studies on superficial distal tubules. In these experiments, the following slopes along the distal tubule were obtained: Control TF/P inulin: ln (TF/P In) = 1.256 + 0.012x, $r = 0.719$, slope $P < 0.01$; CA TF/P inulin: ln (TF/P In) = 0.93 + 0.0184x, $r = 0.846$, slope $P < 0.01$. Control TF/P bicarbonate: ln (TF/P HCO_3) = 1.023 − 0.0065x, $r = 0.028$, slope $P > 0.2$. Carbonic anhydrase TF/P bicarbonate: ln (TF/P HCO_3^-) = −1.793 + 0.000x, $r = 0.295$, slope $P > 0.2$; control TF/P bicarbonate/inulin: ln (TF/P HCO_3^-/ln = −2.293 − 0.0123x, $r = 0.573$, slope $P < 0.01$; carbonic anhydrase: ln (TF/P HCO_3^-/In) = −2.715 − 0.0127x, $r = 0.56$, slope $P < 0.01$. Inspection of Fig. 7 shows that no effect of CA infusion was detected along the proximal tubule. However,

Table III. Functional Parameters in Rats during Control Period and after Intravenous CA Administration

	Before CA ($n = 8$)	After CA ($n = 8$)	P
GFR, *ml/min/kidney*	1.16±0.07	1.01±0.08	NS
Blood pH	7.47±0.01	7.56±0.02	<0.01
Blood P_{CO_2}, *mmHg*	46.7±1.4	45.9±1.3	NS
Plasma (HCO_3^-), *mM*	29.5±1.2	31.3±1.0	NS
Hct	48.1±1.1	46.4±1.4	NS
Urine pH	7.01±0.21	6.98±0.24	NS
(U/P)In	261±40.4	178±19	<0.05
$FE_{HCO_3^-}$, %	0.380±0.110	0.201±0.056	<0.05

Values are mean±SEM. Student's t test for paired data was used. Abbreviations: (U/P)In, urine to plasma inulin ratio; $FE_{HCO_3^-}$, fractional bicarbonate excretion, percent of filtered load; Hct, hematocrit; n, number of rats. The rats' body weight was 298±20 g.

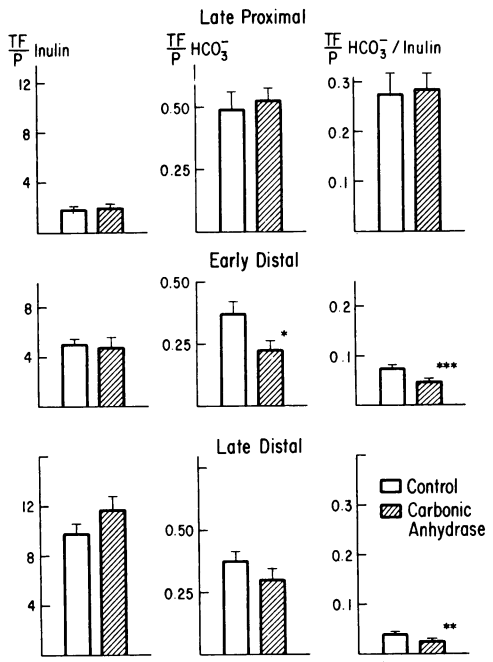

Figure 7. Upper panel: Inulin, HCO_3, and HCO_3–inulin tubule fluid/plasma concentration ratios in late proximal tubule before and after carbonic anhydrase infusion. *Middle panel:* Inulin, HCO_3 and HCO_3–inulin tubule fluid/plasma concentration ratios in early distal tubules before and after carbonic anhydrase infusion. The localization (mean±SEM) of the puncture sites, expressed as percentage of the distal tubular length, was 30±2.5 before and 30.5±2.3 after carbonic anhydrase infusion. *Lower panel:* Inulin, HCO_3, and HCO_3–inulin tubular fluid/plasma concentration ratios in late distal tubule before and after carbonic anhydrase infusion. The localization (mean±SEM) of the puncture site, expressed as percentage of the distal tubular length, was 81±2.5 before and 80.1±3.0 after carbonic anhydrase infusion. *$P < 0.05$; **$P < 0.02$; ***$P < 0.001$.

at the early distal tubular level, CA infusion induced a significant reduction of bicarbonate TF/P and fractional delivery rates. This effect is also observed at the late distal site, although to a lesser extent.

