Integrative physiology of splanchnic glutamine and ammonium metabolism

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Departments of 1Nutrition and 2Surgery, Case Western Reserve University, Cleveland, Ohio 44106; and 3Renal Division, Department of Medicine, St Michael’s Hospital, University of Toronto, Toronto, Ontario M5B 1A6, Canada

Yang, Dawei, Jeffery W. Hazey, France David, Jittendra Singh, Ryan Rivchum, Jason M. Streem, Mitchell L. Halperin, and Henri Brun engraber. Integrative physiology of splanchnic glutamine and ammonium metabolism Am. J. Physiol. Endocrinol. Metab. 278: E469–E476, 2000.—The substrates for hepatic ureagenesis are equimolar amounts of ammonium and aspartate. The study design mimics conditions in which the liver receives more NH4+ than aspartate precursors (very low-protein diet). Fasted dogs, fitted acutely with transhepatic catheters, were infused with a tracer amount of 15NH4Cl. From arteriovenous differences, the major NH4+ precursor for hepatic ureagenesis was via deamination of glutamine in the portal drainage system (rather than in the liver), because there was a 1:1 stoichiometry between glutamine disappearance and NH4+ appearance, and the amide (but not the amine) nitrogen of glutamine supplied the 15N added to the portal venous NH4+ pool. The liver extracted all this NH4+ from glutamine deamination plus an additional amount in a single pass, suggesting that there was an activator of hepatic ureagenesis. The other major source of nitrogen extracted by the liver was 14N alanine. Because alanine was not produced in the portal venous system, we speculate that it was derived ultimately from proteins in peripheral tissues.

urea synthesis is the main route of nitrogen disposal in virtually all terrestrial mammals (for a review, see Ref. 28). Most of this nitrogen in urea results from the complete oxidation of proteins (8, 16, 28). Because of a steady state and a relatively constant protein intake, urea nitrogen excretion during a given day is largely equivalent to the protein intake of that day; however, in fact it is derived only in part from ingested proteins, the remainder being supplied by body proteins that were catabolized on that day (and resynthesized from the amino acids of dietary origin). Humans who consume proteins at 1.0–1.5 g·kg−1·day−1 excrete urea at a relatively constant rate in the 24-h cycle (4). There is good evidence that an amount of urea equal to ~20% of the usual daily urea production rate is hydrolyzed to NH4+ and HCO3− in the lumen of the gastrointestinal (GI) tract by urease derived from local microorganisms in normal subjects (7, 8, 12, 14, 15, 21, 28).

Proteins are essential components of the body’s lean body mass. Although they are macronutrients that are ingested and oxidized daily, their primary function is not to provide energy but rather to be made into structural and contractile elements, enzymes, transporters, hormones, and defense elements such as antibodies. In contrast to carbohydrates and lipids, amino acids contain nitrogen (and sulfur), which must be disposed of. Because true gluconeogenesis (GNG, i.e., the conversion of amino acids to glucose) and ureagenesis share common intermediates until late in this metabolic pathway (argininosuccinate), Jungas et al. (16) and Halperin and Rolleston (10) have emphasized that GNG and ureagenesis are in fact components of the same metabolic process (Fig 1). Moreover, the two nitrogen (N) atoms that are incorporated into the urea molecule are in two different forms, i.e., one via free NH4+ and the other via aspartate (Fig. 1). Nevertheless, under conditions of low-protein intake and continuous splanchnic urea hydrolysis (i.e., a process that generates NH4+ but does not supply aspartate), it is not clear what the source of aspartate will be in the liver. If one-half of the NH4+ load could be converted to aspartate by glutamate dehydrogenase and aspartate-aminotransferase reactions, one would expect to find that the rise in urea appearance would be equal to the rate of supply of NH4+ to the liver, and that both nitrogens would almost equally be 14N enriched with the same molar percent enrichment (MPE) as NH4+. In contrast, if NH4+ were extracted by the liver but not converted to aspartate at a sufficient rate, one would expect to find a higher rate of appearance of urea, because a net supply of aspartate by proteolysis would be obligated, and this aspartate would not be 15N.
enriched. If, indeed, proteolysis is needed in this setting, it could help to explain the protein catabolism seen in patients consuming a low-protein diet (22, 27).

