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Hyperammonaemia

A Variant Type of Deficiency of Liver Ornithine Transcarbamylase

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The specific syndrome arising from an absent or low hepatic ornithine transcarbamylase activity has been termed hyperammonaemia (Russell *et al.*, 1962; Levin and Russell, 1967; Levin, 1968). In the previous communication (Levin *et al.*, 1969) 2 cases occurring in mother and child are described. In this article, we record an infant who during the course of an investigation for the cause of his vomiting had an unexplained episode of illness in which he became lethargic, drowsy, and finally comatose, with convulsions. He was found to have a high plasma and CSF ammonia, and the diagnosis of hyperammonaemia was confirmed by assay of the urea cycle enzymes of the liver.

Studies of the properties of the liver ornithine transcarbamylase in this patient suggest that he represents a variant type of deficiency of this enzyme. This may be correlated with the relative mildness of his condition, and the rapidity and completeness of clinical recovery. The effect of citric acid, glutamic acid, alanine, and arginine on plasma ammonia levels was also studied.

Case Report

A male, born on August 8, 1966, was the first child of unrelated parents in whom there was no family history of fits, mental defect, or other metabolic diseases on either side. The mother developed toxaemia during pregnancy which was terminated by induction at 36 weeks. The infant was normal but slightly immature, weighing 2·4 kg. He was breast-fed at first, then bottle-fed, and was apparently well for 6 months, growing steadily along the 3rd centile for weight (Fig. 1). At 6 months he was admitted to Southend General Hospital on account of bronchiolitis. No other abnormality was noted at this time, and the milestones were normal. He weighed 6·5 kg. and took his milk and weaning diet well.

Very soon after discharge he began vomiting occasionally. It was effortless and unassociated with malaise, anorexia, or constipation. However, it became increasingly troublesome and he ceased to gain weight. He was treated with atropine methonitrate ('eumydrine'), with no improvement, and at 8½ months was readmitted for investigation. He was then a thin, lively infant weighing 7 kg. There were no abnormal physical signs, and the blood chemistry, urine microscopy, and chromatogram were normal. Radiological examination showed no abnormality of the oesophagus, stomach, or upper bowel. The vomiting continued in hospital, occurring mainly in the evening, and often contained much of the day's food.

During the first week he was quite lively, but subsequently became drowsy and increasingly difficult to rouse in the morning. His temperature rose to 39·4 °C., the pulse to 136 per minute, and the liver, on which no comment had been made when he was examined earlier for possible pyloric stenosis, was now enlarged to 3 cm. below the costal margin. His condition deteriorated rapidly. CSF obtained by lumbar puncture gave normal results by the usual tests. Immediately after the lumbar puncture he collapsed and became semicomatose, with twitching. It was thought possible that the procedure had led to coning and medullary pressure consequent upon an intracranial space-occupying lesion, and 2 months after his first admission, May 7, 1967, he was transferred to the London Hospital for neurological investigation. He arrived deeply comatose and convulsing, remaining in this condition for over 36 hours. The fontanelle was tense and bulging. A further lumbar puncture produced a blood-stained fluid containing no excess of white cells and a normal protein content. The liver was now still further enlarged, to 6 cm. below the costal margin. Numerous biochemical investigations, including blood urea (22 mg./100 ml.), gave normal results. The EEG was grossly abnormal and reported as showing 'probably severe structural damage possibly due to severe electrolyte disturbance'. The echogram showed no mid-line shift and an air ventriculogram no abnormality.

He was at first fed by gavage with a milk mixture, but because of regurgitation and convulsions this was replaced by intravenous glucose 18 hours after admission. Shortly after starting this he rapidly improved and was out of coma within 24 hours. Paper chromatography of a specimen of urine taken shortly after

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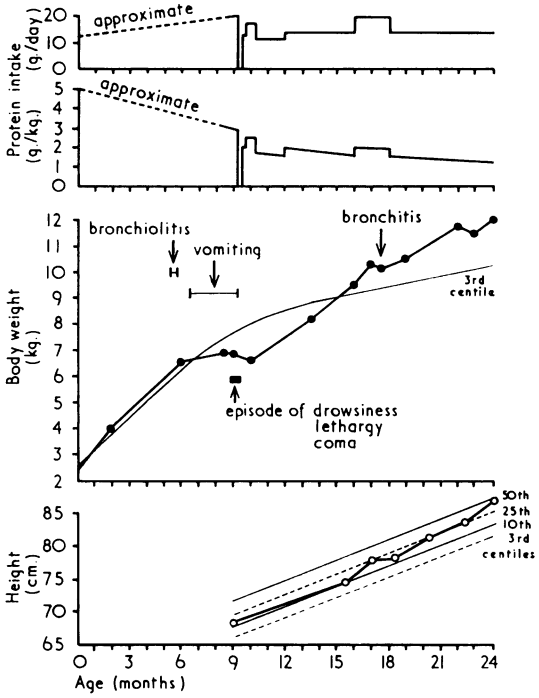

