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TREATMENT OF CARBAMYL PHOSPHATE SYNTHETASE DEFICIENCY WITH KETO ANALOGUES OF ESSENTIAL AMINO ACIDS

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Abstract Congenital carbamyl phosphate synthetase deficiency was diagnosed by liver biopsy in a 13-year-old girl. α-Keto analogues of essential amino acids have been shown to spare nitrogen by reducing urea formation; hence, they were given to this patient in the hope of reducing hyperammonemia and improving protein tolerance. After intravenous infusion of the keto analogues of valine, leucine, isoleucine, methionine and phenylalanine, the corresponding plasma amino acids, including alloisoleucine and tyrosine, rose sharply. Twenty-four hours later, fasting plasma ammonia had fallen from the preinfusion value of 0.050 to 0.026 mM. Protein intake was kept at 0.5 g per kilogram for two weeks. Addition of keto acids by mouth reduced plasma ammonia and alanine to normal or near normal levels. Seizures and episodes of vomiting and lethargy decreased in frequency. Urinary nitrogen decreased, suggesting that nitrogen balance improved. These data indicate that keto acids may be useful in the treatment of congenital hyperammonemia. (N Engl J Med 292:1085-1090, 1975)

CONGENITAL disorders caused by defects in each of the five enzymes of the Krebs-Henseleit urea cycle have been described.[1] The clinical and biochemical manifestations differ somewhat among these syndromes, but all are characterized by hyperammonemia, impaired mental and physical development, and episodes of vomiting, lethargy, and coma after the ingestion of protein. Hyperammonemia is most pronounced in patients with defects of the first two enzymes of this cycle: carbamyl phosphate synthetase and ornithine transcarbamylase.

Treatment of these disorders is unsatisfactory, and most children die in infancy. Protein restriction ameliorates symptoms but does not restore the ammonia concentration of plasma to normal and may prevent adequate growth. Administration of orotic acid[2] has been proposed, but the effectiveness of this substance is not established.

The case of carbamyl phosphate synthetase deficiency described below was treated with α-keto analogues of five essential amino acids: valine, leucine, isoleucine, methionine and phenylalanine. We reasoned that these compounds, upon transamination to the corresponding amino acids, might become incorporated into protein and thereby promote growth and reduce hyperammonemia. Previous observations in adults with hyperammonemia and portal-systemic encephalopathy caused by cirrhosis of the liver provided some support for this approach.[3]

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CASE REPORT

A 13-year-old white girl was the product of an uncomplicated full-term pregnancy, birth, and neonatal period. The family history (Fig. 1) included a female sibling who was stillborn at 32 weeks' gestation and several cases of migraine, all on the maternal side.

At three weeks of age, while on a proprietary milk formula, the patient first manifested vomiting and lethargy, both of which remitted on a diet of clear fluids. She remained asymptomatic, with normal growth and development, until 13 months of age, when postprandial vomiting and lethargy recurred.

At 2½ years of age she had an episode of transient left hemiparesis. Psychometrics at three years revealed severe mental retardation. One year later, she had an episode of vomiting, followed by stupor and coma, which resolved over 36 hours on intravenous fluids. Within one month, she had another episode of vomiting and persistent lethargy associated with right hemiparesis. During the following month, akinetic seizures developed, and persisted to the time of admission. She also continued to have monthly episodes of vomiting and lethargy, often precipitated by high protein meals. She voluntarily restricted herself to a low protein diet (approximately 1.5 g per kilogram per day).

In past hospital admissions, electroencephalograms showed progression from a mild abnormality with depressed left hemispheric activity at two years to bilateral spiking in the posterior regions at 3½ years of age. A pneumoencephalogram showed dilatation of the right ventricle. Brachial arteriography demonstrated moderate cortical atrophy, with some evidence of hydrocephalus. An echoencephalogram, brain scan, and liver-function tests revealed no additional abnormalities. Ketosis was never demonstrated. Venous carbon dioxide levels ranged from 20 to 27 mM, and blood glucose was 79 to 105 mg per 100 ml.