Absolute bicarbonate reabsorption rates along the nephron in control and CA-treated animals are shown in Fig. 8. The upper panel summarizes data during the control period,[4] the lower panel those after CA infusion. At very similar filtered loads, we detected no action of CA along the proximal tubule. Accordingly, the load of bicarbonate delivered out of the proximal tubule is similar in control and CA-treated animals. We notice, however, that following CA infusion, bicarbonate reabsorption between the late proximal and early distal sites is stimulated. These differences are significant for both absolute ($P < 0.001$) and fractional ($P < 0.05$) delivery rates. After CA infusion, bicarbonate reabsorption along the remainder of the distal tubule is lower in absolute amounts ($P < 0.05$), but unaltered when expressed in the percentage of the early distal load (45.7±5.3%

4. The absolute rates of bicarbonate reabsorption in this control group are higher than that in group A. This could be related to the different rate of intravenous infusion of calcium-free solutions in the two sets of experiments: 4.6 ml/h in group C and 6.7 ml/h in group A. In the latter group, higher blood levels of parathyroid hormone, an inhibitor of proximal bicarbonate reabsorption, would have resulted from greater dilution of plasma calcium (26).

Table IV. Micropuncture Data for Rats before and after CA Infusion

	SNGFR		[TF/P]inulin		[TF/P]HCO_3		[TF/P]HCO_3/inulin × 100	
	Control	CA	Control	CA	Control	CA	Control	CA
	nl · min^{-1}							
Late proximal	44.2±5.9	43.7±3.5	1.80±.09	1.8±.11	0.485±.068	0.528±.049	27.4±4.1	28.7±3.1
	(8)	(4)	(8)	(4)	(8)	(4)	(8)	(4)
Early distal	40.4±2.6	40.9±2.4	4.97±.38	4.74±.68	0.370±.052	0.224±.034	7.4±.6	4.8±.4
	(5)	(6)	(5)	(6)	(5)	(6)	(5)	(6)
Late distal	43.7±2.8	40.2±3.1	9.82±.72	11.7±1.1	0.377±.036	0.301±.042	4.1±.4	2.7±.3
	(21)	(15)	(21)	(15)	(21)	(15)	(21)	(15)

(*n*), number of tubules.

in control period versus 44.5±7.1% after CA infusion). Finally, urinary bicarbonate excretion was significantly reduced by CA treatment ($P < 0.05$).

Discussion

Bicarbonate transport along the superficial distal tubule during normal acid-base status. Several free-flow micropuncture studies have indicated that a significant fraction of the filtered bicarbonate load is reabsorbed along the distal convoluted tubule (12–14). This conclusion was based on the following observations. First, free-flow studies using a variety of electrodes have shown that the epithelium of the distal tubule maintains pH levels uniformly lower than blood by some 0.6–1.0 pH units. Importantly, these pH levels did not change along the distal tubule. Recently even a small but significant decrease, of the order of 0.3 pH units, has been measured by glass pH electrodes by DuBose et al. (27). From these studies and from the significant increase in inulin concentrations along the distal tubule (some two- to fourfold), significant net bicarbonate reabsorption can be deduced. The fractional reabsorption rates of bicarbonate that have been reported range between 5–10% of the filtered load (13).

