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ABSTRACT Papillary and surface micropuncture was used to assess the effects of a chronic metabolic acidosis on the renal tubular handling of ammonium by surface nephrons, juxtamedullary nephrons, and the terminal segment of collecting duct. Rats chronically fed ammonium chloride had an expected decline in arterial pH and bicarbonate concentration associated with a doubling in the amount of ammonium excreted and a decline in urine pH. The glomerular filtration rate and absolute delivery of water and sodium to micropuncture sites of surface and deep nephrons was not measurably altered. Ammonium delivery to the end of the proximal tubule increased from 853±102% to 1,197±142% (SE) of the filtered load of ammonium after the induction of metabolic acidosis. This increase was due to a rise in tubular fluid ammonium content from 2.31±0.23 to 4.06±0.28 mM/liter. After the induction of acidosis, absolute and fractional delivery of ammonium ion to the end of the distal tubule was less than to the end of the accessible portion of the proximal tubule. These findings indicate that ammonium is lost in the intervening segment.

Ammonium handling by deep nephrons was profoundly affected by acid loading. Absolute delivery to the bend of the loop of Henle increased twofold while fractional delivery rose from 1,222±108% to 1,780±132% of the filtered ammonium. This was due to a marked increase in ammonia entry. During acidosis, ammonium delivery to the terminal segment of the collecting duct was doubled (709±137% in controls vs. 1,415±150% in acidosis, $P < 0.005$) but did not change between proximal and tip collecting duct sites. In both groups of animals delivery of ammonium to the terminal segment of the collecting duct was greater than to end distal tubular micropuncture sites suggesting that ammonia entry occurred between these two sites. The differences in delivery was greater after the induction of a metabolic acidosis (887±140% vs. 384±144%, $P < 0.05$). Thus, the present study indicates that deep nephrons contribute to the adaptive increase in ammonium excretion seen during the induction of metabolic acidosis. The data also suggest that ammonia leaves the nephrons at a site(s) along the loop of Henle to enter the collecting duct and that the induction of a metabolic acidosis enhances this reentry.

INTRODUCTION

In mammals, the production and secretion of ammonia by the kidney plays a key role in maintaining acid-base balance. Indeed, in face of either an exogenously administered or an endogenously produced acid load the ammonium excretion may increase four to five times. The net effect is that the acid load is excreted with only minimal changes in urine pH (1, 2). While the biochemical events involved in the renal production of ammonia seem clear (1, 2), the secretory site(s) in the nephron are less well defined. Micropuncture studies in rats (3–7) reported that the concentration of ammonium in fluid obtained along the proximal segments of surface nephrons was higher than could be accounted for by water extraction suggesting that ammonium entry occurs in this segment of the renal tubule. Further, these studies reported a higher concentration of this buffer in tubular fluid when rats were

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made acidotic with ammonium chloride. To date, however, no information is available as to the quantitative delivery of ammonium ions out of the proximal tubule in absolute terms or in relation to the filtered load of this buffer.

The role of more distal nephron segments in ammonium production and secretion remains unclear. Preliminary studies using the technique of microperfusion indicate that ammonia is the major buffer entering the distal tubule under conditions of metabolic acidosis (8). In contrast to these results, others have reported that the concentration of ammonium in distal tubular fluid is actually lower than would be predicted if water extraction and ammonium entry continues along this nephron segment (3, 4, 7). Although the absolute and fractional delivery of ammonium to the distal tubule is not known, these data suggest that there is a net loss of this cation between the end of the proximal and beginning of the distal tubule. On the other hand, results of microcatheterization studies indicate that the increase in the ammonium concentration in collecting duct fluid between the corticomedullary junction and the tip of the papilla is more than can be accounted for by water extraction alone (9, 10).

Although no information is available concerning deep nephron handling of ammonium, two roles have been postulated. First, deep nephrons may be involved in delivery of ammonium formed in the proximal tubule to the medulla. Secondly, it has been suggested that the amount of ammonia entering collecting duct fluid is at least 30% more than can be accounted for in fluid delivered to end accessible sites of the proximal tubule (7). In the absence of the enzyme glutaminase I in collecting duct epithelia (11), it is likely that the additional ammonia originates at other sites, perhaps in deep nephrons.

The present study was designed to determine the absolute and fractional delivery of ammonium to end proximal and distal sites of surface nephrons after the induction of a metabolic acidosis and to characterize the role of deep nephrons in the compensatory increase of ammonium secretion seen in this setting. Based on these observations we have attempted to describe the sites of ammonium production, loss, and reentry along the nephron.

METHODS

Approximately 4 d before micropuncture, 13 Münich-Wistar rats were given a solution of ammonium chloride (168 mM/liter) and glucose (5.5 mM/liter) as drinking water. On the 2nd and 3rd d of the study, each rat was tube fed 1 mM NH_4Cl and on the day before micropuncture they were given 3 mM NH_4Cl in divided doses. Approximately 12 h before study, food was withheld and the animals were allowed to drink only the ammonium chloride and glucose solution. A control group of rats (n = 13) was given 5.5 mM/liter glucose to drink for 4 d before study and tube-fed water on successive days. As with the experimental group, these rats were fasted for ~12 h before study. During this interval they were given 5.5 mM/liter glucose to drink.

On the day of the micropuncture study, the rats were anesthetized with Inactin (100 mg/kg body wt). A tracheostomy was performed, the animal was intubated (polyethylene tubing No. 190), placed on a rodent respirator (Harvard Apparatus Co., S. Natick, MA) and mechanically ventilated throughout the interval of the study. Catheterization of the jugular veins and right femoral artery was followed by laparotomy and preparation of the kidney for papillary micropuncture as described previously (12). A catheter was placed in the bladder for collection of urine from the right untouched kidney. Immediately after the abdomen was opened and before the kidney was prepared for study, all rats were given an inulin prime in 0.6 ml of 0.9% NaCl followed by an infusion of normal saline containing inulin in sufficient quantities to maintain plasma levels between 50 and 100 mg/100 ml. The infusion rate was calculated at 30 μl/min per 100 g body wt. In all studies, an equilibration period of 40–60 min was allowed.

Collections from papillary structures were instituted 20–30 min after removal of the ureteral pelvis. In each group of animals one to four loops of Henle were identified visually, and timed tubular fluid samples were collected. Samples were obtained from collecting ducts at sites as close to the base (CD_{prox})[1] of the papilla as possible and from points along the same collecting duct near the tip (CD_{tip}) of the papilla. The distance between the two collecting duct sites was estimated with an eyepiece micrometer. In almost all cases, paired samples were obtained from at least two different collecting ducts.

Either before or after the papillary studies were performed, the cortical surface was illuminated with a fiber optics light guide attached to a quartz rod. The end of the accessible portion of the proximal and distal tubules were identified by small amounts of 5% lissamine green (0.02–0.05 ml) injected intravenously. After the dye had disappeared from the surface of the kidney, timed collections were obtained.

