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### Research Article

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# Contribution of Individual Superficial Nephron Segments to Ammonium Handling in Chronic Metabolic Acidosis in the Rat

## Evidence for Ammonia Disequilibrium in the Renal Cortex

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### Abstract

Ammonia entry along surface nephron segments of rats was studied with micropuncture techniques under control and chronic metabolic acidosis conditions. Tubule fluid was collected successively from sites at the end and beginning of the distal tubule and at the end of the proximal tubule of the same nephron. During chronic metabolic acidosis, ammonium excretion doubled. As anticipated, the ammonium concentration ( $TF_{NH_4}$ ) was significantly higher in proximal tubule fluid during acidosis, and ammonium delivery to end proximal sites increased from  $19.4 \pm 2.3$  to  $34.0 \pm 3.2$  pmol/min ( $P < 0.001$ ). Although chronic acidosis did not affect  $TF_{NH_4}$  at the beginning of the distal tubule, ammonium delivery to the end of the distal tubule increased from  $5.72 \pm 0.97$  to  $9.88 \pm 0.97$  pmol/min. In both control and acidotic groups ammonium delivery was lower ( $P < 0.001$ ) to end distal sites than to end proximal sites, indicating net loss in the intervening segment. This loss was greater during chronic metabolic acidosis ( $23.9 \pm 3.3$  vs.  $13.6 \pm 2.0$  pmol/min in controls,  $P < 0.025$ ). In both groups net entry of ammonia, in similar amounts, occurred along the distal tubule ( $P < 0.05$ ). In situ pH averaged  $6.80 \pm 0.05$  at end proximal tubule sites and fell to  $6.54 \pm 0.08$  at the beginning of the distal tubule ( $P < 0.005$ ). Chronic metabolic acidosis did not affect these measurements. The calculated free ammonia at the end of the proximal tubule rose from  $9.3 \pm 2.2$  to  $21 \pm 9$   $\mu$ M ( $P < 0.005$ ) during chronic metabolic acidosis, and was also higher at beginning distal sites during acidosis ( $8.8 \pm 2.4$  vs.  $2.7 \pm 0.7$   $\mu$ M in controls,  $P < 0.05$ ). In both groups ammonia values for the beginning distal tubule fluid were lower than for end proximal tubule fluid. Thus, loss of ammonium in the loop segment is enhanced by chronic metabolic acidosis. Distal entry of ammonia is markedly less than along the proximal tubule and does not change in chronic metabolic acidosis, and ammonia permeabilities for the proximal and distal segments of surface nephrons seem different.

### Introduction

In most mammals, the acid load that results from the metabolism of dietary protein is largely excreted after it has been

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buffered by ammonia and by titratable acid (1, 2). Of these two buffer systems, the ammonia:ammonium pair predominates with regard to both the relative amount excreted and the ability to adapt to acid-base perturbations (1-3). (In these studies, the term "ammonia" will refer to only the nonionic, while "ammonium" will be used to designate the ionic form of this buffer pair.) The proximal tubule is the major site of ammonia entry and synthesis (3-7). Further, most studies indicate that ammonia moves by diffusion into the tubule lumen where it is ionized and subsequently entrapped as ammonium. Based on both indirect (8, 9) and direct evidence (10), it has long been postulated that there is no limitation to the movement of ammonia across cell membranes and that it exists at diffusion equilibrium conditions. However, evidence suggests that this theory may not apply to all structures within the kidney. First, the concentration of ammonia within the medulla is higher than in the cortex (11-13). Since it is thought that the major sites of ammonia production are in the cortical region of the kidney (3-7, 14), the high ammonia content of the medulla is thought to be due its concentration in the interstitium after delivery from the cortex. Recent micropuncture studies showing that ammonium delivery to the end of the proximal tubule is greater than to the end of the distal tubule support this theory (3-5, 15). Robinson and Owen (11) suggested that the medullary concentration of ammonia occurs by countercurrent multiplication. However, inherent to this process is the existence of a permeability difference between those tubular structures leaving and entering the medulla. Second, studies by Hamm et al. (16) indicate that the ammonia permeability of the isolated perfused rabbit cortical collecting tubule is considerably less than that of proximal convoluted tubule. Further, the studies of Nagami and Kurokawa (17) indicate that the preferential entry of ammonia into proximal tubule fluid cannot be entirely explained by the peritubular and luminal differences in pH. Thus, the tubule fluid concentration of the free base, ammonia, may not exist at the diffusion equilibrium conditions in all nephron segments in the cortex. To date no studies have attempted to directly assess this possibility in vivo.

Chronic metabolic acidosis enhances entry of ammonia along the proximal tubule and its loss from the loop segment of superficial nephrons (3, 4). In the studies demonstrating this, fluid was obtained near the end of the proximal and distal tubule. Thus, the exact magnitude of ammonia loss from the loop segment and the contribution of the superficial distal tubule to the process of buffer production and urine acidification could not be completely differentiated in these studies. Nevertheless, since ammonium delivery to the end of the distal tubule was markedly less than to the end of the proximal tubule, it was predicted that if ammonia entry occurred along the distal tubule, it would be small during control conditions (4), although a compensatory increase during

acidosis remained to be assessed. This latter postulate was partially supported by the observations of Lucci et al. (18) who found that hydrogen ion secretion was only detectable in the superficial distal tubule after the induction of a chronic metabolic acidosis.

The present study was designed to evaluate directly net ammonia entry or loss along the superficial proximal and distal tubule under both control conditions and in chronic metabolic acidosis by successively sampling tubule fluid along the same nephron from end distal, beginning distal, and end proximal tubule sites. In this way we were able to compare directly the contribution of each of the intervening nephron segments to ammonium excretion. Further, the measurement of intraluminal pH and ammonium content of tubule fluid near the end of the proximal and the beginning of the distal tubule, allowed us to determine the concentration of the free base ammonia at these micropuncture sites. By comparing these calculated values, we were able to directly test the hypothesis that ammonia is in diffusion equilibrium throughout the renal cortex.

