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Citrulline
genetic disease
hyperammonemia
metabolic disease
nitrogen metabolism
urea metabolism

Metabolism of Compounds Labeled with ^{15}N by an Infant with Congenital Hyperammonemia

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Extract

The excretion of ^{15}N in the urine following the oral administration of compounds labeled with ^{15}N was evaluated in an infant with congenital hyperammonemia while the infant was receiving a low protein diet. When she received either ^{15}N-glycine or $^{15}NH_4Cl$, the ratio of [^{15}N-NH_3] to [^{15}N-urea] in urine was strikingly elevated with respect to [^{15}N-NH_3]/[^{15}N-urea] found in studies of other infants receiving high protein diets. However, reduction of the protein in the diet of the control infants resulted in elevation of the ratio of [^{15}N-NH_3] to [^{15}N-urea] in urine after the administration of ^{15}N-glycine or $^{15}NH_4Cl$. No mode of metabolism specific for congenital hyperammonemia was demonstrated in the studies of orally administered $^{15}NH_4Cl$. In the metabolism of ^{15}N-glycine, the hyperammonemic infant receiving a low protein diet was different from other infants on both high and low protein diets.

In the hyperammonemic infant, ^{15}N-urea was much more readily formed from ^{15}N-citrulline than from $^{15}NH_4Cl$. On the other hand, the addition of unlabeled citrulline to the diet of this infant did not enhance the synthesis of ^{15}N-urea from ingested $^{15}NH_4Cl$ when compared with the addition of unlabeled ornithine-HCl to the diet.

Speculation

The data presented lead to the conjecture that inborn errors of ammonia detoxification which result in clinical disease in surviving patients are "partial" defects, and that "complete" defects in ammonia detoxification are so rapidly lethal as to be seldom recognized clinically.

Introduction

The excretion of ^{15}N following the administration of ^{15}N-amino acid and ^{15}N-ammonium has been investigated in a number of human metabolic states [2, 8, 11, 12, 16, 19, 21, 23]. When the intake of protein in the diet is normal, the isotope ingested in these studies rapidly and extensively labels urea in urine (Fig. 1) [2, 8, 11, 12, 21, 23]. For ^{15}N-amino acid, the general pathway to ^{15}N-urea involves initial transamination with α-ketoglutaric acid to form glutamic acid [1]. Glutamic acid may then undergo deamination to yield ammonium ion for the synthesis of citrulline via carbamyl phosphate [7] or undergo transamination with oxaloacetic acid to yield aspartic acid which can in turn react with citrulline to form arginino-succinic acid [18].

When ^{15}N-ammonium is ingested, the sequence of metabolic events is less clear. Ammonium ion can participate in the synthesis of glutamine, glutamic acid, and urea (Fig. 1). Studies of the excretion of ^{15}N in adult subjects have been compatible with substantial direct incorporation of ingested ^{15}N-ammonium into urea [21, 23]. In premature infants receiving high pro-

tein diets, the pattern of excretion of ^{15}N following the ingestion of ^{15}N-ammonium chloride suggests little direct incorporation of the nitrogen of ammonium ion into urea [12]. In those infants this nitrogen seems to be incorporated into amino acids and subsequently used, to a large extent, for the synthesis of urea. When nitrogen in the diet is restricted in the premature infant and in other human subjects, the production of ^{15}N-urea from ingested ^{15}N-ammonium ion is reduced [12, 19, 23]. Under these circumstances, the nitrogen of ammonium ion is retained as amino acid nitrogen at the expense of urea production.

The relation of the synthesis of urea to protein intake in diet is particularly significant in considering those inborn errors of metabolism which are associated with hyperammonemia. For infants with these disorders, reduction in protein in diet has been associated with symptomatic improvement [3]. The present report deals with the metabolism of compounds labeled with ^{15}N in an infant with congenital hyperammonemia and reduced activity of hepatic carbamyl phosphate synthetase. She was maintained on a low protein diet, of clinical necessity, throughout these studies. The results are compared with those obtained in similar studies of other infants receiving both high and low protein diets.

Case History

A brief description of this patient, together with some of the data presented here, appeared elsewhere [5], as has a detailed presentation of the clinical history and general laboratory evaluation [6].

JS, a Caucasian female infant, was evaluated at 5 weeks of age for episodic vomiting and lethargy leading to coma, precipitated by formula feedings and alleviated by feeding clear protein-free fluids. Family history revealed that a female sibling had expired at 5 months of age with a clinical history identical with that of the patient.

Physical examination was unremarkable.