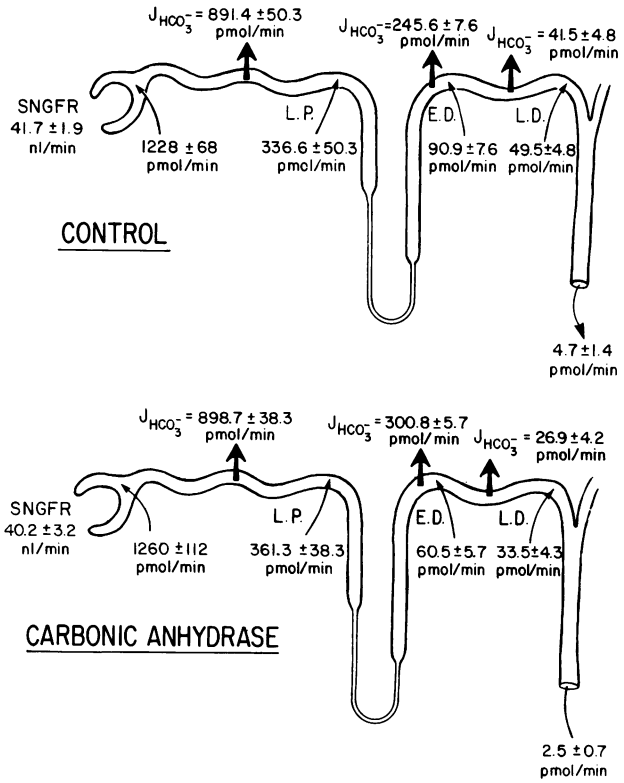

Figure 8. Schematic summary of HCO_3 reabsorption along the nephron before (*upper panel*) and after (*lower panel*) carbonic anhydrase infusion. Filtered load (J) of bicarbonate and delivery of bicarbonate, reabsorption of bicarbonate (mmol · min^{-1}) to various tubule sites and into the urine expressed in pmol · min^{-1}.

A second line of evidence supporting the notion of net bicarbonate reabsorption in the distal tubule derives from stationary microperfusion studies. In such studies, pH in buffered fluid samples, in which transepithelial fluid movement is minimized, is monitored as a function of time. When pH was determined in such experiments, it falls rapidly to similar levels or even below the distal pH values found in free-flow conditions (28, 29). In such studies somewhat lower steady-state pH values are obtained in late distal than in early distal perfusions. Significant bicarbonate reabsorption has also recently been reported in an amphibian distal nephron, in the early and late distal tubule of *Amphiuma* (30, 31).

The measurement of net bicarbonate reabsorption by the Picapnotherm technique employing "in vivo" microperfusion methods and using artificial perfusion solutions has provided different results. Employing microcalorimetric methods to measure total CO_2, two recent perfusion studies have failed to report significant bicarbonate reabsorption along the distal tubule in animals in normal acid-base balance (16, 17). Only during metabolic acidosis was significant bicarbonate reabsorption observed. In another study, an acid disequilibrium pH was also only observed in distal tubules of acidotic animals (22).

In the present free-flow micropuncture study, bicarbonate transport was assessed by means of the microcalorimetric method. The results obtained did not differ from previous experiments in which bicarbonate concentrations were evaluated by measuring the pH of tubule fluid (12–14). The fact that the bicarbonate concentration along the distal tubule was maintained at low levels despite significant fluid reabsorption demonstrates significant net bicarbonate reabsorption along the distal tubule in conditions of normal acid-base balance.

The reason for the discrepancy between our results and those reported by other investigators using the same analytical method to measure bicarbonate concentrations but in which distal tu-

bules were pump-perfused is not entirely clear. Several points deserve comment.

In the perfusion studies, artificial solutions were used which differ in composition from native tubule fluid. Thus, in general, the perfusion fluids used did not contain buffers other than bicarbonate. The absence of such buffers as phosphate, normally present in distal tubule fluid in concentrations varying from 3 to 8 mM (32–34), could lead to a significant disequilibium pH. Given the presence of H-ion secretion and the subsequent reaction with luminal bicarbonate, one would expect formation of carbonic acid in the absence of carbonic anhydrase. Interaction of carbonic acid with alkaline phosphate could generate bicarbonate and acid phosphate, thus reducing the disequilibrium concentration of carbonic acid. As a result, H-ion secretion could proceed against a smaller pH gradient and this may account for the observation that in free-flow distal collections, a sizeable reabsorption rate of bicarbonate is found. In experiments using distal tubule perfusions, however, no phosphate was added to perfusion fluid. Hence, generation of a significant disequilibrium pH could occur. Conceivably, this could impair bicarbonate reabsorption by imposing a steeper pH gradient across the epithelium of the distal tubule as was observed in proximal tubule after c.a. inhibition (35, 36).