## Methods

Chronic metabolic acidosis was induced by giving ammonium chloride to 17 Munich-Wistar rats. 4 d before micropuncture, 168 mM ammonium chloride in 5.5 mM glucose was substituted for the rats' drinking water, and the use of this solution was continued until the time of the experiment. 3 and 2 d before the micropuncture study, each animal also received 1.25–1.75 ml of 1 M ammonium chloride by gastric tube. 1 d before the experiment they were gavaged with similar amounts of ammonium chloride on three occasions. A control group of 16 rats was given 5.5 mM glucose to drink and was gavaged with water on a schedule similar to that of the acidotic group. Food but not the drinking solutions was withheld in both groups for ~12 h before the study.

On the day of the study, the rats were anesthetized with Inactin (Promonta, Hamburg, Federal Republic of Germany) given intraperitoneally (100 mg/kg body wt). After tracheostomy and intubation with polyethylene tubing, each animal was ventilated on a rodent respirator (Harvard Apparatus Co., S. Natick, MA) for the duration of the study. The internal jugular veins and the right femoral artery were catheterized with polyethylene tubing (P.E. #50). A catheter was placed in the bladder for the collection of timed urine samples. The left kidney was exposed by a mid-line abdominal incision. An inulin prime in 0.6 ml of 0.9% saline was administered intravenously and a solution of normal saline containing inulin in sufficient quantities to maintain plasma levels between 50 and 100 mg/100 ml was infused at the rate of 35  $\mu$ l/min per 100 g body wt through the right jugular vein. The kidney was prepared for surface micropuncture according to methods previously described (19).

The animals' body temperatures were determined with a rectal thermometer and maintained between 36.5 and 38°C. Arterial samples and pressure measurements were obtained at ~60-min intervals or immediately after the collection of tubule fluid. Two timed urine collections were obtained during the interval of the study. Tubule fluid samples were processed and analyzed for inulin content as described previously (19) and ammonium and titratable acid concentrations were determined by coulometric titration (3, 4, 20). In an attempt to characterize the effects of the amino acid content of proximal and distal tubule fluid on the ammonium values obtained with this method, recoveries were performed on solutions containing amino acids at concentrations that approximate those found near the end of the proximal tubule (21, 22).<sup>1</sup> The ratio of the recovered to the actual

1. The amino acid content of the solutions used in these recoveries contained the following amino acids ( $\mu$ M): Ala 34, Arg 6.5, Asn 3.0,

value of ammonium in these samples (range, 2–4 mM,  $n = 20$ ) averaged  $1.09 \pm 0.01$  (SE). Samples containing no ammonium averaged 0.22 mM ( $n = 4$ ). Arterial and urine pH and  $p\text{CO}_2$  were determined with a blood gas microsystem. Plasma and urine samples were analyzed for inulin, ammonium, sodium, potassium, and osmolality with previously described methods (4, 19). Titratable acid content of urine was determined with standard titrametric techniques.

In 11 control and 11 acidotic animals, the end distal, beginning distal, and end of the accessible proximal tubule of the same nephron were identified by puncturing random proximal tubules with a pipette of a small outer diameter (4–5  $\mu$ m) and injecting small amounts of a solution containing 5% lissamine green. Once we identified a nephron in which the distal tubule was accessible to micropuncture, timed fluid collections were obtained successively from end distal, beginning distal, and end proximal tubule sites. The distal segment of nephrons selected for study in almost all cases had at least two convolutions and a 4–5-s difference in transit time between the two sites. (The distal tubule as defined here is a heterogeneous segment comprised of cells similar to the thick ascending limb, the "true" distal convoluted tubule, the connecting tubule, and the initial segment of the cortical collecting duct. It is ~1.5 mm in length [23].) In this manner, one to three nephrons were studied in each animal.

The measurement of ammonium and inulin in timed tubule fluid collections allowed the calculation of absolute and fractional delivery of fluid and ammonium to the sites of collection. Single nephron glomerular filtration rate (SNGFR)<sup>2</sup> and absolute ammonium delivery at the site of micropuncture were calculated in the standard fashion. Distal tubule pairs were accepted for inclusion only if the SNGFR measured at the end distal site differed from the mean of the end proximal and beginning distal SNGFR by <20%. In this manner collections from points past the confluence of two cortical collecting tubules could be excluded.

The filtered load of ammonium ( $\text{FL}_{\text{NH}_4^+}$ ) was calculated as:<sup>3</sup>

$$\text{FL}_{\text{NH}_4^+} = \text{SNGFR} \times \text{P}_{\text{NH}_4^+},$$

using the SNGFR determined at each individual site of micropuncture. Thus, fractional ammonium delivery ( $\text{FD}_{\text{NH}_4^+}$ ) to the site of micropuncture was determined from the following formula:

$$\text{FD}_{\text{NH}_4^+} = (\text{TF}/\text{P}_{\text{NH}_4^+} \times \text{P}/\text{TF}_{\text{in}})100.$$

Absolute entry ( $\text{T}_{\text{NH}_4^+}$ ) along the proximal tubule was calculated as:

$$\text{T}_{\text{NH}_4^+} = (\text{TF}_{\text{NH}_4^+} \times \text{V}_f) - \text{FL}_{\text{NH}_4^+}.$$

The entry or loss of ammonium between end proximal and beginning distal micropuncture sites (loop segment of surface nephrons)<sup>4</sup> or

Cit 3.5, Cys 1.4, Glu 10.9, Gln 28.6, Gly 34.4, His 5.9, HOPro 5.4, Ile 5.6, Leu 13.2, Lys 26, Met 2.6, Orn 9.7, Phe 5.6, Pro 17.1, Ser 14.9, Taur 8.3, Thr 14.3, Tyr 3.5, and Val 13.6

2. *Abbreviations used in this paper:*  $\text{D}_{\text{NH}_4^+}$ , plasma ammonium concentration;  $\text{FD}_{\text{NH}_4^+}$ , fractional ammonium delivery;  $\text{FL}_{\text{NH}_4^+}$ , filtered load of ammonium; SNGFR, single nephron glomerular filtration rate;  $\text{T}_{\text{NH}_4^+}$ , absolute entry of ammonium.

