Metabolism of Ingested Ammonium- ^{15}N by Premature Infants

JOHN F. NICHOLSON^[20]

Babies Hospital and the Department of Pediatrics, College of Physicians and Surgeons,
Columbia University, New York, New York, USA

Extract

In premature infants receiving high protein diets (3.2–5.1 g/kg/24 h) and given oral trace doses of $^{15}\text{NH}_4\text{Cl}$, [ammonia- ^{15}N] was initially higher than [urea- ^{15}N] in the urine. Isotope concentrations were equal at the time of maximum urea- ^{15}N concentration, suggesting that the pool of nitrogen sampled as ammonia- ^{15}N in urine was the precursor for urea- ^{15}N under these conditions. In premature infants receiving low protein diets (1.5 and 1.0 g protein/kg/24 h), this precursor-product relation was lost, [ammonia- ^{15}N] remaining higher than [urea- ^{15}N] after the time of maximum urea- ^{15}N concentration. This was also true for an infant receiving 1.5 g protein/kg/24 h when glycine- ^{15}N was administered, suggesting that the alteration in the relation between [urea- ^{15}N] and [ammonia- ^{15}N] in urine, under conditions of lowered protein intake, was a result of a change in the relation of the metabolic pool of nitrogen to urea and not to a specific alteration in the metabolism of ingested ammonium ion.

As protein intake was progressively restricted, overall excretion of urea was reduced as well as excretion of urea- ^{15}N , in response to a trace dose of $^{15}\text{NH}_4\text{Cl}$. No evidence was found to indicate that the mode of metabolism of ammonium- ^{15}N by premature infants receiving a high protein diet was necessitated by a lack of capacity for urea synthesis.

The size (P) and turnover rate (B) of the pool of nitrogen reflected by [ammonia- ^{15}N] in urine was evaluated after ingestion of $^{15}\text{NH}_4\text{Cl}$. The pool size was smaller than that found after ingestion of glycine- ^{15}N . The rate of metabolism, $P \times B$, declined with decreasing protein intake.

In one infant who received both glycine- ^{15}N and $^{15}\text{NH}_4\text{Cl}$ on a high and a low protein diet, estimations of size and rate of turnover of the pool of metabolic nitrogen were made by analyzing $[\text{NH}_3\text{-}^{15}\text{N}]$ and by using the method of SAN PIETRO and RITTENBERG. The results were similar when $^{15}\text{NH}_4\text{Cl}$ was the test compound and dissimilar when glycine- ^{15}N was the test compound.

Speculation

The data presented suggest that the nitrogen of ammonia absorbed via the portal vein is incorporated into the active metabolic pool of nitrogen, thus providing a flexible mechanism for both the conservation of ammonia nitrogen and its incorporation into urea.

Introduction

Through studies in animals on the toxicity of ammonia [5], the fixation of ammonia has come to be regarded as an essential detoxification in mammalian metabolism. It is generally accepted that the principal reactions for the fixation of ammonia nitrogen in mammalian liver are: 1) the synthesis of glutamate from ammonia, and α -ketoglutarate; 2) the synthesis of glutamine from ammonia and glutamate; and 3) the synthesis of carbamyl phosphate from ammonia, carbon dioxide, and adenosine triphosphate. These three reactions are similar in that they use free ammonia, but differ in that the synthesis of carbamyl phosphate is the first step in urea synthesis and can be regarded in large part as an excretory process while the syntheses of glutamate and glutamine are potentially conservative.

The studies of SPRINSON and RITTENBERG [12], and WU and SENDROY [15], on the metabolism of ammonium ion by adult humans showed, in the presence of high protein intake, rapid incorporation of administered ^{15}N ammonium ion into urea. Ammonia in urine, considered representative of the pool of metabolically active nitrogen from which it was derived, contained only small amounts of ^{15}N . It appeared from these studies that a large part of the ingested ammonium ion might be incorporated directly into urea. Studies of the first enzyme in the Krebs-Henseleit cycle supported this view by demonstrating that free ammonia was necessary for the synthesis of carbamyl phosphate [1, 4]. SPRINSON and RITTENBERG [12], however, showed that the nitrogen of ingested ammonia could be conserved when dietary protein was restricted. This aspect of the metabolism of ammonia was emphasized by SNYDERMAN *et al.* [11] in studies of premature infants receiving diets low in protein. The infants showed striking incorporation of the nitrogen of ^{15}N -urea into plasma proteins. Since urea nitrogen becomes available for incorporation into amino acids only after hydrolysis to CO_2 and NH_3 through the action of intestinal bacteria [14], these findings indicate conservation of ammonia nitrogen under conditions of restricted nitrogen intake.