The methods for measurement of the volume of tubular fluid samples, the concentration of sodium, potassium, and inulin and the preparation of micropuncture pipettes have been described previously (12). The osmolality of tubular fluid was measured with a Clifton microosmometer (Clifton Technical Physics, Hartford, NY). Immediately after the volume of the timed samples had been determined, an aliquot (~2.8 nl) was placed in an equal quantity of 25 mM hydrochloric acid which was fixed to the wall of a plexiglass trough. It was not necessary to acidify collecting duct samples because of their high hydrogen ion content. They were diluted 1:6 in water before an aliquot was fixed to the trough wall. At the end of the experiment or immediately the following day, the samples were analyzed for ammonium concentration. The method used was originally described by Solomon and Alpert (13) and modified by Karlmark (14). Briefly, using the titrating apparatus illustrated in Fig. 1, the acidified sample was titrated to the arterial pH of the rat at the time of the collection. This was done by allowing a

[1] *Abbreviations used in this paper*: BHL, bend of Henle's loop; CD_{prox}, CD_{tip}, proximal portion and tip of collecting duct, respectively; $FL_{NH_4^+}$, filtered load of ammonium; SNGFR, single nephron glomerular filtration rate; $T_{NH_4^+}$, renal tubular secretion of ammonium.

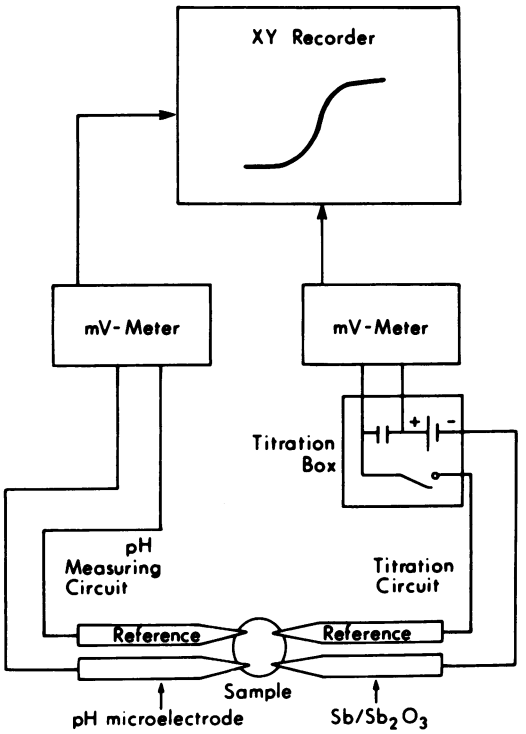

FIGURE 1 A schematic drawing of the electrodes and circuitry used in the coulometric titration of hydrogen ion in samples <3 nl in size. This scheme is a modification of a diagram published in 1973. *Anal. Biochem.* **52:** 69. (By permission).

current to pass through an antimony electrode that resulted in the production of hydroxyl ions according to the following reaction:

$$Sb_2O_3 + 3H_2O + 6e^- \leftrightharpoons 2Sb + 6OH^-$$

at this juncture, ~ 0.8 nl of formic acid, titrated to 7.38, was added to the sample. This resulted in the formation of the organic acid hexamethylene tetramine in the following manner:

$$4\ NH_4^+ + 6CH_2O \leftrightharpoons (CH_2)_6\ N_4 + 4H^+ + 6H_2O.$$

Retitration of the sample generated a current that was stored in low peak capacitors. The charge on these capacitors is stoichiometrically related to the concentration of ammonium ion in the tubular fluid sample. The pH-sensitive microelectrode used in these studies was constructed according to the technique of Pucacco and Carter (15). Using this technique, 32 samples were handled and analyzed for ammonium content in a fashion identical to tubular fluid samples. The measured to calculated ratio was 1.04±0.01 SE, a relationship depicted in Fig. 2.

Blood samples and blood pressure measurements were obtained at hourly intervals or immediately after the collection of tubular fluid. Body temperature was determined with a rectal thermometer and maintained between 36.5° and 38°C. One or two timed urine collections were made in preweighed test tubes for the measurement of volume. At the end of the experiment, aortic puncture was performed

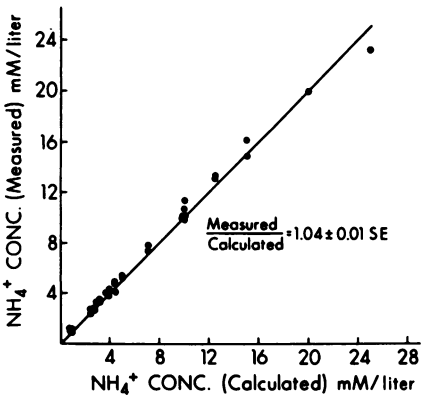

FIGURE 2 Relation of the known (calculated) ammonium concentration to that determined with the micromethod for measuring ammonium described in the text.

and arterial blood (1–2 ml) was collected into a heparinized syringe for ammonium measurement. This sample was placed immediately in a cooled test tube, spun in a refrigerated centrifuge to separate the plasma, and stored until analysis. The serum was analyzed for ammonium ion with an ion specific electrode and a flow through cell (Orion Research Inc., Cambridge, MA). In all cases, plasma analysis for ammonium was performed within 2 h of aortic puncture. The determination of inulin, sodium, potassium, and osmolality in plasma and urine have been described previously (12). Arterial pH and pCO_2 were measured hourly with a blood gas microsystem (BMS-3, Radiometer, London Co., West Lake, OH).

Tubular fluid to plasma inulin ratios (TF/P_{In}) permitted calculation of fractional water reabsorption to the site of micropuncture. Single nephron glomerular filtration rate (SNGFR), fraction of filtered water, sodium, and NH_4^+ delivered to the site of micropuncture were calculated by methods previously described (12).

At proximal sites, renal tubular secretion of ammonium ($T_{NH_4^+}$) was calculated according to the following formula:

$$T_{NH_4^+} = (TF_{NH_4} + V_{tf}) - (FL_{NH_4^+})$$

where $TF_{NH_4^+}V_{tf}$ is the absolute delivery of NH_4^+ to each site of micropuncture and $FL_{NH_4^+}$ is the filtered load of this cation (SNGFR × $P_{NH_4^+}$) for each nephron. Entry of ammonium between the end of the proximal and distal tubule [($T_{NH_4^+}$) loop] was calculated as the difference in the nonfiltered ammonium delivered to these sites.

$$(T_{NH_4^+})_{Loop} = (T_{NH_4^+})_{Proximal} - (T_{NH_4^+})_{Distal}$$

Mean values obtained at proximal and distal sites for each rat were used in these latter calculations. It is assumed that NH_4^+ is equal to the sum of NH_3 and NH_4^+ present in the sample since in each case the sample was acidified before analysis.