3. Inherent in this calculation is the assumption that the concentration of ammonium in glomerular capillary blood is the same as that of systemic arteries. This may be an underestimate since it is likely that ammonia will diffuse into renal arterioles from the surrounding renal parenchyma and thus the ammonium concentration in glomerular filtrate may be as much as 0.5 mM and 1.5 mM in control and acidotic studies, respectively. There is no actual data on the ammonium content of glomerular filtrate, and, therefore, estimates of this variable are not possible. Nevertheless, if one assumes that the major site of ammonia production is the proximal tubule of surface nephrons (6, 7), then it is this segment that is primarily responsible for the higher renal parenchymal concentration of ammonia and its subsequent entry into renal arterioles; that is, entry of ammonia between the proximal tubule micropuncture site and the renal artery is a consequence of ammonium production in the proximal tubule.

4. The term "loop" segment, in the present text, refers to the heterogeneous segment of surface nephrons that exists between the last

between beginning distal and end distal sites was determined as the difference in absolute ammonium delivery between sites. Fractional entry along the proximal tubule was determined as:

$$T_{\text{NH}_4^+}/FL_{\text{NH}_4^+}$$

Fractional entry or loss along the loop segment or distal tubule was the difference in  $FD_{\text{NH}_4^+}$  between sites.

In an additional five control and six acidotic animals, the renal capsule was carefully dissected free from the surface to facilitate tubule puncture with pH microelectrodes. End proximal and beginning distal tubule segments were identified as in the previous series of studies and fluid was collected for the determination of ammonium only. Either before or after this collection, *in situ* pH was measured. For these studies double-barreled pipettes consisting of a glass membrane pH electrode and a reference electrode were used. They were manufactured and tested with methods previously described (24). The measurement of tubule fluid pH and ammonium content at the same tubule site allowed calculation of the tubule fluid ammonia concentration as previously described (25). In these calculations the pK was corrected for the ionic strength of tubule fluid (26). For the proximal tubule a value of 9.04 was used, based on the assumption that the tubule fluid sodium and potassium concentrations were 146 and 4.6 mM, respectively. For the beginning of the distal tubule we used a value of 9.02, which was calculated from concentrations of 50 mM for sodium and 2.3 mM for potassium. The values used to calculate the ionic strength were obtained from control animals studied in this laboratory under conditions that are similar to those described in the present work (27).<sup>5</sup>

Individual values of micropuncture samples were used for statistical analysis. The Student's *t* test for unpaired data was used to examine individual differences in nephron and whole kidney function when comparing groups of rats. The *t* test for paired data was used when comparing values obtained from the different sites along the same nephron. Where indicated, samples were compared statistically after logarithmic transformation (28).

## Results

Since the results from the two series of studies did not differ significantly, they were combined before statistical analysis. Table I presents the base-line information in the control and chronically acidotic rats and is consistent with results obtained in the past in our laboratory (3, 4). As expected, acid loading produced a marked reduction in plasma bicarbonate and arterial pH while the arterial  $p\text{CO}_2$  fell only slightly. Plasma ammonium levels were significantly elevated in the acidotic rats.

The mean glomerular filtration rates (see Table II) of the two groups were not significantly different. However, urine flow rate and thus fractional fluid excretion were higher in the experimental group. Net acid excretion in the acidotic rats nearly doubled ( $2.73 \pm 0.13$  vs.  $1.43 \pm 0.08$   $\mu\text{eq}/\text{min}$  in controls,  $P < 0.001$ ) due to a twofold increase in ammonium excretion. Titratable acid excretion did not increase during chronic acidosis. Urine pH did not differ in the two groups.

*Nephron studies.* Table III presents the data for SNGFR

portion of the proximal and the earliest part of the distal tubule accessible to study with the technique of micropuncture. It includes not only the loop of Henle but also a portion of the *pars convoluta*, and all of the *pars recta* of the proximal tubule.

5. There are no comparable data in the literature for chronic acidotic conditions. If the sodium and potassium concentrations were higher in distal tubule fluid during chronic acidosis, then the pK of ammonia would be slightly higher than in control conditions. It would approach that of the proximal tubule (which would not change in this setting). At the most, such a change would affect the calculated value for ammonia by  $<5\%$  and would reduce the difference between distal and proximal tubule fluid ammonia.

measured at the three sites of micropuncture in the two groups of rats. There was no significant difference in SNGFR between the control and acidotic rats at any site. However, in both groups the SNGFR measured at distal sites tended to be lower than that measured at end proximal sites.

As shown in Fig. 1, in both groups there was a progressive reduction in tubule fluid flow rates from end proximal to end distal micropuncture sites, indicating significant fluid abstraction in the intervening segments. Acidosis had a significant effect on fluid reabsorption along the nephron. This was most evident at distal tubule collection sites, where there was a striking increase in the fractional and absolute delivery of fluid. This effect seemed to relate to the altered handling of water in more proximal segments of the nephron, since there was no significant difference in fluid reabsorption along the distal tubule between chronic acidosis ( $2.71 \pm 0.25$  nl/min) and control conditions ( $2.36 \pm 0.21$  nl/min). In the proximal tubule, absolute fluid reabsorption fell from  $8.66 \pm 0.89$  in controls to  $5.14 \pm 0.64$  nl/min ( $P < 0.005$ ) in chronic acidosis and fractional reabsorption was decreased from  $43.2 \pm 2.7$  to  $31.7 \pm 2.0\%$  ( $P < 0.005$ ). The amount of fluid reabsorbed between the end proximal and beginning distal sites was similar in controls ( $6.57 \pm 0.50$  nl/min) and in animals with chronic acidosis ( $4.93 \pm 0.50$  nl/min).

Table IV presents mean ammonium data obtained in controls and after the induction of a chronic metabolic acidosis. Under control conditions there was a decline in tubule fluid ammonium concentration between the end proximal and early distal sites from  $1.74 \pm 0.18$  to  $1.19 \pm 0.15$  mM ( $P < 0.005$ ). The ammonium concentration then rose to  $4.04 \pm 0.33$  mM ( $P < 0.001$ ) at the end of the distal tubule. Fig. 2 shows individual values for absolute (*left*) and fractional (*right*) ammonium delivery to the site of micropuncture. At the end proximal tubule the amount of ammonium delivered exceeded the filtered load, reflecting a net entry of ammonia proximal to the site of micropuncture ( $17.1 \pm 2.3$  pmol/min). Fractional delivery of ammonium to the end of the proximal tubule averaged  $901 \pm 113\%$ , similar to the fraction of ammonium found in the urine ( $799 \pm 75\%$ ).