Other studies involving undernourished infants [8] and adults with renal disease [3, 9] have also demonstrated conservation of ammonia- ^{15}N .

Studies previously reported [6] dealt with some of the characteristics of the metabolism of glycine-nitrogen by premature infants. The present studies of the metabolism of ammonia-nitrogen were performed to delineate some of the differences and similarities between the metabolism of free ammonia and of amino nitrogen in these infants.

Material and Methods

Subjects

Premature infants of various ages were the subjects for these studies (table I). All were considered to be in good health and growing rapidly. For studies of the metabolism of labeled compounds in association with reduced dietary protein, the infants were fed diets containing the specified amount of protein together with additional fat and carbohydrate so that the total calorie intake remained unchanged. Infants receiving 1.5 and 1.0 g protein/kg/24 h were maintained at this level for 12 and 10 days, respectively, during which time they continued to gain weight, though at reduced rates [16].

Experimental

The infants ingested the test compounds (table I) together with feeding. Spontaneously voided urine specimens were collected and frozen immediately in dry ice. Glycine- ^{15}N (97% ^{15}N) and NH_4Cl - ^{15}N (95.2% ^{15}N) were obtained commercially [17].

Analytical

Urine specimens were analyzed as described [6]. Calculations involving amounts of labeled nitrogen used milliequivalents as the unit of measurement for nitrogen. Tabulated results for pool sizes and rates of protein synthesis and nitrogen excretion were obtained by multiplying the final value in mEq N by 0.01401 g N/mEq N.

Table I. Identifying data for studies of the metabolism of compounds labeled with ^{15}N

Infant	Age at study, days	Birth wt, g	Wt at study, g	Diet/24 h		Test compound	^{15}N dosage, mEq
				Protein, g/kg	Calories, kCal/kg		
<i>Pe</i>	83	1,220	2,820	5.1	150	NH_4Cl	0.515
<i>Sh</i>	41	1,430	2,270	4.6	133	NH_4Cl	0.551
<i>DC</i>	70	1,070	2,430	4.4	121	NH_4Cl	0.184
<i>St</i>	40	1,610	2,430	3.2	124	NH_4Cl	0.429
<i>DC</i>	80	1,070	2,550	1.5	121	NH_4Cl	0.184
<i>Af</i>	21	2,250	2,260	1.0	126	NH_4Cl	0.184
<i>DC</i>	78	1,070	2,540	1.5	121	Glycine	0.643

Results

Ammonia in urine was rapidly and heavily labeled following administration of $^{15}\text{NH}_4\text{Cl}$ (figs. 1 and 2). The concentration of ^{15}N in urea rose more slowly. The curves for $[\text{NH}_3\text{-}^{15}\text{N}]$ and $[\text{total } ^{15}\text{N}]$ in figure 1 show small regions of discontinuity, which were also observed in several other studies. The reason for this finding was not readily apparent but did not appear to be experimental artifact. The descending limbs of the curves for $[\text{NH}_3\text{-}^{15}\text{N}]$ were related to the curves for $[\text{urea-}^{15}\text{N}]$ as precursor to product in the studies of infants receiving a high protein diet and $^{15}\text{NH}_4\text{Cl}$ (table II). In these infants, 16.9, 21.8, and 22.8% of the ^{15}N was excreted as urea in the first 10 h after administration (table II).

When dietary protein was restricted, the relation of the curves for $[\text{NH}_3\text{-}^{15}\text{N}]$ and $[\text{urea-}^{15}\text{N}]$ in the urine were considerably altered (fig. 2), and the precursor-product relation was absent. The absence of a precursor-product relation was also noted when glycine- ^{15}N was administered to an infant receiving a low protein diet (table IV). As dietary protein was progressively restricted, the excretion of urea (table III) and urea- ^{15}N (fig. 3) progressively declined.