The mean values of individual micropuncture samples obtained at similar anatomical sites for each rat were used for statistical analysis. Mean differences in whole kidney and superficial nephron function were examined by the Student's *t* test for unpaired data when comparing the two groups of animals studied, and the Student's *t* test for paired data when comparing superficial and deep nephron function in the same animal or when comparing proximal to tip measure-

TABLE I

Weight, Blood Pressure, and Plasma Parameters in Control and Acidotic Rats

	Body weights			Kidney weights		Plasma levels				
	Initial	Final	ΔWeight/day	Right	Left	Blood pressure	pH	pCO_2 mmHg	HCO_3	NH_4+
		g		*mg*		*mmHg*		*mmHg*	*mM/liter*	
Control rats (n = 13)	75.2 ±1.6	84.3 ±1.7	2.49 ±0.47	394 ±9	387 ±8	118 ±4	7.36 ±0.01	36.5 ±0.9	19.9 ±0.4	0.13 ±0.01
Acidotic rats (n = 13)	81.0 ±1.9	83.9 ±1.9	0.66 ±0.39	457 ±10	436 ±10	112 ±3	7.17 ±0.02	33.0 ±1.2	13.0 ±1.1	0.19 ±0.02
P	<0.05	NS	<0.025	<0.001	<0.005	NS	<0.001	<0.05	<0.001	<0.025

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

ments along the same collecting duct. Where indicated samples were compared statistically after logarithmic transformation (16).

RESULTS

In Table I, base-line information obtained in the two groups of rats is presented. The body weight at the time of initiation into the study was slightly greater in the experimental group. However, at the time of study, the mean weight of the acidotic animals was not different from that of the control group. Thus, the daily weight gain of the experimental group was significantly less than controls (0.66±0.39 and 2.49±0.47 g/d, respectively $P < 0.025$). Blood pressure, hematocrit, and plasma sodium and potassium concentrations were within the normal range for both groups of animals. The plasma bicarbonate of the rats receiving ammonium chloride was significantly reduced and was associated with a marked reduction in both arterial pH and pCO_2. Plasma ammonium levels were significantly higher in experimental animals.

The mean kidney weight of rats receiving ammonium chloride was considerably greater than controls. However, glomerular filtration rate, urine flow, and the fractional water excretion were similar in the two groups of animals (Table II). As expected, the absolute rate of ammonium excretion more than doubled after the administration of ammonium chloride. Thus, in control animals, ammonium excretion averaged 0.39±0.03 μmol/min in contrast to a mean of 0.94±0.06 μmol/min for the acidotic animals. A similar increase is seen when the absolute amount of ammonium excreted in the urine is expressed as a function of filtered load (FE_{NH_4+}). Titratable acid excretion was also greater after the induction of metabolic acidosis. Urine pH values in the control group averaged 5.62±0.04, which was slightly higher but significantly different from the mean obtained in the group given ammonium chloride (5.48±0.04).

TABLE II

Whole Kidney Function in the Two Groups of Rats

	V	GFR	V/GFR	$U_{NH_4}{}^+V$	$FE_{NH_4}{}^+$	$U_{TA}V$	U_{pH}	U_{Osm}
	μl/min		%	*μmol/min*	%	*μeq/min*		*mosmol/kg H_2O*
Control rats (n = 13)	2.99 ±0.39	559 ±42	0.52 ±0.05	0.39 ±0.03	677 ±95	0.209 ±0.020	5.62 ±0.04	1,609 ±100
Acidotic rats (n = 13)	3.94 ±0.50	497 ±32	0.82 ±0.10	0.94 ±0.06	1,179 ±70	0.328 ±0.057	5.48 ±0.04	1,367 ±84
P	NS	NS	<0.025	<0.001	<0.025	<0.05	<0.05	NS

Values are mean±SE. V, rate of urine flow; GFR, glomerular filtration rate; V/GFR, fraction of filtered water excreted; $U_{NH_4}{}^+V$, absolute ammonium excretion; $FE_{NH_4}{}^+$, absolute excretion of ammonium factored by filtered load, $U_{TA}V$, absolute excretion of titratable acid; U_{pH}, urine pH; U_{Osm}, osmolality of urine.

Surface nephron studies. Measurements of tubular fluid obtained from surface nephrons near the end of the proximal and distal tubule for the two groups of rats are summarized in Table III. SNGFR measured at these two sites did not differ in the two groups of rats. Further, when values obtained in controls were factored by kidney weight, the results were similar to what we have reported previously in this species of rat (17). At proximal sites mean SNGFR measurements averaged 29.8±3.3 nl/min per g kidney wt and at distal site the value was 25.4±4.6 nl/min per g kidney wt. In both absolute and fractional terms, delivery of water to micropuncture sites was unaffected by the ammonium chloride ingestion.

The ammonium content was measured in 21 tubular fluid samples obtained near the end of the proximal tubule of control rats. The tubular fluid concentration of ammonium ion averaged 2.31±0.23 mM/liter with the values ranging from 0.86 to 2.96 mM/liter. In absolute terms, delivery of ammonium to end proximal sites averaged 12.3±1.9 pmol/min. $T_{NH_4^+}$ was nearly 10-fold greater than the filtered load of ammonium indicating that the greatest portion of ammonium delivered to the end of the proximal tubule was a consequence of ammonia entry at a site beyond the glomerulus. Thus, in fractional terms, delivery of ammonium to the point of micropuncture was 853% of the filtered load. Distal tubular fluid samples were obtained from nine of these rats. The mean ammonium concentration in these samples tended to be greater than that measured at end proximal sites (4.17±0.95 mM/liter), but this difference did not achieve statistical significance because of the wide range in values (0.80–8.3 mM/liter). Absolute delivery of ammonium to end distal sites was half that measured near the end of the proximal tubule averaging 5.91±1.35 pmol/min ($P < 0.025$). Similarly, fractional ammonium delivery to these sites was lower than to end proximal sites. This relationship is graphically depicted in Fig. 3. Thus, ammonium was lost between the two sites of collection and $T_{NH_4^+}$ became a negative number (−4.93±1.59 pmol/min).

