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Research Article

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Secretion of Bicarbonate by Rat Distal Tubules In Vivo

Modulation by Overnight Fasting

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Abstract

We have performed microperfusion studies on distal tubule bicarbonate reabsorption ($JtCO_2$) of fed and fasted rats to extend our previous observations of in vivo bicarbonate secretion and to resolve certain discrepancies between free-flow and microperfusion data. When rats are fasted overnight, as in previous free-flow studies, distal tubule microperfusion with a 28-mM tCO_2 solution results in significant $JtCO_2$ (53±6 pmol · min^{-1} · mm^{-1}) at normal flow and increases briskly (91±16 pmol · min^{-1} · mm^{-1}) with bicarbonate load. This response is not influenced by the addition of other normal tubular fluid constituents. However, when normally fed rats are used, as in our previous microperfusion studies, distal tubule $JtCO_2$ is not different from zero when a 28-mM tCO_2 solution is perfused at normal flow rates but becomes negative (−54±13 pmol · min^{-1} · mm^{-1}) at high flow rates, which indicates the existence of bicarbonate secretion against a concentration gradient. Alkali loading of fasted rats also elicits bicarbonate secretion at high flow. These results demonstrate for the first time that normal feeding or alkali loading can induce bicarbonate secretion in a mammalian nephron segment in vivo, and resolves previous discrepancies between free-flow and microperfusion data.

Introduction

Recent in vitro studies of cortical collecting tubules and outer medullary collecting ducts have provided unique insights into mechanisms of urinary acidification (1). Perhaps the most striking of these observations is the demonstration that the cortical collecting tubule of the rabbit can reabsorb or secrete bicarbonate in response to alkali or acid loads (2). However, in vivo studies in our laboratory (3), as well as those of Lucci et al. (4), have indicated that microperfused superficial distal tubules of normal rats do not reabsorb bicarbonate, which suggests that this segment does not participate in urinary acidification when acid-base balance is normal. More recently, we have also shown that when flow rate and load are increased, bicarbonate secretion can be demonstrated in in vivo perfused distal tubules (5).

However, in studies employing free-flow collections, Capasso et al. (6) have reported brisk bicarbonate reabsorption ($JtCO_2$)[1] by distal tubules of normal rats, which increases in response to rising bicarbonate load (7). It was suggested by Capasso et al. (6) that failure to demonstrate bicarbonate reabsorption under microperfusion conditions might be due to the absence of some normal tubular fluid constituents in the perfusate (3).

In the present studies we have attempted to extend our previous observations on bicarbonate secretion[2] in this nephron segment and to evaluate the possibility that fasting rats before micropuncture, as undertaken by Capasso et al. (6), might stimulate distal tubule bicarbonate reabsorption ($JtCO_2$).

Methods

These studies were performed on male Sprague-Dawley rats that weighed ~ 300 g and were bred and raised in a climate-controlled facility at the University of Ottawa. The animals were allowed free access to water and rat chow (5012; Ralston-Purina of Canada Ltd., Woodstock, Ontario) that contained 22% protein. Rats were allowed water ad lib. and were either fasted or fed while being housed in individual metabolic cages that permitted urine collection for 16 h before microperfusion. One group of rats (group IA) was allowed free access to food and water but was kept in cages housing three to four animals until the time of experimentation. The rats were anesthetized with 100 mg/kg Inactin (BYK Gulden, Konstanz, Federal Republic of Germany) and were prepared for microperfusion studies as previously described (5). Urine was collected under oil using thymol as a preservative. The data were derived from 150 samples obtained from 50 rats. Paired samples from 75 tubules were collected at two flow rates as previously described (5).

Intratubular perfusion. Table I shows the three perfusion solutions used. Solution 1 simulated early distal tubular fluid (5) and contained gluconate so that chloride concentration could be kept constant when tCO_2 concentration was raised in solution 2. Solution 3 was designed to include other normal tubular fluid constituents and as far as possible, to be comparable to solution 2. All perfusion solutions contained 0.05% FD and C green dye and were gassed with humidified 9% CO_2, 91% O_2 to attain a P_{CO_2} of ~ 65 mmHg. In these experiments, as before (5), the microperfusion pump was calibrated in vitro in every experiment. All in vivo collections were quantitative and timed, and the concentration of [^{3}H]inulin was determined in the initial perfusate and in the collected samples. In this way significant departures from nominal perfusion rates were detected. In the present experiments in vivo perfusion rates were 8.12±0.15 and 25.77±0.36 nl/min for the 150 samples taken.