Discussion

Detoxification of Ammonia Nitrogen by Premature Infants Receiving a Normal Diet

Some of the features of the data obtained in studies of the metabolism of ammonia- ^{15}N by infants receiving high protein diets are presented in table II, along with similar data obtained in a previous study using glycine- ^{15}N as the test compound [6]. It was apparent that ^{15}N

Fig. 1. Concentrations of ^{15}N in ammonia and urea in urine following ingestion of $^{15}\text{NH}_4\text{Cl}$ by infant DC while receiving a high protein diet.

Fig. 2. Concentrations of ^{15}N in ammonia and urea in urine following ingestion of $^{15}\text{NH}_4\text{Cl}$ by infant DC while receiving a low protein diet.

Fig. 3. Cumulative excretion of ^{15}N in urea as percentage of the dose administered to infants receiving various amounts of dietary protein. Grams of protein per kilogram per day as follows: infants Sh, 4.6; DCH, 4.4; Pe, 5.1; St, 3.2; DCL, 1.5, and Af, 1.0.

Table II. Comparison of excretion of $\text{NH}_3\text{-}^{15}\text{N}$ and urea- ^{15}N by premature infants following ingestion of either glycine- ^{15}N or $^{15}\text{NH}_4\text{Cl}$

Infant	Test compound	^{15}N dosage, mEq	Maximum [urea- ^{15}N], A % excess	Maximum [NH $_3$ - ^{15}N], A % excess	Time of maximum measured [urea- ^{15}N], days	Time of equalization of [NH $_3$ - ^{15}N] and [urea- ^{15}N], days	Dose of ^{15}N excreted as urea in initial period postingestion	
							Excreted, %	Time interval, days
<i>FB</i>	Glycine	0.643	0.240	0.972	0.164	0.255	3.89	0.548
<i>McC</i>	Glycine	0.643	0.229	0.914	0.183	0.210	4.66	0.517
<i>DC</i>	Glycine	0.627	0.174	0.446	0.181	0.245	4.63	0.470
<i>DC</i>	NH_4Cl	0.184	0.299	0.471	0.175	0.150	21.8	0.455
<i>Pe</i>	NH_4Cl	0.515	0.630	1.743	0.115	0.098	16.9	0.429
<i>Sh</i>	NH_4Cl	0.551	0.777	1.989	0.197	0.170	22.8	0.430

Table III. Relation of dietary protein to excretion of ammonia nitrogen and ^{15}N and urea nitrogen and ^{15}N following ingestion of $^{15}\text{NH}_4\text{Cl}$ by premature infants

Infant	Dietary protein, g/kg/24 h	Period of urine collection, days	Urea excreted, g N	Urine NH $_3$ -N Urea- ^{15}N	Time of maximum [urea- ^{15}N], days	Time of equalization of [NH $_3$ - ^{15}N] and [urea- ^{15}N], days	Total NH $_3$ - ^{15}N in urine, % of dose	Total NH $_3$ - ^{15}N Urea- ^{15}N
<i>Pe</i>	5.1	0.429	0.295	0.037	0.115	0.098	0.73	0.043
<i>Sh</i>	4.6	0.430	0.298	0.102	0.197	0.170	2.64	0.116
<i>DC</i>	4.4	0.397	0.222	0.098	0.175	0.150	1.49	0.077
<i>St</i>	3.2	0.342	0.045	0.203	0.154	0.136	2.02	0.383
<i>DC</i>	1.5	0.498	0.022	0.464	0.339	0.38-0.45	2.02	1.161
<i>Af</i>	1.0	0.427	0.010	0.912	0.093	> 0.427	3.78	12.09

was incorporated into urea in higher concentrations when ^{15}N -ammonium ion was administered than when glycine- ^{15}N was administered, even though the dose of ^{15}N -ammonium ion was smaller than that of glycine- ^{15}N . Furthermore, the peak concentration of isotope in ammonia in urine was higher when ammonium ion was the test compound. It was also evident that, in urine, the concentration of ^{15}N in ammonia falls to a level very nearly equal to that in urea at the point of maximum [urea- ^{15}N]. This relation between [NH $_3$ - ^{15}N] and [urea- ^{15}N] would be expected if the pool of nitrogen directly sampled by measuring [NH $_3$ - ^{15}N] were also giving rise to urea- ^{15}N . Since ammonia in urine is derived directly from amino acids and amino acid amides [7] it follows that the concentration of ^{15}N in urinary ammonia was related to the concentration of ^{15}N in urea as precursor to product when glycine- ^{15}N was administered. In the present study, such a precursor-product relation was also observed when am-