The induction of a metabolic acidosis with ammonium chloride increased significantly the delivery of ammonium to end proximal sites (21.5±1.5 pmol/min, $P < 0.005$). This was primarily due to an increase in tubular fluid ammonium concentration (4.06±0.28 mM/liter, $P < 0.001$), since absolute delivery of tubu-

TABLE III
Effects of Metabolic Acidosis on Surface Nephron Handling of Ammonium

	End proximal			End distal		
	Control	CMA	*P*	Control	CMA	*P*
n	13	13		9	11	
SNGFR, *nl/min*	11.3 ±1.4	10.7 ±0.7	NS	9.93 ±0.44	9.48 ±0.94	NS
V_{tf}, *nl/min*	5.44 ±0.65	5.11 ±0.51	NS	1.87 ±0.44	1.65 ±0.40	NS
FD_{H_2O}, %	49.0 ±2.0	46.9 ±2.6	NS	18.8 ±2.6	17.3 ±2.8	NS
$TF_{NH_4^+}$, *mM/liter*	2.31 ±0.23	4.06 ±0.28	<0.001	4.17 ±0.95	8.17 ±1.04	<0.025
$FL_{NH_4^+}$, *pmol/liter*	1.47 ±0.21	1.93 ±0.20	NS	1.13 ±0.22	1.75 ±0.15	0.025
$TF_{NH_4^+}V_{tf}$, *pmol/min*	12.3 ±1.9	21.5 ±1.5	<0.005	5.91 ±1.35	9.39 ±1.15	<0.05
$T_{NH_4^+}$, *pmol/min*	10.8 ±1.7	20.3 ±1.5	<0.005	−4.93 ±1.59	−12.2 ±1.6	<0.01
$FD_{NH_4^+}$, %	853 ±102	1,197 ±142	<0.025	545 ±99	642 ±89	NS

Values are mean±SE. CMA, chronic metabolic acidosis; FD, fractional delivery to the site of micropuncture. TF, tubule fluid concentration; V_{tf}, tubule fluid flow rate.

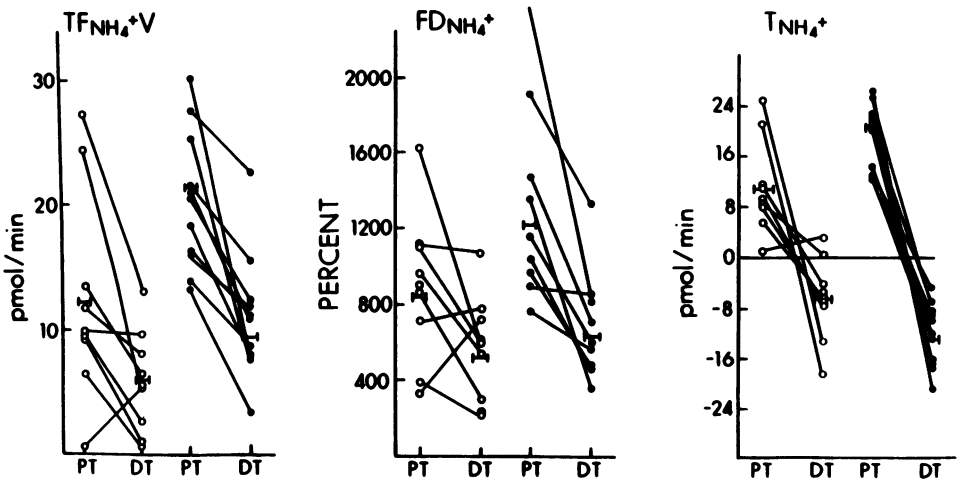

FIGURE 3 Comparison of delivery of ammonium in absolute ($TF_{NH_4^+}V$) and fractional ($FD_{NH_4^+}$) terms to end proximal (PT) and distal tubular (DT) micropuncture sites is shown for controls (O) and after the induction of a chronic metabolic acidosis (●) is depicted in the left and center panels, respectively. To the right ammonium entry ($T_{NH_4^+}$) is presented in a similar fashion. Bars (|—|) designates mean values obtained at each site.

lar fluid was not different from controls. Although slightly greater, the differences in filtered load between control and experimental rats did not achieve significance. This increase did not account for the greater delivery of ammonium to end proximal sites, since $T_{NH_4^+}$ averaged 20.3±1.5 pmol/min, and was twofold greater than the mean value measured under control conditions. Further, when delivery of ammonium ion to end proximal sites was factored by filtered load the value was significantly greater than that obtained in control animals. Thus, entry of ammonia along this tubular segment was greatly enhanced in the presence of a metabolic acidosis. In 11 of these rats fluid was collected near the end of the distal tubule. The concentration of ammonium in these samples was twofold greater than the mean of controls. Absolute delivery to end distal sites also increased with metabolic acidosis. On the other hand, when absolute delivery was factored by filtered load, mean values in the experimental group averaged 642±89%, a value which was not significantly greater than that obtained in controls. As in the control setting, the amount of ammonium delivered to these micropuncture sites was invariably less ($P < 0.001$) than to end proximal sites (Fig. 3). Surprisingly, loss of the ammonium ion between the proximal and distal micropuncture sites doubled. $T_{NH_4^+}$ averaged -12.2 ± 1.6 pmol/min ($P < 0.01$).

Deep nephron studies. As with surface nephrons, SNGFR values of deep nephrons were similar to those reported previously (17) averaging 48.7±4.7 nl/min per g kidney wt. Further, metabolic acidosis did not alter SNGFR or sodium and water handling before the bend of the Henle's loop (BHL). Measurements obtained in 29 samples of tubular fluid, near the BHL, in 12 control rats, are summarized in Table IV. In Fig. 4, ammonium content at this site of micropuncture is compared with mean values obtained near the end of the proximal tubule of surface nephrons. The concen-

TABLE IV

Effect of Metabolic Acidosis on Delivery of Ammonium to the BHL of Deep Nephrons

	SNGFR	V_{tf}	FD_{H_2O}	TF_{Osm}	FD_{Na}	$FL_{NH_4^+}$	$TF_{NH_4^+}$	$TF_{NH_4^+}+V_{tf}$	$T_{NH_4^+}$	$FD_{NH_4^+}$
	nl/min		%	*mosmol/kg H_2O*	%	*pmol/min*	*mM/liter*	*pmol/min*		%
Control rats	18.5	2.85	16.6	1,070	39.3	2.20	11.3	25.5	23.8	1,222
n = 12	±1.5	±0.39	±2.4	±102	±5.5	±0.22	±1.8	±1.8	±1.7	±108
Acidotic rats	19.4	2.96	16.0	1,111	43.4	3.39	20.6	54.6	51.7	1,780
n = 12	±1.4	±0.25	±0.3	±38	±2.7	±1.00	±1.7	±4.7	±5.0	±132
P	NS	NS	NS	NS	NS	<0.005	<0.005	<0.001	<0.001	<0.001

Values are the mean±SE. For abbreviations see Table III and abbreviations list.