A possible explanation for the discrepancy between free-flow and perfusion studies could be the absence of ammonia in the perfusate of the microperfusion studies (Knepper, M., personal communication). Thus, significant amounts of NH_3 could have entered the perfusate from the interstitium, reacted with protons, and formed NH_4^+. These protons are not available to titrate bicarbonate. Consequently NH_3 entry decreases bicarbonate reabsorption by an amount equal to the rate of NH_3 entry into the lumen of perfused tubules. The rate of NH_3 entry might be appreciable, especially at high flow rates. If the total ammonia concentration rises to 1 mM at a flow rate of 10 nl/min, NH_3 entry would be 10 pmol/min (37). Bicarbonate reabsorption would be substantially reduced.

The possibility should also be considered that different distal tubule segments were studied in free-flow and in in vivo microperfusion experiments. The superficial distal tubule is lined by several cells types (15, 38–41). In analogy to observations made on the cortical collecting duct, some cells in the initial collecting tubule ("late distal tubule") may have the ability to secrete bicarbonate ions into the tubular lumen (10). Such bicarbonate secretion could induce an element of variability into studies of distal bicarbonate transport, particularly if later tubule segments, containing the cell type that has been implicated in epithelial bicarbonate secretion, were pumpperfused (37). The present study and the microperfusion experiments were performed in different strains of rats (Wistar vs. Sprague Dawley).

Finally, an important point concerns the strikingly different rates of fluid reabsorption in free-flow conditions and in pump-perfused tubules. During nondiuretic free-flow conditions about two thirds of early distal fluid is reabsorbed (reflected by a threefold increase in inulin concentrations). In sharp contrast, during pump perfusion only about 10–20% of the perfusion fluid are reabsorbed (17). This reduction in fluid transport may reflect functional changes that could be related to the inability of perfused tubules to reabsorb bicarbonate.

Additional quantitative considerations strengthen our conclusion that significant bicarbonate reabsorption occurs along the distal tubule of the rat during free-flow conditions in vivo. The bicarbonate concentration in the fluid entering the distal tubule is of the order of 8–10 mM (see Fig. 2). Given physiologic rates of distal fluid reabsorption, the absence of bicarbonate reabsorption would lead to a dramatic increase of bicarbonate concentrations to levels approaching or exceeding arterial plasma levels. This is a consequence of a roughly two- to fourfold increase in TF/P inulin ratios along the distal tubule (12–14, 42).[5] Similar considerations also apply to pH changes along the distal tubule. In the absence of bicarbonate reabsorption, the pH would have to increase from an early distal value of about 6.8 to values in excess of 7.3 in the late distal tubule. Clearly, an increase of luminal pH of such magnitude has never been reported (12–14, 27). Accordingly, we believe that the results of the reported perfusion studies do not apply to the free-flow situation in vivo.

It is of interest to discuss the possible mechanisms of bicarbonate reabsorption as observed in our work. Bicarbonate reabsorption along most nephron segments has been attributed to hydrogen ion secretion. Evidence for bicarbonate reabsorption by such a mechanism has been obtained also for the thick ascending limb of the rat kidney (44) and the distal tubule of amphibians (30, 31). However, participation of passive bicarbonate reabsorption by electrodiffusion cannot be discarded, especially in a segment where a significant transepithelial potential difference has been observed (45, 46). Assuming distal transepithelial potential differences between −20 mV and −40 mV, bicarbonate TF/P ratios of 0.47 and of 0.22, respectively, are expected if electrochemical equilibrium were reached along this tubule segment. Further studies will be necessary to clarify whether the distal bicarbonate permeability is large enough for passive bicarbonate reabsorption to be significant (47–50).