monium ion was administered. In their study of an adult subject, Wu and SENDROY [15] found that the administration of ammonium- ^{15}N resulted in a precursor-product relation between [NH $_3$ - ^{15}N] and [urea- ^{15}N] in urine. In this adult subject, however, the peak [urea- ^{15}N] occurred so quickly (0.023 days) that it was possible to interpret the results as showing simultaneous direct incorporation of ammonia into urea and into amino acids. In the present series, urea was labeled much more slowly, and the peak [urea- ^{15}N] occurred much later. The metabolism of ammonia by the premature infants was in this respect quite similar to their metabolism of glycine [6].

Table II also shows the relative excretion of ^{15}N in urea following administration of either ammonium- ^{15}N or glycine- ^{15}N to premature infants receiving normal diets. A much larger percentage of the administered dose was excreted as urea when the test compound was ammonium ion than when the test compound was

glycine. The data in table II suggest that, although orally ingested ammonium ion was largely excreted as urea by premature infants receiving a high protein intake, the metabolic sequences of events involved a primary fixation of ammonia in a pool of metabolically active nitrogen which yielded both urea and ammonia in the urine.

If this interpretation is accepted as correct, there are two possible physiological explanations: there is a relative lack of synthetic capacity for urea in the face of large protein load, so that a primary alternative to a direct synthesis of urea from ingested ammonia is required; or this mode of nitrogen metabolism is the normal one, and its prominence in the present study is merely a function of the relatively slow turnover of metabolic nitrogen in the premature infant [6].

To approach these two possibilities experimentally, the metabolism of ammonia in infants receiving diets with lowered protein content was investigated.

Ammonia Metabolism in the Presence of Restricted Protein Intake

Three infants were given $^{15}\text{NH}_4\text{Cl}$ orally while receiving diets containing 3.2, 1.5, and 1.0 g protein/kg/24 h, respectively. In the infant who received 3.2 g protein/kg/24 h [$\text{NH}_3\text{-}^{15}\text{N}$] and [urea- ^{15}N] exhibited a precursor-product relation. This was not true for the two infants receiving 1.5 and 1.0 g protein/kg/24 h (table III).

Figure 2 shows the concentration of ^{15}N in ammonia and urea in urine following ingestion of $^{15}\text{NH}_4\text{Cl}$ by infant DC on day 12 of a diet containing 1.5 g protein/kg/24 h. Not only was a clear precursor-product relation between the concentrations of ^{15}N in ammonia and urea absent but equilibration of the ^{15}N concentration in these two compounds appeared to be a gradual process. In infant Af (table III), similar findings were noted while the infant was receiving a diet containing 1.0 g protein/kg. When infant DC received glycine- ^{15}N on day 10 of a low protein diet (table IV), the concentration of ^{15}N in urinary ammonia remained higher than that found in urea after 48 h.

If a substantial amount of the ^{15}N ingested as ammonium chloride had been directly incorporated into urea, an early peak in the concentration of ^{15}N in urea might have been observed with loss of the precursor-product relation between [$\text{NH}_3\text{-}^{15}\text{N}$] and [urea- ^{15}N]. Equilibration between the isotope concentrations in urea and ammonia in urine might then be expected to be delayed if the synthesis of urea- ^{15}N from ^{15}N -amino acids were reduced. That this interpretation is unlikely to be true for these infants is shown by the study in which infant DC was given glycine- ^{15}N while receiving a low protein diet (table IV). The precursor for labeled urea is α -amino nitrogen. Maximum concentration of ^{15}N in urea occurred 0.435 days after administration of the isotope while equilibration between [$\text{NH}_3\text{-}^{15}\text{N}$] and