Laboratory evaluation revealed hyperammonemia (400 μg/100 ml, determined by the Conway method as described by Faulkner and Britton [4]), modest elevation of glycine in the plasma (5.9 mg/100 ml), and nonprotein nitrogen in the blood within the lower limits of normal (22 mg/100 ml). Glutamine in the plasma ranged from 3.7 to 10.5 mg/100 ml (normal, 6–9). During the course of her illness she exhibited intermittent ketoacidosis and neutrapenia. Methylmalonic acid was not present in her urine.

Reduction of protein to 1.0 g/kg/day was associated with marked improvement in her neurologic state and with relatively normal psychomotor development. This amount of protein was, however, insufficient for growth. She developed hypoalbuminemia and osteoporosis, necessitating an increase in protein to 1.3 g protein/kg/day.

When she was 5 months old, exploratory biopsy of the liver was performed under general anesthesia. By contrast radiography the portal venous system was normal. Assays of enzymes of the urea cycle by methods described elsewhere [6, 22] revealed the following activities (micromoles per hour per gram liver protein):

	Patient	Control (mean of 3)
Carbamyl phosphate synthetase	61	302
Ornithine transcarbamylase	40,000	30,300
Argininosuccinic acid synthesizing system	288	303
Arginase	80,000	127,000

In the immediate postoperative period, she developed severe metabolic ketoacidosis and expired in spite of vigorous supportive efforts. Postmortem examination revealed only fatty metamorphosis of the liver, which was also noted in the biopsy specimen.

Materials and Methods

Urine specimens were analyzed for urea nitrogen and ^{15}N, and ammonia nitrogen and ^{15}N by methods described earlier [11].

Labeled compounds were obtained as follows: $^{15}NH_4Cl$ (95.2 and 99.0% ^{15}N) and ^{15}N-glycine (97% ^{15}N) were obtained from Volk Radiochemical

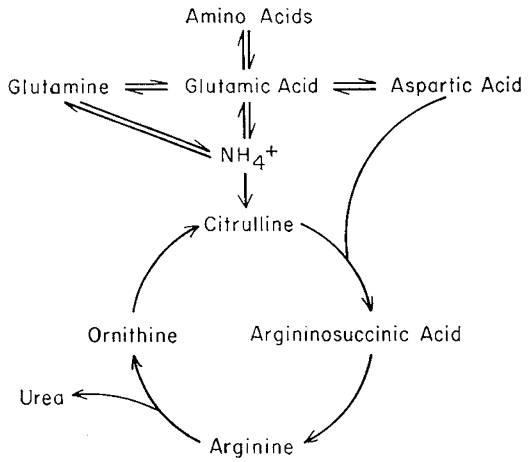

Fig. 1. Schematic representation of the relation of ammonium ion to amino acids and to the urea cycle.

Company; ^{15}N-citrulline was a gift from Professor David Rittenberg.

Experimental Subjects (Table I)

The affected infant is described above. Three control subjects were patients at Babies Hospital: *DC* and *St*, normal premature infants, and *BS*, a normal infant awaiting placement. Two subjects with Down's syndrome (*SA* and *BE*) had been in the Richmond Sanatorium since shortly after birth and were in stable clinical condition.

Experimental Method

The infants were given the labeled compounds in conjunction with feedings [27]. Spontaneously voided urine specimens were collected separately, frozen immediately in Dry Ice, and thawed just prior to analysis. For studies of the effects of low protein diets, the infants were given the specified amount of protein with enough additional carbohydrate and fat to make the diet isocaloric with respect to the previous high protein diet (Table I). The labeled compounds were administered 5–9 days after the low protein diet was instituted.

For studies of the effect of loading doses of citrulline and orthithine-HCl on the metabolism of orally administered $^{15}NH_4Cl$, the infants were given either citrulline or ornithine-HCl (0.3 g/kg/day) in divided doses for 4 consecutive days. The metabolism of orally administered $^{15}NH_4Cl$ was evaluated on the 4th day of amino acid loading (Table I).

Results

As protein in the diet was restricted, the recovery of ^{15}N as urea following the ingestion of $^{15}NH_4Cl$ was reduced in all the infants studied (Table I). The patient, *JS*, excreted the smallest percentage of the ^{15}N administered as urea, but also had the lowest protein intake (Table I). For both *JS* and an infant with Down's syndrome (*BE*), the administration of supplemental ornithine-HCl or citrulline resulted in increased recovery of ^{15}N-urea after the administration

Table I. Studies of the excretion of ^{15}N following the oral administration of compounds labeled with ^{15}N

