Ammonia Production by Individual Segments of the Rat Nephron

David W. Good and Maurice B. Burg
Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, Bethesda, Maryland 20205

Abstract. Ammonia production was measured directly in 10 segments of the rat nephron to determine the relative importance of the segments as sites of renal ammonia production. Tubules were microdissected from normal rats and rats drinking 0.28 M NH_4Cl or 0.28 M $NaHCO_3$ for 3–8 d. The segments were incubated in vitro with and without 2 mM glutamine. Ammonia concentrations in the incubation fluid were measured by microfluorometry to determine ammonia production rates. All segments produced ammonia from glutamine. In normal rats, production with glutamine was highest (>5 pmol/min per mm) in the proximal convoluted (S-1), proximal straight (S-3), and distal convoluted tubules, and lowest (≤2) in cortical and medullary collecting ducts and thin descending limbs. Metabolic acidosis increased production by 60% in the S-1 segment of the proximal convoluted tubule and by 150% in the S-2 segment of the proximal straight tubule without significant effect in any other segment. Bicarbonate loading decreased production by S-1 but had no effect on S-2 or S-3. Thus, acid-base changes altered production only in specific segments of the proximal tubule. We infer that the bulk of ammonia production occurs in the proximal tubules and that production by collecting ducts can account for only a few percent of renal ammonia production and excretion in the rat.

Introduction

Ammonia produced by renal cells and secreted into tubule fluid is the principal urinary buffer in most mammals, including rat, dog, and man (1). During acute or chronic metabolic acidosis the rate of renal ammonia production increases and excess hydrogen ions are excreted in the urine largely in the form of ammonium ions (1–5). This increase in production is usually associated with increased uptake and metabolism by kidney cells of the amino acid glutamine (1, 6, 7). An adaptive change also occurs with metabolic alkalosis that causes renal ammonia production and excretion to decrease (1, 8–10).

To understand renal ammonia handling, it is necessary to identify the specific sites within the kidney where ammonia is produced. Despite the fundamental importance of this question, there have been no extensive, direct studies of ammonia production by single nephron segments. Previous information regarding sites of renal ammonia production has come primarily from indirect studies in which the amounts of ammonia-producing enzymes have been assayed in particular zones or segments of the kidney (11–16). However, interpretation of these studies is complicated by the fact that enzyme activity in intact tubule cells may differ from that measured in broken or freeze-dried cell preparations. In addition, different studies in rats are not in complete agreement regarding the quantitative distribution among different nephron segments of the major ammonia producing enzyme, phosphate-dependent glutaminase (PDG)[1] (12, 15). These studies also differ as to which tubule segments have increased glutaminase activity during metabolic acidosis.

In the present study we have developed methods that permit direct measurement of ammonia production by individual segments of the rat nephron. Microdissection and in vitro incubation techniques were used in conjunction with a microfluorometric assay to measure ammonia production by intact tubule segments. Our aims were to evaluate rates of production among different segments of normal rats and to determine which segments alter their rate of production in response to changes in systemic acid-base balance.

Methods

Male Sprague-Dawley rats (Taconic Farms Inc., Germantown, NY) weighing 155–255 g were allowed free access to food (NIH diet No. 07,

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Address correspondence and reprint requests to Dr. David W. Good, National Institutes of Health, Laboratory of Kidney and Electrolyte Metabolism, National Heart, Lung, and Blood Institute, Building 10, Room 6N307, Bethesda, MD 20205.

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1. *Abbreviations used in this paper:* CAL, cortical thick ascending limb; CCT, cortical collecting tubules; DCT, distal convoluted tubules; DL, descending limbs of Henle's loop; IMCD, inner medullary collecting ducts; MAL, medullary thick ascending limb; OMCD, outer medullary collecting ducts; PDG, phosphate-dependent glutaminase; S-1, S-2, S-3, segment 1, 2, and 3 of proximal tubule.

Ziegler) and were divided into three groups. One group (control) received tap water to drink. A second group (metabolic acidosis) was given 0.28 M NH_4Cl to drink for 3–7 d before experiments. A third group drank 0.28 M $NaHCO_3$ for 4–8 d before experiments. Blood was collected at the time of death into heparinized beakers and, whenever possible, urine in the bladder was collected. Plasma total carbon dioxide concentration was measured immediately using a carbon dioxide analyzer (model E-100, Ericsen, Corning Medical Supply, New York, NY) and urine pH was measured with a microcombination electrode (MI-40, Microelectrodes, Inc., Londonderry, NH).

Solutions. Two solutions were used for experiments. Solution I contained (in mM): 117 NaCl, 25 $NaHCO_3$, 2.5 K_2HPO_4, 1.2 Na_2SO_4, 2.0 Ca lactate, 0.5 Mg citrate, and 5.5 glucose. Solution II was identical except for the addition of 2.0 mM L-glutamine (Sigma Chemical Co., St. Louis, MO). Each solution was equilibrated with 95% oxygen/5% CO_2. Measured pH of the solutions at 37°C was 7.45.

