Metabolism of Glutamine and Glutamate by Rat Renal Tubules

STUDY WITH 15N AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY*

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Gas chromatography-mass spectrometry was utilized to study the metabolism of [1-15N]glutamate, [2-15N]glutamine, and [5-15N]glutamine in isolated renal tubules prepared from control and chronically acidic rats. The main purpose was to determine the nitrogen sources utilized by the kidney in various acid-base states for ammoniagenesis. Incubations were performed in the presence of 2.5 mM 15N-labeled glutamine or glutamate. Experiments with [5-15N]glutamine showed that in control animals approximately 90% of ammonia nitrogen was derived from 5- or glutamine versus 60% in renal tubules from acidic rats. Experiments with [2-15N]glutamine or [15N]glutamate indicated that in chronic acidosis approximately 30% of ammonia nitrogen was derived either from 2- or glutamine or glutamate-N by the activity of glutamate dehydrogenase. Flux through glutamate dehydrogenase was 6-fold higher in chronic acidosis versus control. No 15NH3 could be detected in renal tubules from control rats when [2-15N]glutamine was the substrate.

The rates of 15N transfer to other amino acids and to the 6-amino groups of the adenine nucleotides were significantly higher in normal renal tubules versus those from chronically acidic rats. In tubules from chronically acidic rats, 15N abundance in 15NH3 and the rate of 15NH3 appearance were significantly higher than that of the 6-amino group of adenine nucleotides or 15N-amino acids studied.

The data indicate that glutamate dehydrogenase activity rather than glutamate transamination is primarily responsible for augmented ammoniagenesis in chronic acidosis. The contribution of the purine nucleotide cycle to ammonia formation appears to be unimportant in renal tubules from chronically acidic rats.

The mechanism of stimulation of renal ammonia production in response to metabolic acidosis has posed one of the more complex and unsolved problems in metabolic regulation even after many decades of intense study. An important relationship between glutamine metabolism and renal ammoniagenesis was identified more than 40 years ago when Van Slyke et al. (1) demonstrated that the renal extraction of glutamine could account for the bulk of renal NH3 production. The centrality of glutamine as precursor for renal ammonia nitrogen was documented by Pitts et al. (2, 3), whose investigations represent a landmark in our understanding of renal ammoniagenesis. However, their research is subject to serious criticism, particularly with regard to the methods utilized for isotopic analysis. Thus, Pitts et al. (2, 3) failed to measure directly 15N in urinary and arterial NH3, glutamine (both amide and α-nitrogen), or glutamate. Such direct measurements could not be made with isotopic ratio-mass spectrometry, which quantitates isotopic abundance most accurately in ammonia, the end-product of the reaction. Precise determination of the contribution of all the major amino acid precursors to urinary ammonia nitrogen was not possible with isotopic ratio-mass spectrometry.

Chronic acidosis is accompanied by several metabolic alterations. Decreased concentrations of glutamine, glutamate, and citric acid cycle intermediates in the renal cortex of the intact animal indicate that the biochemical response is more widespread than a direct effect on glutamine utilization alone (4–6). Kinetic and nitrogen balance studies in isolated rat renal mitochondria suggest that glutamate deamination via glutamate dehydrogenase may be important in regulating tissue glutamate levels and, therefore, regulating ammonia production (7, 8).

A full understanding of the metabolic fate of glutamine in acidic states would require the demonstration of the extent to which the amino nitrogen of glutamate is converted to aspartate or ammonia.

Tanen (9) proposed that most glutamine taken up by the kidney is transported into the mitochondrial matrix, where it is metabolized by phosphate-dependent glutaminase to glutamate and NH3. The glutamate so formed can be metabolized by three possible mechanisms.

1. Oxidation to α-ketoglutarate and ammonia by glutamate dehydrogenase in which 1 mol of ammonia is produced per mol of glutamate utilized.
2. Transamination of glutamate with oxaloacetate to form α-ketoglutarate and aspartate.
3. Decarboxylation of glutamate by glutamate decarboxylase to form GABA.

After transamination to aspartate, glutamate-N may enter the PNC. The latter produces ammonia from aspartate in the cytosol by using catalytic amounts of IMP, adenosine, and AMP (10–12). Bogusky and Aoki (13), perfusing rat kidney with [15N]glutamate and employing isotopic ratio-mass spectrometry, showed that the purine nucleotide cycle was the major route for the production of 15NH3 from [15N]glut-
mate in control animals. Extrapolation of these findings to chronic metabolic acidosis may be unwarranted, however, since the experimental rats were pretreated with methionine sulfoximine to inhibit glutamine synthesis. Furthermore, the use of isotope ratio-mass spectrometry did not permit direct determination of isotopic abundance in important intermediates such as [15N]aspartate and [15N]alanine.

GC-MS, the sensitivity of which permits analysis of isotopic enrichment in vitro, represents an extremely powerful analytical tool in studies of this kind. We have already taken advantage of this sensitivity to study the metabolism of 15N in cultured brain cells (14, 15) and we have now applied these methods to the study of renal ammoniagenesis. A central purpose was to measure ammonia formation from [15N]glutamate, [2-15N]glutamine, and [5-15N]glutamine in tubules from normal and chronically acidic rats. In addition, we tested the hypothesis that the PNC has a major role in ammonia production (13).

