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Effect of cortical-medullary gradient for ammonia on urinary excretion of ammonia

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Effect of cortical-medullary gradient for ammonia on urinary excretion of ammonia. Previous studies suggested that a portion of ammonia secreted into the proximal tubule may diffuse directly from Henle's loop into the medullary collecting duct. Since water is absorbed along the course of the descending portion of the loop, it was proposed that the concentration of ammonia increased in loop fluid, and that rapid diffusibility of the free base would facilitate the delivery of ammonia into medullary interstitium where a high level could be maintained by the countercurrent exchange process. In this schema it was proposed that there was an ammonia concentration gradient between medullary structures and cortex, and recovery of ammonia by the medullary collecting duct due to the low pH in tubule fluid at that site. The present study was designed to evaluate this hypothesis by estimating ammonia concentrations in medullary and cortical tissue, and by correlating medullary levels with secretion rate into the inner medullary collecting duct. In control animals the concentration of total ammonia ($NH_4^+ + NH_3^+$) in inner medullary vasa recta was 9.2 ± 1.5 μmoles/ml, a level 100-fold higher than the cortical level of 0.10 ± 0.01. During acute acidosis the medullary level rose to 22.5 ± 2.7 μmoles/ml, but in acute acidosis during mannitol infusion the level fell to 8.0 ± 1.2. The rate of ammonia secretion into inner medullary collecting duct fluid correlated directly with medullary vasa recta ammonia concentration. These data provide evidence for a steep ammonia concentration gradient between the medulla and cortex, and suggest that the diffusion gradient across collecting duct epithelium governs the rate of the addition of ammonia to collecting duct fluid.

Effet du gradient cortico-médulaire d'ammoniaque sur l'excrétion urinaire d'ammoniaque. Des études antérieures ont suggéré qu'une partie de l'ammoniaque sécrété dans le tubule proximal pourrait diffuser directement de l'anse de Henlé jusqu'au canal collecteur médullaire. Puisque de l'eau est réabsorbée le long de la partie descendante de l'anse, on a proposé que la concentration d'ammoniaque augmenterait dans le liquide de l'anse, et que la diffusibilité rapide de la base libre pourrait faciliter l'apport d'ammoniaque dans l'interstitium médullaire où son niveau élevé pourrait être maintenu par le processus d'échange à contre-courant. Dans ce schéma, il a été proposé qu'il y avait un gradient de concentration d'ammoniaque entre les structures médullaires et le cortex et une récupération de l'ammoniaque par le canal collecteur médullaire en raison du faible pH du liquide tubulaire à ce niveau. Cette étude a été conçue pour évaluer cette hypothèse en estimant les concentrations d'ammoniaque dans le tissu médullaire et cortical, et en corrélant les niveaux médullaires avec la vitesse de sécrétion dans le canal collecteur médullaire interne. Chez les animaux contrôles, la concentration d'ammoniaque total ($NH_4^+ + NH_3^+$) dans les vasa recta médullaires internes était de 9,2 ± 1,5 μmoles/ml, une valeur 1 000 fois plus élevée que le niveau cortical de 0,10 ± 0.01. Pendant une acidose aiguë, le niveau médullaire s'élevait à 22,5 ± 2,7 μmoles/ml, mais en acidose aiguë au cours d'une perfusion de mannitol, le niveau chutait à 8,0 ± 1,2. La vitesse de sécrétion d'ammoniaque dans le liquide du canal collecteur médullaire interne était directement corrélée avec la concentration d'ammoniaque dans les vasa recta médullaires. Ces données apportent la preuve d'un fort gradient de concentration d'ammoniaque entre la médullaire et le cortex, et suggèrent que le gradient de diffusion à travers l'épithélium du canal collecteur gouverne la vitesse d'addition de l'ammoniaque au liquide du canal collecteur.

Although the proximal tubule has been shown to be the major site for ammonia production in the kidney [1, 2], early studies [3], as well as more recent reports [2], indicate that some of the ammonia secreted into tubular fluid in the proximal tubule may escape from tubular fluid between proximal and distal segments and gain access to urine without traversing the distal tubule. Microcatheterization studies have shown that the medullary collecting duct is an important site of re-entry, because ammonia secretion accounted for 80% of net acid excretion in controls and 55% in animals during acute acidosis [4].

The mechanism regulating ammonia secretion into medullary collecting duct fluid remains unclear. Pitts [5] postulated that the free base of ammonia escaped from tubular fluid in the loop of Henle into the medullary interstitium because of increases in ammonia concentration and tubular fluid pH due to water absorption. In this formulation tubular fluid in the loop becomes more alkaline because of the increase in concentration of residual bicarbonate while P_{CO_2} is maintained at a constant level. In addition, it was postulated that secretion of acid into the collecting duct results in relative alkalinity of papillary interstitial fluid. This change in pH in loop fluid and interstitial fluid would shift the equilibrium of ammonia toward the dissociation of ammonium to its free base and hydrogen ion. Because the concentration of both species of ammonia would presumably reach a maximum at the tip of the loop, the rapid diffusibility of the free base would facilitate its diffusion from the ascending limb into other medullary structions where a high level of ammonia could be maintained by the countercurrent exchange process. The low pH of tubular fluid in medullary collecting duct, in this formulation, served to produce a steep diffusion gradient for the free base of ammonia and a site for trapping ammonia in the medullary collecting duct. This process therefore, would be characterized by a high gradient for

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total ammonia along the medullary-cortical axis, and the diffusion gradient across the wall of the collecting duct would determine, at least in part, the rate of addition of ammonia to collecting duct fluid.

The present study was conducted to examine this hypothesis by estimating the concentration gradient of ammonia between papillary interstitium and cortex. In addition, since the medullary concentration of ammonia would influence the rate of transfer into collecting duct fluid, the rate of ammonia secretion into the medullary collecting duct was correlated with medullary levels of ammonia.