Collagenase perfusion. The rats were killed by decapitation and the left kidney was perfused in situ with collagenase as follows: 100 mg of collagenase (type 1 or 1A, Sigma Chemical Co.) was dissolved in 50 ml of solution I. The solution was warmed to 37°C in a water-jacketed beaker and bubbled continuously with 95% O_2/5% CO_2. A polyethylene catheter was inserted into the animals' aorta immediately below the left renal artery. The catheter was connected to a peristaltic pump (model 2-6150, Buchler Instruments, Inc., Fort Lee, NJ) that perfused the warmed collagenase solution through the left kidney at 2.2–2.5 ml/min for 15 min. To insure that only the left kidney was perfused, the aorta was ligated between the two renal arteries. In some cases a syringe was used to flush the kidney with 2–3 ml of collagenase solution before pump perfusion. This method of perfusion permits the kidney to remain oxygenated during collagenase treatment and reduces the time required for adequate digestion (17).

Tubule dissection. Following perfusion, two or three slices were cut from the center of the kidney along the corticomedullary axis. The slices were placed in 5–8 ml of solution I that was cooled to 10°C and suffused continuously with 95% O_2/5% CO_2. Nephron segments were dissected using a Wild M8 stereomicroscope (Wild Heerbrugg, Ltd., Heerbrugg, Switzerland). Glomeruli, proximal convoluted (S-1) and distal convoluted tubules (DCT) were dissected from the cortical labyrinth. The S-1 segments were always observed to be attached to a glomerulus and did not exceed 1 mm in length (18). DCT were distinguished from other distal tubule segments by their convoluted shape, bright appearance, and absence of clear definition of single cells under high magnification.[2] Care was taken to exclude the straighter, more granular appearing connecting tubule segment into which the DCT gradually transforms. The S-2 portion of the proximal straight tubule was dissected from medullary rays in the outer half of the cortex; the S-3 portion of the proximal straight tubule was taken from the outer stripe of the outer medulla (18). Cortical portions of thick ascending limb (CAL) and cortical collecting tubules (CCT) were dissected from corticomedullary rays. Thin descending limbs of Henle's loop (DL), medullary portions of thick ascending limb (MAL), and outer medullary collecting ducts (OMCD) were taken from the inner stripe of the outer medulla. Inner medullary collecting ducts (IMCD) were dissected from the inner medulla/papilla. Total dissection time was limited to 30–60 min for each experiment. Lengths of tubule segments were determined immediately following dissection using an ocular micrometer. In most cases only one type of tubule was dissected and studied from each kidney.

2. Collagenase concentration was increased by 30% in some experiments with DCT to facilitate their dissection. Production rate did not differ with the two collagenase concentrations.

In vitro incubation. In each experiment, dissected tubule segments were divided into two groups for incubation in vitro. Each group contained three to seven segments totaling 2.0–5.5 mm in length depending on the anticipated rate of ammonia production. One group was rinsed in the glutamine-free solution (solution I) and the other was rinsed in the glutamine-containing solution (solution II). The rinse solutions were at room temperature and were equilibrated with 95% O_2/5% CO_2. Each group of tubules was then drawn along with its rinse solution into the tip of a variable-stroke piston pipette (Gilson Model P-20, Rainin Instrument Co. Inc., Woburn, MA) that was set to collect a volume of 2.5 μl. The tubules and fluid were deposited from the pipette onto a glass slide that was siliconized and bathed in mineral oil. The oil was warmed to 37°C and was equilibrated with 95% O_2/5% CO_2 and water. It was bubbled vigorously with that gas mixture throughout each experiment and the bubbling oil was propelled continuously over the fluid droplets by means of an air-lift recirculation system. In addition, a jet of the gas was focused on the surface of the oil immediately above the droplets. The gas jet not only maintained an atmosphere of 95% O_2/5% CO_2 above the tubules but also helped stir the fluid within the droplets. This effect, plus vibration of the droplets from bubbling of the oil, produced sufficient mixing to agitate the tubules visibly within the droplets.

The microliter pipette used to transfer tubule segments to the incubation slide was calibrated gravimetrically each day. The volume of incubation droplets varied from 2.2 to 2.7 μl on different days with a coefficient of variation of <10% for repeat measurements on the same day.

Determination of ammonia production rates. Production of ammonia by the tubule segments caused its concentration to rise linearly in the incubation droplets (see Results). This rise was measured by sampling 14-nl volumes from each droplet at 5–10-min intervals. The nanoliter samples were taken using a calibrated constriction pipette. Ammonia in each sample was measured fluorometrically as described below. Total incubation time was 40–60 min/experiment. Ammonia production rate in picomoles per minute per millimeter was determined for each droplet by dividing the slope of the linear regression line relating droplet ammonia content to incubation time by the total length of tubule in the droplet.