Subject	Age, wk	Diagnosis	Wt, kg	Protein in diet, g/kg/day	Test compound	Dose of ^{15}N, mEq	^{15}N recovered in urea, % dose	Collection period, hr
SA	10	Down's syndrome; failure to thrive	3.1	5.1	Glycine	0.643		
SA	11		3.1	1.5	Glycine	0.300		
DC	10	Prematurity	2.4	4.4	Glycine	0.626		
DC	11		2.5	1.5	Glycine	0.643		
JS	10	Hyperammonemia	3.1	1.0	Glycine	0.643		
SA	10	Down's syndrome; failure to thrive	3.1	5.1	NH_4Cl	0.650	10.0	8.4
SA	11		3.1	1.5	NH_4Cl	0.662	2.7	9.8
BE	26	Down's syndrome	5.5	3.6	NH_4Cl	0.874	26.6	8.7
BE	27		5.7	1.5	NH_4Cl	0.874	13.4	11.1
BS	17	Normal	5.9	1.5	NH_4Cl	0.664	5.3	10.9
DC	10	Prematurity	2.4	4.4	NH_4Cl	0.184	19.4	9.5
DC	11		2.5	1.5	NH_4Cl	0.184	1.4	9.2
JS	12	Hyperammonemia	3.2	1.0	NH_4Cl (+ Na benzoate)	0.637	1.2	9.7
BE	28	Down's syndrome	5.8	1.5 (+ ornithine-HCl, 0.3 g/kg/day)	NH_4Cl	0.882	20.8	7.2
JS	19	Hyperammonemia	3.9	1.3 (+ ornithine-HCl, 0.3 g/kg/day)	NH_4Cl	0.642	5.8	12.5
BE	28	Down's syndrome	5.9	1.5 (+ citrulline, 0.3 g/kg/day)	NH_4Cl	0.884	15.5	7.8
JS	21	Hyperammonemia	3.9	1.3 (+ citrulline, 0.3 g/kg/day)	NH_4Cl	0.642	4.2	10.0
JS	15	Hyperammonemia	3.7	1.0	Citrulline	0.031	11.3	10.5

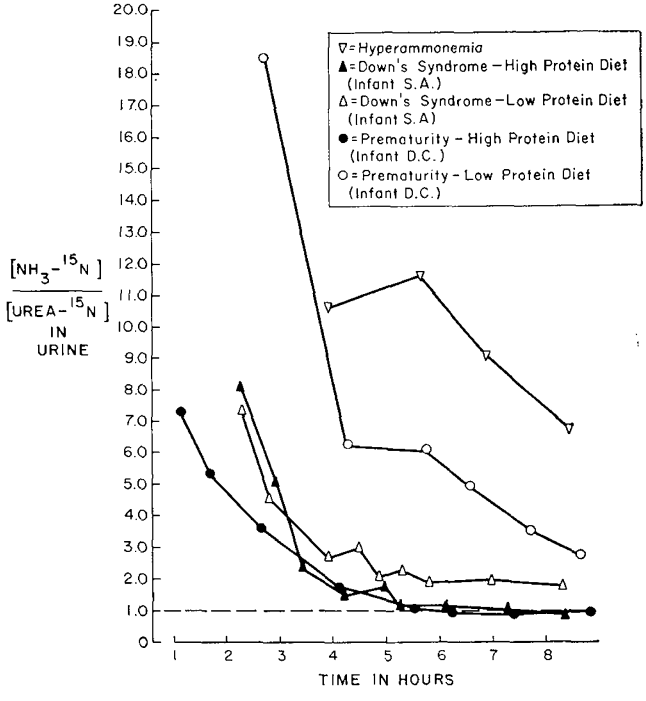

Fig. 2. The ratio of [^{15}N-NH_3] to [^{15}N-urea] in the urine following the ingestion of ^{15}N-glycine by the infant with congenital hyperammonemia and by several other infants.

of $^{15}NH_4Cl$ (Table I). The percentage of the dose of ^{15}N administered which was recovered as urea was greater for *JS* when ^{15}N-citrulline was administered than when $^{15}NH_4Cl$ was administered to her under any circumstance (Table I).

The ratio of [^{15}N-NH_3] to [^{15}N-urea] following the administration of ^{15}N-glycine fell from initially high values to values near 1 after 5.5 hr in both a premature infant and an infant with Down's syndrome when these infants received a normal diet (Fig. 2). When protein was restricted, the ratio did not reach unity during the 8-hr study period and was higher for the premature infant than for the infant with Down's syndrome. In the study of *JS*, the patient, the ratio was even higher than in the case of the premature infant.