At 12 years of age, the patient was readmitted to Johns Hopkins Hospital for further investigation. On physical examination, weight and head circumference were below the third percentile,

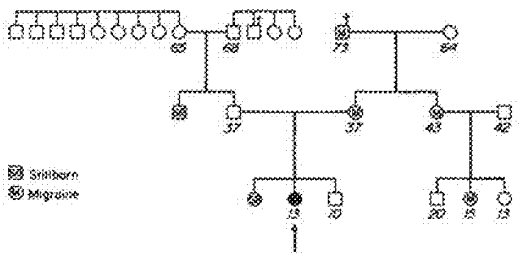

Figure 1. Family Pedigree of the Patient (Indicated by the Arrow), Including Stillborn Babies (SB) and Family Members with Migraine (M).

and height at the 10th percentile. There was a spastic quadriparesis, with hyper-reflexia, ankle clonus, and bilateral Babinski signs. There were no cerebellar signs or asterixis. Muscle mass and strength were diminished, especially on the left. The gait was hemiparetic.

Initial screening of urine and serum amino acids showed a semiquantitative increase in glutamine. Ammonia nitrogen in fasting venous blood was high: 3.4 μg per milliliter by the Seligson method.[4] Blood and cerebrospinal-fluid ammonia rose to 6.0 and 3.0 μg per milliliter respectively six hours after an oral protein load of 0.5 g per kilogram (Fig. 2). Protein tolerance tests on members of her family have not yet been completed. Urine was negative for protein when tested with sulfosalicylic acid.

Psychometrics revealed a mental age of 19 months on the Cattell Infant Intelligence Scale (I.Q. of 13). She used a number of single words and word combinations that were largely memorized units, and her understanding of spoken language was at a level of 16 to 18 months. She was able to help dress herself and could spoon-feed herself.

On an unrestricted diet the blood ammonia was 3.4 to 3.6 μg per milliliter. The serum urea nitrogen was 10 to 12 mg per 100 ml. Liver-function tests gave normal results. Concentrations of glutamine, alanine and lysine were elevated in the blood. Methylmalonic acid was not found in the urine. Orotic acid excretion was within normal limits.

Assay of liver tissue for urea-cycle enzymes was performed within 30 minutes of percutaneous biopsy. Activities of ornithine transcarbamylase and arginosuccinic acid synthetase were measured by the method of Brown and Cohen,[5] with the modification of Schimke.[6] Activity of carbamyl phosphate synthetase was measured by conversion of carbamyl phosphate formed during incubation[5] to urea in an alkaline medium.[7] Normal rat livers[8] were used as simultaneous controls for the assay. Arginosuccinic acid lyase and arginase could not be measured because the quantity of tissue was insufficient. Results (Table 1) showed carbamyl phosphate synthetase activity to be less than 15 per cent of normal.[9]

The child's protein intake was reduced to 1.0 and later to 0.5 g per kilogram per day. Clinical improvement was noted: she slept fewer hours and was more able to concentrate on self-help tasks. Seizure frequency decreased from five to seven per day to an average of one per day, and episodes of lethargy and vomiting ceased during the ensuing three months of protein restriction. Elevated values continued to be obtained of fasting ammonia (mean, 0.7 μg per milliliter, or 0.05 mM by a resin method — normal, 0.1 to 0.4 μg per milliliter), glutamine (mean, 1.1 mM, — normal, 0.53 to 0.73 mM) and alanine (mean, 1.0 mM — normal, 0.19 to 0.54 mM).

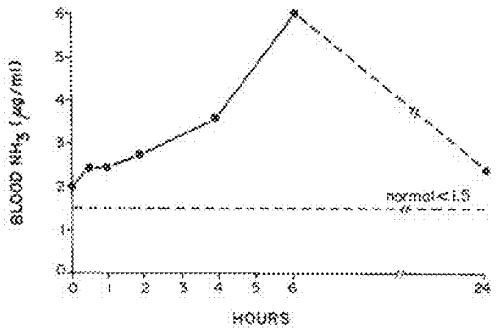

Figure 2. Changes in Blood Ammonia (Seligson Method) after an Oral Protein Load of 0.5 G per Kilogram.

Table 1. Activities of Urea-Cycle Enzymes in Liver.

Enzyme*	Patient	Normal Range†
Carbamyl phosphate synthetase	23	180-615
Ornithine transcarbamylase	7299	3950-6550
Arginosuccinic acid synthetase	25	21-41

*Unit is μmole of product formed/hr/g wet weight of tissue.
†Data of Levin et al.[9]

