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# Metabolic Fate of [<sup>13</sup>N]Ammonia in Human and Canine Blood

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Nitrogen-13- (<sup>13</sup>N) ammonia is a widely used tracer for PET myocardial blood flow studies. Quantification of blood flow using tracer kinetic principles requires accurate determination of [<sup>13</sup>N]ammonia activity in blood. Since [<sup>13</sup>N]ammonia is rapidly metabolized, the arterial input function may be contaminated by labeled metabolites. We, therefore, characterized the <sup>13</sup>N-labeled metabolites in blood after intravenous (i.v.) injection of 20 mCi [<sup>13</sup>N]ammonia in nine healthy volunteers. Utilizing a series of ion exchange resins, <sup>13</sup>N-labeled compounds were separated into four groups: ammonia, neutral amino acids, acidic amino acids, and urea. Analysis of the metabolic fate of [<sup>13</sup>N]ammonia indicates that over 90% of the blood activity within the first two minutes after injection is present as [<sup>13</sup>N]ammonia. However, there is considerable contamination of the blood activity at 3–5 min by [<sup>13</sup>N]glutamine (amide) and urea, which collectively represent 18%–50% of the blood activity. Thus, correction of the arterial input function for <sup>13</sup>N-metabolites is required to accurately quantify the arterial input function of [<sup>13</sup>N]ammonia in myocardial blood flow studies.

**J Nucl Med 1990; 31:163–167**

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**N**oninvasive assessment of regional myocardial blood flow using PET has been performed using a number of blood flow tracers. The accurate measurement of the arterial blood curve, called the input function, is required for quantification of regional myocardial blood flow. This input function is obtained either by withdrawing discrete arterial blood samples followed by well counting or by defining the time-activity curve derived from a region of interest (ROI) over the left atrial or ventricular chambers in dynamic myocardial PET images (1). Routine quantification of blood flow with ammonia assumes that the activity in the blood represents the ammonia input function to the myocardial tissue. Thus, no correction is applied for the presence of labeled metabolites in the blood samples.

Received Apr. 4, 1989; revision accepted Sept. 22, 1989.  
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The distribution and metabolic fate of [<sup>13</sup>N]ammonia has been previously reported for a variety of tissues (tumor, heart, blood, liver, and brain). These studies were performed in a variety of animal species, including man, as a function of the route of administration (i.v., i.p., hepatic portal vein, carotid artery, and rectal administration) (2–11), all of which may alter the metabolic fate of [<sup>13</sup>N]ammonia in blood. Since the circulating levels of ammonia are normally low and exogenously administered, ammonia is rapidly metabolized (12). We hypothesized that metabolites of [<sup>13</sup>N]ammonia are formed rapidly during acquisition of the arterial input function, which will lead to considerable overestimation of the “true” [<sup>13</sup>N]ammonia input function. Thus, the purpose of this study was to define the metabolic fate of intravenously administered [<sup>13</sup>N]ammonia in human and canine blood and its implication on determination of the arterial input function.

## MATERIALS AND METHODS

### Isotope Production

Nitrogen-13-ammonia was produced by the <sup>16</sup>O(p,α)<sup>13</sup>N reaction on water as previously described (13). The specific activity of the [<sup>13</sup>N]ammonia was ~200–400 mCi/μmol (6).