Bicarbonate reabsorption in potassium depletion. Our studies extend a considerable body of evidence suggesting that tubular bicarbonate reabsorption is significantly increased in hypokalemic metabolic alkalosis. Evidence supporting this view is based on several free-flow micropuncture studies in which bicarbonate transport was evaluated (18, 20, 51–54), although Levine et al. (55) have also reported that absolute bicarbonate reabsorption may not be enhanced during selective potassium depletion, particularly when the filtered bicarbonate load is decreased and metabolic alkalosis fails to develop. In other studies, enhanced bicarbonate transport was, however, observed in continuous microperfusions in which tubules and peritubulular capillaries of hypokalemic rats were exposed to bicarbonate concentrations typically observed in low-K rats (18). In addition, studies on brush border vesicles from rats in hypokalemia have also established the presence of accelerated H-ion secretion (19). Our present results confirm these observations by demonstrating a significantly enhanced absolute rate of bicarbonate reabsorption along the proximal tubule. The magnitude of this stimulation is similar to that observed in the in vivo microperfusion studies cited above (18).

In previous studies, Cogan et al. (52) have emphasized the important role of a reduced filtered load of bicarbonate in hypokalemia. Proximal bicarbonate delivery and absolute reabsorption in these studies were thus not different from control values (see also Maddox and Gennari [53]). Our studies extend

5. It is of interest that even in those micropuncture studies in Wistar rats in which osmotic equilibration failed to occur during the passage of tubule fluid along the distal tubule, inulin ratios rose significantly between early and late distal collection sites (43).

the range over which bicarbonate transport can be stimulated. In the present series of experiments, the mean filtered bicarbonate load in low-K rats did not change as compared to that in control animals, yet proximal bicarbonate reabsorption was, nevertheless, enhanced. The increased absolute rate of bicarbonate reabsorption under these conditions suggests that hypokalemia can stimulate proximal tubule bicarbonate reabsorption. Maddox and Gennari (53) have also noted that in certain states of hypokalemic alkalosis a sustained reduction in the filtration rate is not necessary, and that the absolute rate of proximal bicarbonate reabsorption may be significantly increased above control rates in a load-dependent manner. Both a fall in cell pH and activation of a modifier site of the brush border Na/H exchanger may be responsible for the increased rate of H secretion observed (19, 54), although this stimulating effect may be offset, partially at least, by the alkalemia which has been shown to inhibit proximal acidification (56). The development of tubular hypertrophy in chronic potassium depletion may also be responsible for the adaptive response of enhanced bicarbonate reabsorption (57).

As a reflection of enhanced bicarbonate transport in the proximal tubule, decreased delivery of bicarbonate into the distal tubule was observed. Despite this reduction of the distal bicarbonate load, further bicarbonate retrieval was present at this site. Bicarbonate transport continued in hypokalemic rats and actually resulted in an increase of fractional bicarbonate reabsorption (80±4% vs. 52±7%). In another series of experiments in which the rate of bicarbonate delivery was enhanced by loading with bicarbonate, we were able to show that the absolute rate of bicarbonate reabsorption is sharply and even further augmented during hypokalemia (58).

The state of hypokalemic alkalosis in the present conditions was also associated with steeper transepithelial bicarbonate concentration differences. Comparing late distal bicarbonate concentrations (see Fig. 4), a concentration difference between blood and lumen of 27.1 − 7.7 = 19.4 mM was found in control rats, and of 36 − 4.9 = 31.1 mM in low-K rats. If these values are representative of or approaching steady-state (static head) conditions, the steeper concentration gradients in low-K rats may reflect a larger driving force of the distal H-ion secretory pump. An alternative possibility is that the lower early distal flow rates in hypokalemic rats (7 vs. 12 nl · min^{-1} in controls) contributed, as summarized in Table V, to the development of steeper transepithelial bicarbonate concentration gradients.

Table V. Effects of Flow Rate and Contact Time on Bicarbonate Concentration in the Distal Tubule

Tubular flow rate	Distal contact time	End distal (HCO_3^-)
nl/min	*s*	*mM*
1	18.9	4.4
2	9.5	5.6
5	3.8	7.5
10	1.9	8.6
20	0.95	9.3

Contact times calculated as $T = \pi r^2 l/\dot{V}$, where r is tubular radius (10 μm), l tubular length (1 mm), and $\dot{V}$ tubule flow rate. End distal bicarbonate concentrations are calculated from a distal acidification half-time of 5.0 s, early distal bicarbonate concentration of 10 mM, and distal stationary bicarbonate concentration of 4 mM.