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing between 300 and 320 g were obtained from Charles River Breeding Laboratories, Wilmington, DE. The rats were subjected to chronic metabolic acidosis by adding 1.5% NH4Cl and 20% sucrose to the drinking water for 7 days. Control rats received only 20% sucrose in their water. The animals were allowed free access to Purina chow until 15 h prior to killing by cervical dislocation.

In most studies, blood for arterial blood pH, pCO2, electrolytes, and metabolite measurements was obtained from the left renal artery of the unconscious rats.

Preparation and Incubation of Renal Cortical Tubules—Cortical tubules from metabolic acidic and control rats were prepared as described previously (16) using the modification of Guder et al. (17). For the measurement of total tubular metabolites, tubules (25–30 mg wet weight/ml) were incubated for the times indicated at 37 °C in 4 ml Krebs-Henseleit saline solution containing 0.5% albumin (dialyzed bovine fraction V) at pH 7.4 in stopped 25-ml Erlenmeyer flasks in a shaking water bath. The fluid was preheated with 95% O2, 5% CO2. Incubations were initiated by addition of substrate to 4 ml of cell suspension. In a separate series of experiments, the substrates were either 2.5 mM [2-15N]glutamine, [15N]glutamate, or [5-15N]glutamine (99 atom % excess) (MSD Ltd.). Incubation was stopped by adding 0.4 ml of 40% perchloric acid to each flask. The concentration of total metabolites and 15N enrichment were determined on neutralized supernatants.

Analytical Methods—Amino acids were determined with high performance liquid chromatography using a Varian 5060 System on line with the Varian 401 data system. Pre-column derivatization with o-phthalaldehyde and subsequent fluorescent detection was employed. Ammonia was determined spectrophotometrically by the reductive amination of α-ketoglutarate via glutamate dehydrogenase (18). Lactate was measured according to the method of Gutman and Wahlfeld (19), pyruvate by the method of Czok and Lamprecht (20), and α-ketoglutarate by the method of Passonneau and Brown (21). ATP was measured by the method of Lamprecht and Trautschold (22) and ADP and AMP were measured according to Jaworek et al. (23). All enzymes and reagents were obtained from Sigma. Blood pH and CO2 were measured with an Instrumentation Laboratory System 1308 pH/blood gas analyzer. Electrolytes were measured in a clinical laboratory.

15N Analysis—Analysis of 15N in amino acids was done on a Finnigan 4021 GC-MS on line with the INCOS data system. 15NH3 was measured with a slight modification of the method we have described previously (24). To disposable columns of AG-50 resin (H+; X-8; 50–100 mesh), which was conditioned with boiled 1 N NaOH, 0.5 ml of neutralized perchloric acid extract was added. The NH3 was eluted from the columns with 2 ml of 0.01 N NaOH which had been prepared immediately prior to use from the boiled, ammonia-free 1 N NaOH and water. The labeled ammonia was eluted into test tubes containing 2 ml of 0.1 M sodium phosphate buffer, pH 7.0, 0.334 mM NADH, and 5 units/ml of glutamate dehydrogenase. The test tubes then were covered and immediately placed in a water bath for 15 min at 37 °C after which the tubes were placed on ice and sufficient 1.5 N HCl was added to adjust the pH to 4–5. The total contents of each test tube were added to columns of AG-50 resin (H+; X-8; 100–200 mesh; 0.5 × 7 cm). After washing with 5 ml of water, the 15N glutamate formed from the 15NH3 was eluted with 4 ml of 4 N NH4OH. The eluate was dried at 70 °C under N2, and the n-butyl-N-trifluoroacetyl glutamate derivative was prepared (24). GC-MS analysis of the [15N]/[14N] ratio was done as described previously (24). [15N]Glutamine in the perchloric acid extract was analyzed following application of the neutralized extract to an AG-50 column (H+; X-8; 100–200 mesh; 0.5 × 7 cm) and subsequent elution with 4 N NH4OH. The N,N-bis-trifluoroacetylated derivative was formed and isotopic abundance of the amino and amide nitrogens was measured from the 15N/14N and 14N/13N ratios according to a method we have described elsewhere (25).

Isotopic enrichments of [15N]alanine, [15N]serine, and [15N]glycine were measured after isolation of these amino acids from a column of AG-50 resin as described above. The n-butyl-N-trifluoroacetyl derivatives were formed according to Roach and Gehrke (26). The m/z 207/206 and m/z 185/184 ratios were measured.