Methods

Preparation of animals and sample collection

Microcatheterization studies were performed on male Sprague-Dawley rats (Cam Research, New Jersey, USA) weighing approximately 200 g. For micropuncture studies, smaller animals weighing approximately 100 g were used. Animals were allowed free access to food and tap water prior to the acute study. Animals for microcatheterization and micropuncture were each divided into three groups and prepared as follows:

Control group. Animals were fed regular rat chow (Ralston Purina, St. Louis, Missouri, USA), injected with a priming dose of 30 μCi of methoxy-^{3}H-inulin and infused with 0.15 M NaCl, containing 30 μCi/ml of methoxy-^{3}H-inulin, at 0.01 ml/min per 100 g body weight during the study.

Acute metabolic acidosis group. Animals were fed regular rat chow (Ralston Purina) and infused with the same priming and sustaining infusions as the control group. At the initiation of the acute study NH_4Cl was administered by gavage, in a dose of 8 mmoles/kg body wt. Pilot studies showed that this dose of NH_4Cl was sufficient to induce severe acidosis with a fall in blood pH for approximately 3 hr.

Acute metabolic acidosis with mannitol infusion group. Animals were prepared in a manner similar to the acute metabolic acidosis group, but during the acute study they were also infused with 5% mannitol in saline at 0.02 ml/min/100 g body wt. This dose of mannitol was used after preliminary studies indicated that the medullary level of ammonia was reduced to near control values in acidotic animals at this rate of mannitol infusion.

Animals were anesthetized with Inactin (Promonta, Hamburg, West Germany) in a dose of 12 mg/100 g body wt. A tracheostomy was performed, and a jugular vein was cannulated for infusion of fluids. Cannulation of a carotid artery permitted blood sampling. The left experimental kidney was prepared for microcatheterization as previously described by this laboratory [6]. The kidney, exposed by flank incision, was placed in a (Lucite®) cup. The left ureter was dissected free and cannulated (PE-50). The papilla of the left kidney was exposed by partial excision of the dorsal ureteral wall near the renal pelvis. Urine from the left kidney was collected under suction, and from the unexposed right kidney by a bladder catheter. To replace surgical losses of body fluids 0.15 M NaCl, in an amount equivalent to 2% of body weight, was infused at the time of surgery. The equilibration period before sample collection was approximately 1 hr. The transit time of fluid in the proximal tubule was determined following the intravenous injection of a 0.05 ml bolus of lissamine green (5% in water). Animals were discarded if the transit time was longer than 12 sec.

Samples of collecting duct fluid were collected via polyethylene catheters (O.D. 35 μm) inserted into different collecting duct orifices and advanced until slight resistance was encountered. Tubular fluid was collected by suction. Use of a machinist's clock gauge permitted determination of the depth of insertion as the catheter was withdrawn. Following each collection, the catheter was reintroduced into the same duct and a sample was obtained at the orifice of the same collecting duct. Samples containing erythrocytes were discarded. At the conclusion of the experiment, the left kidney was removed, cut sagittally, and the length of the inner medullary collecting duct was measured.

Vasa recta samples were obtained from animals prepared for micropuncture. The surgical preparation of animals was performed in the same manner used for the microcatheterization, except that the left ureter was excised completely to provide adequate visualization. Approximately 1.5 mm of papilla was exposed for micropuncture.

The vasa recta samples were transferred under water-equilibrated mineral oil to a collagen-coated petri dish to permit rapid separation of red blood cells and plasma. Known volumes of plasma were transferred with constant bore pipettes for subsequent determination of ammonia concentration.

Microanalytical method for determination of ammonia

Since the method used in this study to determine the concentration of ammonia in samples of vasa recta plasma and tubular fluid, to our knowledge, has not been reported previously, details of the procedure, and its accuracy and precision are described as follows:

Total ammonia ($NH_3 + NH_4^+$) was measured directly using a modification of the Berthelot color reaction. The anticipated concentrations of ammonia in vasa recta plasma, as compared to peripheral plasma, tubular fluid, and urine were shown to be of sufficient magnitude to justify the elimination of the cumbersome diffusion step used previously [7] in the preparation of microsamples.

The chromogen formed by the reaction of ammonia with phenol in the presence of sodium nitroprusside, as a catalyst, and alkaline sodium hypochlorite was quantified spectrophotometrically at 630 nm. The color produced was stable for 8 hr. The pH of the reagent reaction mixture was 10.8 to minimize the deamination of proteins and amino acids present in the biological samples [8].

Reagents were prepared as follows:

(1) Ammonia-free water was prepared by adding 2 g of AG 50W-X2, 50-100 mesh cation exchange resin (Bio-Rad Laboratories, Richmond, California, USA), to 500 ml of distilled, deionized water. Agitation for 2 min of the resin/water mixture produced water that retained adequate quality for at least 1 week.

(2) Phenol nitroprusside solution was prepared by adding 10 g of crystalline phenol and 0.050 g of sodium nitroferricyanide (sodium nitroprusside) to 1 liter of ammonia-free water. The solution was stored in amber bottles at 4°C and discarded after 2 months.

(3) Alkaline hypochlorite solution was prepared by dissolving 5 g of sodium hydroxide and approximately 8 ml of commercial

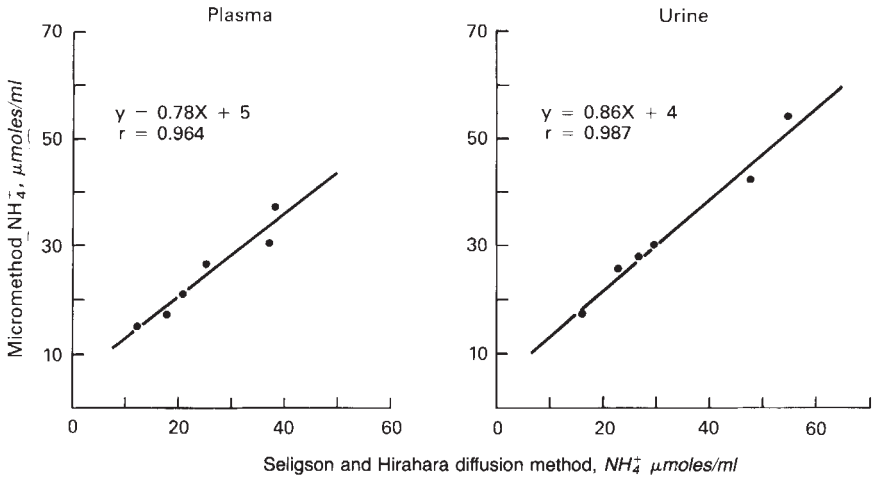

Fig. 1. *Correlation of microanalytical method for ammonia determination in rat plasma and urine with the Seligson and Hirahara microdiffusion method.*

bleach (Chlorox) in 1 liter of ammonia-free water. The solution was stored in amber bottles at 4°C and discarded after 2 months. This solution was allowed to stand for 48 hr prior to use. A yellow color as a reaction endpoint would imply that aged bleach was used in the reagent preparation.