In most tubules examined, absolute and fractional ammonium delivery declined between the end of the proximal and beginning of the distal tubule (see Fig. 2). Net loss of ammonium along the loop segment of surface nephrons averaged  $13.6 \pm 2.0$  pmol/min or  $610 \pm 122\%$  of the filtered ammonium (Fig. 3). Thus, only about one-third of the ammonium that was present at the end of the proximal tubule remained near the beginning of the distal tubule. Loss of ammonium in the loop segment was positively correlated with delivery. This relationship is shown in Fig. 4.

There was a small but consistent increase in ammonium delivery between the beginning and end of the distal tubule. This small increase, from  $5.72 \pm 0.84$  to  $7.40 \pm 0.71$  pmol/min, was due to a net entry of  $1.74 \pm 0.70$  pmol/min (see Fig. 3). Also, fractional ammonium delivery to the end distal site increased by  $185 \pm 50\%$  of the filtered load. Despite this increase, delivery to the end of the distal tubule was markedly less than that found near the end of the proximal tubule ( $P < 0.005$ ). As we have shown in the past (3, 4), in the present study there was evidence of significant entry of ammonium beyond the distal tubule; that is, the fraction of the filtered ammonium found in the urine was  $349 \pm 91\%$  more ( $P < 0.005$ ) than that delivered to end distal micropuncture sites (see Tables II and IV).

Table I. Weight, Blood Pressure, and Blood Parameters in Control and Acidotic Rats

	Body weights			Kidney weights		Blood pressure	Blood parameters				
	Initial	Final	$\Delta$ wt/d	Left	Right		Hct	pH	pCO <sub>2</sub>	HCO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>
	g	g	g	mg	mg		%		mmHg	mM	mM
Control rats (n = 16)	106±1	118±2	4.3±0.4	544±21	552±17	104±2	45±1	7.37±0.01	36.7±0.7	20.9±0.4	0.113±0.007
Acidotic rats (n = 17)	111±1	114±2	1.1±0.3	589±10	629±12	103±3	45±1	7.23±0.01	33.5±1.2	13.5±0.6	0.137±0.007
P	NS	NS	<0.001	NS	<0.001	NS	NS	<0.001	<0.05	<0.001	<0.02

Values are mean±SE. n, number of rats studied in each group;  $\Delta$ wt/d, average weight gain per day during the interval that the rats were fed either 5.5 mM glucose alone or 168 mM ammonium chloride. P, level of significance when the two groups are compared.

The induction of chronic metabolic acidosis significantly affected ammonium handling in all the nephron segments studied (see Table IV). The concentration of ammonium at all three sampling sites was greater in acidosis than in controls. As in the control group, a decline in ammonium concentration occurred in the acidotic group between the end of the proximal and the beginning of the distal tubule, from 3.31±0.25 to 2.04±0.19 mM, followed by a significant rise to 4.97±0.44 mM at the end distal tubule. Chronic acidosis significantly increased delivery of ammonium to the end proximal tubule, due to enhanced entry along this segment, since there was no difference in filtered load (Fig. 3, Table IV). As in controls, fractional delivery to the end of the proximal tubule and to the final urine were not significantly different in the acidotic rats.

In chronic acidosis, ammonium delivery to the beginning distal was less than to end proximal sites (Fig. 2). Net loss of ammonium in the loop segment averaged 23.9±3.3 pmol/min. This was nearly double that seen under control conditions (P < 0.025). Nevertheless, chronic acidosis did not seem to alter the relationship between ammonium delivery to and loss from the loop segment of surface nephrons (see Fig. 4). That is, the greater loss from the loop segment in acidosis was proportional to the increase in ammonium delivery to the end of the proximal tubule.

In acidosis, delivery of ammonium to the distal tubule was higher than in controls. Nevertheless, there was a small but consistent increase in ammonium delivery between the beginning and end of the distal tubule (Fig. 2). In absolute terms, ammonium delivery to the end distal micropuncture site

averaged 12.3±0.9 pmol/min and reflected a net entry of 2.38±1.04 pmol/min (Fig. 3) along this segment.

Fractional delivery to the end of the distal tubule also increased, averaging 726±72% of the filtered ammonium, indicating that fractional delivery increased by 181±62% of the filtered load between these two sites. Though absolute and fractional ammonium delivery to the end distal site was significantly higher during acidosis than under control conditions, entry of ammonium along the distal tubule was not different. As in controls, fractional delivery of ammonium to the end distal tubule was significantly less (P < 0.001) than delivery to the final urine (Tables II and IV). Less than one-half of the ammonium present in the final urine is accounted for by that found at the end distal site. Further, the percentage of the filtered ammonium that entered beyond the superficial distal tubule (836±107%) was significantly greater than that seen under control conditions (P < 0.025).

Individual in situ pH measurements performed at end proximal and beginning distal sites are shown in Fig. 5 and mean values are presented in Table V. As anticipated, the pH measured near the end of the proximal tubule was higher than at beginning distal sites. Acidosis had no significant effect on in situ pH measurements obtained at these sites. The individual calculated values for the ammonia content of fluid obtained near the end of the proximal and beginning of the distal tubule of the same nephron are depicted in Fig. 6. Mean values are shown in Table V. In controls, the average value for proximal tubule fluid was significantly higher than that determined for beginning distal tubule fluid. Further, although acidosis increased the ammonia concentration more than twofold at both

Table II. Whole Kidney Function in Control and Acidotic Rats

	V	GFR	V/GFR	U <sub>pH</sub>	U <sub>TA</sub> V	U <sub>NH<sub>4</sub><sup>+</sup></sub> V	U <sub>N.A.</sub> V	FE <sub>NH<sub>4</sub><sup>+</sup></sub>
	$\mu$ l/min	$\mu$ l/min	%		$\mu$ eq/min	$\mu$ mol/min	$\mu$ eq/min	%
Control rats (n = 16)	3.79±0.21	1,251±61	0.32±0.02	5.56±0.06	0.403±0.023	1.05±0.06	1.43±0.08	799±75
Acidotic rats (n = 17)	6.53±0.32	1,131±46	0.60±0.04	5.53±0.04	0.394±0.022	2.39±0.11	2.73±0.13	1,563±98
P	<0.001	NS	<0.001	NS	NS	<0.001	<0.001	<0.001

Values are mean±SE. V, urine flow rate; GFR, glomerular filtration rate; V/GFR, fraction of filtered water excreted; U<sub>pH</sub>, urine pH; U<sub>x</sub>V, absolute excretion of titratable acid (TA), ammonium (NH<sub>4</sub><sup>+</sup>), and net acid (N.A.); FE<sub>NH<sub>4</sub><sup>+</sup></sub>, fractional excretion of NH<sub>4</sub><sup>+</sup>.