The question we addressed in the studies to be reported is, 'What is the physiological response to a supply of nitrogen to the liver that differs markedly from the 1:1 stoichiometry for the nitrogen precursors (NH₄⁺ and aspartate) illustrated in Fig. 1?' To address this question, we took advantage of a new sensitive technique we developed to measure the concentration and ¹⁵N enrichment of NH₄⁺ in biological fluids (30). This technique also measures the ¹⁵N enrichment of L-[5-¹⁵N]glutamine after isolation and treatment with glutaminase and the average ¹⁵N enrichment of urea nitrogen after isolation and treatment with urease. The study was conducted in anesthetized dogs infused with tracer amounts of ¹⁵NH₄Cl. The data to be presented show that 33% of the glutamine delivered to the portal venous drainage bed was deamidated, and this provided the liver with a large NH₄⁺ load. A near-equal quantity of nitrogen in the form of [¹⁴N]alanine was extracted from the portal venous blood by the liver, but it was not derived from the intestinal tract as judged by the absence of an arterial-portal vein concentration difference for alanine.

METHODS

Animals. All animal experiments were approved by the Animal Care and Use Committee of Case Western Reserve University. After three orientation experiments, seven mongrel dogs (24 h fasted, 20–24 kg) were anesthetized and ventilated with 50% O₂, Pco₂, hemoglobin, and pH were measured to calculate the O₂ content in the arterial, portal venous, and hepatic venous blood (18). The techniques to measure the ¹⁵N enrichments of NH₄⁺, glutamine-amide, and urea nitrogen were recently described (30). Briefly, to measure the ¹⁵N enrichment of plasma NH₄⁺, chilled blood was centrifuged and the plasma was treated with AG-50-X8-Na⁺ resin to neutral pH. After the resin was rinsed with water, NH₄⁺ was eluted with 4 M NaCl. The eluate was treated with formaldehyde and NaOH to form hexamethylenetetramine (HMT). The latter was extracted and its ¹⁵N enrichment assayed by gas chromatography-mass spectrometry (GC-MS). The enrichment of HMT is four times that of plasma NH₄⁺, because the HMT molecule contains 4 nitrogen atoms derived from NH₄⁺. To measure the concentration of NH₄⁺, a second aliquot of plasma was spiked with an internal standard of ¹⁵NH₄Cl and treated as above. Measurements conducted on whole blood and plasma showed that the concentration of NH₄⁺ in whole blood was 1.5 times that of the corresponding plasma. The ¹⁵N enrichment of NH₄⁺ was the same in whole blood and in plasma (30).

To measure the ¹⁵N enrichment of the amide nitrogen of glutamine and the average enrichment of the two nitrogens of urea, aliquots of the water eluate of the resin were treated with glutaminase and urease, respectively. The NH₄⁺ released by the action of these enzymes was converted to HMT, and the ¹⁵N enrichment of the latter was assayed by GC-MS. To measure the ¹⁵N enrichment of the amine nitrogen of glutamine, glutamate derived from the treatment of glutamine with glutaminase was converted to a tert-butyldimethylsilyl derivative and assayed by GC-MS.

Statistical analyses. Data are presented as means ± SE. Statistical differences were evaluated by the two-tailed t-test.