Methods

In the Pediatric Clinical Research Unit concentrations of free amino acids in sulfosalicylic filtrates of plasma were measured by ion-exchange chromatography. Nitrogen intake was estimated from the patient's protein intake. Urine was collected from 8 a.m. to 8 p.m. daily. Neither night-time urine nor stool collections could be obtained reliably because of intermittent incontinence. Plasma and urine urea were determined with diacetylmonoxime as previously described.[10] Creatinine and uric acid were measured in the Clinical Chemistry Laboratory by automated methods using the Jaffe reaction[11] and cupric neocuproine,[12] respectively. Urinary ammonia was determined by the Berthelot reaction.[13] Total urinary nitrogen was assayed with a Coleman Nitrogen Analyzer (Coleman Instruments, Maywood, Illinois). Undetermined urinary nitrogen was calculated as the difference between total nitrogen and the sum of urea nitrogen, creatinine nitrogen, uric acid nitrogen, and ammonia nitrogen. Total keto acids were measured as previously reported.[10]

Determination of Blood Ammonia, Glutamine and Alanine

Initial measurements of ammonia were made by the Seligson method.[4] Before the experimental studies, methods for measuring ammonia, glutamine and alanine on capillary blood were developed as follows:

Ammonia was determined by a micromodification of the resin method of Fenton.[14] Heparinized capillary blood was centrifuged at 4°C. 0.1 ml of plasma was added to 0.2 ml of water, followed by 0.05 ml of a slurry of BioRad AG 50 resin (acid form). After incubation for five minutes, resin was washed twice with water. After 0.1 ml of 4 M sodium chloride was added, color was developed by addition of indophenol color reagents[13] to make a final volume of 0.3 ml and was read at 630 nm. The normal range is 0.007 to 0.028 mM (0.10 to 0.40 μg per milliliter).[15] Glutamine was also determined as ammonia after incubation of plasma with glutaminase[16]: 0.02 ml of plasma was added to 0.1 ml of glutaminase solution, containing 1 unit of enzyme per milliliter (Sigma Chemical Company, Grade V). The blank consisted of addition of 0.1 ml of water in place of the enzyme solution. Also, a blank containing plasma but not enzyme was used. After the reaction mixture was incubated at 37°C for 30 minutes, resin was added, and ammonia was measured as described above. The mean recoveries of ammonia and glutamine added to plasma were 99 and 100 per cent, respectively. Alanine was determined in 0.1 ml of plasma by the alanine dehydrogenase method as described by Williamson[17] with enzyme obtained from the Boehringer Mannheim Company. Deproteinization of plasma was required, since recovery from undeproteinized plasma averaged only about 80 per cent, whereas recovery from sulfosalicylic filtrates was complete.

The synthesis and preparation of keto analogues for oral and intravenous use were as previously described.[18]

Student's t-test was used to examine the significance of differences.

Results

Diurnal Variation in Ammonia, Alanine and Glutamine

During an eight-day control period on a constant daily protein intake (0.5 g per kilogram), capillary-blood samples were obtained at 8 a.m. (before breakfast of a constant composition), 10 a.m. and 12 noon. Considerable day-to-day variation in ammonia, alanine and glutamine was observed (Fig. 3). In addition, there was a significant decline in all three concentrations from 8 a.m. to 12 noon (Table 2). When fasting values were compared to postprandial values, the only statistically significant decline was in ammonia.

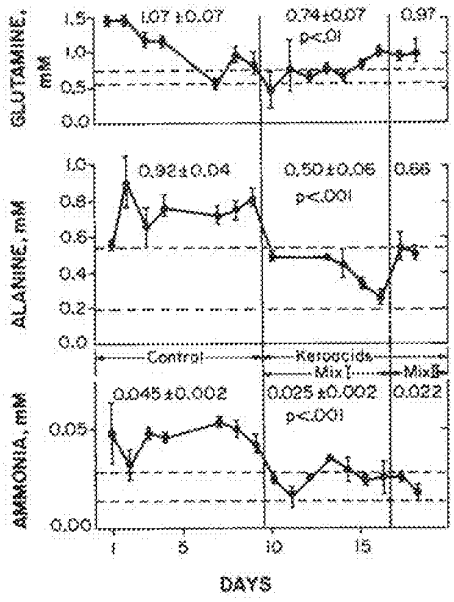

Figure 3. Summary of the Effect of Oral Keto Acids on Blood Ammonia, Glutamine and Alanine.

The dashed lines indicate normal ranges,[15,16a] and the vertical lines represent ±S.E.M.