FIG. 1.—*Progress chart. Note improving weight and height gain on a low protein diet.*

admission revealed an excess of glutamine and uracil, suggesting a possible high blood ammonia. This was confirmed when the plasma ammonia was found to be 182 μg. NH_3-nitrogen/100 ml. more than 48 hours after the start of the infusion. A provisional diagnosis of hyperammonaemia, possibly due to a congenital metabolic disorder, was made, and on May 12, he was transferred to the Queen Elizabeth Hospital for Children for further investigation. On arrival he was alert and hungry and able to smile, but seemed dazed, was hypotonic, and exhibited some indefinite ataxic movements of the arms. No abnormal physical signs were detected apart from the liver enlargement which had diminished. Enzyme assays on a biopsy specimen of the liver confirmed the presence of an enzyme defect of the urea cycle. Histologically there was a mild increase of fibrous tissue in the portal tracts but no evidence of cirrhosis.

Fasting levels of plasma ammonia ranged between 40 and 90 μg.NH_3-N/100 ml., i.e. up to twice the upper limit of normal, and rose to 160-250 μg.NH_3-N/100 ml. after ingestion of protein (see Table II). A low protein diet of SMA ('adapted' milk) in 5 equally spaced feeds was thereafter instituted. Later, egg yolk and rice were added. Within a few days of admission he seemed almost normal, except for persisting liver enlargement which gradually returned to normal during the next 4 weeks. The EEG 4 days after admission showed only slight residual abnormality. All subsequent EEG's have been normal, both between meals and after protein ingestion. He was discharged a month after admission weighing 7·8 kg. and on a diet containing 13–14 g. protein per day. He gained weight rapidly at home and resumed normal mental development. Within a few weeks he had lost all evidence of hypotonia and at 1 year old was beginning to stand and walk. At 15 months he weighed 9 kg. and was between the 3rd and 10th centile for height. He was then able to say a few words. Progress was so good that his protein intake was increased to 18·5 g. daily. However, within two months he had lost a little weight, possibly associated with an attack of bronchitis, and the plasma ammonia level rose. In consequence, though he showed no adverse symptoms, the intake of protein was reduced to about the previous level, at which he has since remained. At 21 months he remained in excellent health and he was walking, running, and talking in short sentences. When last seen, at 2 years of age, he was a normal boy and had grown from below the 3rd centile for weight and the 10th centile for height at 1 year to the 25th centile for both at 2 years (Fig. 1), while the bone age, which had been 6 months retarded at 1 year, was approximately at the chronological age at 2 years.

Results of Laboratory Investigations

The methods used are described in the previous paper (Levin *et al.*, 1969).

Biochemical investigations. Plasma sodium, potassium, chloride, standard bicarbonate, and *p*H determined on a number of occasions were all within normal limits. Serum calcium, magnesium, phosphorus, total protein, albumin, globulin, non-protein nitrogen, cholesterol, and uric acid were also normal. The blood urea ranged from 10 to 22 mg./100 ml. in random estimations, though occasionally levels up to 28 mg./100 ml. were reached 3 or 4 hours after a protein meal. The liver function tests were normal except for the serum GOT which was moderately raised during the acute episode and for a time thereafter. The alkaline phosphatase was also slightly raised.

There was only a slight increase in the urinary excretion of amino acids. Paper chromatography showed a prominent glutamine band. Quantitative analysis confirmed the increased glutamine excretion.