Microsamples of biological fluids were collected and processed under water-equilibrated mineral oil. Volumes collected ranged between 60 to 100 nl. Samples were processed within 2 hr of collection. Known volumes of sample ranging in size from 10 to 50 nl were pipetted directly into 50 μl of water in disposable 500 μl plastic autoanalyzer containers and covered with a plastic cap. The solution was mixed carefully using a vortex mixer (Scientific Products) at a low speed to avoid spreading the sample into the cap. Next, 10 μl of phenol nitroprusside solution was pipetted and mixed rapidly. After 5 min, 10 μl of alkaline hypochlorite were added and mixed. The sample was incubated at 53°C in a water bath for 15 min and then removed. The final volume was 70 μl with a sample to volume ratio between 1:1400 and 1:7000. Absorbances of unknown samples and standards (4.6 to 70 μmoles/ml), prepared in an identical fashion, were determined spectrophotometrically using a microfluorophotometer (model #10-280, Aminco Inc.) with a fiber-optic microcolorimeter accessory (model #4-7407, Aminco Inc.) and a 625 nm filter with a 10 nm one-half band pass (Pomferd Research Optics, Stamford, Connecticut, USA).

Cuvettes containing approximately 5 μl of sample were manufactured using disposable 100 μl pipettes (Drummond) cut to standard size. All glassware was cleaned with concentrated sulfuric acid and rinsed thoroughly prior to use. All samples were read in duplicate or triplicate.

Blood samples obtained for determination of ammonia in peripheral plasma and renal vein plasma were collected in iced glass syringes containing sodium ethylenediaminetetraacetate (EDTA) in a concentration of 90 mg/dl as an anticoagulant. The samples were transferred immediately and centrifuged in a Sorvall RC2B at ×770*g* at 0°C for 10 min. Plasma was separated and appropriately diluted for each assay which was performed within 30 min of sample collection.

The lowest measurable level of total ammonia by the microassay was 105 pmoles using a sample size of 42 nl or 2.5 μmoles/ml, and the sensitivity of the method was 1.4 μmoles/ml.

For each assay standards ranging in concentration from 4.6 to 70 μmoles/ml were run concurrently.

The standard curve was evaluated by using a least-squares linear regression program in a programmable calculator (Olivetti P602). In a series of ten consecutive assays the mean slope was 0.013 ± 0.001 absorbance U/μmoles/ml (mean ± SD) of ammonia and all assays had r values greater than 0.99. Empirical observation demonstrated that the curve was nonlinear for concentrations greater than 75 μmoles/ml.

To determine the accuracy of the micromethod in estimating ammonia concentration in nanoliter samples of vasa recta plasma and collecting duct fluid, the concentrations of ammonia in 30 to 60 nl samples of peripheral plasma and rat urine were determined concurrently by the micromethod and the Seligson and Hirahara [8] microdiffusion method, which uses a sample size of 0.4 ml. Since the micromethod, described above, did not have sufficient sensitivity to measure ammonia in peripheral plasma, ammonium chloride was added to samples of peripheral plasma in concentrations from 10 to 40 μmoles/ml. As in the micromethod, samples determined by the Seligson and Hirahara [8] diffusion method were incubated after addition of reagents at 53°C for 15 min.

The Seligson and Hirahara [8] microdiffusion method was used as the standard for comparison because *(1)* this technique has been demonstrated to provide accurate assessments of whole blood and plasma ammonia concentrations [8] and is comparable to the Conway method in the determination of urinary ammonia [9], and *(2)* the Seligson and Hirahara microdiffusion method was used in this experiment to estimate the concentration of ammonia in samples of peripheral plasma and renal vein plasma, because these fluids contained lower concentrations of ammonia.

The results of this comparison, in which six samples of rat urine and six samples of rat plasma were examined, are shown in Figure 1 and demonstrate a close correlation between the two methods for ammonia determinations in the range of vasa recta plasma and urine values found experimentally. There was no evidence that micromethod overestimated the level of ammonia in the lower range of plasma ammonia concentrations since comparison of peripheral plasma with water by this technique yielded a value that was below 1 μmoles/ml, a value less than the lowest measurable level of 2.5 μmoles/ml.

Since the nanoliter size samples were stored under water-equilibrated oil for as long as 120 min until the Seligson and Hirahara [8] microdiffusion analysis was completed, these data also show that ammonia was not lost to the oil phase during storage and handling of small samples for microassay.

To determine the interassay variation, a single sample containing approximately 40 μmoles/ml was assayed concurrently in ten consecutive experiments. The coefficient of variation was 7%.

To determine the within assay variability, 20 to 40 nl samples of urine with an ammonia concentration in the range of 20 to 50 μmoles/ml and of plasma with a concentration in the range of 15 to 40 μmoles/ml were assayed in sextuplicate. In a series of six experiments the coefficients of variation ranged from 3.6 to 11.2% for urine and 3.2 to 6.7% for plasma. A series of recovery experiments also were performed on 30 to 60 nl samples of peripheral plasma and urine before and after the addition of ammonium chloride in various concentrations; the percent recovery of the added ammonium chloride was calculated. Two to five samples were examined in each of seven experiments. In plasma the mean ± SEM recovery was 99.8 ± 2.0% and in urine the mean recovery was 98.7 ± 2.0%.

These data validate the micromethod for the estimation of ammonia in samples of rat vasa recta plasma and collecting duct fluid with an ammonia content greater than 105 pmoles.