A portion of this work has already appeared in abstract form (1985. *Clin. Res.* 33:487A).

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1. *Abbreviations used in this paper:* BW, body weight; $JtCO_2$, distal tubule total CO_2 reabsorption; tCO_2, distal tubule total CO_2 concentration.

2. In this paper tCO_2 secretion, HCO_3 secretion, and negative values for $JtCO_2$ are used interchangeably.

Table I. Perfusion Solutions

	Solution 1	Solution 2	Solution 3*
tCO_2 (*mM*)	12	28	28
Na (*mM*)	56	56	66
K (*mM*)	2	2	2
Cl (*mM*)	26	26	36
Urea (*mM*)	22	22	0
Gluconate (*mM*)	20	4	0

* Solution 3 also contained (in millimolars) PO_4, 4.0; NH_4, 1.5; alanine, 5.0; glucose, 5.0; acetate, 1.5; Ca, 1.8; Mg, 1.0; SO_4, 1.0.

Balance studies. Because it is not possible to compare urines in the same animal after feeding and fasting when terminal micropuncture experiments are done, metabolic balance studies were undertaken on seven rats to document sequential body weight and 24-h urine pH. The rats were acclimatized for 7 d before the study. 24-h urine collections were obtained after feeding, fasting, and refeeding for 3 d in the same animal.

In a separate study, six rats were taken from group cages after normal overnight feeding and a 5-h urine collection was obtained after each animal was placed in an individual metabolic cage with access to water but not to food. This urine collection represented that of fed rats during the microperfusion experiment. The next morning, another 5-h urine collection was obtained after the overnight fast. This collection corresponded to the experimental period of fasted rats.

Microperfusion studies. Seven fed rats (group 1A) and nine fasted rats (group 1B) were perfused with solution 1 (12 mM tCO_2). As noted above, these fed rats were housed in group cages.

Seven fed rats (group 2A) and seven fasted rats (group 2B) were perfused with solution 2. Solution 3 (group 2C) was perfused in six fasted rats to determine if other constituents such as NH_4, PO_4, etc. could alter the tCO_2 flux.

In two groups of fasted rats, a 1-M solution of either $NaHCO_3$ (group 3A) or NaCl (group 3B) was gavaged before overnight fasting. Eight fasted rats (group 3A) thus were gavaged with ~ 6 ml of 1 M $NaHCO_3$ (2 mM/100 g body weight) and they drank a solution of 15 mM $NaHCO_3$ and 10 mM $KHCO_3$. Six fasted rats (group 3B) were given an equivalent amount of a 1-M NaCl solution and drank a solution containing 15 mM NaCl and 10 mM KCl.

All animals except group 3A were infused with 0.5% body weight (BW) of donor plasma followed by 1% BW/h of isotonic saline. Group 3A rats were infused with 0.5% BW donor plasma followed by a solution containing 120 mM NaCl, 30 mM $NaHCO_3$, and 4 mM KCl at 1% BW/h.

Analytical methods and statistical analyses. In these studies tritiated inulin was dialyzed against distilled water at 4°C for 48 h and aliquots were lyophilized and frozen until use. All other analytical techniques have been recently detailed (5). The statistical analyses used were the *t* test, analysis of variance, and analysis of covariance, where appropriate, and followed the considerations previously outlined (5). In Table II, significance values for the four groups of means were obtained by assessing the *t* values with Bonferroni probabilities.

Results

Whole animal data. Seven rats were followed in balance cages during the phases of feeding, fasting, and refeeding. During fasting, mean 24-h urine pH and body weight fell in every animal (6.66±0.09 vs. 6.26±0.06 and −27±4 g, respectively, $P < 0.01$). Urine collections were made from six other rats (see Methods) after overnight feeding for the 5 h corresponding to those of the microperfusion experiment in fed rats, which were at the end of the overnight fast, and during the subsequent 5 h that represented the time of the experiment in fasted animals. Urine pH and urine bicarbonate excretion rates fell progressively with each of the three collections in every rat ($P < 0.01$).

However, in the rats prepared for microperfusion, as shown in Table II, each animal was not used as its own control so that although weight was significantly lower in fasted rats, bicarbonate excretion rates and urine pH tended to be lower than that of fed rats, but did not reach statistical significance.