RESULTS

Acid-base data. The acid-base effects of the infusion of the tracer of NH₄Cl are shown in Table 1. The plasma [HCO₃⁻] was close to 20 mM before the infusion, a

<table>
<thead>
<tr>
<th>Time, h</th>
<th>0</th>
<th>3</th>
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<tbody>
<tr>
<td>pH</td>
<td>7.40 ± 0.02</td>
<td>7.36 ± 0.01*</td>
</tr>
<tr>
<td>Pco₂, mmHg</td>
<td>32 ± 1.4</td>
<td>32 ± 1.0</td>
</tr>
<tr>
<td>Hco₃⁻, mM</td>
<td>20 ± 0.5</td>
<td>19 ± 0.5</td>
</tr>
<tr>
<td>Lactate, mM</td>
<td>1.8 ± 0.32</td>
<td>1.9 ± 0.15</td>
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Values are reported as means ± SE (n = 7). For details, see METHODS. *Significantly different from value at t = 0 (P = 0.05).
normal value for this parameter in the dog (11). There was a small and not statistically significant decline in the plasma [HCO₃⁻] to 19 mM at the end of the 3-h experimental period. There was no significant change in arterial blood lactate level.

Levels of nitrogenous metabolites in arterial blood and their ¹⁵N enrichment. The concentration of NH₄⁺ in arterial blood was 35 ± 4 µM during the tracer infusion and did not vary significantly over the 3-h time of observation (Fig. 2A, Table 2). The NH₄⁺ infused was ¹⁵N enriched to help define the sources of nitrogen incorporated into urea. The profile for the ¹⁵N enrichment of NH₄⁺ in the artery, hepatic vein, and portal vein blood during the tracer infusion is shown in Fig 2B. The ¹⁵N enrichment of NH₄⁺ was virtually stable after 1 h in both of the venous sampling sites, whereas a plateau was not quite achieved in the arterial compartment.

The other major circulating nitrogenous metabolite that was enriched with ¹⁵N was glutamine. There was a progressive rise in the MPE of the amide nitrogen of glutamine in arterial blood with time during the tracer infusion (Fig 3). In contrast, there was no detectable ¹⁵N enrichment of the amine nitrogen of glutamine. The ¹⁵N enrichment of the amide nitrogen of glutamine in the hepatic vein was 70% that of the weighted enrichment of glutamine entering the liver.

Table 2. Concentrations of NH₄⁺, glutamine, and alanine across liver

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Artery</th>
<th>Portal Vein</th>
<th>Hepatic Vein</th>
</tr>
</thead>
<tbody>
<tr>
<td>[NH₄⁺]</td>
<td>35 ± 4</td>
<td>140 ± 14*</td>
<td>16 ± 2†</td>
</tr>
<tr>
<td>[Glutamine]</td>
<td>420 ± 23</td>
<td>280 ± 14*</td>
<td>310 ± 26</td>
</tr>
<tr>
<td>[Alanine]</td>
<td>760 ± 150</td>
<td>790 ± 140</td>
<td>690 ± 190</td>
</tr>
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</table>

All concentrations, integrated over 3-h periods, are expressed in µM (means ± SE). *Significantly different from artery (P < 0.001); †significantly different from portal vein (P < 0.001).

Urinary excretion. The urinary excretion of nitrogen (urea + NH₄⁺) was 10.5 µmol N·min⁻¹·kg⁻¹ (Table 3), and this rate remained constant over the 3-h period of observation (Fig. 4). More than 96% of the nitrogen excreted was in the form of urea (Table 3). Because plasma concentrations of urea remained between 3.5 and 3.7 mM (Fig. 4), the excretion of urea is equivalent to its net production.