The activity of methoxy-^{3}H-inulin in collecting duct fluid, urine and plasma were determined on a liquid scintillation counter (Tri-Carb, Packard Instruments, United Technology, Downers Grove, Illinois, USA); pH in blood and urine was determined using an acid-base analyzer (Model pHM 71, Radiometer) with microelectrode unit (Model E5021, Radiometer).

The value of renal blood flow (RBF) was determined from the expression:

$$RBF = \frac{C_{In}}{E_{In}}/1\text{-Hct} \qquad (1)$$

where E_{In} represents the extraction of inulin.

The rate at which ammonia was added to renal venous blood was calculated as:

$$RBF \times (ammonia_{RV} - ammonia_{A}) \qquad (2)$$

where RV and A indicate renal venous and carotid artery blood, respectively. Although urine flow rate may influence the concentration of ammonia in renal venous blood, urine flow rate was not included in this formula since its influence was negligible at the flow rates encountered. The rate of ammonia production was derived from the rate at which ammonia was added to renal venous blood plus the urinary excretion rate of ammonia.

To determine net movement of ammonia along the inner medullary collecting duct, the difference in ammonia concentration in each pair of samples, between a site within the collecting duct and the orifice of the duct of Bellini, was analyzed as:

$$\frac{\Delta\ (TF_{NH_4} + (TF/P_{In})}{\Delta X} \qquad (3)$$

where X represents the distance separating the samples sites in millimeters. This value, therefore, represents the change in ammonia concentration corrected for water movement as a function of tubular length. In these experiments the average length of inner medullary collecting duct was 3 mm. To test whether the proportions of samples in which net secretion or absorption occurred were equal, we compared the slopes of individual paired samples to zero by the χ^2 test with 1° of freedom.

The fractional contribution of ammonia secreted by the inner medullary collecting duct to the amount present at the orifice (final urine) was calculated from the expression:

$$1 - (NH_4+/(TF/P)_{In}\ X/NH_4+/(TF/P)_{In}\ orifice) \qquad (4)$$

where X indicates the sample collected at the more proximal site.

Values are expressed as mean ± SEM.

Table 1. Summary of renal function in animals studied by micropuncture of the renal papilla[a]

	Control	Acute acidosis	Acute acidosis plus mannitol
Number of animals	4	6	5
Body weight	97.3 ±4.4	113.3 ±5.7	108.8 ±2.9
C_{In}, *μl min^{-1} 100 g body wt^{-1}*	848 ±157	783 ±46	745 ±32
RBF, *μl min^{-1} 100 g body wt^{-1}*	3790 ±686	3552 ±223	3262 ±154
$\dot{V}$, *μl min^{-1} 100 g body wt^{-1}*	1.0 ±0.2	2.7[b] ±0.4	4.7[b] ±0.3
UpH	6.10 ±0.17	5.45[b] ±0.08	5.68[b] ±0.11
Ammonia excretion, *μmoles min^{-1} 100 g body wt^{-1}*	0.06 ±0.17	0.30[b] ±0.08	0.19[b] ±0.02
Ammonia production, *μmoles min^{-1} 100 g body wt^{-1}*	0.19 ±0.05	0.66[b] ±0.07	0.56[b] ±0.01

[a] Values are mean ± SEM and were obtained from the right kidney.
[b] $P < 0.05$ compared to the control group.

Results

Analysis of the cortico-medullary gradient of ammonia

This series of studies was performed in young animals, weighing approximately 100 g each, to permit adequate exposure of the papilla for aspiration of samples of vasa recta blood. A summary of whole kidney function measurements from the right, contralateral kidney is shown in Table 1. Compared to the control group there was no difference in C_{In} or renal blood flow (RBF) levels in the two experimental groups with acidosis. Induction of acidosis resulted in a marked and comparable fall in arterial pH in both experimental groups (Table 2) from 7.41 ± 0.02 to 7.26 ± 0.02 ($P < 0.001$), and reduction in urinary pH (Table 1) from the control value of 6.10 ± 0.17 to 5.45 ± 0.08 and 5.68 ± 0.11 in the acidotic and acidotic plus mannitol

Table 2. Summary of plasma ammonia concentration and arterial pH in animals studied in micropuncture and microcatheterization experiments[a]

	Control	Acute acidosis	Acute acidosis plus mannitol
Peripheral arterial plasma, *μmoles ml⁻¹*	0.06 ±0.01	0.09[b] ±0.01	0.09[b] ±0.01
Renal venous plasma ammonia, *μmoles ml⁻¹*	0.10 ±0.01	0.20[b] ±0.02	0.20[b] ±0.02
Vasa recta plasma ammonia, *μmoles ml⁻¹*	9.2 ±1.5	22.5[b] ±2.7	8.0 ±1.2
Peripheral artery pH	7.41 ±0.02	7.26[b] ±0.02	7.26[b] ±0.02

[a] Values are mean ± SEM.
[b] $P < 0.05$ compared to the control group.

groups, respectively. As previously reported renal ammonia production and urinary ammonia excretion increased significantly above control within 60 min after administration of NH_4Cl in both experimental groups [9].

In these experiments the ammonia concentration in renal venous plasma was used to estimate the ammonia level in the interstitium of the cortex, as previously suggested [10], and the ammonia concentration in papillary vasa recta was used to estimate the level in the interstitium of the inner medulla. The average concentrations of ammonia in arterial and renal venous plasma and in vasa recta plasma are shown in Table 2.

In control animals there was a steep concentration gradient for ammonia between vasa recta plasma and renal venous plasma. The vasa recta level of 9.2 ± 1.5 μmoles/ml was nearly 100-fold greater than the estimated level of 0.10 ± 0.01 μmoles/ml in renal venous plasma. Following induction of acute acidosis the renal venous level increased to 0.20 ± 0.02 μmoles/ml in both experimental groups, while the vasa recta plasma level was influenced by the presence or absence of mannitol infusion. In the absence of mannitol total ammonia concentration in the medulla rose twofold above the control value to 22.5 ± 2.7 μmoles/ml. In contrast, the medullary level of 8.0 ± 1.2 μmoles/ml was not significantly different from control in acidotic animals infused concurrently with mannitol.