Intravenous Keto Acids

To determine the possible utility of keto acids in the treatment of this child, a mixture of 8.5 g of the α-keto analogues of five essential amino acids (valine, leucine, isoleucine, methionine and phenylalanine) was given intravenously as sodium salts. Plasma valine, methionine, leucine, tyrosine, alloisoleucine, phenylalanine and isoleucine all increased sharply at the end of the four-hour infusion, with a mean increase of 0.12 ±0.03 mM (Fig. 4). Fasting capillary ammonia concentration was 0.050 mM before and 0.078 mM immediately after infusion and 0.028 mM 20 hours later. Alanine concentration decreased from 1.17 to 0.64 mM. Glycine and lysine also decreased, whereas histidine, arginine and other amino acids remained unchanged after keto acid infusion. Glutamine was not measured during this study.

Table 2. Diurnal Variations in Ammonia, Glutamine and Alanine Concentrations in Capillary Blood.*

Period of Change	Ammonia	Glutamine	Alanine
	mM	*mM*	*mM*
8 am–10 am	−0.006 ± 0.002†	−0.12 ± 0.09	−0.01 ± 0.04
8 am–12 m	−0.015 ± 0.003‡	−0.19 ± 0.07§	−0.26 ± 0.06†
10 am–12 m	−0.001 ± 0.003	−0.13 ± 0.04§	−0.27 ± 0.03¶

*Means ± SEM.
†Significantly different from 0, $p < 0.02$ ($n = 6$).
‡Significantly different from 0, $p < 0.01$ ($n = 9$).
§Significantly different from 0, $p < 0.05$ ($n = 7$).
¶Significantly different from 0, $p < 0.001$ ($n = 7$).

Oral Keto Acids

After the control period, the same keto acids were given by mouth. The initial mixture (Mix I) contained the following: sodium α-keto-isovalerate, 1.44 g; sodium α-keto-β-methylvalerate, 1.46 g; sodium α-keto-isocaproate, 1.94 g; sodium phenylpyruvate, 2.00 g; and sodium α-keto-γ-methylthiobutyrate, 2.12 g. After six days, the plasma amino acid analysis showed low phenylalanine and markedly elevated alloisoleucine. Accordingly, the mixture was changed to contain 4.0 g of phenylpyruvate, and the keto analogue of isoleucine was discontinued (Mix II). During treatment with Mix I, capillary ammonia and alanine concentrations showed significant reductions to normal levels (Fig. 3). Glutamine fell by t-test, but the significance of this change is questionable, since the value fell progressively during the control period. Total plasma keto acids were 0.5 mM or less.

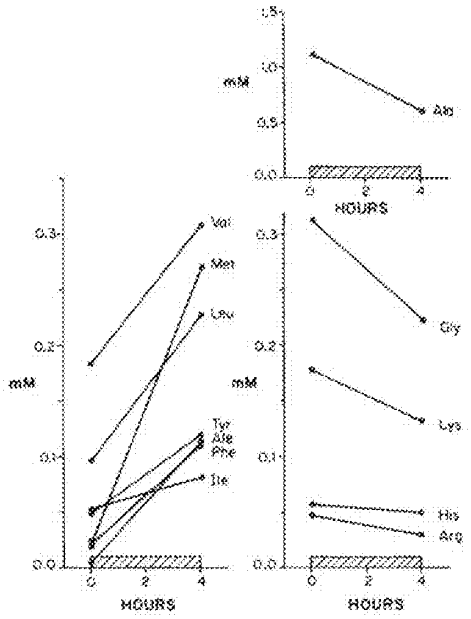

Figure 4. Changes in Circulating Amino Acids after Infusion of the Keto Acid Mixture.

The left-hand panel shows the increases in plasma amino acids corresponding to the infused keto acids, and the right-hand panel the amino acids that fell.

The patient improved clinically. She had no episodes of lethargy or vomiting and was able to complete self-help skills previously beyond her abilities. Seizure frequency decreased to one or two per week, although the electroencephalogram showed no improvement.

Nitrogen Excretion

Table 3 compares the values for control and keto acid periods. All values are expressed in relation to creatinine nitrogen to correct for errors in collection. The significant decrease in urea nitrogen/creatinine nitrogen excretion in comparisons of control with both keto acid periods is suggestive of improved nitrogen balance while the patient was on keto acid therapy. None of the other measurements changed significantly. When keto acid Mix I was compared with Mix II, there was a further significant decrease in urea nitrogen/creatinine nitrogen as well as a significant reduction in total nitrogen/creatinine nitrogen, suggesting further improvement in nitrogen balance on the second keto acid mixture.