Portal drainage system. Three sets of data provide evidence concerning the metabolic source of NH₄⁺ added to the portal venous blood. First, NH₄⁺ was produced because its concentration was close to 100 µM higher in portal venous than in arterial blood (Table 2). Second, the source of this NH₄⁺ could be glutamine, because the concentration of glutamine was ≥100 µM lower in portal venous than in arterial blood (Table 2). Third, the use of ¹⁵N enrichment suggested a precursor (glutamine)-product (NH₄⁺) relationship. In more detail, because the concentration of NH₄⁺ was fourfold higher in the portal venous than in the arterial blood (Table 2), one would anticipate the MPE of ¹⁵NH₄⁺ in this drainage system to fall to 25% if the source of NH₄⁺ was an

Fig. 2. Plasma concentration (A) and ¹⁵N enrichment of NH₄⁺ (molar percent enrichment, MPE, B) in femoral artery (ART), portal vein (PV), and hepatic vein (HV) of dogs infused for 3 h with tracer ¹⁵NH₄⁺. Data are presented as means ± SE (n = 7).

Fig. 3. ¹⁵N enrichment of glutamine amide in plasma of the same dogs as in Fig. 2.
14N precursor (e.g., urea). In fact, the MPE only fell by close to 50% in the portal vein (Fig. 2B). These data are best explained by having a major precursor of NH₄⁺ that was predominantly 15N enriched. It is of interest that the amide nitrogen of glutamine was 15N enriched (Fig. 3) with an MPE of 40% of that of arterial NH₄⁺ by the end of the experimental period (compare Figs. 3 and 2B). Moreover, as mentioned above, the amine nitrogen of glutamine was not enriched with 15N.

The liver produced 10.5 µmol urea N·min⁻¹·kg⁻¹, and the GI tract produced 3.4 ± 0.14 µmol NH₄⁺·min⁻¹·kg⁻¹, largely from the amide nitrogen of glutamine (3.2 ± 0.21 µmol·min⁻¹·kg⁻¹, Table 3). Hence glutamine metabolism in the GI tract could supply close to one-third of the nitrogen converted to urea in the liver, or almost two-thirds of the free NH₄⁺ needed (i.e., one-half of the 10.5 µmol urea N·min⁻¹·kg⁻¹; see Table 3). We could not identify the fate of the amine nitrogen of glutamine because there was no significant appearance of alanine (Table 2) or glutamate in portal venous blood (not shown).

The O₂ uptake by the gut was 15.2 ± 4.5 µmol·min⁻¹·kg⁻¹, a value that was four- to fivefold higher than the uptake of glutamine. This amount of O₂ would be needed to completely oxidize glutamine (Eq. 1) if glutamine were the exclusive fuel for the entire portal drainage system.

\[
\text{glutamine} + 5 \text{O}_2 \rightarrow 5 \text{CO}_2 + 3 \text{H}_2\text{O} + 2 \text{NH}_3 \tag{1}
\]

Events in the liver. The amount of NH₄⁺ extracted by the liver was close to 4 µmol·min⁻¹·kg⁻¹ (Table 3). The other major source of nitrogen extracted by the liver was alanine (3 µmol·min⁻¹·kg⁻¹), most of which was derived from a systemic source because there was no significant difference in alanine concentrations between the artery and portal vein (Table 2). Thus NH₄⁺ and alanine supplied close to 75% of the total nitrogen converted to urea. The concentration of NH₄⁺ was very low in the hepatic vein (16 µM, Table 1), reflecting the complete extraction of the NH₄⁺ derived from glutamine and more than one-half of the arterial NH₄⁺ delivered to the liver (Table 2).

The 15N enrichment of arterial urea N increased linearly by 0.3%/h throughout the experiment (Fig. 5). Note that this is an average enrichment of the two N of urea, because the assay required the conversion of urea N to NH₄⁺ by urease before the making of HMT.