Three rates of ammonia production were calculated from each experiment: (*a*) total ammonia production measured in the presence of 2 mM glutamine; (*b*) ammonia production measured in the absence of glutamine and; (*c*) the difference between (*a*) and (*b*), which is the increment in ammonia production with added glutamine.

Ammonia in nanoliter samples was determined by microfluorometry as previously described (19). In brief, the method is based on the enzymatic conversion of ammonium ion and α-ketoglutarate to glutamate with associated oxidation of NADH. The decrease in NADH fluorescence is used to quantitate sample ammonia content. The method requires only that samples be injected into a flowing reagent stream and allows samples to be measured at intervals of 2–3 min without prior processing. For a sample volume of 14 nl, the method resolves differences of 0.02 mM between samples containing 0–0.5 mM ammonia. When 0.3 or 0.75 mM ammonia was added to solution I, the ammonia was recovered completely with a relative error of <2% (19).

Ammonia standard solutions containing 0, 0.1, and 0.5 mM ammonium chloride were measured alternately with samples from tubule incubation droplets. A standard curve was constructed for each experiment. The standard curves were linear over this concentration range (19). There was no detectable loss of ammonia from 2.5-μl droplets of standard solutions that were incubated in the recirculating oil bath for 40–60 min. Also, droplets of both experimental solutions were incubated without tubules during each experiment. In those, final ammonia con-

centrations were negligibly small. They averaged 0.035±0.014 mM (SD) (n = 113) for the glutamine-containing solution (solution II) and 0.018±0.011 (n = 111) for the glutamine-free solution (solution I). Even in experiments with segments that produced little ammonia in the absence of glutamine, the microfluorometric method was sufficiently sensitive to detect the ammonia production. Without glutamine in the incubation fluid, the measured ammonia concentration at the end of the experiments ranged from 0.04 to 0.30 mM, depending on the particular segment studied.

Statistical analysis. Rates of ammonia production for proximal tubule segments in the presence and absence of glutamine were compared among the three treatment groups using analysis of variance. For all other tubule segments, control and acidotic groups were compared using the unpaired t test. A P value of <0.05 was regarded as indicating significance.

Results

Mean body weight, plasma total carbon dioxide concentration, and urine pH measured on the day of experiments are summarized for all animals in Table I. The weight of the rats did not differ significantly before treatment. Control rats and rats drinking $NaHCO_3$ gained ~20–40 g during the period of treatment before experiments. The weight of acidotic rats remained stable during the period of NH_4Cl intake. In rats drinking NH_4Cl, both plasma total CO_2 concentration and urine pH were reduced significantly compared with controls. In rats given $NaHCO_3$, plasma total CO_2 and urine pH were significantly increased.

Two experiments are shown in detail in Fig. 1 to illustrate how ammonia production rates were determined. Panel *A* shows results for a segment (S-3) producing ammonia at a relatively high rate; panel *B* shows data for a segment (OMCD) producing ammonia at a relatively low rate. As detailed in Methods, production rates in the presence and absence of glutamine were determined in each experiment from the slope relating droplet ammonia content to time. Coefficients of determination (r^2) for this relation were calculated for each experiment (Tables II–IV). For tubules studied in the presence of glutamine, the mean value of r^2 ranged between 0.909 and 0.989. The linearity suggests that ammonia production was not importantly affected by the rise in droplet ammonia concentration that occurred over the 40–60-min experimental period.[3] Rates of ammonia production per millimeter tubule length did not correlate with the weight of the animals in any treatment group or with the total length of tubule contained in the incubation droplet.

Table I. Effect of NH_4Cl or $NaHCO_3$ on Body Weight, Plasma Total Carbon Dioxide Concentration, and Urine pH

Treatment group	Body weight	Plasma Total CO_2	Urine pH
	g	*mM*	
Control	216±4 (48)	27.9±0.3 (35)	6.54±0.10 (19)
NH_4Cl	180±3* (50)	17.5±0.5* (48)	5.71±0.03* (25)
$NaHCO_3$	200±6‡ (13)	30.0±0.4§ (10)	7.87±0.08* (11)

Values are means±SE. Numbers in parentheses indicate numbers of rats. P values vs. control (analysis of variance):
* $P < 0.001$.
‡ $P < 0.05$.
§ $P < 0.025$.

Ammonia production in control rats. Rates of ammonia production by nephron segments of control rats are summarized in Table II and Fig. 2. Ammonia was produced by all segments studied. The rate of production was always greater in the presence than in the absence of glutamine (Table II). However, total production rate (that with 2 mM glutamine) varied considerably among the different segments (total bar length, Fig. 2; Table II). This rate exceeded 5 pmol/min per mm in the S-1 and S-3 segments of proximal tubule and in the DCT. In contrast, total production averaged 2 pmol/min per mm or less in the three segments of collecting duct and in the thin DL. Total production rates were intermediate in the S-2 segment and in CAL and MAL.