Table II shows that animals gavaged with either chloride or

Table II. Blood and Balance Data

Measurements	Fed rats $n = 23$	Fasted rats $n = 22$	Fasted rats ($NaHCO_3$ gavaged) $n = 8$	Fasted rats (NaCl gavaged) $n = 6$
pH	7.43±0.01	7.38±0.01*	7.47±0.01	7.40±0.01‡
P_{CO_2} (*mm Hg*)	44.8±0.7	41.2±1.3*	40.7±1.1	44.7±1.4‡
tCO_2 (*meq/liter*)	30.2±0.5	24.9±0.6*	30.0±0.5	27.2±0.2‡
Na (*meq/liter*)	144±1	146±1*	147±1	146±1
K (*meq/liter*)	4.9±0.1	4.5±0.1*	3.7±0.1	4.0±0.1‡
Cl (*meq/liter*)	104±1	110±1*	102±1	106±1‡
Hematocrit (%)	46.5±0.6	51.8±1.2*	46.1±1.2	45.3±0.6
Protein (*g/dl*)	4.8±0.1	5.0±0.2	4.6±0.3	5.1±0.1
Δ Body weight (*g*)	+12±2	−19±1*	−22±2	−21±1
Urine				
Volume (*ml/16 h*)	15.2±3.1	21.3±2.4	28.6±3.6	28.1±2.3
pH	6.86±0.06	6.83±0.06	7.42±0.05	6.44±0.10‡
HCO_3 (*μeq/16 h*)	374±122	145±24	2,529±211	150±21‡
Na (*μeq/16 h*)	1,690±223	493±54*	5,069±334	5,184±323
K (*μeq/16 h*)	5,022±603	1,316±85*	1,670±82	1,497±94
Cl (*μeq/16 h*)	2,212±238	438±48*	930±62	5,642±280‡

Different by analysis of variance and post *t* testing with Bonferroni significance level > 0.05: *fed vs. fasted rats, ‡$NaHCO_3$-loaded vs. NaCl-loaded rats.

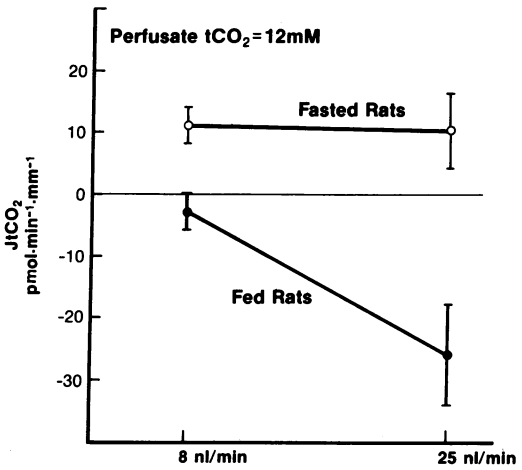

Figure 1. Distal tubule $JtCO_2$ in fed and fasted rats. Values are mean±SEM. Perfusate simulated early distal tubular fluid composition (see Methods) and contained 12 mM tCO_2. Perfusion at 8 and 25 nl/min. See Results for statements of statistical significance.

bicarbonate solutions also had significant weight loss when compared with normally fed animals. Fasted animals show a significant reduction in plasma tCO_2 concentrations and blood pH when compared with fed animals or bicarbonate gavaged animals ($P < 0.01$). Finally, Table II shows that plasma potassium concentration of the bicarbonate gavage group (3A) is significantly lower than that of other groups; despite this, bicarbonate secretion did occur (see below).

Perfusion with 12 mM tCO_2 solution (groups 1A and 1B). Fig. 1 and Table III show microperfusion data from fasted and fed rats perfused with a solution simulating endogenous early distal fluid composition with respect to Na, K, Cl, HCO_3, and osmolality (5). In fasted rats, at normal flow rates, there is a modest but statistically significant $JtCO_2$ of 10±3 pmol·min^{-1}·mm^{-1} ($P < 0.01$ vs. 0), which does not increase at high flow. In fact, at high flow $JtCO_2$ is not different from zero, $P > 0.05$. For fed rats, $JtCO_2$ is not different from zero at normal flow but becomes significantly negative at high flow.