Isotopic abundance in [15N]glutamine and [15N]aspartate, these amino acids were separated from glutamine and asparagine by adding 0.5 ml of the neutralized perchloric acid extract (2 ml of 0.1 M Tris-HCl, pH 6.7, and adjusting this mixture to a concentration of AG-1 (Cl-; X-8; 50–100 mesh; 0.5 × 2.5 cm). The columns then were washed with 3 ml of deionized water and the glutamate and aspartate were eluted with 3 ml of 1 N HCl. Enrichment in [15N]glutamate and [15N]aspartate was measured in the respective n-butyl-N-trifluoroacetyl derivatives from the m/z 255/254 and m/z 185/184 ratios.

Calculation—In each of the experiments described above, we measured metabolite concentration and 15N atom % excess. The concentrations were normalized to wet weight of the tubular fragments. The concentration of 15N in a particular metabolite was derived from the product of 15N abundance (atom % excess) and metabolite concentration, as μmol or mmol/g of wet tissue. The rate of appearance of 15N-labeled metabolites was determined from the linear segment of the concentration curve versus time of the 15N metabolite in question. Linear regression analysis was used to ascertain the slope of the line corresponding to the rate of appearance of 15N metabolite produced/g of wet tissue min−1. The utilization rate of 15N-labeled substrate was determined by the rate constant and zero time intercept of the exponential decay curve. Curve fitting was done on a HP-85 desk top computer.

The Student’s t-test was used for the statistical analysis.

RESULTS

Acid-base Status of Animals—The arterial blood acid-base parameters are given in Table I. Rats with metabolic acidosis for 7 days had significantly lower pH values and HCO3− concentrations in comparison with controls. Plasma ammonia, aspartate, alanine, glycine, and serine concentrations showed little change. Only glutamine and glutamate were significantly decreased in metabolic acidosis versus control rats (Table I).

Metabolism of [5-15N]Glutamine—The contribution of the 5-N of glutamine to renal ammonia formation was assessed by incubating isolated renal cortical tubules with 2.5 mM [5-15N]glutamine. As shown in Fig. 1, the isotopic abundance (atom % excess) of 15NH3 was similar in control and acidic tubules for the first 30 min, but was higher at 60 min in control compared with acidic tubules. However, total 15NH3 production was considerably higher in tubules obtained from acidic compared with control animals (Fig. 2). In both acidic and control preparations, the appearance of 15NH3 was linear (r = 0.99) during the initial 30 min of the incubation.
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(Fig. 2): 1.3 ± 0.21 versus 0.62 ± 0.11 μmol/g of wet tissue min$^{-1}$ ($p < 0.01$), respectively (Table II). The rate of [5-$^{15}$N] glutamine utilization was similar (0.70 ± 0.14 versus 0.98 ± 0.18 μmol/g of wet tissue min$^{-1}$) in control and acidosis, respectively (Table II). Total ammonia production was higher in acidosis: 2.1 ± 0.14 versus 0.71 ± 0.19 μmol/g of wet tissue min$^{-1}$ ($p < 0.01$) for acidosis and control, respectively. The ratio between the rates of $^{15}$NH$_3$ and total NH$_3$ production indicated that in the control tubules approximately 90% of ammonia was derived from 5-N of glutamine while in chronic acidosis only 60% of ammonia was so formed.

The transfer of N from [5-$^{15}$N]glutamine to other amino acids is illustrated in Figs. 1 and 3. The rates of $^{15}$N amino acids formation from [5-$^{15}$N]glutamine are shown in Table III. $^{15}$N enrichment in the amino acids was higher in control than in acidotic tubules (Figs. 1 and 3). Similarly, the rates of $^{15}$N-amino acid formation were remarkably higher in renal tubules from control versus chronic acidotic rats (Table III). The higher rates of $^{15}$N incorporation into amino acids probably reflect enhanced transamination activity in control versus acidosis. The $^{15}$N enrichment in glutamate and the rate of [$^{15}$N]glutamate formation exceeded by as much as 15-fold that of other amino acids studied in both normal and acidotic states.

Little change was found during the incubation in the levels of α-ketoglutarate, pyruvate, and lactate in either normal or acidotic tubules (Fig. 4). The ratio of lactate/pyruvate is between 8:1 and 12:1 in both control and metabolic acidosis.

Metabolic Fate of [2-$^{15}$N]Glutamine—To study the contribution of 2-N of glutamine to ammonia nitrogen in metabolic acidosis, we incubated renal cortical tubules from control and chronic acidotic rats with 2.5 mM [2-$^{15}$N]glutamine. As shown in Figs. 2, 5, and 6, no $^{15}$NH$_3$ could be detected in control

![Graph](image)