Ammonia production measured in the absence of glutamine also varied among segments (open bar length, Fig. 2; Table II). Average values ranged from 5.5 pmol/min per mm in the DCT to <1 pmol/min per mm in the IMCD and thin DL.[4]

The increment in ammonia production with added glutamine (solid bar length, Fig. 2; Δ, Table II) was clearly highest in the S-1 and S-3 segments of the proximal tubule. The increment measured in these segments (2.8 pmol/min per mm in S-1; 5.3 in S-3) was three to five times higher than that observed in the remaining segments.

Although glomeruli produced ammonia, the rates were low relative to values obtained with tubule segments.

Effects of NH_4Cl administration. Ammonia production rates by nephron segments of rats given NH_4Cl are summarized in Table III and Fig. 2. Total ammonia production was increased by 150% in the S-2 segment and by 60% in the S-1 segment in response to metabolic acidosis. Administration of NH_4Cl did not increase ammonia production by these segments in the

3. In some experiments with S-1, the rate of rise in droplet ammonia content with time often decreased after 30 min. This decrease occurred in the presence and absence of glutamine and was observed with tubules from normal, acidotic, and alkalotic animals. Production rates for this segment were calculated from data obtained over the initial 20–30 min of the experiment, by which time the droplet ammonia concentration was 0.20–0.35 mM. The reason for the decline in production rate in this segment has not yet been determined.

4. With the three collecting duct segments and descending limbs, ammonia production in the absence of glutamine was very low and it was difficult to be sure that production was linear with time in some individual experiments. Nevertheless, in 29 of the 38 experiments performed with these segments the slope of the regression line differed significantly from zero and there was, on average, significant ammonia production without glutamine in each of these segments ($P < 0.025$; Tables II and III).

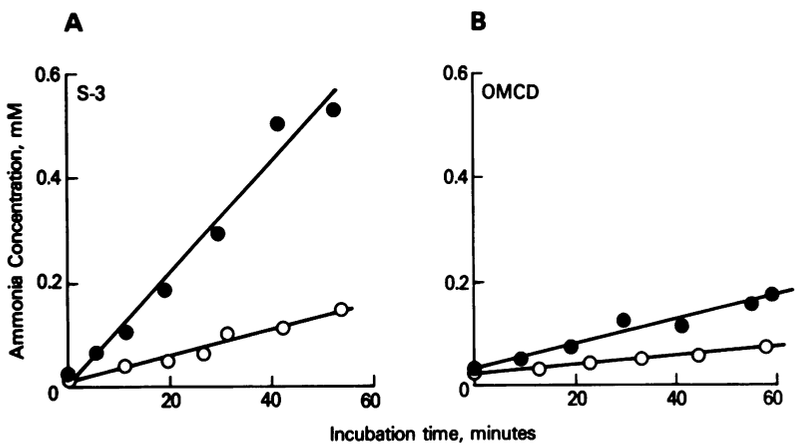

Figure 1. Increase in incubation droplet ammonia concentration with time in experiments with S-3 (panel *A*) and OMCD (panel *B*) segments. Filled circles (●) are for tubules incubated with 2 mM glutamine; open circles (○) are for tubules incubated without glutamine. The lines are least-squares regressions calculated for the filled or open circles. Sample calculation for panel *A*, filled circles: droplet volume = 2.4 μl; slope = 22.7 pmol/min; r^2 = 0.974; total tubule length = 3.0 mm; ammonia production rate = 7.6 pmol/min per mm.

absence of glutamine. Therefore, the increase in total ammonia production with acidosis resulted from increased synthesis of ammonia from added glutamine. Acidosis caused the increment in ammonia production with added glutamine to double in S-1 and to increase nearly five times in S-2 (solid bar length, Fig. 2).

With the exception of the DCT, metabolic acidosis had no significant effect on ammonia production in any other segment. In the DCT, total ammonia production rate did not differ from controls but production in the absence of glutamine was reduced by a small but significant amount.

Effects of $NaHCO_3$ administration. Ammonia production rates by nephron segments of rats given $NaHCO_3$ are shown in Table IV and Fig. 2. Only proximal tubule segments were studied. In the S-1 segment, total ammonia production was decreased by 20% compared with controls ($P < 0.05$, analysis of variance). $NaHCO_3$ administration did not reduce production measured in the absence of glutamine. Thus, the decrease in total production reflects a reduced rate of ammonia synthesis from added glutamine. Metabolic alkalosis had no detectable effect on production by either the S-2 or S-3 segments of the proximal straight tubule.