<table>
<thead>
<tr>
<th>Portal Drainage System</th>
<th>Liver</th>
<th>Urinary Excretion</th>
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<tbody>
<tr>
<td>NH₄⁺</td>
<td>Glutamine</td>
<td>NH₄⁺</td>
</tr>
<tr>
<td>-3.4 ± 0.14</td>
<td>3.2 ± 0.21</td>
<td>4.1 ± 0.08</td>
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All rates, integrated over 3-h periods, and expressed in µmol nitrogen·min⁻¹·kg body wt⁻¹ for glutamine amide nitrogen, alanine, urea, and NH₄⁺, and in µmol NH₄⁺ equivalents·min⁻¹·kg body wt⁻¹ for urea, are presented as means ± SE. Negative organ balances correspond to substrate releases into blood. Balances across liver were calculated by assuming a blood flow ratio (portal vein)/(hepatic artery) of 4.0. Rate of tracer ¹⁵NH₄Cl infusion was 0.7 µmol·min⁻¹·kg⁻¹. Note that, because plasma concentrations of urea did not change significantly throughout experiments, excretion of urea is equivalent to net urea production.

Fig. 4. Profiles of plasma urea concentration (●) and urea excretion (■).

Fig. 5. Average labeling of plasma urea nitrogen in the same dogs as in Fig. 2.
DISCUSSION

The principal findings in this study were that the major sources of NH$_4^+$ extracted by the liver of fasted dogs appeared to be the amide nitrogen of glutamine after its deamidation in the portal drainage system rather than in the liver. The other major source of nitrogen extracted by the liver was from alanine, whose ultimate source was the systemic rather than the portal drainage system. The $^{15}$N portion of the protocol strongly suggested that the $^{15}$N incorporated into urea was derived primarily from $^{15}$NH$_4^+$ produced from the amide nitrogen of glutamine and that the alanine nitrogen did not mix appreciably with the $^{15}$NH$_4^+$ pool.

NH$_4^+$ is a critically important, yet toxic, metabolic intermediate in the degradation of amino acids (Fig. 1). Therefore, its concentration in plasma and cells must be maintained at very low levels. Accordingly, the metabolism of NH$_4^+$ is difficult to monitor in vivo with tracer techniques, because 1) multiple samples are needed in view of its rapid turnover, and 2) current techniques for measuring the $^{15}$N enrichment of plasma NH$_4^+$ require fairly large volumes of blood. For these reasons, we developed a technique that is capable of measuring the concentration and a low $^{15}$N enrichment of NH$_4^+$ on smaller blood samples (30). With this technique, the metabolism of NH$_4^+$ and glutamine can be followed in organs in vivo. When combined with the traditional arteriovenous measurements of metabolites, along with an index of blood flow rate to specific organs, valuable new insights about this metabolism in vivo can be obtained.

The stoichiometry involved in hepatic NH$_4^+$ metabolism played a central role in our thinking (Fig. 1). An equal number of NH$_4^+$ and aspartate molecules must be provided within the liver for the combined synthesis of new glucose molecules plus urea, because hepatic true GNG and ureagenesis are different ways of naming the same metabolic pathway (10, 16). We reasoned that a relatively large amount of NH$_4^+$ but a small exogenous amount of aspartate would be provided to the liver in patients who eat a low-protein diet. This could create a metabolic problem if aspartate could not be made quickly enough in the liver from exogenous NH$_4^+$ plus fumarate (Fig. 1) and/or oxaloacetate formed via pyruvate carboxylase. With use of the above points as linchpins for our approach, there were unexpected findings, challenges to existing hypotheses for the control of glutamine metabolism, and a novel mechanism was suggested as a partial explanation for the protein catabolic state found in patients with end-stage renal disease. In quantitative terms, there could be a large supply of NH$_4^+$ delivered to the liver in patients with poor renal function if a large amount of urea were to enter the colon [because of the high urea concentration in body fluids and a urea transport system in the intestinal mucosa (14)], where bacterial urease would convert it to NH$_4^+$ + HCO$_3^-$ (or NH$_3^+$ + CO$_2^+$). After absorption, NH$_4^+$ is delivered via the portal vein to the liver. Moreover, with a portal blood flow of close to 1,000 l/day in adult human subjects and the daily hydrolysis of 100 mmol of urea (20% of production) in their colon (8, 28), the [NH$_4^+$] in the portal vein would rise by an average of close to 200 µM. This should lead to a supply of NH$_4^+$ that greatly exceeds exogenous aspartate when a low-protein diet is consumed.