Table III. Single Nephron Glomerular Filtration Rate in Control and Acidotic Rats

	End proximal	Beginning distal	P*	End distal	P
Control rats	18.9±1.2	17.4±1.3	NS	15.3±1.1	<0.001
n	21	21		20	
Acidotic rats	15.7±1.0	14.3±1.0	NS	13.1±1.1	NS
n	19	20		17	
P	NS	NS		NS	

Values are the mean±SE in nl/min.

\* P, level of significance for the difference between the two preceding sites of collection; n, number of individual measurements.

sites, values measured at proximal tubule micropuncture sites remained significantly higher than at beginning distal tubule sites. Thus, in both experimental settings the ammonia content of fluid near the beginning of the distal tubule is not in equilibrium with that near the end of the proximal tubule.

## Discussion

As in previous studies (3–5), in the present work the induction of a moderate metabolic acidosis in rats by chronic ammonium chloride loading resulted in a nearly twofold increase in the renal production and excretion of ammonium. The present work confirms these earlier studies in that significant ammonia entry was evident by end proximal micropuncture sites. The fractional delivery of ammonium to the end of the proximal tubule exceeded the filtered load by nine times in control, and 17 times in acidotic animals (Table IV). Further, there was no significant difference between the fractional ammonium delivery to this site and to the final urine. Thus, these results are in accord with the view that the proximal tubule is a major site of ammonia production under control conditions and after the administration of a chronic acid load (3–7).

The results presented here are important because they clarify the role of other nephron segments in the adaptive increase in ammonium excretion seen during chronic acidosis. Ammonium handling by the loop segment was studied exclusive of the modifying effects of the distal tubule; that is, in earlier

works (3–5), delivery of ammonium to the end proximal micropuncture site was compared with that at the end of the distal tubule. The data presented here indicate that fractional and absolute ammonium delivery to the distal segment was always less than to the end of the proximal tubule. In controls, about one-third of the ammonium delivered to the end of the proximal tubule was present in the filtrate that reached the distal tubule. In chronic metabolic acidosis, the absolute and fractional loss of ammonium from the loop segment was enhanced. However, this increase was proportional to the increase in ammonium delivered to this segment (see Fig. 4). Although the present studies do not assess directly the mechanisms by which ammonia loss from the loop segment occurs or is enhanced by acidosis, there are several factors that seem to contribute to these phenomena. As postulated previously (1–4), part of this loss is probably due to an increase in the pH of tubule fluid as it enters the medulla, resulting in the dissociation of ammonium to ammonia and hydrogen ion. Free ammonia would then diffuse out of the tubule lumen and come into equilibrium with the papillary interstitium to be ultimately reentrapped in the acidic fluid of the collecting duct. This process is likely to be enhanced by the augmentation in collecting duct acidification known to occur after chronic acid loading (3–5, 29). It is possible that part of the loss of ammonium from the loop segment may be due to its reabsorption through a mechanism other than simple diffusion of ammonia. Data supporting such a mechanism in the thick ascending limb of Henle's loop has recently been reported (30). Finally, chronic metabolic acidosis may affect the hemodynamic patterns of cortical and medullary blood flow. To date no information is available on this possibility, although Roy et al. (31) have presented preliminary data suggesting that medullary blood flow is increased in acute respiratory acidosis. Whether similar events occur in chronic metabolic acidosis is unknown at this time. The present work indicates that the amount of ammonium lost from the loop segment is load-dependent (see Fig. 4). However, this finding does not lend particular support to any of the proposed mechanisms for enhanced ammonium loss in the loop of Henle. Proportional increases in either ammonium reabsorption in the thick ascending limb or in collecting duct hydrogen ion secretion may underlie the unaltered load-dependent reabsorption of ammonium in this segment during chronic acidosis.

The present study shows that there is a small but consistent increase in absolute and fractional ammonium entry along the distal tubule. Under control conditions the absolute and fractional entry of ammonia averaged 1.74 pmol/min and 180% of the filtered load, respectively. This was less than a fifth of the entry before the end of the proximal tubule of the same nephron and, as a consequence, delivery of ammonium to the end of the distal tubule remained significantly less than to the end of the proximal tubule (Figs. 2, 3). Thus, the distal tubule of superficial nephrons does not have a major role in the adaptive response to a chronic acid load.

Although the mechanisms that govern the movement of ammonia into the distal tubule cannot be completely elucidated from our findings, several factors are readily apparent. First, the secretion of ammonia into the distal segment was not influenced by the induction of a chronic metabolic acidosis. Second, there was no evidence that distal ammonia entry was affected by the differences in the rate of flow found in the present study. Delivery of tubule fluid to the distal tubule increased by more than 25% during chronic acidosis, and

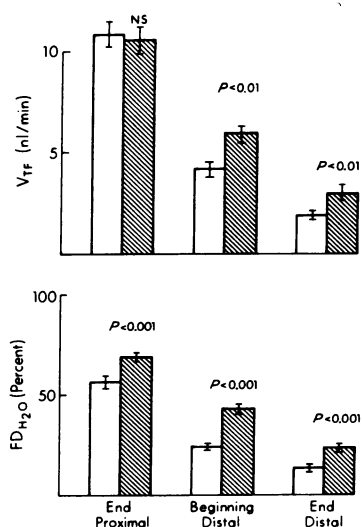


Figure 1. Mean values±SE for the absolute ( $V_{TF}$ , top) and fractional delivery ( $FD_{H_2O}$ , bottom) of tubule fluid to the collection sites under control conditions (□) and after the induction of a chronic metabolic acidosis (▨).