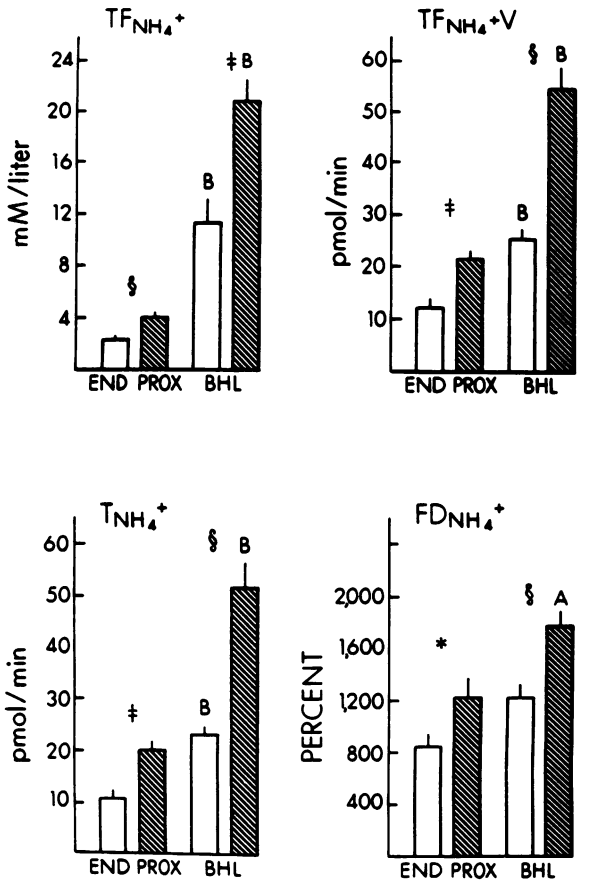

FIGURE 4 The effect of chronic metabolic acidosis on the tubular fluid concentration, delivery, and entry of ammonium by the end of proximal tubule (END PROX) and the BHL are presented as hatched bars and compared with values obtained under control conditions (clear bars). Level of significance is * < 0.025, ‡ < 0.005, § < 0.001 when comparing the control with the experimental group and A < 0.005 and B < 0.001 when comparing end proximal values with those obtained near the BHL.

tration of ammonium in loop fluid averaged 11.3±1.8 mM/liter, this value was nearly threefold greater than that obtained near the end of the proximal tubule of surface nephrons. Absolute ammonium delivery to the BHL averaged 25.5±1.8 pmol/min and was more than double the mean value obtained at end proximal micropuncture sites. As in surface nephrons, delivery to this micropuncture site was primarily a consequence of ammonia entry at a site beyond the glomerulus; thus, $T_{NH_4^+}$ was 10-fold greater than the filtered load of ammonium. Further, when absolute delivery was factored by filtered load, the values measured at this site of collection averaged 1,222±108%, significantly greater than the mean value measured near end proximal sites of surface nephrons.

After the induction of a chronic metabolic acidosis, ammonium delivery to the BHL increased strikingly. Mean values presented in Table IV reflect measurements made on 28 tubular fluid samples. The absolute delivery averaged 54.6±4.7 pmol/min—more than twofold greater than that obtained under control conditions. This was the consequence of a marked increase in the concentration of ammonium in tubular fluid. Ammonia entry before the site of micropuncture ($T_{NH_4^+}$) averaged 51.7±5.0 pmol/min, indicating that renal tubular entry of this buffer more than doubled during metabolic acidosis. As expected, the rise in absolute delivery was associated with a marked increase in fractional delivery (1,780±132%). As depicted in Fig. 4, metabolic acidosis increased absolute and fractional ammonium delivery to the BHL of deep nephrons to a greater extent than to the end of the proximal tubules of surface nephrons.

Collecting duct studies. The induction of chronic metabolic acidosis did not affect the capacity of the terminal segment of the collecting duct to reabsorb water and sodium or generate high osmolalities. Mean values for these parameters are presented in Table V and were not significantly different from those obtained in the control group.

The concentration of ammonium in fluid collected near the base of the collecting duct averaged 94.0±11.8 mM/liter and rose significantly to 122±17 mM/liter near the tip of the papilla. This latter value did not differ significantly from the mean measured in the final urine of the contralateral kidney. The increase in the concentration of ammonium in collecting duct fluid was primarily a consequence of water extraction. Thus, fractional delivery of ammonia to the two sites of collection were not significantly different. Surprisingly, fractional delivery of ammonium to CD_{prox} was significantly greater than the value obtained at end distal sites. The difference in fractional delivery to these two sites is graphically depicted in Fig. 5. Fractional delivery to CD_{prox} averaged 384±144% more than to end distal sites (Fig. 6). After the induction of a chronic metabolic acidosis, the tubular fluid ammonium concentration was twofold greater and increased between base and tip sites. As in controls, this increase in ammonium concentration could be accounted for by water extraction. Thus, delivery to the two sites of collection was not significantly different. On the other hand, fractional delivery to CD_{prox} was twofold greater to this site than during control conditions. This difference appeared to be due to a greater entry of ammonia between end distal micropuncture sites and the base of the papilla. During chronic metabolic acidosis the increase in fractional delivery between these two sites of collection was more consistent and significantly more profound than that seen under control conditions (Fig. 5). The mean difference in delivery was 888.7±140%, more than two times that found in the control setting (Fig. 6).

TABLE V

Effects of Chronic Metabolic Acidosis on Terminal Collecting Duct Function

	TF/P_{Osmol}		FD_{H_2O}		FD_{Na}		$TF_{NH_4^+}$		$FD_{NH_4^+}$		
	Prox	Tip	Prox	Tip	Prox	Tip	Prox	Tip	Prox	Tip	Distance
			%		%		*mM/liter*		%		*mm*
Control rats	3.56	4.01*	1.86	1.12*	1.66	0.94‡	94.0	122§	709	763	0.742
n = 13	±0.22	±0.27	±0.46	±0.25	0.56	±0.26	±11.8	±17	±137	±115	±0.036
Acidotic rats	4.10	4.49§	2.11	1.71§	1.79	1.24§	196	246‡	1,415	1,431	0.799
n = 13	±0.43	±0.36	±0.63	±0.52	±0.55	±0.38	±25	±32	±150	±144	±0.057
P	NS	NS	NS	NS	NS	NS	<0.005	<0.005	<0.005	<0.005	NS

Values are the mean±SE. TF/P_{Osmol} is the ratio of tubular fluid osmolality to that of plasma. Distance refers to the mean distance between proximal and tip collecting duct sites. See Table III and abbreviations list for remaining abbreviations. Significantly different from CD_{prox} at * $P < 0.01$ and ‡ $P < 0.005$; § $P < 0.025$.

DISCUSSION

As has been reported previously (1, 2), the major compensatory response to the chronic ingestion of a large acid load is a marked increase in the excretion of ammonium ion. In this study, ammonium excretion was more than threefold greater in rats ingesting NH_4Cl than in the control group, while titratable acid excretion and urine pH changed only slightly (Table II). Further, the increased ammonium excretion was a consequence of an increase in renal production; that is, the filtered load of ammonium was unchanged while fractional excretion rose nearly 600%. The importance of the present study is that it represents the first attempt to quantitate ammonium handling by surface nephrons, deep nephrons, and the terminal collecting duct. It provides measurements of absolute and fractional delivery of ammonium to end proximal and