Role of carbonic anhydrase in distal acidification. The role of CA in acidification of tubular fluid is well established (54, 59–61). There is general agreement that the presence of CA in the brush border membrane of the proximal tubule (S_1 and S_2 segments) is an essential component of effective bicarbonate reabsorption in this nephron segment (21, 36, 60, 61). Hence, no further stimulation of bicarbonate reabsorption would be expected by exposing the proximal lumen to CA. Our results confirm this notion.

The situation is different in more distally located nephron segments. The existence of a disequilibrium pH in distal tubules has been described by early studies and was taken to indicate the absence of CA in the luminal membrane (14, 21). Recently it was observed that addition of CA to the luminal perfusion fluid in stationary microperfusion experiments increased luminal pH, especially in early distal segments. This observation is compatible with dissipation of a disequilibrium pH at this site (62). However, studies using an equilibrium electrode have not confirmed the generation of a disequilibrium pH in the distal tubule (22).[6] Recent observations in isolated perfused rabbit outer medullary proximal straight tubule segments (S_3) have also demonstrated the ability of this nephron segment to generate a spontaneous disequilibrium pH (63). This is consistent with the absence of brush border CA in this initial segment of Henle's loop.

Our present observations show that after CA infusion the absolute rate of bicarbonate reabsorption was significantly increased between late proximal and early distal collection sites. This supports the view that CA is not sufficiently available for the dissipation of a disequilibrium pH at some site along the straight portion of proximal tubule, loop of Henle, and early distal tubule. Our data are compatible with both the reports of Kurtz et al. (63) and of Good et al. (64) and Good (44). As pointed out above, the in vitro perfusion studies of Kurtz et al. (63) implicated the S_3 segment of the proximal tubule in hydrogen-ion secretion whereas Good et al. (44, 64) observed significant bicarbonate reabsorption along the thick ascending limb of Henle in the rat and provided evidence for Na^+–H^+ exchange. The data of Lönnerholm and Ridderstrale (38) are also of interest. These investigators did not detect significant amounts of CA in the luminal membrane of the S_3 segment and of early distal tubule cells of the rat. In contrast, they did observe staining indicative of the presence of CA in the apical membrane of intercalated cells of late distal tubule. This heterogeneity of the distribution of apical CA along the distal tubule is consistent with our observations that the absolute rate of bicarbonate reabsorption was stimulated along the S_3 segment, the loop of Henle, and the early distal tubule but not increased above control rates between early and late distal segments, i.e., where tubular fluid has access to CA. However, the lower absolute distal bicarbonate absorption rates after CA infusion could also be related to the lower bicarbonate load delivered to the early distal as consequence of higher bicarbonate reabsorption along tubule segments located upstream of the early distal puncture sites.

6. The presence of a disequilibrium pH does not introduce a significant error into the measurements of bicarbonate transport by the picapnotherm technique. The disequilibrium concentration of carbonic acid is in the micromolar range, and hence will not significantly affect total CO_2 measurements in the millimolar range.

In conclusion, we have used the microcalorimetric method to evaluate bicarbonate reabsorption along the nephron. We demonstrated the presence of significant bicarbonate reabsorption along the distal tubule in normal acid-base conditions. Furthermore, we have shown that hypokalemia stimulates proximal bicarbonate transport, even under conditions in which the filtered load of bicarbonate is maintained at control levels. Distal bicarbonate reabsorption persists in this group of animals and leads to even steeper concentration differences by the time the fluid has reached the end of that tubule segment. Finally, we have demonstrated a significant enhancement of bicarbonate reabsorption after intravenous administration of CA along segments between the late proximal and early distal tubule. In contrast, bicarbonate transport was not affected by CA along the proximal tubule and beyond the early distal tubule. These results are consistent with the uneven distribution of apical CA along the straight portion of proximal tubule, the loop of Henle, and in early and late distal tubule cells. The significant stimulation of bicarbonate reabsorption by CA can best be explained by the dissipation of a disequilibrium pH along either the medullary straight proximal tubule (S_3 segment) or along the thick ascending limb of Henle's loop and early distal tubule.

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