Portal drainage system. There was a surprising finding at the outset in the portal drainage system, because we did not expect to find a much higher [NH$_4^+$] in the portal venous than in the arterial blood in fasted dogs unless its source was $^{14}$N urea that was hydrolyzed in the colon by bacterial urease. Three lines of evidence suggested that the source of the extra portal vein NH$_4^+$ was the amide ($^{15}$N) nitrogen of glutamine (Fig 6). First, the decline in the concentration of glutamine was similar to the rise in the $[^{15}]$NH$_4^+$ when portal venous and arterial blood are compared (Table 2). Second, the concentration of NH$_4^+$ in the portal drainage system increased fourfold (Table 2), whereas its MPE only halved (Fig. 2B), implying that most of the NH$_4^+$ produced was derived from a pool with a $^{15}$N MPE similar to $^{14}$NH$_4^+$. This in effect suggests that the amide (or $^{15}$N nitrogen) and not the amino (or $^{14}$N nitrogen) of glutamine or the nitrogens in urea (which are mostly $^{14}$N) was the source of this $^{15}$NH$_4^+$. Third, given the stoichiometry of glutamine oxidation to CO$_2$ + NH$_3$, there could only be complete oxidation of this fuel if glutamine were the only fuel oxidized by the entire portal venous drainage bed (Eq. 1); this is an unlikely possibility (29).

The site for glutamine deamidation in fasted dogs was in the portal drainage bed, and this differs from the scheme proposed by Häusssinger et al. (13). On the basis of data gathered in isolated rat livers perfused with NH$_4$Cl or glutamine, Häusssinger inferred that the hydrolysis of glutamine in vivo occurs in periportal hepatocytes (17) because they contain high activities of glutaminase and carbamoyl phosphate synthetase (CPS). The fate of the amine nitrogen of glutamine was not established in the present study because there was no detectable release of glutamate into the portal vein, and the major transamination product, alanine (Table 3), did not appear in significant amounts in the portal venous blood. One possible explanation is that glutamate was transformed into compounds such as ornithine or citrulline in the intestinal tract. These compounds are poorly extracted by the liver and could be converted to arginine in the proximal convoluted tubule of the kidney (for a review, see Ref. 1). This allows arginine to avoid immediate hepatic hydrolysis and be delivered to other organs, where it can serve as the substrate for protein synthesis and for the production of the critically important messenger nitric oxide (24).

The deamidation of glutamine in the intestinal tract seems to be a paradox if one focuses only on NH$_4^+$ rather than glutamine (5, 6, 26). A derivative of glutamate, N-acetyl glutamate, is the physiological activator of CPS (23), the first enzymatic reaction that removes toxic NH$_4^+$ in the process of converting it into the nontoxic nitrogenous end-product, urea. Therefore, if some of this intestinally derived glutamate had reached the hepatocytes, it could have led to a high level of
N-acetyl glutamate without requiring the production of glutamate by hepatocytes.

Events in the liver. The liver removed even more than all the extra NH$_4^+$ (derived from glutamine amide) delivered to it in portal venous blood in a single pass (the hepatic vein [NH$_4^+$] was significantly lower than the [NH$_4^+$] in arterial blood, Table 2). Second, only one-half of the nitrogen in newly synthesized urea appeared to be $^{15}$N enriched. Our basis for this impression is derived from the fractional synthetic rate (FSR) of urea, which was calculated in two different ways. First, we compared the output of urinary urea with the urea body pool size (calculated from plasma concentration and a volume of distribution of 2/3 of body weight). This yielded an FSR of 10–12%/h, which is similar to published values in humans, where the pool size is close to 200 mmol (5 mM × 40 liters) and the excretion of urea is 480 mmol/day or 20 mmol/h (8, 28). Second, after finding very low $^{15}$N enrichment of plasma aspartate (<0.2%), as had also been found in humans infused with tracer $^{15}$NH$_4$Cl (25), we assumed that urea was labeled only from free $^{15}$NH$_4^+$. Accordingly, we divided the linear rate of labeling of plasma urea nitrogen (Fig. 5) by one-half the $^{15}$N enrichment of NH$_4^+$ entering the liver, and the result was an FSR of 10%. The similarity between the stoichiometric and the isotopic calculations of the FSR for urea supports the assumption made in the latter calculation. Under the conditions of tracer infusion, it was not possible to detect doubly labeled $[^{15}$N$^2]$urea by GC-MS of the intact molecule. If both urea N atoms were equally labeled at 0.9% at 3 h (Fig. 5), the molar fraction of doubly labeled urea would be <0.008%, which would not have been detected.