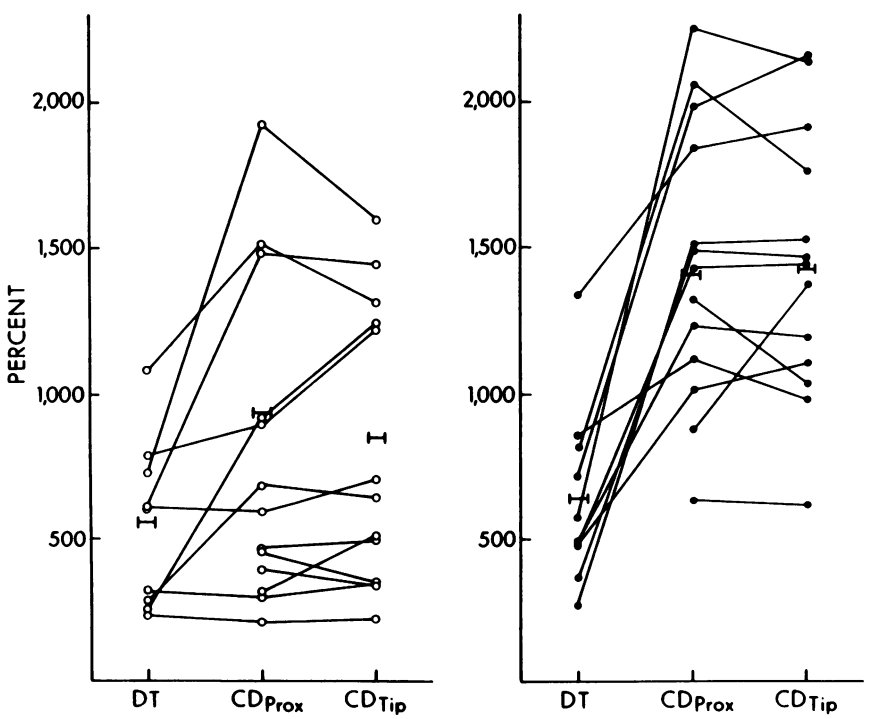

FIGURE 5 Comparison of fractional ammonium delivery to the end of the distal tubule (DT) to values obtained near CD_{prox} and CD_{tip} under control conditions (O) and after the induction of a chronic metabolic acidosis (●).

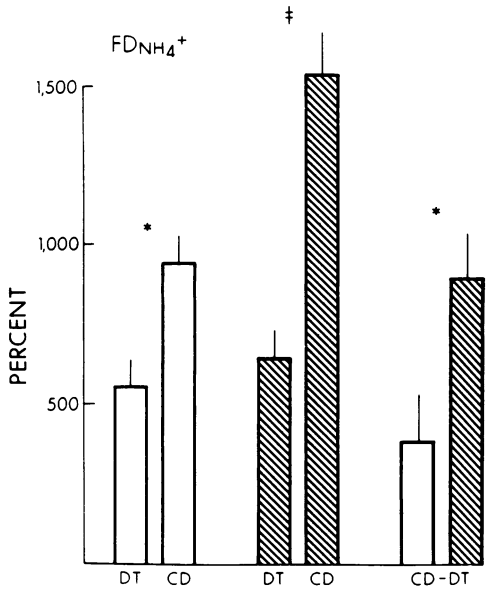

FIGURE 6 Comparison of fractional delivery to end distal tubular sites (DT) and to the base of the collecting duct (CD) under control conditions (open bars) and after the induction of a chronic metabolic acidosis (hatched bars). The columns to the left represent mean differences between fractional delivery to end distal sites and the base of the collecting duct. The level of significance when comparing distal with collecting duct sites was $*P < 0.05$, $\ddagger P < 0.001$.

distal sites of surface nephrons and compares these values to those obtained near the BHL of deep nephrons under hydropenic conditions and after the chronic ingestion of an acid load. These studies are possible primarily because of the modification of a microtechnique described by Karlmark (14), which has the advantage that the ammonium content of tubular fluid can be determined with a fair degree of accuracy (~5%) on samples < 3 nl. Thus, tubular fluid samples obtained near the BHL of deep nephrons as well as at the end of the distal tubule of surface nephrons are of sufficient size to allow for the analysis of inulin, sodium, osmolality, and ammonium content.

In the present study, delivery of ammonium to the end of the accessible portion of the proximal tubule was more than 10 times the amount filtered and indicates that the major portion of ammonium delivered to this site is a consequence of renal tubular secretion. The magnitude of the ammonia entry along the accessible proximal tubule is profound; in fractional terms, the value averaged 853% and was actually more than the mean value measured near the tip of the collecting duct (Tables III, V, and Fig. 7). Thus, the proximal tubule of surface nephrons is a major site for ammonia production and secretion. When the hydrogen load of the animal was increased by allowing them to drink ammonium chloride, the net effect was predictable. Ammonium delivery to end proximal sites rose strikingly. This increase occurred at a time when absolute delivery of tubular fluid was unchanged and was therefore due to the nearly twofold increase in the tubular fluid concentration of ammonium. Although plasma ammonium levels were significantly higher in acidotic rats, the increase in ammonium delivery to the end of the proximal tubule was not a consequence of a change in filtered load; but to enhanced entry of ammonia along the renal tubule. Thus, $T_{NH_4^+}$ more than doubled and fractional delivery of ammonium to this site was >1,200%. Despite the fact that these values reflect a significant increase in ammonium delivery, the contribution of the proximal tubule to the amount of ammonium present in the final urine was less than that found in controls. That is, under control conditions, the mean value for fractional delivery of ammonium to the end of the proximal tubule was 23.6±11.6% ($P < 0.01$) more than what was found at sites along the terminal segment of collecting duct, but after the induction of a chronic metabolic acidosis, fractional delivery of ammonium to end proximal sites was only 75.1±14.5% ($P < 0.025$) of the mean value measured near the tip of the collecting duct. Thus, the increase in ammonium excretion following the chronic administration of an acid load is also a consequence of enhanced production of ammonium at sites beyond

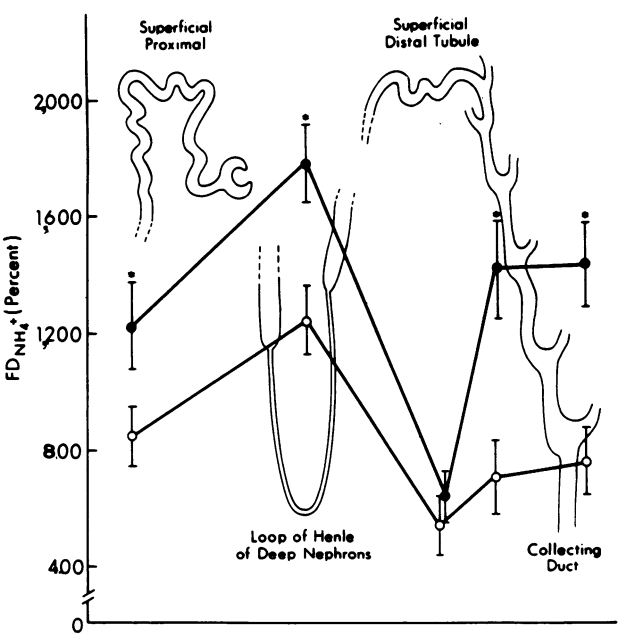

FIGURE 7 Profile of fractional delivery of ammonium to the micropuncture sites in controls (○) and after the chronic ingestion of an acid load (●). Asterisk denotes that the two values are significantly different.

the proximal tubule of surface nephrons. These observations are in agreement with the findings reported by Sajo et al. (7) in acidotic rats. He measured the ammonium index, the ratio of ammonium content in tubular fluid to the tubular fluid to plasma inulin ratio ($TF_{NH_4^+}$:TF/P_{In}), near the end of the proximal tubule of surface nephrons and found it to be 60% of the value measured in fluid obtained near the beginning of the medullary collecting duct.