### Animals

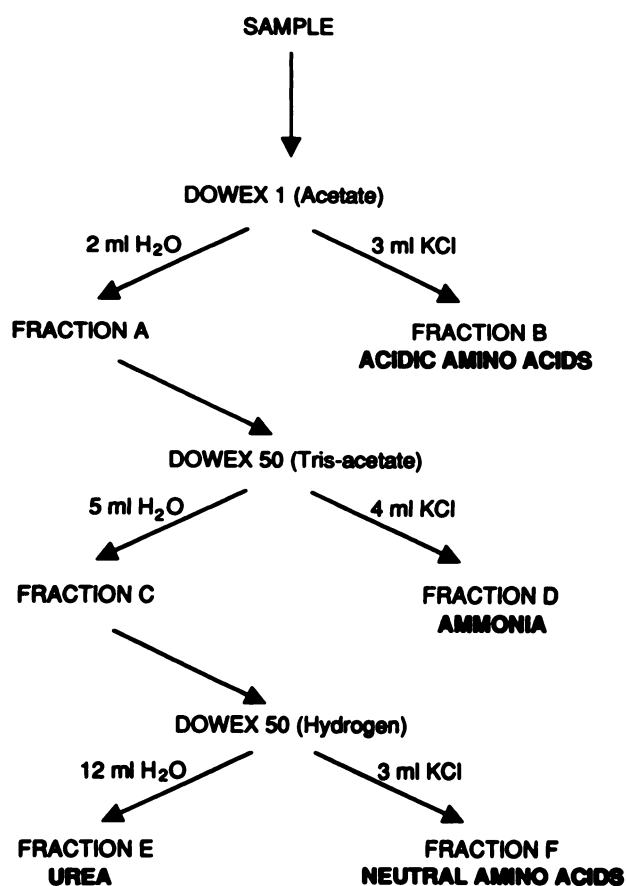
Two mongrel dogs (20–25 kg) anesthetized with pentobarbital, were injected in the femoral vein with 5 mCi of [<sup>13</sup>N]ammonia in 5 ml normal saline over 30 sec. Heparinized arterial blood samples were removed from the femoral artery at 2, 5, 7 and 10 min after injection. Two dogs (15 and 24 kg) were injected with 80 and 150 mCi of [<sup>13</sup>N]ammonia, respectively, with blood samples taken as above. Blood samples from these dogs were used for metabolic identification of the neutral amino acid and urea fractions using enzyme treatment (see below).

### Human Volunteers

After obtaining informed consent, nine healthy male volunteers (ages 19–25) were injected in the antecubital vein with 20 mCi of [<sup>13</sup>N]ammonia in 5 ml normal saline as a 30-sec bolus. Heparinized blood samples (3–5 ml) were removed from the contralateral vein at 1, 2, 3, 4 and 5 min after the end of injection.

## Blood Sample Preparation and Analysis

Human and canine whole blood was deproteinized using 5-sulfosalicylic acid (100 mg/ml) followed by centrifugation at  $12,000 \times g$  for 30 sec. The most accurate method for metabolite identification is radio-HPLC (14). However, the short half-life of  $^{13}\text{N}$  limits this technique because of insufficient counts in the blood samples from the 20-mCi [ $^{13}\text{N}$ ] ammonia administration allowed in humans at our institution. Thus, we used a batch elution method on a series of small ion exchange columns as described by Cooper et al. (6).  $^{13}\text{N}$ -metabolites in the supernatant were separated into four  $^{13}\text{N}$ -labeled fractions: ammonia, urea, neutral amino acids (NAA) (i.e., glutamine, asparagine), and acidic amino acids (AAA) (i.e., glutamate, aspartate) (Fig. 1). Briefly, the separation of metabolites was performed as follows. The whole-blood supernatant was applied with pressure to a Dowex 1 column (acetate form,  $0.5 \times 1$  cm) (Bio-Rad Laboratories, Richmond, CA). The pass-through with a 2-ml water wash (fraction A) was collected. [ $^{13}\text{N}$ ]AAA were retained and eluted with 3 ml 1M KCl (fraction B). Fraction A was then applied to a Dowex 50 column (Tris-acetate form,  $0.5 \times 4$  cm) (Sigma Chemical Co., St. Louis, MO) and washed with 5 ml water (fraction C). This column retains [ $^{13}\text{N}$ ]ammonia, which is



**FIGURE 1**

Schematic depiction of the sequence followed for  $^{13}\text{N}$ -labeled metabolite separation with ion exchange resins using the method of Cooper et al. (6). The process resulted in the  $^{13}\text{N}$  activity from blood being separated into acidic amino acids, ammonia, urea, and neutral amino acids.