Net ammonia transport in the inner medullary collecting duct

Water absorption along the inner medullary collecting duct was estimated from the $(TF/P)_{In}$. In control animals the fractional delivery of water decreased from 3% at the beginning of the inner medullary collecting duct to 0.9% at the orifice ($y = 69x + 39$, $r = 0.53$). Acute metabolic acidosis induced a significant increase in urinary flow rate and decreased fractional water absorption in the terminal nephron segment. Fractional delivery of water was 5% at the origin and 2% at the end of the inner medullary collecting duct in acidotic animals ($y = 40x + 24$, $r = 0.61$); corresponding values in the acidotic animals with mannitol infusion were 6 and 3%, respectively ($y = 15x + 17$, $r = 0.38$).

To correlate the findings obtained from the microcatheterization studies with medullary ammonia levels, obtained in a separate and smaller group of animals, it was important to show that C_{In}, RBF, urinary acidification, and renal ammonia metabolism were comparable between the two groups. The summary of average values of these parameters in animals studied by microcatheterization are shown in Table 3 and can be compared to Table 1 which shows the same parameters in animals studied by micropuncture. After correction for body weight there were no substantial differences in the function of the right kidney between the two animal groups of differing body weight. It seemed reasonable to assume therefore, that renal function and renal ammonia metabolism were also comparable between the left kidney of animals studied by micropuncture, in which measurements were not performed due to the absence of the ureter; the left experimental kidney of animals was prepared for microcatheterization. Since C_{In} is reflected by E_{In}, it should be noted that the value of E_{In} in the left micropunctured kidney was 0.37 ± 0.01 and 0.35 ± 0.01 in the microcatheterized kidney. The similarity between E_{In} values provides additional support for the suggestion that overall renal function, when corrected for body weight, was comparable in the two animal groups of differing body weight used in these experiments. As previously reported the C_{In} was reduced in the left experimental kidney prepared for microcatheterization compared to the contralateral kidney [11], and in this study the RBF was decreased proportionately. The absolute level of C_{In} in the left experimental kidney of approximately 400 μl min^{-1} 100 g body wt^{-1} was also comparable to the values reported by this and other laboratories [2, 4, 11], although C_{In} in the right contralateral kidney was somewhat higher than previously reported [11]. Since urine was exposed briefly to air during collection under suction from the left kidney, and therefore was associated probably with loss of carbon dioxide, the urine pH of the microcatheterized kidney is not reported. This assumption is supported by evidence of water evaporation in urine collected, under these conditions, from the microcatheterized kidney, since inulin concentration increased approximately twofold between fluid collected at the orifice of the duct and samples of collected urine.

Changes in tubular fluid concentration of ammonia along the inner medullary collecting duct in controls and the two groups with acute acidosis are shown in Figure 2. In controls the ammonia levels rose from approximately 2 μmoles/ml at the beginning of the ductal segment to 18.9 ± 3.2 μmoles/ml at the orifice of the duct. In acidotic animals the estimated concentration of ammonia at the origin of the inner medullary collecting duct was 3.6 μmoles/ml and rose sharply to 50.1 ± 6.1 μmoles/ml. During administration of mannitol to acidotic animals, in contrast, the rise in ammonia concentration along the duct was blunted and the concentration of ammonia at the origin of the tubular segment was 15.6 and 25.8 ± 3.8 μmoles/ml at the orifice. The levels of ammonia at the origin and end of the inner medullary collecting duct in control and acidotic groups are in the same range that were reported by Graber et al under similar experimental conditions using different assay techniques (4).

Net movement of ammonia along the inner medullary collecting duct in control and experimental animals is shown in Figure 3. These data represent tubular fluid ammonia levels, corrected

Table 3. Summary of renal function in animals studied by microcatheterization of the medullary collecting duct[a]

	Control		Acute acidosis		Acute acidosis plus mannitol	
Number of animals	6		6		6	
Body weight, *g*	200.3 ±20.9		197.7 ±4.7		216.2 ±9.1	
	(R)	(L)	(R)	(L)	(R)	(L)
C_{In}, *μl min^{-1} 100 g body wt^{-1}*	763 ±63	373 ±62	729 ±38	392 ±33	696 ±44	434 ±28
RBF, *μl min^{-1} 100 g body wt^{-1}*	3430 ±282	1674 ±282	3478 ±145	1844 ±137	3180 ±191	2089 ±91
$\dot{V}$, *μl min^{-1} 100 g body wt^{-1}*	1.0 ±0.1	1.5 ±0.7	3.1[b] ±0.5	4.3[b] ±1.0	6.2[b] ±0.4	9.5[b] ±1.1
UpH	6.07 ±0.17		5.40[b] ±0.05		5.57[b] ±0.08	
Ammonia excretion, *μmoles min^{-1} 100 g body wt^{-1}*	0.04 ±0.004	0.03 ±0.003	0.42[b] ±0.07	0.21[b] ±0.04	0.26[b] ±0.03	0.22[b] ±0.02
Ammonia production, *μmoles min^{-1} 100 g body wt^{-1}*	0.18 ±0.01	0.10 ±0.02	0.77[b] ±0.07	0.40[b] ±0.04	0.62[b] ±0.04	0.46[b] ±0.01

Abbreviations: (R), (L), right and left kidneys, respectively.
[a] Values are mean ± SEM.
[b] This value represents a comparison with values in the control group.