It is not likely that the distal tubule is a significant site for the production of the additional ammonia found in the final urine in response to a chronic acid load. Under control conditions fractional and absolute ammonium delivery to the end of the distal tubule was consistently less than to the end of the proximal tubule. Further, after the induction of a chronic metabolic acidosis, absolute delivery to the distal tubule rose only slightly while fractional delivery did not change (Table III and Fig. 7). These results unequivocally indicate that ammonium leaves rather than enters the tubular fluid between the end of the proximal and distal tubule of surface nephrons. Indeed, the present studies indicate that loss of ammonium between these two sites of the collection is greatly enhanced during acidosis. These findings are consistent with the observations of Sajo et al. (7) who found that the ammonium index measured at beginning distal sites is considerably less than that measured at end proximal sites of surface nephrons.

The role of deep nephrons in the process of urine acidification is clearly significant. In the control studies, delivery of ammonium to the BHL of deep nephrons averaged 25.5 pmol/min, twofold greater than the mean obtained at end proximal sites of surface nephrons. In fractional terms, the difference was ~500%. Further, fractional delivery to this site of collection exceeded values present in the urine by nearly 300% ($P < 0.05$). After the induction of a chronic metabolic acidosis, absolute delivery to the BHL increased more than twofold and was a consequence of a marked increase in the concentration of ammonium in tubular fluid. The increase was more profound than that seen at end proximal sites, but again was due to ammonia entry along the renal tubule rather than an increased filtered load. In fractional terms, ammonium delivery to this site averaged 1,780%. The mechanisms which underlie the increased delivery of ammonium to the BHL of deep nephrons cannot be entirely determined from the present study; although, three possibilities exist. First, ammonia could be recycled from the collecting duct. Second, it is possible that the greater delivery of ammonium to the BHL of deep nephrons than to end proximal sites of surface nephrons is a consequence of continued secretion along the remaining portion of the convoluted proximal tubule and the pars recta. Third, the capacity of deep nephrons to produce ammonia may be greater than that of superficial nephrons. It seems unlikely that ammonium is being recycled from the collecting duct since it has been reported that the tubular fluid pH is higher near the BHL than at the end of the proximal tubule (1, 2). Thus, if anything, this would favor the dissociation of ammonium to ammonia which would then diffuse out of the tubule lumen and ammonium delivery to the BHL would be a minimum estimate of proximal juxtamedullary ammonium formation. If one assumes that (*a*) ammonium entry does not occur along the descending limb of the loop of Henle, (*b*) the TF/P_{Na} ratio at the end of the proximal tubule of deep nephrons is 1.0 and (*c*) that no sodium is lost between this site and the BHL (17), then the tubular fluid concentration of ammonium at the end of the proximal tubule of deep nephrons can be estimated using the following equation:

$$\left[\frac{TF_{NH_4^+}}{P} \div \frac{TF_{Na}}{P}\right] P_{NH_4^+}.$$

The mean control group value for this estimate was 4.99±0.81 mM/liter indicating that the ammonium concentration near the end of the proximal tubule of surface nephrons is half that of the value estimated at the end of the proximal tubule of deep nephrons. Under conditions of a metabolic acidosis, the concentration of ammonia at this site averaged 8.2±0.7 mM/liter, more than double the amount measured at end proximal sites. Thus, assuming that fractional fluid delivery to end proximal sites of surface and deep nephrons are similar (17), these estimates would strongly suggest that in response to an acid load either the capacity to secrete ammonia by the remaining portion of the convoluted tubule and the pars recta is markedly greater than the accessible portion of the proximal tubule or that deep nephrons have a greater capacity to produce and secrete ammonia than do surface nephrons.

If ammonium production along the proximal tubule is to play a major role in buffering the acid load excreted by the kidney, it must reenter the nephron at a site beyond the distal tubule of surface nephrons. There is considerable evidence to suggest that the medullary collecting duct may be a site of ammonium reentry. The work of Finkelstein and Hayslett (18) indicates that the capacity of the kidney to produce ammonia is impaired after papillectomy. Early studies using stop flow and whole kidney clearance techniques indicate that a major site of ammonium entry is along the most distal segment of the nephron (19). Finally, studies utilizing the technique of microcatheterization, in both rats (9) and hamsters (10), have indicated that the concentration of ammonium in fluid obtained

along the medullary collecting duct increases as a function of the distance from the cortical medullary border to the tip of the papilla. This increase in ammonium concentration is more than would be predicted on the basis of water extraction alone. The mechanism for this reentry remains unclear. One theory that has been proposed for the ammonia entry into collecting duct fluid is based on the premise that intratubular pH changes near the BHL (1, 2). As fluid traverses this segment, water is extracted and the bicarbonate concentration in loop fluid rises at a rate faster than the pCO_2 of the medullary interstitium. Thus, luminal pH becomes more alkaline as fluid moves distally. This change would result in the dissociation of ammonium to ammonia and hydrogen ions. Free ammonia would then diffuse out of the tubule lumen and come into equilibrium with papillary interstitum to ultimately be reentraped in the acidic fluid of the collecting duct. Although there is no direct evidence to support this hypothesis, a number of observations favor it. First, the presence of glutaminase activity is scant in the collecting duct segments of the rat but high in proximal tubular epithelium (11). Further, the activity of this enzyme in the proximal tubule increases significantly when rats are made acidotic (11). Since glutaminase is thought to be essential in ammonia synthesis, this observation suggests that the collecting duct is not a major site for ammonia production. Second, it has been reported that the pH of loop fluid is more alkaline than fluid obtained at end proximal micropuncture sites (1, 2). Lastly, the concentration of ammonia in renal tissue increases from cortex to medulla (20, 21) and ammonium formation in collecting duct fluid seems dependent on this gradient. Thus, while the concentration in cortical tissue is not altered by an osmotic diuretic the gradient from cortex to medulla disappears (20) and ammonia entry into the collecting duct is diminished (21).