Ammonia levels in plasma and CSF. The plasma ammonia level estimated 4 days after the onset of coma was 182 μg.NH_3-N/100 ml. (normal 10-45 μg.NH_3-N/100 ml.). Thereafter it was determined on many random occasions, the levels ranging from 40-237 μg.NH_3-N/100 ml., depending

TABLE I

Effect of Varying Protein Intakes on Plasma Ammonia; Levels at 3-hourly Intervals Throughout Day

Protein Intake (g./kg. body weight)	Plasma Ammonia (μg.NH_3-N/100 ml.)						
	0 hr.	3 hr.	6 hr.	9 hr.	12 hr.	15 hr.	18 hr.
1·8		64	65	171	126	111	
1·4		48	93	121	142	83	
1·2	46	41	68	77	57	88	68

presumably on the relation to the previous meal. The CSF ammonia level, measured only once, was 32 μg.NH_3N/100 ml. (normal up to 10 μg.NH_3-N/100 ml.). This was 1½ hours after a protein meal, when the plasma levels 30 minutes before and 30 minutes afterwards were 108 and 132 μg.NH_3-N/100 ml., respectively.

Dietary protein and plasma ammonia. The effect of different levels of protein intake was assessed by determining the plasma ammonia at 3-hourly intervals over a period of 15 hours during the day at different levels of protein intake. The results (Table I) show that in general the less the protein intake, the lower the ammonia levels. In two series, at two different age periods, in which though the total protein was unaltered, the intake calculated per kg. body weight was less as he grew older, plasma ammonia levels were lower with the lower intake per kg. This suggests that the apparent increase in protein tolerance with age may be related to a decreasing protein intake per kg. body weight.

TABLE II

Plasma Ammonia Levels After Protein Ingestion; Effect of Citric and Glutamic Acids

Protein Load	μg.NH_3-N/100 ml.				
	Fasting	Hours after Protein Load 1	2	3	4
10 g.	45	108	132	163	152
10 g.	75	104	138	237	111
9 g. + 0·5 g. citric acid ..	87	123	145	251	223
10 g.	88	—	155	189	148
10 g. + 1·5 g. citric acid	71	86	94	200	167
10 g. + 2·0 g. glutamic acid	76	170	240	246	137
Urea mg./100 ml.	(12)	(16)	(17)	(20)	(21)
10 g. alone	112		143	128	116
Urea mg./100 ml.	(14)		(15)	(17)	(18)

Protein loading tests. Plasma ammonia levels were also determined after fasting and 1, 2, 3, and 4 hours after a protein feed. There was a marked rise, maximal at 3 or 4 hours after the meal, greatly in excess of the slight rise occurring in normal infants under similar conditions. This rise in plasma ammonia was not abolished or diminished by giving citric acid (0·5 g. or 1·5 g.) just before the protein meal, nor was glutamic acid (2 g.) any more effective (Table II).

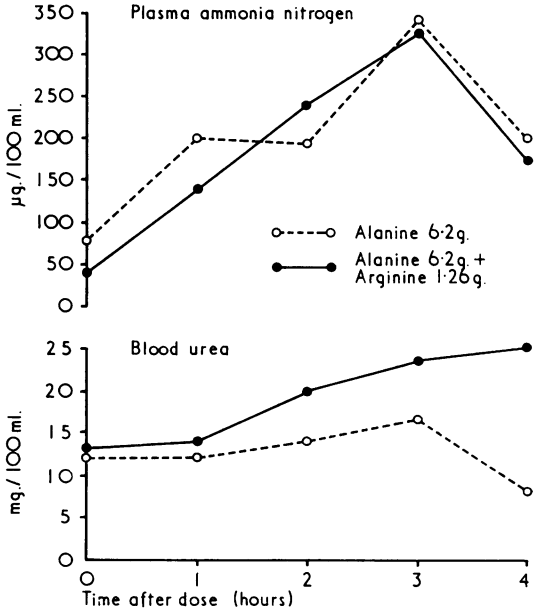

FIG. 2.—*Effect of ingestion of* (a) L-*alanine* (*0·6 g./kg.*), (b) L-*alanine* (*0·6 g./kg.*) *and arginine* (*0·125 g./kg.*) *together on plasma ammonia and blood urea levels. Note that the addition of arginine to* L-*alanine does not reduce the rise of plasma ammonia.*

Effect of ingestion of alanine and arginine. In some cases of protein intolerance associated with raised ammonia levels the latter are lowered by administration of arginine. Plasma ammonia and blood urea levels were therefore determined at hourly intervals for 4 hours after oral ingestion of L-alanine (0·6 g./kg.), and after L-arginine (0·125 g./kg.) given with alanine (0·6 g./kg.) The results are shown in Fig. 2. After alanine, the plasma ammonia rose to the high level of 338 μg.NH_3-nitrogen/100 ml. after 3 hours, with a relatively small increase in the blood urea. L-alanine and arginine together induced an identical rise in plasma ammonia, but the increase in blood urea was