then eluted with 4 ml 1M KCl (fraction D). Fraction C is then applied to a Dowex 50 column (hydrogen form,  $0.5 \times 1$  cm) (Bio-Rad Laboratories, Richmond, CA) and washed with 12 ml water to remove [ $^{13}\text{N}$ ]urea (fraction E). [ $^{13}\text{N}$ ]NAA were removed with 3 ml 1M KCl (fraction F). Each of the four remaining fractions (B, D, E, F) from the columns (representing [ $^{13}\text{N}$ ]AAA, ammonia, urea, and NAA) as well as the ion exchange columns were counted for radioactivity and then decay corrected, with results expressed as percent radioactivity in each fraction for each blood sample. The total activity in the four fractions was taken as 100% of the radioactivity. Less than 2% of the radioactivity remained on the columns and was, thus, not accounted for in calculating the percent radioactivity in the four fractions. In addition, because the blood supernatant is acidic, no precautions were necessary to ensure against volatilization. The time required for each study, from time of injection to counting the metabolite fractions from four blood samples, was 20–40 min.

The extraction efficiency (i.e., the percent radioactivity extracted into the supernatant from the tissue) was determined by counting the radioactivity in the supernatant and pellet samples after centrifugation.

## Enzymatic Assay

We confirmed the identity of the NAA and urea fractions by treating them with glutaminase and urease, respectively. Since these fractions were found to represent a considerable portion of the total blood activity with time, we felt it necessary to identify them especially in light of potential myocardial uptake.

Glutaminase was used to confirm the identity of label in the NAA fraction (fraction F) from the one dog injected with 150 mCi of [ $^{13}\text{N}$ ]ammonia (6). Glutaminase hydrolyzes the amide group of glutamine, resulting in the formation of ammonia and glutamate. The deproteinized supernatant from the five-minute blood sample was passed through a Dowex 1 column (acetate form,  $0.5 \times 1$  cm) (in addition to the Dowex 1 column described above and in Figure 1) to remove the 5-sulfosalicylic acid prior to enzyme treatment. The pass-through from the column with a 2-ml water wash was collected.  $^{13}\text{N}$ -labeled acidic amino acids were retained by this column and were eluted with 3 ml 1M KCl for counting. The pass-through combined with the water wash was divided into two aliquots, which were used for the determination of glutamine. The fraction used for glutamine analysis was adjusted to pH 4.9 and then divided into two fractions. One fraction was treated with 250  $\mu\text{g}$  glutaminase (Grade V) (Sigma Chemicals Co., St. Louis, MO) in 200  $\mu\text{l}$  0.05M acetate buffer. The untreated pH 4.9 fraction served as a control. The fractions were incubated at 37° C for 10 min. The samples were then analyzed on the three Dowex columns as described above and separated into four fractions: AAA, ammonia, urea, and NAA. The difference in the ammonia fractions of the glutaminase-treated and control samples represents the label originally in the amide position of glutamine. The difference in the AAA fractions of the glutaminase-treated and control samples represents label originally in the amino group of glutamine. Label remaining in the NAA fraction from the glutaminase-treated sample represents label as NAA other than glutamine.

Urease was used to confirm the identity of the urea fraction (fraction E) by converting urea to ammonia and carbon dioxide upon urease treatment (5). This study was performed

since components other than urea may co-elute. A dog was injected with 80 mCi of [<sup>13</sup>N]ammonia. A 5-min blood sample was collected, deproteinized, and passed through a Dowex 1 column as described above for glutaminase treatment, except the sample was adjusted to pH 8. The pH 8-adjusted sample was divided into two fractions. One fraction was treated with 5500 units of urease (Type IX) (Sigma Chemical Co., St. Louis, MO), and the second fraction served as a control. Following a 3-min incubation at 37° C, the samples were applied to the three ion exchange columns as described above. The differences between the percent in the ammonia fractions of the urease-treated sample and the control represents label originally present as urea. Label in the urea fraction of the urease-treated sample represents nonurea components co-eluting with urea or unreacted urea.

### Statistical Analysis

Significance was determined using the Student's t-test. Data are expressed as means ± standard deviation.