Perfusion with 28 mM tCO_2 solution (groups 2A and 2B). In contrast to the results of perfusion with the 12-mM bicarbonate solution, Fig. 2 shows that at normal flow, perfusion with 28 mM tCO_2 in fasted rats results in significant tCO_2 reabsorption ($JtCO_2$ = 53±6 pmol·min^{-1}·mm^{-1}) and rises briskly to 91±16 pmol·min^{-1}·mm^{-1} at high flow. These values are significantly different from each other and from the corresponding values with the 12-mM tCO_2 perfusate. The results of perfusion with the same solution in fed animals are strikingly different: no significant reabsorption occurs at normal flow whereas brisk secretion occurred at high flow ($JtCO_2$ = −54±13, $P < 0.01$). As noted in Fig. 3, the negative value for $JtCO_2$ at high flow is associated with a significant rise of tubular fluid tCO_2 concentration from 28.3±0.8 to 34.4±0.6 mM ($P < 0.01$). This addition of tCO_2 into the lumen, against a tCO_2 concentration that is higher than that of plasma, suggests active secretion, as we have previously proposed (5).

As Table III shows, JH_2O at high flow in fasted rats (2B) is higher than that of fed rats (2A), which suggests some linkage to the brisk tCO_2 reabsorptive flux in the fasted group. Analysis of covariance of $JtCO_2$ and JH_2O at both flow rates for groups 1A, 1B, 2A, and 2B reveals a highly significant effect ($P < 0.001$) of feeding/fasting on $JtCO_2$, even when the contribution of H_2O movements are taken into account.

Perfusion with other normal tubular fluid constituents (group 2C). Fig. 4 *A* and Table III give the results of our studies designed to test the proposal of Capasso et al. (6) that normal tubular fluid constituents that are not usually present in our perfusates may augment bicarbonate reabsorption. As Fig. 4 *A* shows, at normal and high flow rates the presence in the perfusate of alanine, glucose, acetate, magnesium, calcium, phosphate, and ammonium result in $JtCO_2$ values that were not

Table III. Summary of Microperfusion Data

Protocol	No. tubules/No. rats	Tubular length	Perfusion rate	Perfusate [tCO_2]	Collected [tCO_2]	JH_2O	$JtCO_2$
		mm	*nl/min*	*mM*		*nl/min·mm*	*pmol/min·mm*
1. A. Fed rats	13/7	1.4±0.1	8	12.2±0.6	17.9±0.9	2.1±0.3	−2±3
			25		15.8±0.7	2.8±0.4‡	−26±8‡
B. Fasted rats	14/9	1.4±0.1	8	12.1±0.4	15.9±0.7	2.0±0.2	10±3
			25		14.0±0.5	3.0±0.4‡	11±6
2. A. Fed rats	10/7	1.4±0.1	8	28.3±0.8	36.3±1.1	1.4±0.2	6±4
			25		34.4±0.6	1.8±0.3	−54±13‡
B. Fasted rats	10/7	1.4±0.1	8	28.9±0.9	37.0±0.6	2.5±0.2	53±6
			25		31.7±1.1	4.2±0.4‡	91±16‡
C. Fasted rats*	10/6	1.5±0.1	8	27.2±0.5*	34.8±3.0	2.2±0.2	49±6
			25		29.0±1.2	3.0±0.4	71±20
3. A. Fasted + HCO_3-loaded rats	10/8	1.5±0.1	8	27.2±0.5	37.3±1.0	1.8±0.3	12±8
			25		34.7±1.0	2.8±0.3‡	−27±14‡
B. Fasted + Cl-loaded rats	8/6	1.6±0.1	8	30.2±0.4	34.0±1.8	1.2±0.2	28±5
			25		32.8±0.8	2.1±0.4‡	33±7

* Plus (in millimolars): $PO_4^=$, 4; NH_4^+, 1.5; alanine, 5.0; glucose, 5.0; acetate, 1.5; Ca^{++}, 1.8; Mg^{++}, 1.0; $SO_4^=$, 1.0. ‡ Significant at $P < 0.05$ vs. 8 nl/min by paired *t* testing.