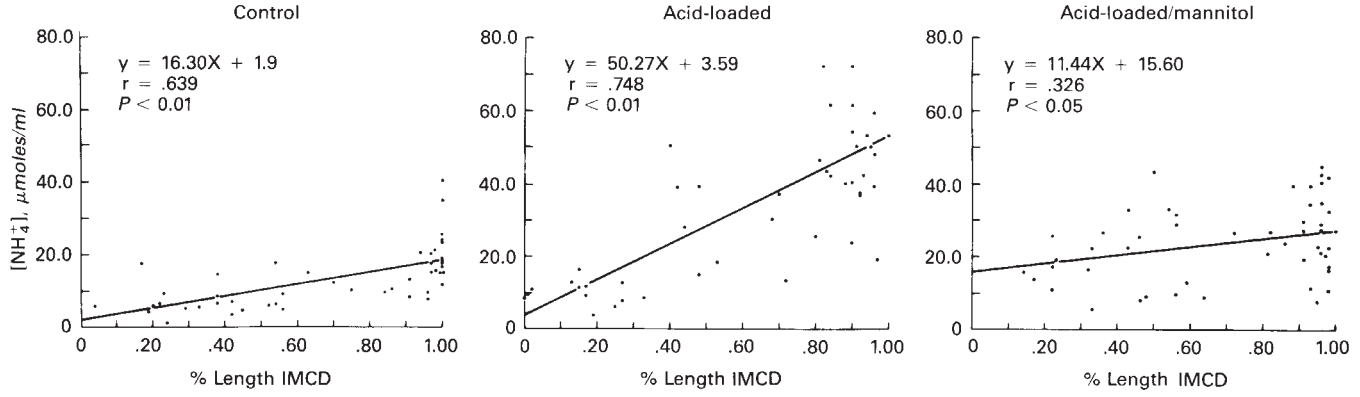

Fig. 2. *Ammonia concentration along the length of the inner medullary collecting duct (IMCD).* The value zero percent on the *abscissa* indicates the beginning of the IMCD and 100% indicates the orifice of the duct. The *regression line*, by the least-squares method, is shown for each group.

for water movement, in all paired collections in which samples were obtained at some site along the inner medullary collecting duct and at the ductal orifice: The average length between collection sites was approximately 3.0 mm. For the purpose of illustration the scale of values along the ordinate in Figure 3 is reduced in the two experimental groups compared to control.

In control animals there was no evidence of net movement of ammonia in the inner medullary collecting duct. The rise in ammonia concentration was therefore, due to water absorption. The mean slope of ammonia delivery between collection sites, computed from the slope of individual paired collections, was +0.01 ± 0.01 per mm; this value was not statistically different from zero by the χ^2 test. Inspection of Figure 3, however, indicates that acute acidosis resulted in net secretion of ammonia into tubular fluid along the terminal portion of the collecting duct. The mean slope, computed from the individual pairs, was +0.19 ± 0.05 per mm; this value was statistically different from zero ($P < 0.01$). In the acidosis plus mannitol group of animals the mean slope of the paired samples of −0.08 ± 0.06 per mm was not statistically different from zero, indicating that the net addition of ammonia did not occur in the inner medullary collecting duct.

Because net transport of ammonia was not detected along the inner medullary collecting duct in either controls or in the acidotic plus mannitol group, there was no evidence that secretion of ammonia by this nephron segment contributed to urinary ammonia in these groups. In the absence of mannitol infusion, however, acidosis was associated with a substantial contribution of the inner medullary collecting duct to urinary excretion of ammonia. Using Eq. *(4)*, presented in **Methods,** ammonia secretion in the inner medullary collecting duct accounted for approximately 44% of urinary ammonium.

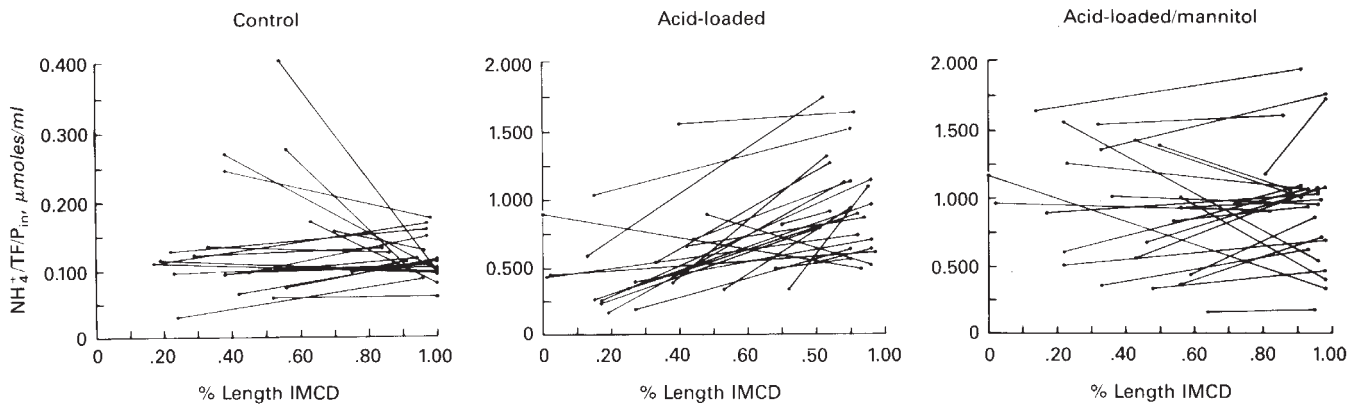

Fig. 3. *Ammonia concentration, corrected for water movement, along the inner medullary collecting duct.* Individual paired collections of tubular fluid are connected by *lines*. The scale of the *abscissa* is the same as in Figure 2.

Discussion

Urinary ammonia is derived from the renal pool of ammonia, which is formed from ammonia entering the kidney in arterial blood and from ammonia produced in renal tubular cells. Hayes et al [1] and Sajo et al [2] demonstrated that 70 to 100% of urinary ammonia is present in late portions of the proximal tubule in both control and acidotic animals. In past years investigators suggested that some of the ammonia secreted into the proximal tubule may gain access to urine by directly diffusing from the loop of Henle into collecting duct fluid, thus bypassing intervening portions of the distal nephron [3, 5]. According to this hypothesis, the alkalinity of tubular fluid in the loop increases due to an increased concentration of residual base as a consequence of progressive water absorption. The fall in hydrogen ion concentration results in the generation of NH_3 from NH_4^+ which diffuses into medullary interstitium, and due to the countercurrent exchange process in the medulla a high diffusion gradient for the free base would occur to facilitate ammonia trapping in acidified collecting duct fluid.