## RESULTS

### Metabolism of [<sup>13</sup>N]Ammonia in Dog

Table 1 shows the results of the metabolic analysis in dog blood, expressed as a relative fraction of total blood activity at each time. The [<sup>13</sup>N]ammonia fraction remains over 80% within the first 2 min before it rapidly decreased to only ~15% of the total blood activity as ammonia at 7 min and 10 min after injection. Urea is the predominant metabolite being formed, representing over 40% of the blood activity at 5 min and over 60% at 10 min after injection. [<sup>13</sup>N]NAA are the second most predominant metabolite with ~25% of the total blood activity at 5 min and 20% at 10 min.

The results of glutaminase treatment show that over 99% of the NAA activity was converted to [<sup>13</sup>N]ammonia, indicating that the radioactivity is present in the NAA fraction as [<sup>13</sup>N]glutamine (amide). Likewise,

urease treatment resulted in >93% of the <sup>13</sup>N in the urea fraction being converted to [<sup>13</sup>N]ammonia.

### Human Metabolism

Table 2 shows the average time course of [<sup>13</sup>N]ammonia metabolism determined in blood from nine individuals. [<sup>13</sup>N]Ammonia is the principle component of the blood activity during the first two minutes after injection; however, the relative contribution of ammonia to the total blood activity declines rapidly thereafter. The decrease of the [<sup>13</sup>N]ammonia component appears slower than in the dog, as there is ~50% of the activity in blood as [<sup>13</sup>N]ammonia at five minutes in humans compared to ~30% in dogs. This difference, however, does not reach significance (*p* > 0.10).

As in the animal studies the principle metabolites found in human blood are [<sup>13</sup>N]urea and [<sup>13</sup>N]NAA. There is less than 1% of the total blood activity present as <sup>13</sup>N-labeled acidic amino acids (i.e., glutamate or aspartate).

In Figure 2, integrals of the concentration of both [<sup>13</sup>N]ammonia and total <sup>13</sup>N (ammonia and metabolites) in blood are plotted as a function of time. The total <sup>13</sup>N curve (curve A) was generated from an ROI placed over the left ventricle in a kinetic ammonia PET study following the i.v. injections of 20 mCi of [<sup>13</sup>N]ammonia. The integral of the [<sup>13</sup>N]ammonia (curve B) in the arterial blood was devised by correcting the <sup>13</sup>N curve A by the average metabolite fractions in Table 2. The uncorrected activity integral overestimated the true [<sup>13</sup>N]ammonia input by 8% at 2 min increasing to 27% at 10 min following i.v. injection.

## DISCUSSION

The presented data demonstrate the rapid metabolism of [<sup>13</sup>N]ammonia following i.v. injection in canine

**TABLE 1**  
Distribution of Radioactivity in Canine Whole Blood after [<sup>13</sup>N]Ammonia Administration\*

Metabolite	% Metabolite†			
	2 min	5 min	7 min	10 min
Ammonia	81.1 ± 16.9 (61.7–92.5)	31.7 ± 17.2 (17.7–50.9)	14.6 ± 5.0 (10.0–19.9)	14.5 ± 6.5 (7.6–20.9)
Urea‡	16.2 ± 19.1 (1.4–37.9)	42.0 ± 16.5 (26.5–59.5)	61.0 ± 21.4 (47.3–85.7)	64.6 ± 14.8 (49.9–79.4)
Neutral amino acids§	2.1 ± 3.3 (0–5.9)	26.0 ± 14.5 (13.5–41.9)	23.8 ± 20.8 (0.1–39.6)	20.7 ± 20.9 (0.1–41.9)
Acidic amino acids	0.6 ± 0.5 (0.2–1.2)	0.3 ± 0.3 (0–0.5)	0.7 ± 0.4 (0.4–1.1)	0.3 ± 0.3 (0.1–0.6)

\* Mean ± s.d.; N = 3; range below in parentheses.

† Based on % of total blood radioactivity extracted in the supernatant; the extraction efficiency was 95.4 ± 0.8 (mean ± s.d.; n = 4).

‡ Analysis of this fraction revealed that >93% of the radioactivity was due to [<sup>13</sup>N]urea.

§ Analysis of this fraction revealed that >99% of the radioactivity was due to L-[amide-<sup>13</sup>N]glutamine.