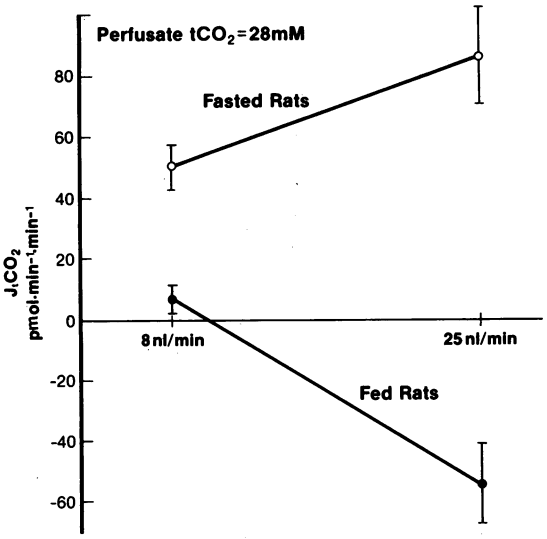

Figure 2. Distal tubule $JtCO_2$ in fed vs. fasted rats. Values are mean±SEM. Perfusate contained 28 mM tCO_2 and was perfused at 8 and 25 nl/min. See Results for statements of statistical significance.

significantly different from those obtained with our more simple perfusates.

Fasted rats gavaged with bicarbonate or chloride solutions (groups 3A and 3B). This series of experiments was undertaken to determine if the difference between fasted and fed rats could be attributed to the effects of ingesting rat chow, which produces weight and acid-base changes when compared with overnight fasted rats (Fig. 1 and Table II). As Fig. 4 *B* and Tables II and III show, bicarbonate gavage and infusion of fasted rats raised plasma [tCO_2], alkalinized the urine (pH = 7.42), completely suppressed $JtCO_2$ at normal flow, and stimulated secretion at high flow. $JtCO_2$ at high flow was -27 ± 14 pmol·min^{-1}·mm^{-1}, a value that was not different from the corresponding fed rat value $JtCO_2 = -54\pm13$ pmol·min^{-1}·mm^{-1} ($P > 0.05$). Thus, bicarbonate gavage and infusion of fasted rats transformed their distal tubule response to those of fed animals. However, in view of the weight loss associated with fasting, and notwithstanding a similar weight loss in the bicarbonate-loaded rats, we were concerned that the cation load of the bicarbonate loading protocol might have contributed to the bicarbonate secretory response. For this reason, six additional rats were studied and given the identical cation loads with chloride, which resulted in $JtCO_2$ values of 28 ± 5 and 33 ± 7 pmol·min^{-1}·mm^{-1} at normal and high flow, respectively. These values (Table III) indicate that suppression of $JtCO_2$ at normal flow, and the negative $JtCO_2$ at high flow after bicarbonate loading, is reasonably attributed to the alkali rather than the cation load.

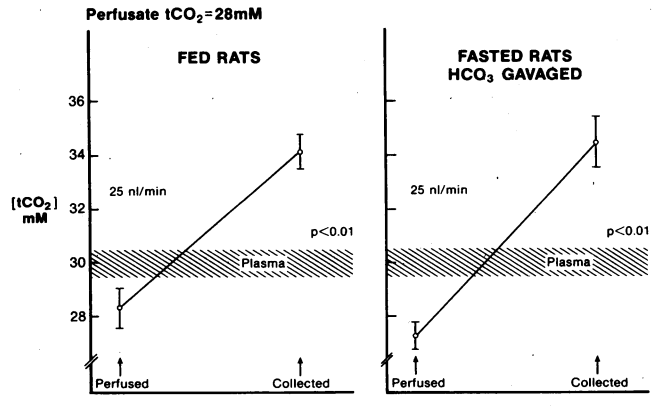

Figure 3. Initial and collected perfusate tCO_2 concentrations from distal tubules of fed and fasted rats loaded with bicarbonate. Values are mean±SEM. Perfusate tCO_2 was 28 mM. Shaded area is range of plasma [tCO_2] expressed as 2× SEM. Perfusion rate was 25 nl/min. Collected tCO_2 concentrations are significantly higher than initial concentrations ($P < 0.01$). Also see text.

Discussion

Our results show that (*a*) microperfused distal tubules from normally fed rats do not reabsorb bicarbonate at normal flow; at high flow, bicarbonate secretion occurs. (*b*) When rats are fasted, as in the free-flow studies of Capasso et al. (6, 7), significant bicarbonate reabsorption occurs with microperfusion at normal flow rates but $JtCO_2$ is not increased by the addition of ammonium, phosphate, calcium, magnesium, alanine, acetate, and glucose to the perfusate. (*c*) In fasted rats, perfused at high flow with a 28-mM tCO_2 solution, $JtCO_2$ strikingly increases when load is in the 500-pmol/min range. However, in fed animals bicarbonate secretion persists. (*d*) Bicarbonate loading of fasted rats results in dramatic suppression of bicarbonate reabsorption at normal flow and reversal of net reabsorption to net secretion of bicarbonate at high flow.