**Fig. 1.** Formation of $^{15}$NH$_3$ and $^{15}$N-amino acids by tubular fragments isolated from normal (○) and acidotic (△) rats during incubation with 2.5 mM [5-$^{15}$N]glutamine. Each point is the mean ± S.E. of 4–6 experiments.
tubules from acidotic rats, the isotopic abundance of $[6^{-15}{\text{NH}}_2]$ was analyzed to $[6^{-15}{\text{NH}}_2]$adenine nucleotides. In which was significantly higher in acidosis with formation of $[15]{\text{NH}}_3$ was $[15]{\text{N}}$glutamate production, $[15]{\text{NH}}_3$ was approximately 6 atom excess in tubules from chronic acidotic animals. The higher rate of $[15]{\text{N}}$glutamate formation presumably the rate of $[15]{\text{N}}$ammonia formation was approximately 100-fold higher than the rate of $[6^{-15}{\text{NH}}_2]$adenine nucleotide appearance (Tables II and IV and Fig. 6). These observations indicate that no precursor-product relationship obtains between ammonia and the adenine nucleotides, implying that the PNC cannot account for augmented ammonia formation in chronic acidosis. In tubules from normal rats, the isotopic abundance of $[6^{-15}{\text{NH}}_2]$adenine nucleotides was approximately 10 atom % excess at the 60-min time point or about 30% of that noted in $[15]{\text{N}}$aspartate (Fig. 5). The $[15]{\text{NH}}_3/[6^{-15}{\text{NH}}_2]$adenine nucleotide ratio could not be measured because no $[15]{\text{NH}}_3$ was detected in tubules from control rats. The lower limit of detection is about 0.5 atom % excess.

Concentrations of ATP, ADP, and AMP during incubation with 2.5 mM glutamine are shown in Fig. 7. Neither the ATP/ADP ratio nor the concentrations of ATP, ADP, and AMP showed any significant change in either control or acidotic tissue during the course of incubation.

Experiments with $[15]{\text{N}}$Glutamate—To determine the role of glutamate dehydrogenase in augmented ammonia forma-

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### Table II

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$[15]{\text{N}}$-substrate metabolism</th>
<th>$[15]{\text{N}}$Ammonia production</th>
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<tr>
<td></td>
<td>Control</td>
<td>Acidosis</td>
</tr>
<tr>
<td>2.5 mM $[5^{-15}{\text{N}}]$Gln</td>
<td>0.70 ± 0.14</td>
<td>0.98 ± 0.18*</td>
</tr>
<tr>
<td>2.5 mM $[2^{-15}{\text{N}}]$Gln</td>
<td>0.83 ± 0.11</td>
<td>1.10 ± 0.13*</td>
</tr>
<tr>
<td>2.5 mM $[5^{-15}{\text{N}}]$Glu</td>
<td>0.26 ± 0.05</td>
<td>0.35 ± 0.04*</td>
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</table>

* $p < 0.05$ compared with controls.

* $p < 0.01$ compared with controls.

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To determine the role of the PNC in ammonia formation, we analyzed $[15]{\text{N}}$ transfer to $[6^{-15}{\text{NH}}_2]$adenine nucleotides. In tubules from acidotic rats, the isotopic abundance of $[6^{-15}{\text{NH}}_2]$adenine nucleotides was approximately 6 atom % excess at 60 min (Fig. 5), only ½ of the $[15]{\text{NH}}_3$ enrichment. Furthermore, the rate of $[15]{\text{N}}$ammonia formation was approximately 100-fold higher than the rate of $[6^{-15}{\text{NH}}_2]$adenine nucleotide appearance (Tables II and IV and Fig. 6). These observations indicate that no precursor-product relationship obtains between ammonia and the adenine nucleotides, implying that the PNC cannot account for augmented ammonia formation in chronic acidosis. In tubules from normal rats, the isotopic abundance of $[6^{-15}{\text{NH}}_2]$adenine nucleotides was approximately 10 atom % excess at the 60-min time point or about 30% of that noted in $[15]{\text{N}}$aspartate (Fig. 5). The $[15]{\text{NH}}_3/[6^{-15}{\text{NH}}_2]$adenine nucleotide ratio could not be measured because no $[15]{\text{NH}}_3$ was detected in tubules from control rats. The lower limit of detection is about 0.5 atom % excess.

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Experiments with $[15]{\text{N}}$Glutamate—To determine the role of glutamate dehydrogenase in augmented ammonia forma-
Fig. 3. Concentration of $^{15}$N-amino acids during incubation of tubules from control (○) or acidic (□) rats with 2.5 mM [5-15N]glutamine. Concentration calculated as defined in legend to Fig. 2. Each point represents the mean ± S.E. of 4-6 experiments.

TABLE III

<table>
<thead>
<tr>
<th>$^{15}$N-metabolite</th>
<th>Rate of production $\text{nmol/g wet tissue min}^{-1}$</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>Glutamate</td>
<td>51.6 ± 4.3</td>
</tr>
<tr>
<td>Aspartate</td>
<td>8.2 ± 1.3</td>
</tr>
<tr>
<td>Alanine</td>
<td>6.0 ± 2.1</td>
</tr>
<tr>
<td>Serine</td>
<td>1.9 ± 0.6</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.7 ± 0.3</td>
</tr>
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</table>

*p < 0.01 compared with controls.