Discussion

Individual segments of the rat nephron were incubated in vitro to assess the relative importance of the segments as sites of renal ammonia production. The results show that all segments pro-

Table II. Ammonia Production by Nephron Segments of Control Rats

		Ammonia production rate			Coefficient of determination (r^2)	
		Glutamine			Glutamine	
Segment	*n*	2 mM	None	Δ	2 mM	None
		pmol/min/mm	*pmol/min/mm*	*pmol/min/mm*		
Glom*	3	0.6±0.06	0.3±0.09	0.3±0.01	0.984±0.002	0.873±0.090
S-1	6	5.6±0.3	2.8±0.5	2.8±0.5	0.955±0.004	0.931±0.020
S-2	8	2.8±0.2	1.5±0.2	1.2±0.3	0.974±0.007	0.858±0.053
S-3	5	7.5±1.0	2.3±0.2	5.3±0.9	0.987±0.004	0.917±0.026
DL	3	1.3±0.1	0.3±0.1	0.9±0.1	0.968±0.007	0.516±0.128
MAL	4	4.6±0.4	3.5±0.4	1.1±0.1	0.986±0.005	0.967±0.016
CAL	6	2.8±0.2	1.7±0.3	1.1±0.1	0.957±0.016	0.873±0.056
DCT	5	6.7±0.3	5.5±0.3	1.2±0.3	0.986±0.003	0.961±0.003
CCT	5	1.8±0.3	1.2±0.4	0.6±0.2	0.935±0.018	0.849±0.054
OMCD	5	2.1±0.5	1.0±0.2	1.1±0.3	0.957±0.010	0.852±0.044
IMCD	5	2.1±0.2	0.6±0.1	1.5±0.1	0.966±0.009	0.551±0.118

Values are means±SE, *n* indicates number of rats and experiments. Δ was calculated for each experiment as the difference with and without glutamine. All Δ values differ significantly from zero ($P < 0.05$), indicating that production with glutamine was greater than that without glutamine in each segment. Coefficients of determination are from linear regression analysis of droplet ammonia content vs. incubation time. * Production rates for glomeruli (Glom) are picomoles per minute per glomerulus.

Table III. Ammonia Production by Nephron Segments of Acidotic Rats

Segment	n	Ammonia production rate: Glutamine 2 mM	Ammonia production rate: Glutamine None	Ammonia production rate: Δ	Coefficient of determination (r^2): Glutamine 2 mM	Coefficient of determination (r^2): Glutamine None
		pmol/min/mm	*pmol/min/mm*	*pmol/min/mm*		
Glom*	3	0.4±0.07	0.2±0.05	0.3±0.07	0.964±0.015	0.739±0.175
S-1	4	8.8±0.7‡	2.6±0.2	6.1±0.8‡	0.984±0.007	0.901±0.028
S-2	6	7.0±0.9‡	1.3±0.3	5.6±0.9‡	0.984±0.005	0.820±0.061
S-3	4	7.6±0.5	2.3±0.1	5.3±0.6	0.978±0.017	0.953±0.015
DL	4	1.1±0.2	0.3±0.1	0.8±0.1	0.937±0.029	0.625±0.106
MAL	6	4.8±0.4	3.3±0.6	1.5±0.3	0.961±0.012	0.906±0.027
CAL	6	3.2±0.3	1.9±0.3	1.3±0.2	0.984±0.005	0.893±0.042
DCT	8	7.3±0.3	4.5±0.3§	2.8±0.5‖	0.989±0.003	0.966±0.017
CCT	5	1.8±0.3	1.0±0.2	0.7±0.3	0.909±0.028	0.872±0.072
OMCD	6	2.6±0.2	1.4±0.2	1.2±0.3	0.954±0.008	0.845±0.053
IMCD	5	2.5±0.3	0.9±0.1	1.6±0.3	0.936±0.025	0.774±0.073

Values are means±SE. Rats received 0.28 M NH_4Cl in drinking water for 3–7 d. *, n, Δ, and r^2 values same as in Table II. ‡ $P < 0.001$ vs. control (analysis of variance). § $P < 0.05$ vs. control (unpaired t test). ‖ $P < 0.025$ vs. control (unpaired t test).

duced ammonia from glutamine. Thus, all portions of the nephron could contribute to ammonia excretion in normal rats. Adaptive changes in ammonia production in response to changes in systemic acid-base balance occurred only in specific parts of the proximal tubule. Production by the S-1 segment of the proximal convoluted tubule was increased by metabolic acidosis and decreased by metabolic alkalosis. Production by the S-2 segment of the proximal straight tubule was increased by metabolic acidosis but was unaffected by metabolic alkalosis. Production by the S-3 segment of the proximal straight tubule was unaffected by either treatment. Although the remaining segments of the nephron all produced ammonia, production by these segments did not change significantly with metabolic acidosis.