Although there is no direct evidence to prove the validity of this hypothesis, a number of observations provide support for this process in renal transport of ammonia. First, Balagura and Pitts [3] demonstrated that after injecting similar small amounts of labeled ammonium lactate and creatinine directly into the renal artery of the dog, ammonia appeared in the final urine before creatinine, indicating that filtered ammonia bypassed some intermediate segment(s) of the nephron. This observation has been extended by micropuncture analysis of surface nephrons and microcatheterization studies of the inner medullary collecting duct. These studies have shown that while the bulk of ammonia which ultimately appears in urine is found in the late portion of the proximal tubule of surface nephrons, only 20 to 30% of excreted ammonia is present in the distal tubule; this observation is consistent with loss of tubular fluid ammonia during passage through the loop of Henle [2]. Furthermore, fractional delivery of ammonia to the bend in the loop of Henle exceeds the amount of ammonia in final urine in both control and acidotic animals [12], and net secretion of ammonia into the inner medullary collecting duct has been demonstrated under the same experimental conditions [4]. Second, Robinson and Owen [13] demonstrated a fivefold gradient in ammonia concentration in slices of dog kidney from nondiuretic animals between cortex and papillary tip. Because this gradient was found in animals with alkaline urine as well as with acid urine, it seemed unlikely that the observed gradient was explained by ammonia present in tubular urine in the medullary slices. Following the induction of an osmotic diuresis to reduce the corticomedullary solute gradient, the ammonia gradient was reduced to zero. Third, recent studies have demonstrated an increase in the pH of tubular fluid and rise in total carbon dioxide between end proximal tubule and the bend in the loop of Henle. DuBose et al [14], for example, reported that in control animals the pH and total carbon dioxide were 6.78 ± 0.03 and 8.1 ± 1.2 mM at end proximal tubule, compared to values of 7.39 ± 0.4 and 20.5 ± 1.5 mM, respectively, in loop fluid. The ratio TFP/P_{In} in the same control animals was 2.56 ± 0.06 at end proximal tubule and 7.70 ± 1.15 in loop fluid. In another report by Buerkert, Martin, and Trigg [15], the pH values were 6.87 ± 0.08 (end proximal tubule) and 7.39 ± 0.06 (loop of Henle) in control animals; corresponding levels of total HCO_3 were 8.76 ± 1.26 (end proximal tubule) and 22.2 ± 2.2 mM (loop of Henle). In acidotic animals the pH rose from 6.81 ± 0.05 to 7.16 ± 0.06 and total HCO_3 increased from 5.08 ± 0.47 to 16.6 ± 1.7 mM between end proximal tubule and the bend of the loop of Henle.

The present study was performed to examine this hypothesis in more detail. Because transport of ammonia from loop fluid into medullary interstitial fluid would be expected to result in a medullary concentration of ammonia higher than that in cortex, we sought to determine the gradient in controls, and in animals with acute metabolic acidosis in which the amount of ammonia delivered by deep loops is increased [12] and ammonia excretion is stimulated markedly by an increase in ammonia secretion by the collecting duct [4]. In this study we used the concentration of ammonia in mixed renal vein plasma as an index of ammonia concentration in cortical interstitial fluid and in vasa recta plasma as an index of medullary interstitial fluid. Denis, Preuss, and Pitts [10] provided evidence that the free base of ammonia is in equilibrium between the water phases in the cortex and that the pNH_3 in renal venous blood is an approximation of the mean value in the several populations of cells in the cortex. Recent in vitro studies have provided support for that suggestion although ammonia may not be in

equilibrium throughout all cortical structures. In isolated perfused tubule segments of the rabbit ammonia reached diffusion equilibrium across the proximal tubule, which constitutes a major portion of cortical mass, but not across the cortical collecting tubule [16]. Because the pH values of renal arterial blood and renal venous blood are not statistically different [9] and it seems reasonable to assume that there is no pH gradient between capillary blood and interstitial fluid, the concentration of total ammonia in renal venous plasma should provide an approximation of levels in cortical interstitial fluid. Similarly, it seems likely that the total ammonia concentration in papillary vasa recta plasma provides a reasonable estimate of levels in papillary interstitial fluid, as well as its components (NH_3 and NH_4^+). Because the ionic composition of the two phases is nearly identical, the values of pK_a should be similar in both vascular and interstitial compartments; there is no evidence that vessels can generate a pH gradient. It should be noted that these considerations would not have been true of whole blood, since red blood cell: plasma ratio for ammonia has been reported to be 3:5 [17].

These studies demonstrated an increase in ammonia concentration from 0.10 ± 0.01 μmoles/ml in renal venous blood to 9.2 ± 1.5 μmoles in the inner medulla of control animals, reflecting a concentration gradient of nearly one hundred-fold. Since exposure of the papilla by excision of the ureter reduces maximum concentrating ability and papillary osmolality [18], it seems probable that the measured levels of medullary ammonia underestimate the concentration in the unaltered kidney by at least 50%. During acute acidosis the medullary level rose to 22.5 ± 2.7 μmoles/ml, an increase of twofold above the control levels. These observed values of ammonia concentration in vasa recta plasma agree closely with reported ammonia levels in tubular fluid at the bend of the loop of Henle of deep nephrons of 11.3 μmoles/ml in control animals and 20.6 in animals with acute acidosis [12]. In this study, as will be noted later, and in previous studies [2, 4, 19] evidence has been provided for net secretion of ammonia into the medullary collecting duct. Therefore, it seems likely that the source for the high medullary levels of ammonia is derived from ammonia delivered by long loops, rather than primarily from backdiffusion from collecting duct fluid.

The concentration of medullary ammonia in acidotic animals was reduced during mannitol infusion to 8.0 ± 1.2 μmoles/ml, a value that was not statistically different from control, although nephrogenous production of ammonia and overall renal function were not altered by the infusion of mannitol compared to values in the group with acidosis alone. Presumably the concentration of ammonia and rise in pH in loop fluid were blunted during mannitol diuresis since under this condition net water reabsorption has been shown to decrease in the loop of Henle [20]. These changes in the composition of loop fluid would tend to reduce the generation of the free base of ammonia and its movement from loop fluid to medullary interstitium. In addition, a mannitol-induced increase in medullary blood flow may have contributed to the reduction in medullary ammonia concentration by increasing the mass flow of solutes toward the cortex.