The effect of metabolic acidosis on glutamate metabolism and the distribution of $^{15}$N from glutamate to other solutes is shown in Figs. 2, 8, and 9 and Tables II and V. In tubules from acidic and control rats, $^{15}$NH$_3$ formation proceeded at 0.22 ± 0.04 versus 0.027 ± 0.001 μmol/g of wet tissue min$^{-1}$, respectively. Chronic acidosis significantly stimulated the rate of glutamate metabolism: 0.26 ± 0.05 versus 0.35 ± 0.04 μmol/g of wet tissue min$^{-1}$ (p < 0.05) (Table II). The ratios between $^{15}$N ammonia formation and [15N]glutamate disappearance were 0.1 in control and 0.63 in chronic acidosis (Table II). This demonstrates that the activity of glutamate dehydrogenase in the tubules was approximately 6-fold higher in tubules from metabolic acidosis than in tubules from control rats, which is consistent with our data involving incubation with [2-15N]glutamine. As shown in Figs. 8 and 9, $^{15}$NH$_3$ isotopic abundance exceeded that of any other metabolite except [2-15N]glutamine in tubules prepared from acidic rats.

The rate of [5-15N]glutamine appearance was 7.5 ± 0.8 versus 1.6 μmol/g of wet tissue min$^{-1}$ in tubules from control versus acidic animals. The rate of glutamine synthesis was 27% of the rate of net NH$_3$ appearance in control cells and 10% in acidic cells. Hence, there is a 3-fold higher flux through the glutamine synthetase pathway in control tubules. Glutamine has long been known to be synthesized in the kidneys of certain mammalian species (28, 29). It has been suggested that in the rat, the kidney is a major source of tissue and blood glutamine (29). The current observation is the first direct measurement of the glutamine synthetase flux in cells obtained from renal cortex of normal and acidic rats.

Enrichment of [2-15N]glutamine exceeds that of ammonia and [5-15N]glutamine in both control and acidic incubations. The current observation is the first direct measurement of the glutamine synthetase flux in cells obtained from renal cortex of normal and acidic rats. Aspartate formation was 41.8 ± 4.5 in control versus 17.6 ± 2.1 μmol/g wet tissue min$^{-1}$ in chronic acidosis (Table V) (p < 0.01). This observation is in good agreement with previous studies of Schoolwerth et al. (8) and Kunin and Tannen (7), who found that aspartate formation in mitochondria from acidic rats was depressed by approximately 50% compared with control experiments. Similarly, the rates of $^{15}$N appearance in alanine, glycine, serine, and [6-15N]adenine nucleotides are 3- to 5-fold higher in tubules from acidic rats.

Another major pathway in tubular glutamate metabolism is via transamination with oxalacetate. The rate of [15N]aspartate formation was 2.5 ± 0.8 versus 1.6 μmol/g of wet tissue min$^{-1}$ in tubules from control versus acidic animals. The rate of glutamine synthesis was 27% of the rate of net NH$_3$ appearance in control cells and 10% in acidic cells. Hence, there is a 3-fold higher flux through the glutamine synthetase pathway in control tubules. Glutamine has long been known to be synthesized in the kidneys of certain mammalian species (28, 29). It has been suggested that in the rat, the kidney is a major source of tissue and blood glutamine (29). The current observation is the first direct measurement of the glutamine synthetase flux in cells obtained from renal cortex of normal and acidic rats.

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compared with tubules from chronic acidotic rats (Fig. 9 and Table V).

Enrichment in [6-15NH2]adenine nucleotides exceeded that in 15NH3 in tubules from control rats whereas the opposite was found in tubules from chronic acidotic rats (Fig. 8). At 60 min, the ratio of [6-15NH2]Ado/15NH3 was 1.5 in control tissue and 0.25 in acidotic tubules. A similar observation was reported by Bogusky and Aoki (13) in kidneys from normal rats perfused with 2 mM [15N]glutamate. In contrast, in tubules from acidotic animals, most ammonia nitrogen appeared to have been derived from glutamate dehydrogenase activity.

In agreement with the experiments with [2-15N]glutamine, no significant differences of baseline ATP, ADP, or AMP levels were noted between tubules from chronic acidotic or control rats (Fig. 7). However, during incubation of control, but not acidic, tissue, there was an increase of approximately 30% of the ATP/ADP ratio associated with an increase of 30% in the ATP concentration 45 min after the incubation was started ($p < 0.05$). Concentration of ADP and AMP remained constant during the course of incubation (Fig. 7). This change in ATP concentration correlates with the rise in 15N incorporation into the 6-amino group of adenine nucleotides (Figs. 8 and 9). In contrast, no significant change was found in acidic tissue during the course of incubation in either the ATP/ADP ratio or the ATP, ADP, and AMP concentration (Fig. 7).

Despite the high activity of glutamate dehydrogenase in tubules from acidotic rats, no accumulation of α-ketoglutarate was found during the course of incubation (Fig. 4). Similarly, in both control and acidic tissue, pyruvate levels changed little but lactate levels in tubules from acidic rats fell approximately 40% during the first 15 min of the incubation and then returned to baseline by 45 min (Fig. 4). The lactate/pyruvate ratio also decreased during the first 15 min in the acidic tissue.