**TABLE 2**  
Distribution of Radioactivity in Human Whole Blood after [<sup>13</sup>N]ammonia Administration\*

Metabolite	% Metabolite†				
	1 min (n = 6)	2 min (n = 7)	3 min (n = 8)	4 min (n = 8)	5 min (n = 5)
Ammonia	93.1 ± 4.9 (85.7–98.9)	94.0 ± 2.8 (90.0–95.9)	81.8 ± 10.5 (63.1–99.1)	74.7 ± 13.4 (56.9–97.5)	50.2 ± 18.9 (26.0–75.0)
Urea	3.1 ± 4.6 (0–10.0)	1.5 ± 1.9 (0–6.2)	7.2 ± 7.3 (0–23.2)	11.1 ± 9.2 (0–28.3)	32.7 ± 10.0 (23.8–49.1)
Neutral amino acids	3.0 ± 2.4 (0–6.5)	4.0 ± 1.5 (1.7–6.1)	10.4 ± 4.5 (0.9–15.5)	13.8 ± 7.5 (1.0–21.6)	16.2 ± 11.2 (0.2–27.9)
Acidic amino acids	0.8 ± 0.7 (0–1.7)	0.4 ± 0.5 (0–1.5)	1.1 ± 1.4 (0–1.5)	0.4 ± 0.3 (0.1–0.8)	0.9 ± 0.7 (0–1.7)

\* Mean ± s.d. with range below in parentheses.  
† Based on % of total-blood radioactivity.

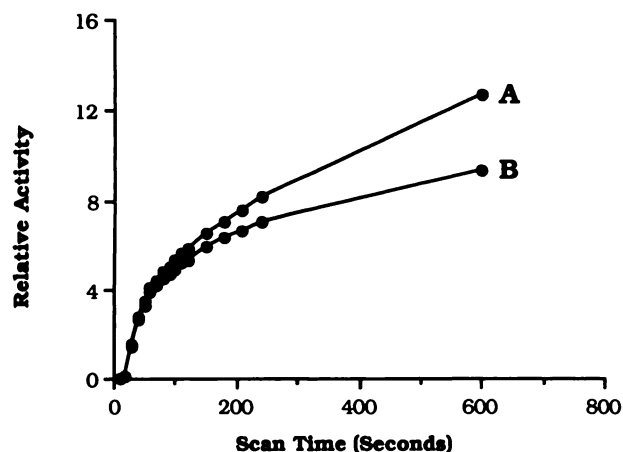
and human studies. Although [<sup>13</sup>N]ammonia contributes over 80% to the arterial input function during the first two minutes, <sup>13</sup>N-labeled metabolites appear rapidly in blood and represent ~50% of the total blood activity at five minutes after tracer injection. This contamination represents ~25% overestimation of the [<sup>13</sup>N]ammonia input function for a 10-min duration study.

The principal labeled metabolites from [<sup>13</sup>N]ammonia found in humans and dogs are urea and glutamine (amide). Qualitatively, these findings are similar to the results reported in rats and mice using intraperitoneal, intravenous and carotid injections (3,5,8). Urea is pro-

duced by a five-enzyme step in the liver (15). Glutamine is produced by glutamine synthetase in a variety of tissues, including muscle and liver (16). The rates of these reactions are rapid. The half-time of blood-borne [<sup>13</sup>N]ammonia conversion to L-[amide-<sup>13</sup>N]glutamine in the brain is <3 sec (6). The half-time of portal vein-derived <sup>13</sup>N in rat liver to be converted to labeled glutamine and urea is 10–11 sec (7).

Qualitatively, it is difficult to compare our findings with the results of others since they were obtained in different species and used different routes of administration. There are species differences in metabolism of <sup>13</sup>N-labeled amino acids (8). Also, it is known that different routes of administration can alter the metabolic fate (17). After an i.p. injection, all of the material is absorbed into the circulation via the mesenteric vascular bed, which drains through the liver via the hepatic portal system. The liver contributes significantly to ammonia metabolism (12), thus allowing more metabolites to be produced than from an i.v. injection. Thus, it appears from our study that the metabolic fate of [<sup>13</sup>N]ammonia is similar in dog and man; only the rate of metabolism is different.