Table IV. Effect of Metabolic Acidosis on Nephron Handling of Ammonium

	End proximal	Beginning distal	<i>P</i> *	End distal	<i>P</i> *
<b>TF<sub>NH<sub>4</sub><sup>+</sup></sub></b> (mM)					
Control rats	1.74±0.18	1.19±0.15 (26)	<0.005	4.04±0.33 (19)	<0.001
Acidotic rats	3.31±0.25	2.04±0.19 (26)	<0.001	4.97±0.44 (23)	<0.001
<i>P</i>	<0.001	<0.005		NS	
<b>FL<sub>NH<sub>4</sub><sup>+</sup></sub></b> (pmol/min)					
Control rats	2.21±0.18	1.99±0.21	NS	1.74±0.15	<0.025
Acidotic rats	2.10±0.13	1.88±0.11	<0.05	1.82±0.13	NS
<i>P</i>	NS	NS		NS	
<b>TF<sub>NH<sub>4</sub><sup>+</sup></sub></b> V <sub>TF</sub> (pmol/min)					
Control rats	19.4±2.3	5.72±0.84	<0.001	7.40±0.71	<0.05
Acidotic rats	34.0±3.2	9.88±0.97	<0.001	12.3±0.9	<0.025
<i>P</i>	<0.001	<0.005		<0.001	
<b>FD<sub>NH<sub>4</sub><sup>+</sup></sub></b> (percent)					
Control rats	901±113	288±47	<0.001	472±55	<0.005
Acidotic rats	1,697±152	544±218	<0.001	726±72	<0.01
<i>P</i>	<0.001	<0.005		<0.005	

Values are mean±SE. TF, tubule fluid concentration; FL, filtered load; V<sub>TF</sub>, tubule fluid flow rate; FD, fractional delivery to the site of micropuncture. \* *P*, level of significance for the difference between the two preceding values. In parentheses is the number of individual pairs of values.

despite this, ammonia entry along this segment did not change. Thus, when distal ammonium entry is expressed as a function of flow rate, it was lower during chronic acidosis than under control conditions. Third, ammonia entry in both conditions must be partially a consequence of diffusion along its concentration gradient; that is, the ammonia concentration difference between proximal tubule fluid and that of the beginning distal tubule (see below) would favor its entry along the distal tubule segment. Fourth, while the pH gradient between the tubule lumen and the blood near the beginning of the distal tubule would favor the diffusion of ammonia into tubule fluid, this

cannot be the sole mechanism underlying its entry along this segment. In control conditions 55% of the tubule fluid delivered to the distal segment is reabsorbed. If proton secretion and buffer entry did not occur along this segment, then free hydrogen ion concentration would increase by 354 nM and tubule fluid pH would fall to 6.19 by the end of the distal tubule. If the pH along the distal tubule does not decline (15, 32) and this were due only to protonation of ammonia as it enters the distal tubule, then the increase in ammonium delivery between beginning and end distal micropuncture sites would increase by only 0.67 fmol/min. Entry along this

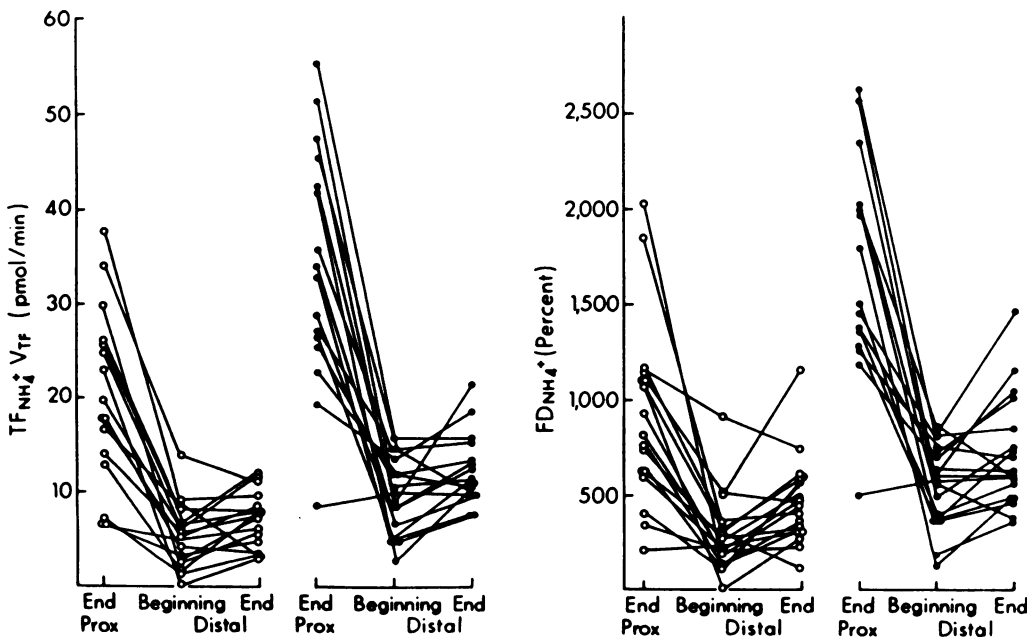


Figure 2. Left, individual measurements for the absolute delivery of ammonium (TF<sub>NH<sub>4</sub><sup>+</sup> + V<sub>TF</sub>) to the three sites of micropuncture under control (○) and chronic acidosis conditions (●). Right, individual values for fractional ammonium delivery (FD<sub>NH<sub>4</sub><sup>+</sup>) to the same three sites.</sub></sub>

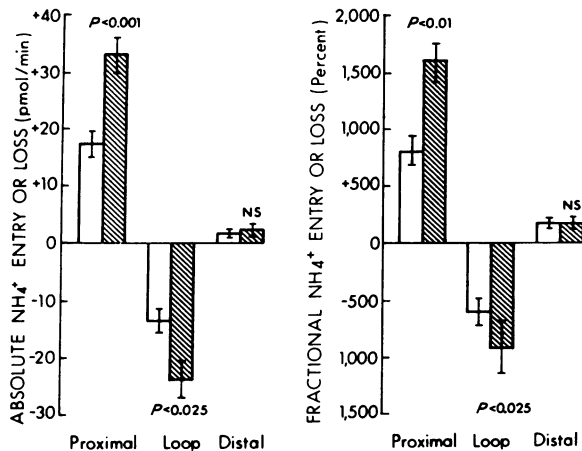


Figure 3. Absolute (left) and fractional (right) entry/loss of ammonium (mean±SE) before the end of the proximal tubule and between the different sites of micropuncture during control conditions (□) and after the induction of a chronic metabolic acidosis (▨).