Tubule segments were incubated in the presence and absence of L-glutamine as the only amino acid substrate. Glutamine was chosen since regulation of renal ammonia production in response to changes in acid-base balance has been shown to involve changes in cellular uptake and metabolism of this amino acid (1, 6, 7). Although ammonia production was always greater in the presence than in the absence of glutamine (Table II), the tubule segments produced ammonia without amino acids in the incubation fluid. This production presumably results from metabolism of endogenous amino acids.[5] Renal cortical slices, cortical tubule suspensions, and isolated perfused kidney all produce ammonia when no free amino acids are provided in incubation or perfusion fluids (20–22). Importantly, acid-base changes altered ammonia production only when glutamine was present. Increased production in S-1 and S-2 with metabolic acidosis was due to increased production from added glutamine; decreased production in S-1 with metabolic alkalosis was due to decreased production from added glutamine. These results are consistent with the observation in vivo that glutamine is the only amino acid extracted from renal blood in increased amounts during chronic metabolic acidosis (4, 23).

It is possible to weight the production of the different nephron segments using their measured rates of production per unit length (Tables II and III) and the total length of each segment per nephron.[6] By comparing the weighted production of each segment with total ammonia produced per nephron (obtained by summing the weighted productions of all the segments), it is possible to estimate the percent contribution of each segment to total renal ammonia production. This analysis permits the following general conclusions to be drawn regarding the relative importance of the different segments as sites of renal ammonia production: (*a*) Because of their relatively high rates of production and large tubule mass, the proximal tubules account for the bulk of renal ammonia production. Production by the

5. In our experiments, production measured without glutamine could not have been due to release of preformed ammonia from cells because: (*a*) ammonia permeates cell membranes rapidly (37) so that any ammonia present within the cells would already have been lost into the ammonia-free solution during dissection; (*b*) initial cell ammonia concentrations of at least 70–100 mM would be required to account for even the lowest production rates observed.

6. The following tubule lengths per nephron (in millimeters) were assumed based on prior observations (18, 28) and on observations made during the course of the present study: S-1 (3 mm), S-2 (5), S-3 (2), DL (4), MAL (2), CAL (3), DCT (1.5), CCT (0.5), OMCD (0.3), and IMCD (0.3). The latter three values were obtained by assuming that an average of six nephrons merge to form one collecting duct (28).

Table IV. Ammonia Production by Proximal Tubule Segments of Alkalotic Rats

Segment	n	Ammonia production rate			Coefficient of determination (r^2)	
		Glutamine			Glutamine	
		2 mM	None	Δ	2 mM	None
		pmol/min/mm	*pmol/min/mm*	*pmol/min/mm*		
S-1	7	4.5±0.2*	3.1±0.3	1.4±0.3*	0.950±0.010	0.952±0.014
S-2	4	2.5±0.2	0.9±0.2	1.7±0.2	0.967±0.010	0.832±0.058
S-3	4	6.7±0.6	1.6±0.3	5.1±0.6	0.989±0.003	0.936±0.034

Values are means±SE. Rats received 0.28 M $NaHCO_3$ in drinking water for 4–8 d. * $P < 0.05$ vs. control (analysis of variance). *n*, Δ, and r^2 values same as in Table II.

S-1, S-2, and S-3 segments could account for at least 60–70% of ammonia produced per nephron from normal rats and at least 70–80% of production per nephron from acidotic rats. In contrast, in nephrons from both control and acidotic animals the combined contribution of the CCT, OMCD, and IMCD was only 2–3%. (*b*) Ammonia produced by medullary collecting ducts in vitro can account for only a small fraction of ammonia excretion by rats in vivo. The total length of OMCD plus IMCD in a single rat kidney is ~16,000 mm (Knepper, M., unpublished observations). With a production rate of 2 pmol/min per mm (Tables II and III), these segments could account for ~0.032 μmol/min ammonia in normal and acidotic rats. When compared with ammonia excretion rate, which averages 0.2–0.6 μmol/min in normal rats and 1.0–1.9 μmol/min in acidotic rats (2, 3, 5, 24–26), the data suggest that ammonia produced by medullary collecting ducts could account for, at most, 2–10% of ammonia excretion. (*c*) It is likely that the above considerations underestimate the importance of the cortical segments (particularly S-1 and S-2) and overestimate the contribution of the medullary segments. This is so because ammonia production by renal cells and the activity of PDG have been shown to be reduced when extracellular ammonia concentration is increased (1, 7, 9). Therefore, production by segments such as MAL, DL, and medullary collecting ducts should be inhibited in vivo by the high ammonia concentrations in the renal medulla, which may reach 10–20 mM (26, 27). In our experiments the extracellular ammonia concentrations did not exceed 0.5 mM and, thus, the effect of ammonia to inhibit production in medullary tubules should have been much less than expected in vivo. (*d*) By summing the contributions of the different segments it is possible to compare ammonia production per nephron obtained from our data with ammonia production measured in vivo. A single rat kidney contains ~30,000 nephrons (28) and normally produces 500–750 nmol/min ammonia (4, 5). Thus, ~20–25 pmol/min are produced per nephron in vivo. In our experiments, ~80 pmol/min of total ammonia was produced per nephron, of which 35 pmol/min resulted from increased production from added glutamine. One difference in our in vitro situation is that we used 2 mM glutamine in incubation fluid whereas plasma glutamine of normal rats is 0.4–0.7 mM (5, 23, 29, 30). Nu-