Lockwood et al. (9) performed a study similar to ours on human volunteers, whereby the deproteinized blood samples were made alkaline and the volatile <sup>13</sup>N (i.e., [<sup>13</sup>N]ammonia) was blown off with the nonvolatile remaining radioactivity representing metabolites. Their results showed detectable levels of <sup>13</sup>N-labeled metabolites at 3 min and with all the <sup>13</sup>N activity in blood as metabolites by 10 min. However, their analysis omitted important early time-points before five minutes, when the metabolites were being formed. They found that <sup>13</sup>N-labeled metabolites accounted for 21%–24% of the <sup>13</sup>N integral blood curve (0–10 min) in five individuals (8). Our results are consistent with these previously published results.



**FIGURE 2**  
Integral curves of the total <sup>13</sup>N activity and [<sup>13</sup>N]ammonia in human blood. The integral of the <sup>13</sup>N activity (curve A) in blood was calculated by drawing a ROI over the left ventricle of a healthy volunteer. The integral of the [<sup>13</sup>N]ammonia (curve B) in blood was generated by correcting the total <sup>13</sup>N curve by mean metabolite function observed in this study.

The determination of  $^{13}\text{N}$ -labeled metabolites was performed on whole blood rather than plasma, since ammonia in plasma has been shown to be in rapid equilibrium with the red blood cell (Reiman RE, *personal communication*, 1989). In the case of glutamine, there is no significant difference between glutamine concentrations in whole blood and plasma (18).

Not only is it important to define the metabolic fate of [ $^{13}\text{N}$ ]ammonia in blood to correct the arterial input function, but it is also important to know the myocardial uptake characteristics of the  $^{13}\text{N}$ -labeled metabolites. [ $^{13}\text{N}$ ]glutamate is known to be taken up avidly by the human myocardium (19). Thus, if a large component of [ $^{13}\text{N}$ ]glutamate were formed *in vivo*, the PET image then would reflect  $^{13}\text{N}$  uptake from both [ $^{13}\text{N}$ ]ammonia as well as [ $^{13}\text{N}$ ]glutamate. However, this is not the case as <1% of the total blood activity is present as AAA. [ $^{13}\text{N}$ ]glutamine (amide), on the other hand, can comprise up to 16% of the total-blood activity. Its distribution has been studied in humans (Gelbard AS, *personal communication*, 1989), but very little heart uptake has been observed qualitatively. [ $^{13}\text{N}$ ]urea is the principle metabolite found in blood, however, little has been reported about the quantitative distribution of urea in man (20). The major proportion of urea is excreted in the urine (22.5% and 89.6% at 2 and 130 hr, respectively), with a minor amount diffused uniformly through total body water (20,21).

A simplified analysis for [ $^{13}\text{N}$ ]ammonia could readily be performed using only a Dowex 50 (hydrogen form) column. [ $^{13}\text{N}$ ]ammonia would be retained by this column allowing all  $^{13}\text{N}$ -labeled metabolites (urea, NAA, AAA) to pass through with the water wash. This would be followed by a KCl wash to elute [ $^{13}\text{N}$ ]ammonia.

In summary, we have found that there are significant  $^{13}\text{N}$ -labeled metabolites in blood following *i.v.* administration of [ $^{13}\text{N}$ ]ammonia. The metabolites in blood rapidly appear during the imaging interval and, thus, can adversely affect the arterial input function. We have identified the  $^{13}\text{N}$ -labeled metabolites in blood as [ $^{13}\text{N}$ ]urea and [ $^{13}\text{N}$ ]glutamine (amide). Correction of the arterial  $^{13}\text{N}$  activity is necessary for the accurate definition of the [ $^{13}\text{N}$ ]ammonia input function used for quantification of myocardial kinetics by PET.

#### ACKNOWLEDGMENTS

The authors thank Leslie Shaw, Jill Rothley, Annette Betley, and Anne Holub for their help with sample analysis, Ms. Vi Rhodes and Ms. Jill Anderson for manuscript preparation, and other members of the Division of Nuclear Medicine for assistance.

This work was supported by American Heart Grant # 88-0699-JI and NIH Grant # 89-2359Ja and HL 27555.

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