segment was greater by more than 1,000-fold. Thus, although small in magnitude, hydrogen secretion must occur in the distal tubule during control conditions and after the induction of a chronic acidosis. These findings contrast with those of Lucci et al. (18) and DuBose et al. (32), who used bicarbonate reabsorption as an index of hydrogen ion secretion. The present studies suggest that ammonia rather than bicarbonate is the major proton acceptor in the distal segment of surface nephrons. Thus, titration of ammonia in the superficial distal segment seems to be a more sensitive indicator of hydrogen ion secretion.

Measurement of in situ pH at end proximal and beginning distal sites indicates that in both control and acidotic conditions, there is a fall in pH between the two sites. Acidosis had no effect on these measurements. Similar results were reported

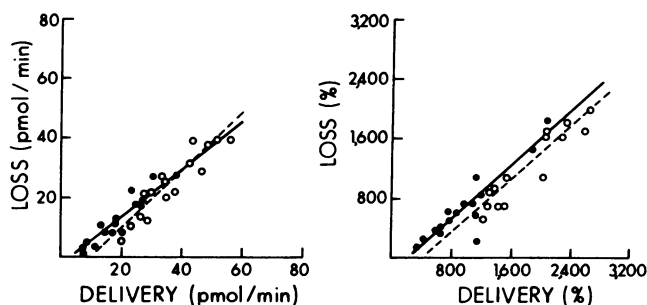


Figure 4. Left, relation between the absolute delivery of ammonium to the end of the proximal tubule and its loss from the loop segment of surface nephrons during control conditions (○) and chronic acidosis (●). For controls the slope of the line is defined by the equation  $y = 0.81x - 2.2$  ( $r = 0.92$ ,  $P < 0.01$ ). In chronic acidosis the slope of the line is defined by the equation  $y = 0.98x - 9.7$  ( $r = 0.93$ ,  $P < 0.01$ ), which was not statistically different from the control value. In the right panel, this relationship is depicted in terms of the filtered load of ammonium. Again, the slopes of the lines derived for the two groups of rats were statistically the same. The line was defined by the equation  $y = 0.92x - 205$  ( $r = 0.91$ ,  $P < 0.01$ ) in controls and by  $y = 0.92x - 395$  ( $r = 0.92$ ,  $P < 0.01$ ) in chronically acidotic rats.

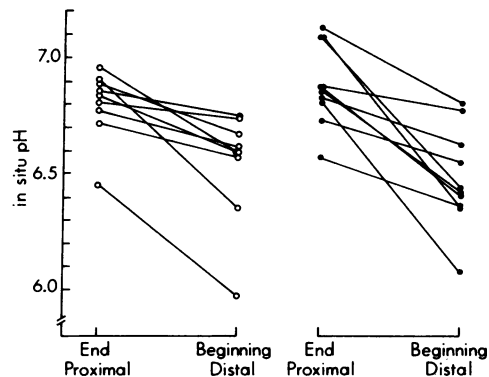


Figure 5. Individual in situ pH measurements made near the end of the proximal and beginning of the tubule in control (○) and acidotic (●) rats.

previously from this laboratory: in situ pH did not change at end proximal and end distal sites after chronic acid loading (3). Taken together, the results of these two studies indicate that the pH profile along the superficial distal tubule is not altered by chronic acid loading. Despite the lower pH at beginning distal sites, the ammonium concentration in both control and in acidosis conditions was markedly lower here than at the end of the proximal tubule. As a consequence, the calculated value for free ammonia was lower in this early segment than at the end of the proximal tubule in both conditions.

The finding that the ammonia content of fluid obtained near the beginning of the distal tubule is lower than that of the proximal tubule has not been reported previously and suggests that the permeabilities of the nephron segments proximal to these two sites of micropuncture are different. These results are in contrast to previous studies (8–10), which suggested that ammonia was in diffusion equilibrium throughout the cortex. Indeed, Oelert et al. (10) reported that the ammonia concentrations in renal venous blood, proximal tubule, and distal tubule fluid were not measurably different. The basis for the apparent difference in the results obtained in the present study and that of Oelert et al. (10) may be in the methodology employed in the two efforts. First, tubule fluid samples were obtained from different segments of the same nephron in the

Table V. Mean Values of In Situ pH and the Ammonia Content of End Proximal and Beginning Distal Tubule Fluid during Control and Acidotic Conditions

	End proximal	Beginning distal	P
TF <sub>pH</sub>			
Control rats	6.80±0.05	6.54±0.08	<0.005
Acidotic rats	6.88±0.03	6.48±0.07	<0.001
P	NS	NS	
TF <sub>NH<sub>3</sub></sub> (μM)			
Control rats	9.28±2.19	2.66±0.68	<0.01
Acidotic rats	21.0±8.5	8.81±2.39	<0.005
P	<0.005	<0.05	

Values are mean±SE.