The results of the present study support the theory that ammonia reentry occurs along the medullary collecting duct. Fractional ammonium delivery to the base of the collecting duct was significantly greater than to end distal sites under both control conditions and after the induction of metabolic acidosis. In the control setting, delivery to CD_{prox} was nearly 400% greater than to the end of the distal tubule. After the induction of a metabolic acidosis, the difference in fractional delivery between the two sites of collection was even more striking and consistent. In this group delivery to CD_{prox} was almost 900% more than to the end of the distal tubule. However, the findings of the present study are also consistent with the possibility that deep nephrons are responsible for the differences in delivery of ammonium to these two micropuncture sites. If it is true that the pH of tubular fluid increases as it proceeds along the descending limb of the loop of Henle, then ammonia formation should occur in this segment of the loop and move into the interstitium. However, as fluid flows axially up the ascending limb and moves into the cortical region where pCO_2 values are higher (22) and pH will fall preventing further formation of ammonia. The consequence of these two likely events is that if ammonia loss from loop fluid is only passive, then the major site of ammonium loss in the loop of Henle must occur along those nephron segments that lie in the medulla. From the present studies, it can not be determined if the loss of ammonium from loop segments of deep nephrons is similar to that of surface nephrons. Thus, it is quite possible that a significant portion of the ammonia that enters the collecting duct between the end of the distal tubule of surface nephrons and proximal collecting duct sites is derived from the effluent of deep nephrons.

In controls, fractional delivery of ammonium was more to the end of the proximal tubule of surface nephrons and to the BHL of deep nephrons than to CD_{prox}. These differences in delivery could be due to the loss of interstitial ammonia in the circulation. Further, it seems that the conditions involved in the production of a metabolic acidosis result in a more efficient reentrapment in tubular fluid. Thus, fractional delivery of ammonium to CD_{prox} is more than to end proximal sites of surface nephrons and the same as that to the BHL of deep nephrons in acidosis. In the present studies, no ammonia entry was demonstrated along the terminal segment of the collecting duct. Technical factors are not likely to contribute to these results since the concentration of ammonium in tubular fluid rose between the base and tip of the collecting duct while water and sodium delivery fell between the two micropuncture sites. Thus, the present observations are more consistent with the suggestion that the terminal segment of the collecting duct is not a major site of ammonia reentry or production.

In conclusion, the present study characterizes ammonium handling by the nephron segments of both surface and deep nephrons that are available to micropuncture. A significant role for deep nephrons in the compensatory increase in ammonium production seen in response to an acid load given on a chronic basis is proposed. We have shown that ammonia leaves the nephron at a site along the loop of Henle and suggest that a significant portion of it is reentraped in the acidotic collecting duct fluid.

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REFERENCES

1. Pitts, R. F. 1973. Production and excretion of ammonia in relation to acid-base regulation. *Hand. Physiol.* (Sect. 8, Renal Physiology) 455–496.
2. Warnock, D. G., and F. C. Rector. 1981. Renal acidification mechanisms. *In* The Kidney. B. Brenner and F. C. Rector, editors. W. B. Saunders Company, Philadelphia, PA. 440–494.
3. Glabman, S., R. M. Klose, and G. Giebisch. 1963. Micropuncture study of ammonia excretion in the rat. *Am. J. Physiol.* **205:** 127–132.
4. Hayes, C. P., J. S. Mayson, E. E. Owen, and R. R. Robinson. 1964. A micropuncture evaluation of renal ammonia excretion in the rat. *Am. J. Physiol.* **207:** 77–83.
5. Karlmark, B., and B. G. Danielson. 1974. Titratable acid, pCO_2, bicarbonate, and ammonium ions along the rat proximal tubule. *Acta Physiol. Scand.* **91:** 243–258.
6. Oelert, H., E. Uhlich, and A. G. Hills. 1968. Messungen des Ammoniakdruckes in den corticalen Tubuli der Rattenniere. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **300:** 35–48.
7. Sajo, I. M., M. B. Goldstein, H. Sonnenberg, B. J. Stinebaugh, D. R. Wilson, and M. L. Halperin. 1981. Sites of ammonia addition to tubular fluid in rats with chronic metabolic acidosis. *Kidney Int.* **20:** 353–358.
8. Wilcox, C. S., G. Giebisch, G. Kirk, and D. Gordon. 1981. Titratable acid and ammonia formation by the superficial distal tubule of the acidotic rat during saline infusion. *Clin. Res.* **29:** 480a.
9. Sonnenberg, H., S. Cheema-Dhadli, M. R. Goldstein, B. J. Stinebaugh, D. R. Wilson, and M. L. Halperin. 1981. Ammonia addition into the medullary collecting duct of the rat. *Kidney Int.* **19:** 281–287.
10. Ullrich, K. J., H. H. Hilger, and J. D. Klumper. 1958. Sekretion von Ammoniumionen in den Sammelrohren der Saugetierniere. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **267:** 244–250.
11. Curthoys, N. P., and O. H. Lowry. 1973. The distribution of glutaminase isoenzymes in the various structures of the nephron in normal, acidotic, and alkalotic rat kidney. *J. Biol. Chem.* **248:** 162–168.
12. Buerkert, J., M. Head, and S. Klahr. 1977. Effects of acute bilateral ureteral obstruction on deep nephron and terminal collecting duct function in the young rat. *J. Clin. Invest.* **59:** 1055–1065.
13. Solomon, S., and H. Alpert. 1969. A method for determining titratable acidity in nanoliter samples of biological fluids. *Anal. Biochem.* **32:** 291–296.
14. Karlmark, B. 1973. The determination of titratable acid and ammonium ions in picomole amounts. *Anal. Biochem.* **52:** 69–82.
15. Pucacco, L. R., and N. W. Carter. 1976. A glass-membrane pH microelectrode. *Anal. Biochem.* **73:** 501–512.
16. Snedecor, G., and W. Cochran. 1967. Statistical Methods. Iowa State University Press, Ames, Iowa. 6th edition. 91–119.
17. Buerkert, J., D. Martin, J. Prasad, S. Chambless, and S. Klahr. 1979. Response of deep nephrons and the terminal collecting duct to a reduction in renal mass. *Am. J. Physiol.* **236:** F454–F464.
18. Finkelstein, F. O., and J. P. Hayslett. 1974. Role of medullary structures in the functional adaptation of renal insufficiency. *Kidney Int.* **6:** 419–425.
19. Sullivan, L. P. 1965. Ammonium excretion during stopped flow: a hypothetical ammonium countercurrent system. *Am. J. Physiol.* **209:** 273–282.
20. Robinson, R. R., and E. E. Owen. 1965. Intrarenal distribution of ammonia during diuresis and antidiuresis. *Am. J. Physiol.* **208:** 1129–1134.
21. Stern, L., K. A. Backman, and J. P. Hayslett. 1981. Cortical-medullary gradient for ammonia: mechanism regulating formation of ammonium in medullary collecting duct. 14th Annual Meeting of the American Society of Nephrology, Wash., D.C. 113 (Abstr.).
22. DuBose, T. D., Jr., and L. R. Pucacco. 1981. Evidence that an acid disequilibrium pH is a determinant of papillary collecting duct pCO_2. *Kidney Int.* **19:** 238 (Abstr.).