[urea- ^{15}N] had not occurred after 2 days. Therefore, it appeared that the relation between the pool of metabolic nitrogen yielding ammonia and that yielding urea had been altered by the marked reduction in dietary protein. Such an alteration would occur if there was a source of urea in urine not derived from nitrogen in equilibrium with the metabolic pool of nitrogen as a whole. This is possible, if, in the process of urea synthesis, arginine synthesized *de novo* mixes with arginine released in the breakdown of dietary and endogenous protein. For short-term studies it can be reasonably expected that the arginine of proteins will have a lower concentration of ^{15}N than the arginine synthesized *de novo* from the metabolic pool. Therefore, the urea produced from a mixture of the two arginine pools will have a lower ^{15}N concentration than that of the metabolic pool of nitrogen which produces arginine *de novo*. If this is the case for infants receiving diets very low in protein, [urea- ^{15}N] may be unrepresentative of urea synthesized *de novo* at any moment in time, and the curve for [urea- ^{15}N] versus time may be unrepresentative of the relative rate of synthesis of urea- ^{15}N *de novo*. The measured time of maximum [urea- ^{15}N] is valid only if the achievement of an isotopic steady-state between unlabeled arginine and arginine synthesized *de novo* from the metabolic pool is more rapid than the achievement of maximum concentration of isotope in arginine synthesized *de novo*, and the contribution of unlabeled urea to total urea excretion is relatively constant with time.

As shown in figure 3, the percentage of the dose of ^{15}N that was excreted as urea declined progressively as dietary protein was restricted. Although retention of arginine- ^{15}N may lower urea- ^{15}N excretion somewhat under these conditions, it is clear that the overall synthesis of urea was also reduced as dietary nitrogen was reduced (table III). There is, therefore, no evidence that an overload of nitrogen in the high protein diet is operative in determining the mode of ammonium metabolism of the premature infants in this study.

Analysis of Urinary [Ammonia- ^{15}N]

As protein is reduced in the diet, the ratio of ammonia N to urea in the urine increases as does the ratio of ammonia- ^{15}N to urea- ^{15}N (table III). In the infant whose diet was lowest in protein, Af, more nitrogen was excreted as ammonia than as urea and much more ^{15}N as ammonia- ^{15}N than as urea- ^{15}N . The percentage of the dose of ^{15}N excreted as ammonia does not show the same systematic variation with change in diet. It appears, therefore, that the relations between urea- ^{15}N and ammonia- ^{15}N are primarily functions of changes in the excretion of urea- ^{15}N rather than alterations in the characteristics of ammonia- ^{15}N production. An attempt was made to evaluate the size and turnover rate of the

Table IV. Excretion of ^{15}N in urea and ammonia in urine following the ingestion of glycine- ^{15}N

Sample no.	Collection period, days	Urinary urea A % excess	Urinary ammonia
1	0-0.035	0.001	0.359
5	0.091-0.135	0.088	1.625
7	0.223-0.253	0.124	0.747
12	0.411-0.459	0.177	0.304
14	0.504-0.622	0.138	0.251
17	0.841-1.039	0.035	0.127
20	1.296-1.435	0.053	0.087
23	1.911-1.983	0.035	0.073

¹ All samples were obtained from *infant DC* while he was fed a low protein diet.

Table V. Magnitude (P) and rate of turnover (B) of the pool of metabolic nitrogen calculated from the disappearance of ^{15}N from ammonia following ingestion of $^{15}\text{NH}_4\text{Cl}$ by premature infants

Infant	Amount of ^{15}N ingested, mEq	(P) Meta-bolic pool size, g N	(B) Turn-over rate, 1/24 h	Protein in diet, g/kg	B $\times$ P, g N/24 h
Pe	0.515	0.283	12.6	5.1	3.57
Sh	0.551	0.196	9.7	4.6	1.91
DC	0.184	0.081	14.6	4.4	1.191
St	0.429	0.126	16.4	3.2	2.07
DC	0.184	0.084	9.9	1.5	0.827
Af	0.184	0.067	6.4	1.0	0.434

Table VI. Turnover rates and magnitude of the pool of metabolic nitrogen estimated by the disappearance of ^{15}N from ammonia in urine and by SAN PIETRO and RITTENBERG method¹

Compound ingested	Amount ingested, mEq ^{15}N	Dietary protein, g/kg	Rate of turnover, days		Size of metabolic pool, g N	
			Meth- od 1 ²	Meth- od 2 ³	Meth- od 1 ²	Meth- od 2 ³
Glycine	0.626	4.4	15.1	7.2	0.315	1.096
NH_4Cl	0.184	4.4	15.5	14.6	0.092	0.081
Glycine	0.643	1.5	5.8	6.6	0.406	0.301
NH_4Cl	0.184	1.5	9.6	9.9	0.056	0.084

¹ All samples obtained from *infant DC*.