DISCUSSION

The current study represents the first use of GC-MS in an in vitro system to develop a comprehensive picture of renal glutamine and glutamate metabolism. The use of [2-15N]glutamine and [5-15N]glutamine as precursors permitted direct measurement of the rate of glutamine or glutamate utilization and the subsequent distribution of their nitrogens to ammonia, other amino acids, and the 6-amino group of the adenine nucleotides (Figs. 2 and 9 and Tables II and V). Thus, in tubules from acidic animals, most ammonia nitrogen appeared to have been derived from glutamate dehydrogenase activity.

In agreement with the experiments with [2-15N]glutamine, no significant differences of baseline ATP, ADP, or AMP levels were noted between tubules from chronic acidotic or control rats (Fig. 7). However, during incubation of control, but not acidic, tissue, there was an increase of approximately 30% of the ATP/ADP ratio associated with an increase of 30% in the ATP concentration 45 min after the incubation was started ($p < 0.05$). Concentration of ADP and AMP remained constant during the course of incubation (Fig. 7). This change in ATP concentration correlates with the rise in 15N incorporation into the 6-amino group of adenine nucleotides (Figs. 8 and 9). In contrast, no significant change was found in acidic tissue during the course of incubation in either the ATP/ADP ratio or the ATP, ADP, and AMP concentration (Fig. 7).

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The current study represents the first use of GC-MS in an in vitro system to develop a comprehensive picture of renal glutamine and glutamate metabolism. The use of [2-15N] glutamine and [5-15N]glutamine as precursors permitted direct measurement of the rate of glutamine or glutamate utilization and the subsequent distribution of their nitrogens to ammonia, other amino acids, and the 6-amino group of the adenine nucleotides. Our central aim was to ascertain in vitro the principal precursors of ammonia in both basal and acidic states and to assess the hypothesis (9) that in acidosis both 2-N and 5-N of glutamine became important sources for ammonia formation.

The results show (Table II) that in tubules from normal rats more than 90% of ammonia originates from 5-N of glutamine. Thus, the amide group of glutamine in the basal state is the primary source of amino-N via the activity of phosphate-dependent glutaminase. This conclusion is supported strongly by the fact that no 15NH3 could be detected in incubations of control tubules with 2.5 mM [2-15N]glutamine. In contrast, in tubules from chronic acidotic rats, the rates of ammonia formation from either [15N]glutamate or [2-15N]glutamine were 20–30% the production rate from [5-15N]glutamine (Table II). Hence, in acidosis, approximately 60% of ammonia was derived from 5-N of glutamine by the activity of phosphate-dependent glutaminase and between 20% and 30% came from 2-N of glutamine by the activity of glutamate dehydrogenase. In acidosis, net renal ammonia production is enhanced, as noted repeatedly by previous investigators (30–32), but the fraction of this excess
Fig. 5. $^{15}$N enrichment in ammonia, amino acids, and [6-$^{15}$NH$_2$]adenine nucleotides in renal tubular fragments isolated from normal (●) and acidicotic (○) rats during incubation with 2.5 mM [2-$^{15}$N]glutamine. The [6-$^{15}$NH$_2$]Ade enrichment represents the average $^{15}$N abundance in ATP, ADP, and AMP. Each point is the mean ± S.E. of 4-6 experiments.
ammonia derived from 5-N of glutamine is smaller than is true in normal acid-base balance. These findings are in accord with earlier conclusions that the rate of glutamate deamination is accelerated substantially in mitochondria from acidotic rats (7, 8).

In Fig. 10 are outlined the primary routes of nitrogen metabolism in either control or acidic tubules. A stoichiometric relationship was noted between rates of isotope utilization and subsequent appearance of $^{15}$N in major metabolites. Thus, in the study with $[2-^{15}$N$]$glutamine in the acidic tubules (Tables II and IV), the rate of isotope disappearance was 1.10 pmol/g of wet tissue/min and the total recovery of $^{15}$N in glutamate, ammonia, alanine, aspartate, and adenine nucleotides (sum of $1a + 2 + 7 + 4 + 5$ in Fig. 10) was 1.12 pmol/g of wet tissue/min. Similarly, in control tubules, $[2-$

\[ \text{Glu} \] \[ \text{Asp} \] \[ \text{Ser} \] \[ \text{Gly} \] \[ \text{Ade} \] \[ \text{Gly} \]

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
\textbf{$^{15}$N-metabolite} & \textbf{Rate of production} & \\
\hline
 & \text{Control} & \text{Acidosis} \\
\hline
Glutamate & 540.2 ± 35.3 & 830.2 ± 45.3$^a$ \\
Aspartate & 40.4 ± 2.2 & 12.5 ± 2.1$^a$ \\
Alanine & 16.3 ± 1.4 & 3.4 ± 0.6$^a$ \\
Serine & 5.3 ± 0.4 & 1.3 ± 0.1$^a$ \\
Glycine & 5.5 ± 0.3 & 1.5 ± 0.2$^a$ \\
Adenine nucleotides & 6.3 ± 0.4 & 2.3 ± 0.5$^a$ \\
\hline
\end{tabular}
\caption{Production rate of $^{15}$N-amino acids and $[6-^{15}$N$]$$^6$Ade in renal cortical tubules from chronic acidotic and control rats incubated with $2.5$ mM $[2-$

$^a$ p < 0.01 compared with controls.