Table 3. Effect of Keto Acid Administration on Urinary Partition of Nitrogen.*

Period	CN	UN/CN	AN/CN	UAN/CN	TN/CN	UDN/CN
	g/72 hr					
Control	0.09 ±0.01	6.73 ±0.53	1.76 ±0.09	0.47 ±0.06	11.2 ±1.00	1.25 ±0.81
Mix I (5)	0.07 ±0.004	4.96 ±0.44†	1.70 ±0.13	0.69 ±0.14	10.9 ±0.36	2.56 ±0.63
Mix II (6)	0.07 ±0.003	3.31 ±0.19‡§	1.44 ±0.22	0.63 ±0.04	9.16 ±0.68	2.73 ±0.54

*Values are means ± SEM. CN denotes creatinine nitrogen, UN urea nitrogen, AN ammonia nitrogen, UAN uric acid nitrogen, TN total nitrogen, & UDN undetermined nitrogen.

†Significantly different from control ($p < 0.005$).

‡Significantly different from control ($p < 0.001$).

§Significantly different from Mix I ($p < 0.01$).

Table 4 compares calculated nitrogen intake with estimated daily urinary nitrogen output, as an index of nitrogen balance. A significant increase in this difference is noted when the control period is compared with both keto acid treatment periods. Again, these data suggest that improvement in nitrogen balance occurred. The small increase in nitrogen intake on keto acid therapy was not due to diet but rather to the nitrogen in the gelatin capsules containing the medication.

Plasma Free Amino Acids

Table 5 shows the changes in plasma amino acid concentrations during protein restriction and during trials of keto acid administration. Initial abnormalities included a mild elevation in glycine (0.313 mM, the normal range being 0.158 to 0.302 mM) as well as marked elevation in glutamine and alanine (measured by enzymatic methods, as noted above). Phenylalanine was abnormally low (0.023 mM, the normal range being 0.039 to 0.071 mM). During the period of oral keto acid administration, there was an increase in the amino acids corresponding to the administered keto acids. Large quantities of alloisoleucine appeared when the keto analogue of isoleucine was administered in Mix I but disappeared when this component was discontinued in Mix II.

Table 4. Effect of Keto Acids on Apparent Nitrogen Balance.*

Period	Intake (N_i)	Urinary Output (N_u)	Difference ($N_i - N_u$)
	g of nitrogen/day		
Control	2.49 ± 0.06	1.94 ± 0.20	0.56 ± 0.22
Mix I	2.82 ± 0.15	1.52 ± 0.11	1.29 ± 0.19†
Mix II	2.76 ± 0.03‡	1.40 ± 0.23	1.38 ± 0.22‡

*Means ± SEM.

†Significantly different from control ($p < 0.05$).

‡Significantly different from control ($p < 0.005$).

Despite the administration of 2.00 g daily of phenylpyruvate in Mix I, the plasma phenylalanine level remained low on the sixth day. The plasma phenylalanine concentration returned to normal after the dose of phenylpyruvate was increased to 4 g per day.

Discussion

This child has many of the clinical features found in other patients with a congenital defect in carbamyl phosphate synthetase.[3,19-22] Yet of the six previously reported cases, all were identified in children well below one year of age. Furthermore, three died before reaching the age of 12 months, and the others have not been followed beyond 15 months of age. Hence, our patient is unusual in that she has survived to adolescence. She voluntarily reduced her protein intake, but nevertheless is profoundly retarded and hemiparetic, with seizures and recurrent episodes of lethargy.

The pattern of inheritance of hyperammonemia Type II (carbamyl phosphate synthetase deficiency) is un-

Table 5. Plasma Amino Acid Concentrations (Fasting).

Amino Acids	Concentration on Indicated Day of Therapy (nM)				Normal Range*
	Control	Mix I	Mix I	Mix II	
		1st	6th	6th	
Amino acids corresponding to ingested keto acids:					
Valine	0.181	0.293	0.181	0.220	0.156-0.272
Leucine	0.096	0.124	0.114	0.075	0.079-0.159
Isoleucine	0.051	0.051	0.052	0.041	0.038-0.090
Methionine	0.021	0.025	0.026	0.022	0.016-0.036
Phenylalanine	0.023	0.056	0.027	0.047	0.039-0.071
Alloisoleucine	0.004	0.123	0.105	0.003	—
Other amino acids:					
Taurine	0.048	0.077	0.078	0.048	0.000-0.240
Aspartic acid	0.022	0.033	0.028	0.019	0.000-0.014
Proline	0.148	0.187	0.137	0.154	0.058-0.286
Citrulline	0.020	0.011	0.023	0.019	0.019-0.047
Glycine	0.313	0.233	0.291	0.324	0.158-0.302
Cystine	0.121	0.090	0.106	0.102	0.058-0.134
Tyrosine	0.047	0.068	0.067	0.100	0.041-0.084
Ornithine	0.059	0.059	0.054	0.056	0.019-0.075
Arginine	0.049	0.038	0.045	0.047	0.044-0.124
Histidine	0.057	0.046	0.055	0.039	0.064-0.104
Lysine	0.178	0.153	0.142	0.136	0.108-0.220