The fact that only one of the urea nitrogens seemed to be labeled was not anticipated, because NH$_4^+$ in the portal compartment was $^{15}$N enriched, and it is the most abundant source of the $^{15}$N in urea. Moreover, to be incorporated into urea, $^{15}$NH$_4^+$ had to enter the mitochondrial compartment and gain access to CPS (Fig. 6). Unless there was a surprising streaming, one would anticipate that this $^{15}$NH$_4^+$ would label the mitochondrial glutamate pool via glutamate dehydrogenase. Moreover, intramitochondrial aspartate should also have $^{15}$N labeling. Therefore, to explain why mitochondrial $[^{15}$N$]$glutamate did not label aspartate used for the synthesis of urea, we favor the following scheme. The pathway of aspartate metabolism for the synthesis of argininosuccinate is located primarily in the cytosolic compartment (Fig. 6). The major source of its nitrogen was alanine (Table 3), which would un-

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**Fig. 6. Compartmentation of $^{15}$N enrichment of urea precursors labeled from exogenous $^{15}$NH$_4^+$ in liver, with cytosolic compartment (left) and mitochondrial compartment (right of vertical double lines). In mitochondrial compartment, $^{15}$NH$_4^+$ is incorporated into aspartate, carbamoyl-phosphate, and then citrulline. The $[^{15}$N$]$aspartate does not appear to equilibrate with $[^{14}$N$]$aspartate in the cytosol. The other important mitochondrial reaction converts pyruvate to malate via pyruvate carboxylase. The exit of citrulline is linked to the entry of ornithine, and the exit of malate occurs via the dicarboxylate carrier in exchange for inorganic phosphate (P). Transamination reactions in cytosol generate $[^{14}$N$]$aspartate needed for synthesis of argininosuccinate. The precursor of malate, oxaloacetate (OAA), could be formed via pyruvate metabolism or from fumarate via cytosolic steps as illustrated. aKG, α-ketoglutarate; PEP, phosphoenolpyruvate; GDH, glutamate dehydrogenase.**
This page discusses the metabolism of glutamine and ammonium in the dog, focusing on the process of ammonia metabolism in the liver. The text highlights the role of aspartate and its labeling with ammonium (NH₄⁺) to study the contribution of endogenous sources to the ureagenesis pathway. The metabolic processes are described in the context of hepatic zonation and the enzyme urea cycle. The text also mentions the importance of amino acid metabolism and the contribution of ammonia to the nitrogen balance in the liver. The discussion includes the use of urea as a tracer and the involvement of bacterial urease in the ammonium metabolism. The text provides insights into the regulation of ammonia production and the impact of dietary protein on nitrogen metabolism. Overall, the page integrates experimental data with theoretical considerations to provide a comprehensive understanding of ammonia metabolism in fasting dogs.
GI tract would be a physiologically advantageous event. Moreover, spreading this supply of NH₄⁺ over more than
half of the 24-h period is an additional means of having
smaller fluctuations in the daily fluxes in the GNG/GIR
and, as a result, an unwanted urea-induced osmotic diuresis.

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