$^b$ Refers to average $^{15}$N in ATP, ADP, AMP (see "Materials and Methods").

We propose (Fig. 10) that the intramitochondrial phosphate-dependent glutaminase pathway (glutaminase I) plays a major role in the regulation of renal ammoniagenesis. However, previous studies in rat (33), dog (34), and human kidney (35, 36) have documented an alternate route of glutamine deamidation (glutaminase II) involving the sequential action of glutamine keto-acid aminotransferase (GKA) and $\omega$-amidase, both of which are located primarily in the cytosol (9).

Thus, according to Cooper and Meister (33), some ammonia could be formed from glutamine in renal tubules from control and acidic rats by the combined action of two enzymes:

\[ \text{L-glutamine} + \alpha\text{-keto acid} \xrightarrow{\text{GKA}} \alpha\text{-ketoglutamate} + \text{ammonia} \]  
\[ \alpha\text{-Ketoglutamate} \xrightarrow{\omega\text{-amidase}} \alpha\text{-ketoglutarate} + \text{ammonia} \]
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Study With Glutamate

![Graph showing ATP, ADP, and AMP concentration in renal tubular fragments isolated from normal (○) and chronic acidotic (○) rats during experiments with 2.5 mM $^{15}$N-glutamate or $^{15}$N-glutamine. Each data point is the mean ± S.E. of 4–6 experiments.]

Nevertheless, the observations that 6-diazo-5-oxo-L-norleucine, an inhibitor of phosphate-dependent glutaminase, glutaminase I, diminished ammonia production by renal slices from the rat (37) and that actinomycin D inhibited glutaminase II activity but not ammoniagenesis in the acidotic rat (38), suggest that the glutamine transaminase pathway does not have an important role in augmented ammoniagenesis.

Our experiments with $[5-^{15}$N$]$glutamine or $[2-^{15}$N$]$glutamine do not rule out the possibility that some $[^{15}$N$]$alanine or $[^{15}$N$]$glutamate was formed by direct reductive amination of pyruvate and α-ketoglutarate, the keto-acids of alanine and glutamate. O’Donovan and Lotspeich (39) studied renal $[5-^{15}$N$]$glutamine metabolism and proposed that the amide group might be used to aminate keto-acids directly. They found more $[^{15}$N$]$alanine was formed in guinea pig kidney homogenate in the presence of pyruvate + $[5-^{15}$N$]$glutamine than pyruvate + $^{15}$NH$_4$Cl. Thus, the formation of $[^{15}$N$]$alanine by both control and acidic tubules incubated with $[5-^{15}$N$]$glutamine or $[2-^{15}$N$]$glutamine (Figs. 1 and 5) could reflect either reductive amination of pyruvate or transamination with $[^{15}$N$]$glutamate. However, in experiments with $[5-^{15}$N$]$glutamate or $[2-^{15}$N$]$glutamine, $^{14}$N enrichment in glutamate exceeded that in alanine (Figs. 1 and 5), implying a precursor-product relationship between $[^{15}$N$]$glutamate and $[^{15}$N$]$alanine. Thus, it is more likely that the major portion of $[^{15}$N$]$alanine was formed by alanine aminotransferase activity. $[^{15}$N$]$Serine presumably was formed from $[^{15}$N$]$alanine via serine-pyruvate transaminase. $[^{15}$N$]$Glycine then would be derived from $[^{15}$N$]$serine via the glycine-serine cleavage system which is known to be very active in renal tissue (40).

Our results in tubules from acidotic rats incubated with $[1^{15}$N$]$glutamate or $[2-^{15}$N$]$glutamine show that the enhanced ammonia production is inversely related to the rate of glutamate transamination (Tables II–V). Thus, in control tissue, transamination of glutamate was relatively higher and ammonia formation was relatively lower than was true of acidotic tissue. In acidosis, deamination rather than transamination predominates in the mitochondria, resulting in ammonia production via glutamate dehydrogenase. This conclusion confirms earlier observations of Kunin and Tannen (7) and Schoolwerth et al. (8), that glutamate deamination by renal mitochondria isolated from chronically acidotic rats was markedly stimulated and contributed approximately 50% of the observed augmented ammonia production. However, in contrast to the conclusion of Kunin and Tannen (7), that in the control state isolated mitochondria metabolize glutamate almost exclusively by deamination, our data (Figs. 8 and 9 and Table V) demonstrate that glutamate transamination to aspartate predominates over glutamate deamination. This observation is in agreement with that of Schoolwerth et al.
Fig. 8. Time course of $^{15}$N (atom % excess) appearance in ammonia, amino acids, and $^{6-^{15}}$N adenine nucleotides in renal tubular fragments isolated from normal (○) and chronically acidotic (Δ) rats. Each point is the mean ± S.E. of 4-6 experiments.
Fig. 9. $^{15}$N appearance in amino acids and [6-15NH$_2$]adenine nucleotides in renal tubular fragments isolated from normal (●) and acidotic (○) rats. The substrate was 2.5 mM [15N]glutamate. Concentration of $^{15}$N-metabolites is the product of $^{15}$N (atom % excess) and concentration (µmol/g of wet tissue) of each compound. Each point is the mean ± S.E. of 4–6 experiments.
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TABLE V