When ^{15}N-ammonium chloride was administered, the ratio of [^{15}N-NH_3] to [^{15}N-urea] fell to values less than 1 at the end of 3 hr in studies of two infants with Down's syndrome receiving both high and low protein diets, a normal infant receiving a low protein diet, and a premature infant receiving a high protein diet (Fig. 3). In both infants with Down's syndrome, the ratio was greater than 1 for a longer period of time when protein was restricted in the diet than when protein was normal. For the premature infant receiving a low protein diet [^{15}N-NH_3]/[^{15}N-urea] remained greater than 1 for 8 hr, and was very similar to that found in the study of the hyperammonemic infant who was also receiving a low protein diet (Fig. 3).

Substantial incorporation of ^{15}N from administered $^{15}NH_4Cl$ into the glycine moiety of hippuric acid was noted when sodium benzoate was administered to-

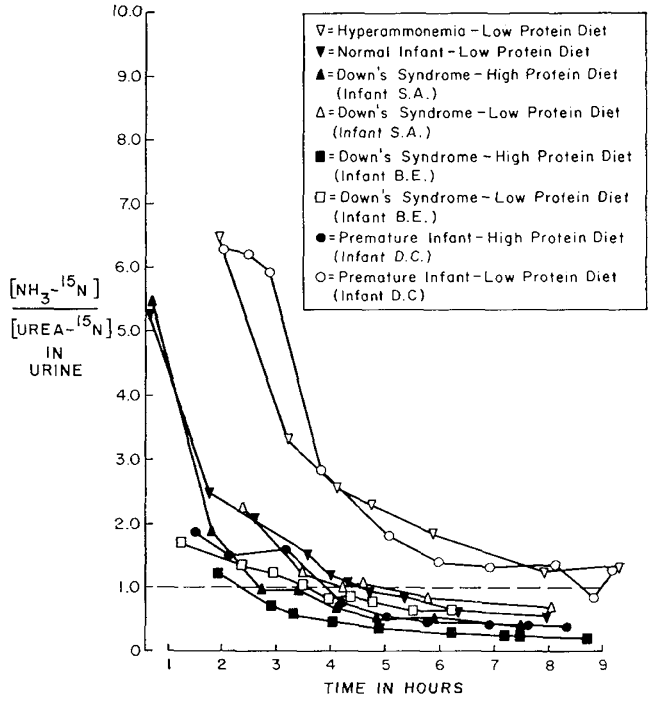

Fig. 3. The ratio of [^{15}N-NH_3] to [^{15}N-urea] in the urine following the ingestion of $^{15}NH_4Cl$ by the infant with congenital hyperammonemia and by several other infants.

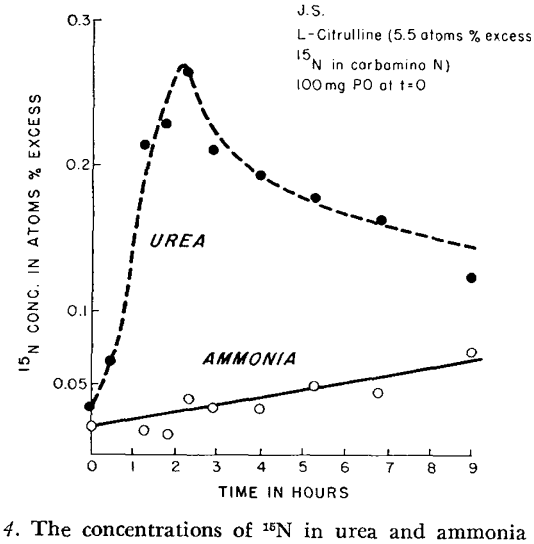

Fig. 4. The concentrations of ^{15}N in urea and ammonia in the urine following the ingestion of ^{15}N-citrulline by the infant with congenital hyperammonemia.