When distal tubules from fasted rats with normal acid-base status are perfused at normal flow, with a 12- and 28-mM tCO_2 perfusate, $JtCO_2$ is in the range of ~ 10 and 50 pmol·min^{-1}·mm^{-1}, respectively. This reabsorptive flux is not influenced by the addition of normal tubular fluid constituents such as calcium, magnesium, phosphate, alanine, acetate, and glucose. However, when fed rats are perfused with the same solutions, little or no bicarbonate is reabsorbed at normal flow, while net secretion occurs at high flow. Our data also indicate that when perfusion tCO_2 concentration is 12 mM, increasing flow rate and load does not augment $JtCO_2$, even though the load is substantially elevated. Indeed, at high flow, $JtCO_2$ is not different from zero. Table III shows that when a 12-mM tCO_2 solution is perfused at 25 nl/min, $JtCO_2$ is only 11 pmol·min^{-1}·mm^{-1} with a load of 300 pmol/min. However, $JtCO_2$ is almost fivefold higher (53 pmol·min^{-1}·mm^{-1}) when a 28-mM CO_2 solution is perfused at 8 nl/min even though the load is lower (224 pmol/min). Our animals subjected to overnight fasting show lower urine pH and consistent weight loss. The lowering of urine pH in response to fasting or, alternatively, the higher urine pH during feeding, suggests the possibility that an alkaline residue diet and its associated higher plasma bicarbonate concentration and urine pH may represent a source of alkali that stimulates distal tubules or cortical collecting tubules to secrete bicarbonate. Very recently, Kunau and Walker (8) reported that feeding rats an acid residue (high protein) diet overnight elicited an increase in distal tubule $JtCO_2$ and a drop in urine pH. We presume this manoeuvre corresponds to fasting rats before micropuncture experiments or to starving rabbits, in which a significant correlation of the $JtCO_2$ of cortical and medullary collecting tubules with urine pH has been reported (9). In referring to our data (3) and those of Lucci et al. (4), Kunau and Walker (8) speculated that our control rats should have urine pH values of ~ 6.9, which would be associated with little distal tubule bicarbonate reabsorption. We believe our data from fed rats (Table II) confirms

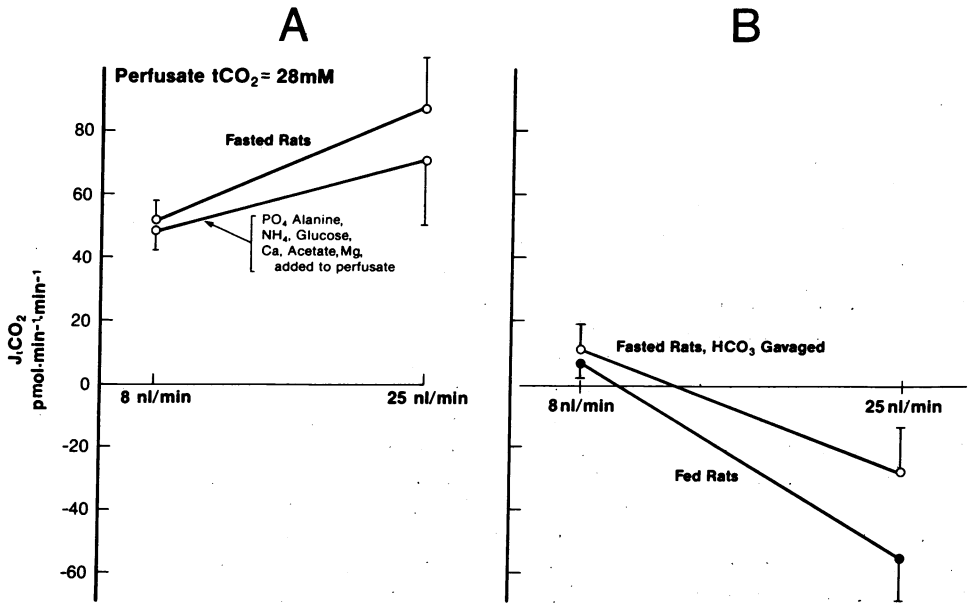

Figure 4. Effects of perfusion with additional constituents (*A*), and of bicarbonate loading of fasted rats (*B*). Values are mean±SEM. (*A*) addition of PO_4, alanine, NH_4, Ca, Mg, glucose, and acetate to 28 mM tCO_2 perfusate. Fasted-rat values are those from Fig. 2. (*B*) Bicarbonate loading of fasted rats perfused with 28 mM tCO_2. Fed-rat values are those from Fig. 2. Also see text.