*95% confidence limits calculated from data of Armstrong & Stave.[25]

known. However, there is evidence that hyperammonemia Type I (ornithine transcarbamylase deficiency) is X-linked.[25,26] Our patient's family history is consistent with a pattern of inheritance that could be either X-linked or autosomal recessive. There are several cases of migraine on the maternal side of our patient's family. It would be of interest to determine whether these family members are protein intolerant.

The neurologic sequelae of this disease, as well as of the other urea cycle enzyme deficiencies, are thought to be consequences of high ammonia levels in blood and brain. The rationale for using keto acids to reduce hyperammonemia and the possible dangers may be stated as follows: keto analogues of methionine and phenylalanine reduce glutamine levels by reacting in the liver with glutamine.[16] Branched-chain keto acids may reduce alanine release from muscle.[10] Thus these compounds direct labile nitrogen to essential amino acids, which could then be used for protein synthesis. Plasma ammonia levels might subsequently fall as glutamine, alanine and other labile nitrogenous compounds are reduced toward normal. On the other hand, a transient rise in ammonia levels after keto acids might occur according to the following series of reactions, if the deamidation of α-ketoglutaramate (step 2) is relatively rapid as compared to the amination of α-ketoglutarate (step 3):

$$1)\ \text{keto acid} + \text{glutamine} \xrightleftharpoons{\text{glutamine transaminase}} \alpha\text{-ketoglutaramate} + \text{amino acid}$$

$$2)\ \alpha\text{-ketoglutaramate} \xrightarrow{\text{omega amidase}} \alpha\text{-ketoglutarate} + NH_3$$

$$3)\ \alpha\text{-ketoglutarate} + NH_3 \xrightleftharpoons{\text{glutamate dehydrogenase}} \text{glutamate.}$$

Furthermore, untoward metabolic effects of these compounds might occur at high concentrations, such as inhibition of gluconeogenesis from certain substrates.[27] In branched-chain ketoaciduria, the keto analogues of valine, leucine and isoleucine accumulate in plasma, and there is some evidence that these compounds are responsible for the symptoms of this disease.[28] However, toxic side effects have not been found with the clinical use of keto acids in portal-systemic encephalopathy, chronic renal failure, and starvation.[8,18,29]

In our patient, the increase in plasma concentrations of valine, methionine, leucine, alloisoleucine, isoleucine, phenylalanine and tyrosine after the infusion (Fig. 4) provides evidence that these keto acids are transaminated to their corresponding amino acids. Concentrations of several amino acids fell. A transient rise in plasma ammonia was indeed seen, but by the next day, the level had fallen to normal.

During the 16-day study of oral keto acid therapy, marked day-to-day variations in plasma ammonia, glutamine and alanine were observed despite a constant diet. The apparent nocturnal rise in plasma ammonia, glutamine and alanine may reflect gluconeogenesis during the early-morning hours.

The oral administration of keto analogues was associated with decreases in plasma concentrations of ammonia and alanine to normal levels. The choice of the initial mixture of the five analogues was empirical. The mixture was changed when amino acid chromatograms indicated an imbalance. We suspect that further changes in the mixture will be needed, possibly including the addition of threonine, tryptophan, histidine, lysine and arginine. The final mixture of keto acids for chronic therapy will be determined by monitoring of the plasma amino acids and clinical response.

The use of keto acids in the therapy of hyperammonemia represents a logical approach to the management of inborn errors of metabolism. The administration of a substrate, in the form of keto acids, permits the incorporation of the presumed toxic compound, ammonia, into normal tissue. The eventual success in our patient can be measured only in terms of palliation — i.e., improved seizure control, attention span, and weight gain, and decreased episodes of vomiting and lethargy. Early diagnosis and therapy in an infant might offer maximum benefit.

We are indebted to Dr. K.-W. Chow for assistance with the assay of liver enzymes and to Sylvia Butler, Ellen Gordes, Valerie Hammond, Robert Ward and the nursing staff of the Pediatric Clinical Research Unit of Johns Hopkins Hospital for technical assistance.

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