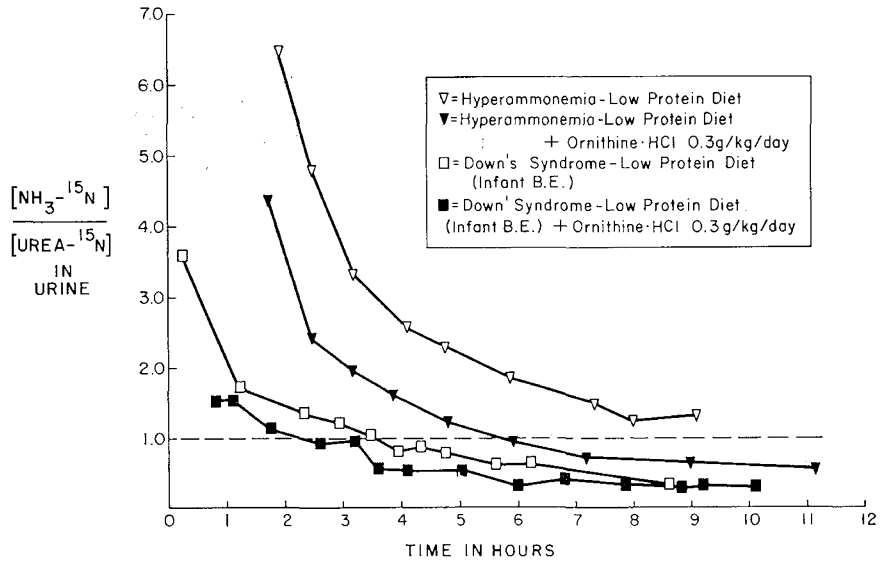

Fig. 5. The ratio of [^{15}N-NH_3] to [^{15}N-urea] following the oral administration of $^{15}NH_4Cl$ to the infant with congenital hyperammonemia and to an infant with Down's syndrome: the effect of ornithine-HCl added to a diet low in protein.

gether with the labeled salt to *JS* (Table II). This was also noted in a control study of a premature infant (Table II).

When ^{15}N-citrulline was administered to the patient, *JS*, urea was rapidly labeled and ammonia was only slowly labeled (Fig. 4).

When either ornithine-HCl (Fig. 5) or citrulline (Fig. 6) was added to the diet of *JS* or to the low protein diet of an infant with Down's syndrome, [^{15}N-NH_3]/[^{15}N-urea] after oral administration of $^{15}NH_4Cl$ reached unity more rapidly than it did in the absence of the added amino acid. For both infants, maximum

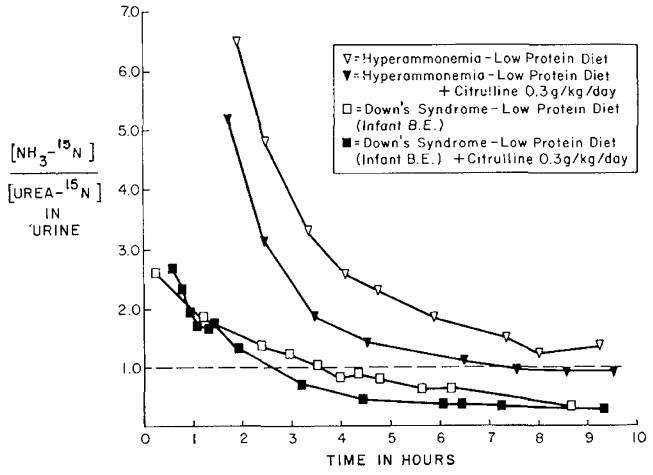

Fig. 6. The ratio of [^{15}N-NH_3] to [^{15}N-urea] following the oral administration of $^{15}NH_4Cl$ to the infant with congenital hyperammonemia and to an infant with Down's syndrome: the effect of citrulline added to a diet low in protein.

Table II. Concentration of ^{15}N in ammonia, urea, and hippuric acid in urine following oral administration of $^{15}NH_4Cl$ and sodium benzoate

Patient	Collection interval after administration, hr	Concentration of ^{15}N, atoms % excess		
		Ammonia	Urea	Hippuric Acid
JS: hyperammonemia; low protein diet (1 g protein/kg/day)	0	0.015	0.018	0.000
	0.0–1.0	0.775	0.076	0.160
	1.5–1.8	4.100	0.388	1.348
	2.9–3.7	1.824	0.553	1.178
St: premature infant; normal diet (3.2 g protein/kg/day)	0	0[1]	0	0
	0.0–1.0	3.030	0.752	1.156
	1.5–1.7	1.596	0.817	1.407
	2.2–3.4		0.796	1.212

[1] Base line values not determined. Infant had not previously received ^{15}N compound.

^{15}N concentration was higher in ammonia and lower in urea in the urine when citrulline was administered than when ornithine-HCl was administered (Table III).

Discussion

Quantitative Excretion of ^{15}N following the Ingestion of $^{15}NH_4Cl$, without Supplemental Ornithine-HCl or Citrulline

When $^{15}NH_4Cl$ was administered orally to the infants, without supplemental ornithine-HCl or citrulline, *JS*, the hyperammonemic patient, excreted a smaller percentage of the dose of ^{15}N than did the

other infants in the study (Table I). However, for the control infants, reduction of protein in the diet was accompanied by reduction in the excretion of ^{15}N-urea. On a low protein diet, the premature infant excreted a percentage of the dose very similar to that excreted by the hyperammonemic infant. Since the diet of the hyperammonemic infant was lower in protein than were the other restricted diets, it seems unlikely that the small amount of ^{15}N-urea excreted by the hyperammonemic infant can be ascribed specifically to her disease.