The hypothesis concerning movement of ammonia from loop fluid to collecting duct fluid involves the concept of non-ionic diffusion whereby the free base, NH_3, readily diffuses across cell membranes, probably as a result of its high lipid solubility, to reach equality of concentration on the two sides of the membrane while the ionized form, NH_4^+, in equilibrium with free base at the existing hydrogen ion concentration, accumulates on the more acid side. According to this concept the net rate of diffusion across cell or tubular membranes will depend on the difference in concentration of NH_3 across the membrane and if the pH and total ammonia concentration remain relatively constant on one side of the membrane, then the ammonia concentration on the other side will be a logarithmic function of the pH on that side. Under the condition of an existing pH gradient across the wall of the collecting duct therefore, where the pH of tubular fluid is lower than that of interstitial fluid, the rate of net ammonia secretion should depend on the level of ammonia in the interstitial compartment. To determine whether these predictions obtained in the inner medulla are valid net movement of ammonia into duct fluid was correlated with the level of ammonia in vasa recta plasma, used as an index of interstitial ammonia.

Although there was no evidence of net ammonia secretion in the inner medullary collecting duct in controls with a medullary ammonia level of approximately 10 μmoles/ml, ammonia secretion promptly occurred during acute acidosis in association with a doubling in the level of medullary ammonia. The increase in ammonia secretion probably occurred in association with a fall in pH of tubule fluid in inner medullary collecting duct, because urinary pH fell significantly in the right kidney. Previous reports indicate parallel changes in tubule fluid pH, measured in situ in the experimental kidney, and in urine of the contralateral kidney [4, 15]. A fall in tubular fluid pH, however, was not the major factor responsible for the change in net movement of ammonia, because reduction in the medullary level of ammonia in acidotic animals with a mild osmotic diuresis, in the absence of a significant change in urinary pH, eliminated net ammonia secretion. These results, therefore, are consistent with the view that net ammonia secretion depends on elevation of interstitial ammonia concentration to a critical level to maintain a favorable concentration gradient for NH_3, concurrently with a rise in ammonia concentration in tubular fluid due to water absorption.

It should be noted that the absence of net secretion of ammonia in control animals is at variance with observations made by other investigators in which net transport of ammonia in the medullary collecting duct was examined by the microcatheterization technique [4, 19], but agrees with a recent report by Buerkert, Martin, and Trigg in which micropuncture techniques were used [12]. The disagreement between these studies in control animals is probably accounted for by technical differences involving the length of the tubular segment examined, reduction in papillary solute concentration, diet, and similar variables. Both this and previous studies, in which the microcatheter technique has been used agree, however, that acute acidosis stimulates ammonia secretion in the medullary collecting duct [4].

Since pH was not measured in vasa recta plasma or collecting duct fluid, a quantitative assessment of the driving forces acting on ammonia transport cannot be made. If changes in urinary pH of the right kidney, however, reflect the acidity of tubular fluid in the experimental kidney, it is apparent that the concentration of ammonia in tubular fluid was not proportional to the level predicted by the concept of non-ionic diffusion. According to

this concept the ammonia concentration in tubular fluid would increase tenfold for each decrease in pH of 1 U. Previous attempts to apply the principles of non-ionic diffusion to the mechanism of ammonia secretion in the kidney also have resulted in findings which show that equilibrium is not achieved. In studies in the rat by Leonard and Orloff [21], for example, a decrease in urine pH of 3 pH U was associated with an increase in ammonia excretion of only five- to tenfold, in contrast to the 1000-fold increase predicted.

At least three factors must be considered for diffusion equilibrium to occur, *(1)* permeability of the tubular epithelium to free base, *(2)* impermeability of the epithelium to ammonium ions, and *(3)* the rate of formation of ammonia. It is possible, therefore, that free-base permeability may be rate limiting so that in the length of tubular segment involved and during the time of fluid contact with tubular membrane the free base does not reach equilibrium across the collecting duct wall. Alternatively, if the epithelium is relatively permeable to the ionic form of ammonia, back diffusion of ammonium ions may limit accumulation in tubular fluid. It is relevant that Goldstein, Claiborne, and Evans [22] have reported recently that the free base of ammonia is not lipid soluble, because at 25°C the $CHCl_3/H_2O$ partition coefficient for NH_3 is only 0.04, compared to other compounds that are considered to be lipid soluble which exhibit a partition coefficient of greater than 1. In summary, the reason for the failure of ammonia secretion to follow the quantitative predictions of the theory of non-ionic diffusion is not clear, but the discrepancy has been observed previously in the dog [23], as well as in the rat.

Since medullary re-cycling of urea has been shown to play an important role in urinary concentrating ability and impairment of that process reduces maximal concentrating capacity, what role can medullary ammonia cycling have in the renal excretion of ammonia? Although the present study provides no direct information on this issue, it is tempting to speculate that at low rates of urinary flow medullary cycling of ammonia may serve to maintain high urinary ammonia levels and hence, enhance absolute excretion. In the absence of this process the return of loop fluid with a high concentration of ammonia to cortical regions of the kidney may result in back diffusion of ammonia due to the low concentration in cortical interstitium. In addition, these studies are consistent with the concept that corticomedullary nephrons with long loops of Henle make a substantial contribution to ammonia excretion, by delivering ammonia to medullary interstitial fluid as a source for secretion by the medullary collecting duct. Pathological states associated with damage or loss of deep cortical nephrons, therefore, could be expected to result in impaired ability for maximal excretion of ammonia. It is of interest, that reduced ammonia excretion has been demonstrated in patients with chronic obstructive nephropathy [24] and in subjects with sickle cell nephropathy [25], by a mechanism not explained by a decrease in renal mass or urinary acidification. In addition in experimental animals papillectomy has been reported to result in impaired ammonia excretion in the absence of changes in urinary acidification [26].

In conclusion, these studies demonstrate a steep corticomedullary gradient for ammonia and provide support for the hypothesis that ammonia formed in the proximal tubule diffuses from the loop of Henle into collecting duct fluid, thus bypassing intermediate nephron segments. In addition these studies provide evidence that the level of ammonia in medullary interstitium is a determinant of net movement of ammonia across the wall of the inner medullary collecting duct.

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