<table>
<thead>
<tr>
<th>$^{15}$N-metabolites</th>
<th>Rate of production (mmol/g of wet tissue min$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>[2-$^{15}$N]Glutamine</td>
<td>63.3 ± 7.4</td>
</tr>
<tr>
<td>[5-$^{15}$N]Glutamine</td>
<td>7.5 ± 0.8</td>
</tr>
<tr>
<td>Aspartate</td>
<td>41.8 ± 4.5</td>
</tr>
<tr>
<td>Alanine</td>
<td>27.9 ± 2.6</td>
</tr>
<tr>
<td>Glycine</td>
<td>5.9 ± 1.3</td>
</tr>
<tr>
<td>Serine</td>
<td>8.1 ± 1.1</td>
</tr>
<tr>
<td>Adenine nucleotides$^*$</td>
<td>15.4 ± 2.3</td>
</tr>
</tbody>
</table>

* $p < 0.01$ compared with controls.
$^*$ Refers to average $^{15}$N in ATP, ADP, AMP (see "Materials and Methods").

(8) that metabolism of glutamate in renal mitochondria occurred exclusively by transamination.

A metabolic fate of glutamate which occurs in rats and sheep, but not in humans or dogs (29), is conversion into glutamine in the presence of ATP and NH$_3$ by cytoplasmic glutamine synthetase. Studies of the flux through the glutamine synthetase reaction in the rat kidney in vivo show decreased activity of this pathway during acidosis and increased activity during alkalosis (41). Our data show that the rate of [5-$^{15}$N] glutamine formation in tubules from normal rats was 3-fold higher than was observed in tubules from chronically acidotic rats (Table V). With [15N]glutamate as precursor, net flux into [5-$^{15}$N]glutamine was approximately 30% of net flux into $^{15}$NH$_3$ in normal tissue and approximately 10% in tubules isolated from acidotic animals. Although the glutamine synthetase pathway appeared relatively more active in control tissue, it seems to play little role in the enhanced ammoniagenesis associated with chronic acidosis. Evidence for this conclusion is the observation that enrichment of $^{15}$NH$_3$ in control tubules exceeded that of [5-$^{15}$N]glutamine, indicating that even in the basal state a precursor-product relationship between [5-$^{15}$N]glutamine and $^{15}$NH$_3$ did not obtain with [15N]glutamate as substrate (Fig. 8). In acidosis, enrichment of [5-$^{15}$N]glutamate versus $^{15}$NH$_3$ is even lower (Fig. 8).

In addition to glutamine synthetase, the data show that formation of [6-$^{15}$N]adenine nucleotides via the purine nucleotide cycle is an important fate for glutamate-N in the kidney. The cycle uses catalytic amounts of purine nucleotides and GTP to convert aspartate to NH$_3$ and fumarate. In chronic acidosis, there is a significant increase in the activity of adenylosuccinate synthetase (11). However, inhibition of aspartate formation from glutamine (42) or glutamate (7) by amino-oxacetylate has no effect on ammoniagenesis (7). Bogusky and Aoki (13) concluded that in normal kidney perfused with [15N]glutamate or [15N]aspartate the turnover of the PNC could account for the ammonia formed and is equal to the rate of ammonia formation from glutamate via glutamate dehydrogenase (13).

Our findings show that in tubules from acidotic rats the rates of $^{15}$NH$_3$ formation from [2-$^{15}$N]glutamate or [15N]glutamine were 100- and 80-fold higher, respectively, than the rates of [6-$^{15}$N]adenine nucleotide formation (Tables IV and V). Furthermore, no precursor-product relationship was found to exist between [6-$^{15}$N]adenine nucleotides and $^{15}$NH$_3$ enrichment. Our data suggest that ammonia formation via the purine nucleotide cycle could not account for the enhanced ammoniagenesis from glutamate or glutamate in tubules from chronically acidotic rats. In contrast, in tubules from rats in normal acid-base status, the enrichment of [5-$^{15}$N]adenine nucleotide formation from [2-$^{15}$N]glutamine or [15N]glutamine exceeded that of $^{15}$NH$_3$ (Figs. 5 and 8). In addition, the rates of [6-$^{15}$N]adenine nucleotide formation from [2-$^{15}$N]glutamine exceeded that of $^{15}$NH$_3$ (Fig. 6 and Tables II and IV). These findings are in good accord with the conclusion of Bogusky and Aoki (13) that in normal acid-base status the turnover of the adenine nucleotides can account for the ammonia formed from glutamate. Additional studies with [15N]aspartate in the presence and absence of PNC inhibitors should define more accurately the role of the PNC in augmented ammoniagenesis in different acid-base states.

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REFERENCES
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