[^{15}N-NH_3]/[^{15}N-Urea] *in Urine after the Oral Administration of* ^{15}N-*Glycine*

When ^{15}N-glycine is administered to humans, the labeled nitrogen is distributed among many amino acids in the total pool of amino acids by transamination and other reactions [20]. Ammonia in urine, derived directly from amino acids and amino acid amides [17] is rapidly labeled following the ingestion of ^{15}N-glycine, and serves, to a limited extent, as a direct indicator of the level of the label present in the total pool of amino acids. Since the urea pool is very large compared with the excretory pool of ammonia nitrogen, the concentration of label rises more slowly in urea than in ammonia. As ^{15}N disappears from the pool of amino acids, [^{15}N-NH_3] declines, whereas [^{15}N-urea] rises. That the two be equal at the point of maximum [^{15}N-urea] is a criterion for the derivation of urea nitrogen from amino acids [26]. In man, the achievement of this precursor-product relation is influenced by complicating factors, particularly the time of equilibration between ^{15}N-glycine and the total amino acid pool [11, 24]. Nevertheless, equality of [^{15}N-urea] and [^{15}N-NH_3] is achieved promptly in humans receiving ^{15}N-glycine in association with normal diets. As shown in Figure 2, the ratios of [^{15}N-NH_3] to [^{15}N-urea] fall to values approximating 1.0 within 6 hr following the administration of ^{15}N-glycine to a premature infant and to an infant with Down's syndrome. For the infant with congenital hyperammonemia receiving a low protein diet, the ratio of [^{15}N-NH_3] to [^{15}N-urea] is greater than 10.0 at 6 hr and is still greater than 6.0 by the end of the study (8.4 hr).

That the protein content of the diet can influence the achievement of isotopic equality between ammonia and urea in urine is shown by the studies in which the premature infant and the infant with Down's syndrome received ^{15}N-glycine after 10 and 7 days, respectively, of lowered protein intake.

In each of these infants, [^{15}N-NH_3]/[^{15}N-urea] in urine was considerably greater than 1 at the end of the study (8 hr), although much lower at this time than [^{15}N-NH_3]/[^{15}N-urea] for the hyperammonemic infant. Although the values for *JS* occupy an extreme position, suggesting an impairment in the incorporation of amino acid nitrogen into urea, it must be noted that the protein content of her diet (1.0 g/kg/day) was lower than that of the controls (1.5 g/kg/day). It is also possible that the hyperammonemic infant, who had elevated levels of glycine in plasma and urine, exhibited some specific abnormality in the metabolism of glycine. A reduction in the conversion of glycine to serine has been demonstrated in the ketotic glycinemia of Nyhan and Childs [15], a disease which is considered to result from a primary defect in the metabolism of propionic acid and to which the present case shows many similarities. Such an impairment in glycine metabolism could account for a reduction in the availability of glycine nitrogen for urea synthesis.

Table III. Maximum concentrations of ^{15}N in ammonia and urea in urine following the oral administration of $^{15}NH_4Cl$: effect of ornithine-HCl and citrulline in the presence of low protein intake

Infant	Diagnosis	Amino acid added to diet, 0.3 g/kg/day	Maximum concentration of ^{15}N, atoms % excess	
			Ammonia	Urea
BE	Down's syndrome	Ornithine-HCl	1.34	1.30
BE		Citrulline	1.84	1.24
JS	Hyperammonemia	Ornithine-HCl	1.81	0.59
JS		Citrulline	2.82	0.53

Incorporation of ^{15}N *from Ingested* $^{15}NH_4Cl$ *into Glycine*

Since *JS* was able to synthesize ^{15}N-hippuric acid from $^{15}NH_4Cl$, it is clear that metabolic pathways leading from ammonia to glycine were utilized by *JS* (Table II). Although quantitative data were not obtained, the finding of similar labeling of hippuric acid by a premature infant fed $^{15}NH_4Cl$ suggests that *JS* was not abnormal in this respect.

[^{15}N-NH_3]/[^{15}N-Urea] *after Oral Administration of* $^{15}NH_4Cl$

When adult humans ingest $^{15}NH_4Cl$, there is rapid incorporation of ^{15}N into both ammonia and urea in urine. In the few appropriate studies available [13, 23, 25], the concentration of ^{15}N-NH_3 falls rapidly and becomes less than the [^{15}N-urea] soon after administration of the labeled compound (0.6–3.0 hr), regardless

of the level of protein in the diet. By contrast, it is noted in Figure 3 that [^{15}N-NH_3] remained higher than [^{15}N-urea] for 9 hr after the administration of $^{15}NH_4Cl$ to the infant with congenital hyperammonemia (Fig. 3). This finding was originally interpreted [5] as evidence of a defect in the direct incorporation of the nitrogen of ingested ammonia into urea, with consequent incorporation of abnormally large amounts of ammonium nitrogen into the pool of amino acids (presumably largely glutamine), giving rise to ammonia in the urine. This interpretation seems reasonable, particularly in view of the finding by Levin and co-workers [10] that glutamine is increased in both plasma and urine in patients with deficient hepatic ornithine transcarbamylase. However, in our patient, *JS*, glutamine in the plasma was not consistently elevated, nor was there any evidence of consistently elevated glutamine in the urine.