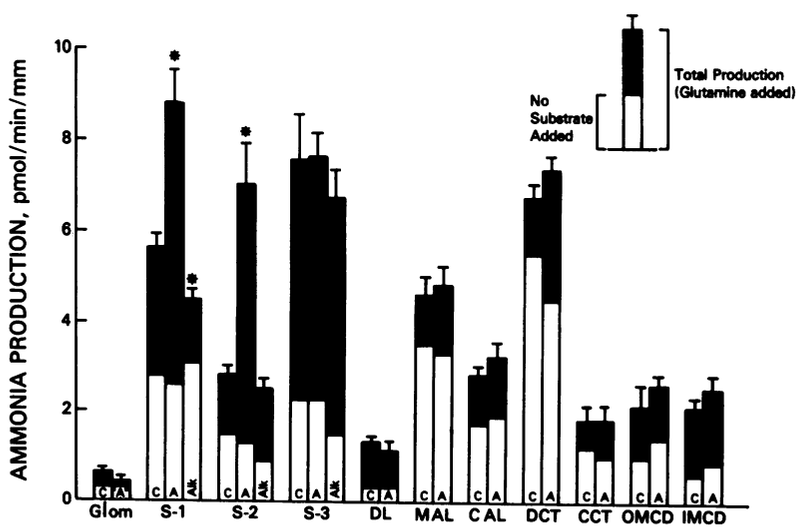

Figure 2. Ammonia production by nephron segments of control (C), acidotic (A), and alkalotic (Alk) rats. Total bar lengths and error bars indicate total ammonia production rates±SE measured in the presence of 2 mM L-glutamine. Open bar lengths indicate rates of production measured in the absence of glutamine. Shaded bar lengths indicate the increments in ammonia production with added glutamine. Asterisk indicates total production rate significantly different from control.

merous studies have shown that ammonia production is directly dependent on the concentration of extracellular glutamine (1, 20, 31). Considering, in addition, that production by medullary tubules is likely to be inhibited in vivo by the high ammonia concentrations in the medulla, the production rates that we measured are in reasonable agreement with those of the intact kidney.

Previous information regarding sites of renal ammonia production has come from measurement of enzyme activities in different regions and segments of the kidney. Much attention has been focused on localization of PDG since the glutaminase pathway is thought to account for most of renal ammonia production (6, 7). Glutaminase activity has been found in all zones and segments of the rat kidney (11–13, 15), an observation consistent with our finding that all nephron segments produced ammonia from glutamine. There has not been agreement, however, regarding the quantitative distribution of this enzyme among different tubule segments. Karnovsky and Himmelhoch (12) found PDG activity in normal rats was highest in the proximal straight tubule, intermediate in the proximal convoluted tubule and collecting ducts, and lowest in the DCT. Curthoys and Lowry (15) found PDG activity to be highest in the DCT and thick ascending limb, intermediate in the proximal convoluted tubule, and lowest in the proximal straight tubule. Although collecting ducts were not examined specifically in this study, PDG activity measured per gram dry weight was greater in the inner medulla than in the proximal convoluted tubules.

Our measured rates of ammonia production do not exactly match either of these patterns of glutaminase activity. We found total ammonia production in normal rats was highest in the S-3 segment of the proximal straight tubule and in the DCT and was lowest in the three segments of the collecting duct. The increase in production with added glutamine was clearly highest in the S-1 and S-3 segments of the proximal tubule (Table II). Thus, PDG activity measured under assay conditions in vitro does not correlate closely with ammonia production rates measured in intact tubule segments.

Although renal ammonia production and excretion are known to increase dramatically during metabolic acidosis, there has not been agreement regarding the specific nephron sites at which the increased ammonia production occurs. Curthoys and Lowry (15) found that NH_4Cl loading caused PDG activity to increase only in the early proximal convoluted tubule whereas Karnovsky and Himmelhoch (12) found that metabolic acidosis caused a generalized increase in PDG activity along the rat nephron. In the latter study, glutaminase increased in the DL, the DCT, and the papillary collecting duct in addition to the proximal convoluted tubule. In a subsequent study, Curthoys and Lowry (32) found that metabolic acidosis caused a decrease in cellular glutamine content in all proximal and distal tubule segments in the rat cortex. They suggested that all cortical segments may develop an increased capacity to degrade glutamine (32). Vinay et al. (33), using cortical tubule suspensions, which were enriched either in proximal or distal tubule fragments, found that metabolic acidosis caused ammonia production to increase only in the proximal enriched fraction but that glutaminase activity increased in both fractions.