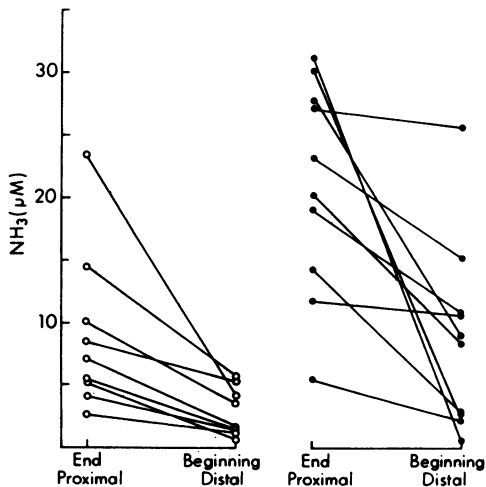


Figure 6. Individual calculations for the  $\text{NH}_3$  content of fluid obtained near the end of the proximal and beginning of the distal tubule in control (○) and chronic metabolic acidosis conditions (●).

present study. This results in a very sensitive method of assessing alterations in the pH and ammonium content of tubule fluid as it moves past the end of the proximal tubule and along the distal segment. In the study of Oelert et al. (10), tubule fluid was collected and pH measurements were determined along proximal and distal tubules of different nephrons. Further, distal tubule fluid was obtained without regard for location. The results presented here indicate that the ammonium content of fluid rises as it traverses the distal tubule, which is compatible with the premise that this segment has a high permeability for ammonia. As a consequence, it seems likely that the ammonia content of distal tubule fluid at the end of this segment approaches or is not different from that of the proximal tubule. Thus, any differences in the ammonia content of fluid obtained near the end of the proximal and the beginning of the distal tubule could easily be masked by inclusion of samples obtained beyond this initial segment. Similarly, given the variability in pH values obtained at end proximal and distal micropuncture sites, it would be more difficult to discern differences in tubule fluid pH at these two sites if one compared measurements obtained randomly from different nephrons instead of those obtained at specific sites along the same nephron. It is therefore not surprising that Oelert et al. (10) found similar tubule fluid pH and ammonia values at proximal and distal micropuncture sites. Another difference in the two studies is that the determinants for estimating the free ammonia concentration (tubule fluid pH, and total ammonium concentration) were measured by different techniques. The pH of tubule fluid in the study of Oelert et al. (10) was measured with quinhydrone electrodes *ex vivo* after equilibration with 5%  $\text{CO}_2$ . The accuracy of results obtained with this method will be adversely affected by the different bicarbonate concentrations of proximal and distal tubule fluid, and by the fact that  $\text{pCO}_2$  of the gas mixture chosen for equilibration is lower than that of renal cortical tissue under eucapnic conditions and which may change with alterations in the acid-base status of the animal being studied (33). In the present study these variables had no effect since

pH was measured *in situ* with double-barreled glass pH sensitive electrodes. The second determinant, tubule fluid ammonium concentration, was assayed by a colorimetric method based on the Berthelot reaction in the work of Oelert et al. (10) and with a titrametric technique (20) in the present study. As in other early studies (34, 35) that used a micromethod based on the Berthelot reaction, the proximal tubule fluid ammonium concentrations were quite low. In this (10) and the other early studies (34, 35), it was below 1 mM and was frequently undetectable under eucapnic conditions. It seems likely that these lower values are partially a consequence of ammonia loss from the tubule fluid samples. That is, if tubule fluid samples are allowed to stand longer than an hour they will become alkaline due to loss of  $\text{CO}_2$  (unpublished observations) and as a consequence, ammonium will dissociate to ammonia, which will then escape from the sample. On the other hand, it does not seem likely that the higher values found with the titrametric technique, which are reported here and in recent studies (3, 4, 15, 25, 36), are due to the amino acid content of tubule fluid. Wilcox et al. (15) have performed ammonium recoveries in tubule fluid using this method and have shown that the method does not overestimate ammonium in tubule fluid. Nevertheless, in an effort to specifically address this issue we performed ammonium measurements in the presence of amino acids in amounts similar to those thought to exist near the end of the proximal tubule (21, 22) and found that within the range of values obtained in the present study these substances contributed to the measured values by <10%. Further, since the amino acid content of fluid does not change between the end of the proximal and the beginning of the distal tubule (21, 22), any error introduced by amino acid interference would tend to diminish the difference in the apparent concentration of ammonium in proximal and distal tubule fluid.

The results presented here represent the first *in vivo* demonstration that not all structures of the cortex are in diffusion equilibrium for ammonia. This implies that the tubule segments immediately preceding the beginning of the distal tubule have an ammonia permeability that is relatively low. In contrast it seems likely that the proximal tubule has a relatively high ammonia permeability and its tubule fluid ammonia is in diffusion equilibrium with that of the cortex, while that of the beginning distal tubule fluid is not. This postulate is supported by several observations. First, the proximal tubule is the major site of ammonia production (6, 7, 14) in normal acid-base and acidosis conditions. Second, Hamm et al. (16) have shown in the isolated rabbit proximal tubule that the permeability of this segment is quite high, and as a result, diffusion equilibrium for ammonia was present at perfusion rates that greatly exceeded those found *in vivo*. Therefore, it is not unreasonable to assume that the highest concentration of free ammonia would exist within the proximal tubule and in its immediate environment. Further, since the renal cortex is comprised primarily of these tubule segments (37), the prevailing cortical free ammonia concentration (1, 8) should approximate that of proximal tubule fluid. Given this information, it seems likely that the free ammonia content of fluid in segments before the beginning of the distal tubule is not in equilibrium with the surrounding interstitium.

The reason why the concentration of the ammonia:ammonium buffer pair is low in fluid collected near the beginning of the distal tubule remains conjectural. There is no evidence that tubule fluid in the ascending limb is in contact with an interstitial fluid having an ammonia concentration lower than that of cortical tissue. In fact, the available evidence suggests that there is a progressive increase in ammonia from cortex to medulla (11, 12). Thus, simple diffusion of ammonia from the tubule lumen cannot be the explanation for the lower value found near the beginning of the distal tubule. Instead, not only must this segment demonstrate a finite permeability to ammonia but there must also exist a mechanism by which this buffer pair is removed from it. Evidence suggests that acidification continues in the thick ascending limb of Henle and that it is accompanied by ammonium reabsorption (30). Data now available are not sufficient to determine the relative importance of ammonia diffusion vs. ionic transport of ammonium in the countercurrent multiplication of this buffer pair in the medullary interstitium.

In summary, the proximal tubule is a major site of ammonia entry that is enhanced by chronic metabolic acidosis. Ammonia loss from the loop segment has been shown directly and likewise, is increased by metabolic acidosis. Ammonia is not in equilibrium throughout all structures of the cortex, being lower in the beginning distal than in end proximal tubule fluid, and this implies that the segments before the beginning distal tubule have a relatively low ammonia permeability. This is consistent with the requirements for the countercurrent multiplication of this buffer pair.

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