Subsequent studies in other infants (Fig. 3) have cast additional doubt on this interpretation. In all these studies except one, equality of ^{15}N concentration in ammonia and urea in urine was achieved within 5 hr after administration of the isotope. However, for both control infants studied while receiving high and low protein diets, [^{15}N-NH_3]/[^{15}N-urea] remained greater than 1 for a longer period of time when protein of the diet was low. The values obtained in the study of the premature infant in association with reduced protein intake are quite similar to those for *JS*. The interpretation of these findings is complicated by factors which affect the appropriateness of the controls. The infant with congenital hyperammonemia was born at term and was maintained for a long period of time on a diet very low in protein. Although the protein content of the diet was maintained at the highest level tolerated from a neurologic point of view, she exhibited protein malnutrition and failure to thrive. No single infant in the control group is appropriate from all these points of view, and no infant in the control group suffered from potein malnutrition. The studies may indicate that the hyperammonemic infant metabolized $^{15}NH_4Cl$ abnormally with respect to proper controls for age, maturity, and general clinical state, but it is clear from the studies of the premature infant that this mode of metabolism is not unique.

Two points concerning the conceptual basis of these studies deserve comment. The first involves the concept of direct incorporation of the nitrogen of ingested ammonium ion into urea. Studies of the metabolism of ingested $^{15}NH_4^+$ in premature infants are compatible with a mode of metabolism in which the nitrogen of ingested ammonium ion is first incorporated into amino acids and then used for the synthesis of urea [12]. The metabolism of ^{15}N-glycine in these infants indicates that the rate of turnover of the total amino acid pool is quite slow by comparison with that of the adult human [11]. It is therefore possible that the incorporation of ingested ammonium ion first into amino acids and then into urea is the normal sequence of events in human metabolism, made apparent in premature infants by the slow turnover of amino acids and ambiguous in adults by the rapid turnover of amino acids. This possibility raises a serious question about the validity of measuring urea synthesis from ingested ammonium ion as a test of the direct incorporation of ingested ammonium into urea.

The second point involves the failure of [^{15}N-NH_3]/[^{15}N-urea] to reach unity for prolonged periods of time in several studies performed while infants were receiving diets restricted in protein. This failure occurred in both the hyperammonemic infant and the premature infant following the ingestion of $^{15}NH_4Cl$, and in the hyperammonemic infant, the premature infant, and the infant with Down's syndrome following the ingestion of ^{15}N-glycine. Since [^{15}N-NH_3] remains higher than [^{15}N-urea] for rather long periods of time after maximum [^{15}N-urea] is achieved, it is unlikely that the pool of nitrogen yielding ammonia in urine is the same as the pool of nitrogen yielding urea in these studies. We have suggested elsewhere [12] that the relatively low [^{15}N] in urea in urine is likely to be due to the mixing of arginine synthesized *de novo* from amino acid nitrogen with arginine derived from the breakdown of endogenous and dietary protein. In this case, the urea derived from the mixed arginine would have a lower concentration of ^{15}N than that of the arginine synthesized *de novo*, and failure of equalization of [^{15}N-NH_3] and [^{15}N-urea] would not indicate a metabolic "disconnection" between the pool of amino acids and urea.

Metabolism of ^{15}N-Citrulline

When ^{15}N-citrulline was administered to the hyperammonemic infant, the recovery of ^{15}N as urea in urine was approximately 9 times that achieved following the administration of $^{15}NH_4Cl$ (Table I) to this infant. Both the quantitiy of ^{15}N recovered as urea and the rapidity of labeling of urea (Fig. 4) indicate that metabolic reactions involving citrulline as reac-

tant were not primarily limiting in the synthesis of urea from ammonium ion or from glycine by *JS*.