In our study ammonia production was measured directly in tubule segments from control rats and rats given NH_4Cl or $NaHCO_3$. Since all segments were incubated under the same conditions, changes in production in response to changes in systemic acid-base balance reflect adaptive changes within tubules that persist when the external stimuli of acidosis or alkalosis are removed.

Our observation that metabolic acidosis increased ammonia production in the S-1 segment of the proximal convoluted tubule is in agreement with the increase in glutaminase found by Curthoys and Lowry (15). It seems likely, therefore, that increased glutaminase activity contributes to the increase in production in S-1. However, we also found a large increase in production in the S-2 segment of the proximal straight tubule whereas no effect of acidosis on glutaminase in the proximal straight tubule was reported (12, 15). Since, combining the results of all the studies, ammonia production correlated with glutaminase activity in S-1, but not in S-2, it is possible that the mechanisms regulating ammonia production may differ between these segments. Perhaps other enzymes such as phosphoenolpyruvate carboxykinase or glutamine synthetase (6, 7, 13, 14, 16, 29) play an important role in regulating production in the S-2 segment of the proximal tubule in the rat. It is also possible that glutaminase activity in S-2 increases with acidosis but that this increase is not detected when enzyme activity is assayed under optimal conditions in vitro.

In our measurements in the DCT, acidosis caused a small decrease in ammonia production measured in the absence of amino acids but no change in the total rate of ammonia production (Table III). Considering the equivocal nature of this change, and that the DCT constitutes a small fraction of total renal mass, we consider it unlikely that production by this segment plays a significant role in regulating renal ammonia excretion in the rat.

Metabolic alkalosis reduces renal ammonia production and excretion in the rat (1, 8–10). We found that administration of $NaHCO_3$ significantly decreased production in the S-1 segment but not in other parts of the proximal tubule (Table IV). The magnitude of this decrease (20%) was considerably less than the stimulation observed with acidosis. This result is consistent with that of Curthoys and Lowry (15) who found that PDG activity in the proximal convoluted tubule increased 20-fold with chronic metabolic acidosis but decreased only 40% with metabolic alkalosis. The observation that alkalosis caused a decrease in ammonia production in S-1 suggests that production by this segment is stimulated in normal, untreated rats.

It is of interest to compare sites of renal ammonia production with sites of ammonia secretion into tubule fluid. Since free base ammonia (NH_3) diffuses readily among different compartments of the kidney, secretion of ammonia by a tubule segment in the intact kidney does not necessarily reflect ammonia production by that segment. Accumulation of ammonia in tubule fluid may reflect "base trapping" as a result of luminal

acidification rather than actual production. Thus, sites of ammonia production cannot be determined accurately from the sites of entry of ammonia into tubule fluid in vivo.

Micropuncture studies in rats have shown that ammonia delivered to the end of the superficial proximal convoluted tubule can account for most or all of the ammonia appearing in the final urine and that proximal tubule secretion increases during metabolic acidosis (24–26, 34). These results are consistent with our finding that the bulk of ammonia production occurs in the proximal tubules and that metabolic acidosis causes production to increase in the S-1 and S-2 segments of the proximal tubule.[7] Punctures at the tip of the loop of Henle of deep nephrons of normal rats have shown that ammonia delivered to this site exceeds the amount delivered to micropuncture sites at the end of the surface proximal tubule (26). Also, ammonia delivery to the tip of Henle's loop increased with metabolic acidosis (26). During acidosis, the absolute delivery of ammonia to the loop tip increased more than that to the end of the accessible proximal tubule (26). Considering our results, the increase in delivery to the bend of Henle's loop with acidosis could be due, at least in part, to increased production of ammonia by the S-2 segment of the proximal straight tubule.

A number of micropuncture and microcatheterization studies have shown that substantial amounts of ammonia are secreted along the collecting ducts and that this secretion increases dramatically during metabolic acidosis (25–27, 35, 36). We found, however, that ammonia produced by collecting ducts could account for only a small fraction of total ammonia production and that there was no adaptive increase in production in collecting ducts with metabolic acidosis (Fig. 2). Evidently, a large fraction of the ammonia secreted into collecting ducts originates from the proximal tubules. Ammonia produced by proximal tubules is believed to be absorbed from the loops of Henle and secreted directly into the collecting ducts, by-passing the distal tubules (1, 26, 34). Thus, although the collecting ducts are an important site of ammonia secretion, that ammonia most likely is synthesized by the cells of the proximal tubules.

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