this suggestion since in both of our previous studies (3, 5) our control rats also had access to the standard protein rat chow, which Table II shows to be associated with urine pH of 6.86. However, as already noted in Results, urine pH from fed and fasted animals may overlap if each animal is not used as its own control. Thus, while the pH of 24-h urine collections fell significantly from baseline in every rat during fasting, the mean pH and bicarbonate excretion rates of our 16-h urine collections of *different* groups of fed and fasted rats (Table II) show a lesser difference. Most striking are the results of bicarbonate loading of fasted rats which leads to frankly alkaline urine (pH = 7.42) and bicarbonate secretion similar to the classic results of McKinney and Burg (2) on in vitro rabbit cortical collecting tubules.

To test the hypothesis that the alkali content of the diet mediated secretion, we undertook bicarbonate loading of fasted rats. As shown in Table II, the bicarbonate gavage protocol, which included drinking a bicarbonate solution overnight and subsequent HCO_3 infusion, resulted in an increase in both plasma [tCO_2] and urine pH of fasted rats, but weight loss persisted. Associated with this was a dramatic reversal of $JtCO_2$ at high flow from net reabsorption to net secretion (Table III). The mean $JtCO_2$ at high flow (-27 ± 14 pmol·min^{-1}·mm^{-1}) was not significantly different from the fed rat value (-54 ± 13 pmol·min^{-1}·mm^{-1}). It is likely extracellular fluid volume changes induced by bicarbonate gavage and infusion did not mediate bicarbonate secretion in view of the results of our chloride gavage protocol (see Results). If we assume that the lumen negative transepithelial potential difference of the distal tubule persists during feeding and alkali loading, then the addition of bicarbonate against a concentration gradient (see Table II, Fig. 3) represents active transport of bicarbonate into the lumen, as we have previously suggested (5).

Three further questions are of interest. What accounts for the flow effects we observed? We speculate that the effects of high tubular flow rate or load on $JtCO_2$ in fasted or fed rats can be explained by the dissipation of limiting concentration gradients. In the fasted rat, we presume that increasing the bicarbonate load threefold results in a higher rate of bicarbonate reabsorption by proton-secreting intercalated cells, concomitant with the attenuation of the uphill hydrogen ion gradient that tends to develop. For the case of fed rats and bicarbonate-secreting cells, high flow would tend to dissipate a limiting alkaline boundary gradient, as we have previously proposed (5). Further, it is possible that high flow would increase chloride delivery and stimulate the chloride/bicarbonate exchanger thought to be located in B-type intercalated cells (10, 11).

Secondly, what cellular events could underlie the bicarbonate reabsorption and secretion observed? There is little doubt that our perfused distal tubules include connecting segments and initial collecting tubules because we often observe branching (12). These portions of the distal tubule are also known to contain intercalated cells (12, 13). It is possible that in response to feeding or alkali loading, B-type intercalated cells are stimulated to secrete bicarbonate (14, 15) or, alternatively, proton secretion by A-type intercalated cells may be suppressed. Clearly, our studies cannot differentiate between the possibility that feeding or alkali gavage may decrease proton secretion by selected intercalated cells rather than enhancing HCO_3 secretion in another group of intercalated cells.

Finally, what is the in vivo significance of our observations? In a recent article, we have already considered the in vivo implications of HCO_3 secretion at high flow (5). However, more relevant to the in vivo state, where very high distal tubular flows are unlikely to be attained, is the suppression of HCO_3 reabsorption at normal flow rates by feeding or alkali loading.

In summary, we demonstrate for the first time that rat distal tubules can secrete bicarbonate in vivo in response to feeding or bicarbonate loading. These findings appear to complement the classic description by McKinney and Burg (2) 10 yr ago of in vitro bicarbonate secretion by rabbit cortical collecting tubules. Further, our data indicate that reported differences between free-flow (6, 7) and microperfusion studies (3, 4, 5) are most likely due to the fact that the free-flow studies involved fasted rats while the microperfusion studies involved normally fed animals.

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