TABLE III

Plasma Amino Acid Levels, Fasting and After Oral Ingestion of Protein or Amino Acids

Amino Acid	Fasting (mg./100 ml.)	3 hr. After Protein Load (mg./100 ml.)	Fasting (mg./100 ml.)	3 hr. After Alanine (6·2g.) (mg./100 ml.)	Fasting (mg./100 ml.)	2 hr. After Alanine (6·2g.) Arginine (1·26 g.) (mg./100 ml.)	15 Normal Adults (Fasting) (mg./100 ml.) Mean ± SD
Glutamine	20·0	23·0	17·2	18·5	15·3	22·1	10·8±0·8
Glutamic acid ..	1·0	0·8	0·61	1·7	1·0	2·5	0·51±0·22
Citrulline	0·10	0·10	0·08	Nil	0·10	0·16	0·45±0·17
Ornithine	0·58	0·71	0·36	0·38	0·45	1·5	0·67±0·09
Arginine	0·84	0·75	0·29	0·51	0·65	2·3	1·5 ±0·42
Glycine	1·7	1·5	1·9	1·3	2·7	2·6	1·7 ±0·27
Alanine	3·4	4·4	2·0	5·1	4·2	18·5	2·8 ±0·63
Leucine	1·3	2·2	0·89	0·48	0·79	0·55	1·5 ±0·22
Urea	14	17	12	16	13	20	14–50 (range)
Ammonia (μg. NH_3–N/100 ml.) ..	112	128	77	338	40	241	10–45 (range)

greater. The significance of these results is discussed below.

Plasma amino acid levels. These were also determined on a number of occasions, and some of the results are shown in Table III. As in the other cases of hyperammonaemia, fasting glutamine levels were very high, and glutamic acid was also raised. Both arginine and citrulline levels were low compared with the normal, whereas ornithine was within normal limits. After ingestion of protein, there was a marked rise in glutamine but little change in the other amino acids. Alanine ingestion resulted in a rise in plasma glutamine and glutamic acid, with little change in the other intermediates of the urea cycle. It also caused a marked rise in plasma alanine. The effect of alanine and arginine given together was similar to that of alanine alone. The rise in glutamine and, more marked, in glutamic acid, was consistent with the failure of arginine to reduce the rise in plasma ammonia resulting from the alanine. As expected, there was a rise in plasma alanine and arginine.

Amino acids in CSF. These were estimated on one occasion only, and the relevant levels are given in Table IV, together with those from normal children. Both glutamine and glutamic acid levels were higher than in the normal, whereas arginine was lower.

Urinary amino acids. These were determined quantitatively on a specimen of urine obtained during his initial episode while he was still having glucose electrolyte infusion, as well as on urine obtained just before a protein loading test and 4 hours after the ingestion. The results are shown in Table V, together with those from normal urine for comparison. The increased glutamine and glutamic acid excretion confirmed the qualitative results of paper chromatography. In the first specimen examined, the excretion of ethanolamine was very high. No explanation can be given for this; in the other two urines, the level of ethanolamine appeared normal. Despite the low plasma arginine, the urinary concentration was normal in amount.

TABLE IV

Levels of Some Amino Acids in Cerebrospinal Fluid

Amino Acid	Patient (mg./100 ml).	17 Controls 8 mth.–11 yr. (mg./100 ml). Mean ± SD
Glutamine	14	8·3±1·2
Glutamic acid	0·15	0·02 (<0·04)
Citrulline	0·02	0·015 (<0·035)
Ornithine	0·10	0·07±0·05
Arginine	0·08	0·34±0·10

TABLE V

Levels of Some Amino Acids in Urine

Amino Acid	During Episode of Illness (mg./g. N)	Fasting (mg./g. N	After Protein Ingestion (mg./g. N)	Normal Children (mg./g. N)
Glutamine ..	110·0	67	38	16, 12
Glutamic acid ..	0·85	1·7	5·7	0·5, 0·2
Ornithine ..	0·53	0·3	0·62	0·15, 0·17
Arginine	0·23	0·58	0·82	0·22, 0·72
Ethanolamine ..	27·0	3·5	3·6	1·4, 1·3
Glycine	22·0	50·0	64·0	8·0, 5·7
Alanine	9·7	6·9	9·5	5·0, 2·2
Histidine ..	44·0	56·0	34·0	8·0, 12·0
Lysine	2·4	2·7	3·4	0·75, 2·1