² SAN PIETRO and RITTENBERG method.

³ Disappearance of ammonia method.

pool of metabolic nitrogen reflected by the excretion of ammonia- ^{15}N by plotting the natural logarithms of the initial descending values of $[\text{NH}_3\text{-}^{15}\text{N}]$ against time in order to obtain both initial slopes and intercepts at $t = 0$. In such an analysis, the slope is equal to the initial turnover rate, and the intercept at $t = 0$, together with the dose of isotope administered, gives the size of the metabolic pool by the principle of isotope dilution [6]. When glycine- ^{15}N is administered to premature infants, such plots are linear for a long enough period of time to give reasonably reliable initial rates of turnover and intercepts [6]. Although the initial slopes in the present study were less mathematically reliable, the values obtained (table V) indicate that the estimated pool sizes reflected by the excretion of $\text{NH}_3\text{-}^{15}\text{N}$ were considerably smaller when ammonium- ^{15}N was administered than when glycine- ^{15}N was administered [6]. The product of the turnover rate and the pool size, *i.e.*, the rate of metabolism, appeared to diminish as dietary protein was restricted. Since the pool of nitrogen involved in ammonia production in urine appeared to contribute largely to urea synthesis when dietary protein was present in abundance, it was not surprising that the total rate of metabolism of this pool appeared to diminish when protein intake and urea synthesis were diminished.

Comparison of the Size and Turnover Rate of the Metabolic Pool as Determined by the Analysis of [Ammonia- ^{15}N] and by the Method of San Pietro and Rittenberg

When glycine- ^{15}N was administered to premature infants, the size and turnover rate of the metabolic pool were quite different when estimated by the excretion of urea- ^{15}N in the urine (method of SAN PIETRO and RITTENBERG [10]), and by excretion of ammonia- ^{15}N in the urine [6].

This difference was interpreted to mean that equilibration between ^{15}N from ingested glycine and the pool of nitrogen which yields ammonia in the urine is not instantaneous [6]. The ammonia-yielding pool of nitrogen can be presumed to be comprised largely of glutamate and glutamine [7]. Ammonia incorporated into amino acids in the liver can be presumed to be incorporated largely into glutamate and glutamine [2, 13]. If both urea and ammonia are derived from this pool of amino acid nitrogen, it can be predicted that the results of the estimation of pool size and turnover rate for this pool by the two methods should show closer agreement when $^{15}\text{NH}_4^+$ is ingested than when glycine- ^{15}N is administered.

For one of the infants in this study, who received both glycine- ^{15}N and ammonium- ^{15}N -chloride, it was possible to make this comparison on both high and low protein diets. Table VI shows reasonable agreement between the two methods of analysis for ammonium-

^{15}N ingestion on high and low protein diets. With glycine- ^{15}N as the test compound, agreement between the methods was better when tested in the presence of a low protein diet. This might be interpreted as indicating that the label of glycine- ^{15}N was more completely equilibrated between the pool of nitrogen yielding urea and that yielding ammonia in the presence of a low protein diet. There is a degree of uncertainty, however, as noted above, in the interpretation of the individual values of [urea- ^{15}N] and of the curve [urea- ^{15}N] versus time in the presence of a low protein diet. The results with both high and low protein diets for ingestion of $^{15}\text{NH}_4\text{Cl}$ were consistent with a sequence of metabolic events in which ingested ammonium was incorporated into a pool of metabolically active nitrogen that yielded both ammonia and urea.

Summary

The excretion of ^{15}N in urinary urea and ammonia was evaluated in healthy premature infants following ingestion of $^{15}\text{NH}_4\text{Cl}$. The pattern of excretion by infants receiving a high protein diet was compatible with a mode of metabolism in which the nitrogen of ingested ammonium ion is first incorporated into amino acids and then largely used for urea synthesis. This pattern was absent when dietary protein was restricted and urea synthesis markedly reduced.

A lack of capacity for urea synthesis does not appear to be operative in determining the mode of ammonia metabolism in these infants.

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