Metabolism of Ingested $^{15}NH_4Cl$ in the Presence of a Low Protein Diet Supplemented with Ornithine-HCl or Citrulline

In an effort to improve the clinical course of *JS*, the effects of supplemental ornithine-HCl and citrulline on the metabolism of ingested $^{15}NH_4^+$ were examined. The percentage of label excreted as urea was greater when the diet was supplemented with ornithine-HCl than when it was supplemented with citrulline (Table I). With either ornithine-HCl or citrulline added to the diet, the hyperammonemic infant excreted only very little ^{15}N-urea, but the percentage of the dose excreted was considerably greater in each case than that excreted when $^{15}NH_4Cl$ was given without the supplemental amino acid. The total nitrogen intake of *JS* was greater during the studies of supplementation (1.3 g protein/kg + amino acid supplement/day) than during the original study of $^{15}NH_4Cl$ metabolism (1.0 g protein/kg/day).

For the control infant with Down's syndrome receiving a diet containing 1.5 g protein/kg/day, results were similar in that supplemental ornithine-HCl and citrulline both increased the excretion of ^{15}N-urea after the ingestion of $^{15}NH_4Cl$ and that the increase in ^{15}N-urea was greater with added ornithine-HCl than with added citrulline (Table I). The percentage of the dose administered which was excreted as ^{15}N-urea was, however, much greater for this infant than for *JS*.

For both the hyperammonemic infant and the infant with Down's syndrome, the values for [^{15}N-NH_3]/[^{15}N-urea] were reduced in the presence of the supplemental amino acids (Figs. 5 and 6). For both infants, equality of [^{15}N-NH_3] and [^{15}N-urea] was achieved earlier in the presence of ornithine-HCl than in the presence of citrulline, and, for both, peak [^{15}N-NH_3] was higher and peak [^{15}N-urea] was lower with added citrulline than with added ornithine (Table III).

Although the substantial quantitative differences between the excretion of ^{15}N-urea by the hyperammonemic infant and excretion by the infant with Down's syndrome seem out of proportion to the difference in nitrogen intake by the two infants, the effect on this study of prolonged nitrogen deprivation in the hyperammonemic infant and of individual variation in nitrogen requirement cannot be ascertained. As in the other studies of the metabolism of compounds labeled with ^{15}N, no unique abnormality attributable to congenital hyperammonemia was detected.

Concluding Remarks

Although some abnormality in the synthesis of ^{15}N-urea from ^{15}N-glycine and ^{15}N-ammonium by the infant with congenital hyperammonemia may be seen in the studies reported here, it is clear that no absolute qualitative difference in the metabolism of nitrogen was demonstrated in the comparison of this infant with controls. If a reduction in the activity of carbamyl phosphate synthetase was the cause of *JS*'s hyperammonemia, as suggested by the enzyme analyses, the studies of the metabolism of compounds labeled with ^{15}N could be interpreted as showing qualitatively normal, but quantitatively abnormal, metabolism. Crane and co-workers [2] have shown alterations of this nature in the synthesis and elimination of ^{15}N-urea following administration of ^{15}N-ammonium to a patient with arginino-succinic aciduria.

This concept is further supported by the observation in mammalian species that the levels of activity of the enzymes of the urea cycle rise and fall coordinately when protein in the diet is raised and lowered [14, 22]. Therefore, a selective reduction in the level of hepatic carbamyl phosphate synthetase would seem, *a priori*, to be abnormal. However, Kirkman and Kiesel [9] have obtained evidence that the activity of carbamyl phosphate synthetase may be selectively lowered in infants receiving low protein diets. If this is true, an inborn genetic lowering of the activity of this enzyme may be indistinguishable from adaptive change when a low protein diet is ingested.

Summary

The excretion of ^{15}N in the urine following oral administration of compounds labeled with ^{15}N was evaluated in an infant having congenital hyperammonemia associated with reduced activity of hepatic carbamyl phosphate synthetase. Although the pattern of excretion of ^{15}N in the affected infant was different from that in controls appropriate for age, sex, and presence of chronic disease, no unique mode of metabolism, specific for congenital hyperammonemia, could be defined.

Addendum

Since completion of the manuscript, interesting enzymatic evidence has been obtained which bears on our

speculation concerning the nature of clinical disease associated with partial and complete enzymatic defects in the elimination of nitrogen. Campbell and coworkers studied a male infant who died on the 5th day of life of intractable hyperammonemia. Liver tissue obtained shortly after death contained no activity of ornithine transcarbamylase. A brother, born earlier, had died similarly. These cases are in marked contrast to females affected by this apparently sex-linked disorder. The latter show mild to severe hyperammonemia over a period of years, and have levels of hepatic ornithine transcarbamylase which are readily detectable but generally less than 10% of normal (*cf.* [10]). (Campbell, A. G. M., Rosenberg, L. E., Snodgrass, P. J., and Nuzum, C. T.: Complete ornithine transcarbamylase deficiency: a cause of lethal hyperammonemia (Abstract). Pediat. Res., *5:* 394 (1971).)

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