TABLE VI

Levels of Enzymes of the Urea Cycle in Liver

	Patient (units)	Normal (mean and range) (units)
Carbamyl phosphate synthetase	250	320 (182–615)
Ornithine transcarbamylase at *p*H 7·0	1288	5183 (3950–6650)
at *p*H 8·0	4332	5787 (3900–9090)
ASA synthetase	13·5	37
ASA cleavage enzyme	115	177
Arginase	24,833	38,420 (24,600–56,300)

1 unit = 1 μmole product formed/hr. per g. wet weight of tissue.
ASA = argininosuccinic acid.

TABLE VII

Properties of Ornithine Transcarbamylase

	Patient	Normal Controls
Km for ornithine	0·89 mM	1·26 mM (mean)
Km for carbamyl phosphate	0·34 mM	1·45; 0·95 mM (mean)
Activity at *p*H 7·0 (tris buffer)	1288 units*	5183 (mean)
at *p*H 8·0 (glycyl glycine buffer)	4332 units*	5787 (mean)
Ratio of the 2 activities	3·3	1·44 (mean)

* 1 unit = 1 μmole product formed/hr. per g. wet weight of tissue.

Urea cycle enzymes in liver. These were assayed in a biopsy specimen of the liver obtained at open operation, and the results are shown in Table VI. Initially, ornithine transcarbamylase activity was measured by the method of Kulhánek and Vojtíšková (1964), using a glycyl glycine buffer at *p*H 8·0, and the result was just within the normal range. However, when it was determined by the method of Brown and Cohen (1959), using a tris buffer, *p*H 7·0, as in some of the earlier cases of hyperammonaemia, the activity was only 25% of the mean normal value, and the ratio of enzyme activities at *p*H 8·0 and 7·0 was double the normal value. As the reduction in activity was smaller than in other instances of hyperammonaemia (Levin, 1968; Levin *et al.*, 1969), the Km values of the enzyme for its two substrates, ornithine and carbamyl phosphate, were determined (Table VII). The Km value for carbamyl phosphate was low, indicating a marked increase in the affinity for this substrate compared with the normal enzyme. The significance of these results is discussed later.

The activities of other enzymes of the cycle were within normal limits, except for that of argininosuccinic acid synthetase which was reduced to about 33% of a normal control.

Effect of enzyme block on pyrimidine synthesis. As in other instances of hyperammonaemia, there is an increased alternative utilization of ammonia to form orotic acid, uridine, and uracil, intermediates in the pathway of pyrimidine synthesis and breakdown (Levin, 1968; Levin *et al.*, 1969). The amount of these substances excreted daily varied according to the protein intake. Initially, when he was on a very low protein diet, 18 mg. orotic acid and 18 mg. uracil were excreted daily, the latter being the earliest indicator of the defect in the urea cycle; even greater amounts were found when the protein intake was increased slightly. Uridine, which was absent from the urine when protein intake was very low, was also excreted in relatively high amount.

Discussion

The clinical course of this patient differed from that of other cases of hyperammonaemia presenting in infancy, in the relative mildness of the condition. Vomiting for no ascertainable cause together with failure to thrive from the age of 6 months were the only features until an acute episode of illness characterized by lethargy, drowsiness, and coma and again with no obvious cause. The similarity to the episodic stupor occurring in hyperammonaemia suggested the possible diagnosis, afterwards confirmed. In two other cases presenting in infancy (Russell *et al.*, 1962; Levin, 1968), severe brain damage was already apparent by the time they presented. In the present instance, there has been no evidence of this, and recovery on a low protein diet has, so far as can be judged, been complete.

Protein requirements. The treatment of this condition has been fully discussed elsewhere (Levin, 1967; Levin and Russell, 1967). Unlike the other cases of hyperammonaemia, neither citric acid nor glutamic acid appeared able to reduce post-prandial rise in plasma ammonia. The only effective method of treatment in this case was found to be a strictly controlled reduction of protein intake.

Studies over half a century on man's protein requirements have been largely concerned to establish optimal rather than minimal needs for the individual. For adults a daily intake of 1 g./kg., for growing children 2-3 g./kg., and for infants 3-4 g./kg. body weight have been widely accepted as optimal, allowing an adequate margin for individual variation and the varying biological

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