Hyperammonaemia: a Deficiency of Liver Ornithine Transcarbamylase

Occurrence in Mother and Child

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The biosynthesis from ammonia of urea, the major end product of nitrogen metabolism in man, involves five stages. Four of them comprise the urea cycle proper (Fig. 1), and inherited metabolic disorders involving three of these steps have recently been characterized by the demonstration of a deficiency of the particular enzyme concerned. The first to be described was argininosuccinic aciduria, resulting from a congenital absence of liver argininosuccinase (Allan et al., 1958; Levin, Mackay, and Oberholzer, 1961). The second was citrullinuria, arising from a deficiency of liver argininosuccinic acid synthetase (McMurray et al., 1962, 1963, 1964). The third was hyperammonaemia, a specific syndrome due to a severe deficiency of liver ornithine transcarbamylase, found in two affected female first cousins, and reported in a preliminary account (Russell et al., 1962; Levin and Russell, 1967).

We here describe an infant with the characteristic clinical and biochemical features of this inherited metabolic disorder in its most severe form; and her mother, who is asymptomatic, except for having had an aversion to protein-containing foods almost from birth, and who has high plasma ammonia levels after an overnight fast, with abnormal increases induced by protein ingestion. The diagnosis was confirmed in both by estimating the activity of the enzymes of the urea cycle which revealed a gross deficiency of ornithine transcarbamylase in the liver.

Case History

Case 1. A girl, the only child of unrelated parents, was delivered on 6 October 1967, by forceps at 41 weeks' gestation, after a pregnancy which was uneventful apart from hyperemesis during the first 3 months and some diarrhoea and vomiting in the last 3 months. The birthweight was 3-24 kg., the length was 50·8 cm., and the head circumference 37·4 cm. She was breast-fed for three weeks and then given dried cows' milk feed, whereupon she began to vomit after each feed and was therefore changed to a succession of proprietary cows' milk preparations, with no improvement.

She was first seen in hospital at the age of 3½ months for persistent vomiting. She appeared a well-nourished girl but weighed only 4·81 kg., i.e. on the 10th centile (Fig. 2). A rash, thought to be that of exanthem subitum, was noticed on the trunk and limbs. She was taking feeds vigorously but would vomit ½-1 hour later. Initial investigations excluded a local cause for the vomiting: barium meal showed only slight reflux and there was no pyloric obstruction.

Renal tubular acidosis was suspected because the plasma standard bicarbonate was initially 18 mEq/l. when the corresponding urine was pH 9, and an attempt was therefore made to determine the hydrogen ion clearance index (Peonides, Levin, and Young, 1965). She was given ammonium chloride (0·9 g.) orally in divided doses over 2 days, i.e. 1·5 g./sq.m. per day. After the last dose the patient refused feeds, became gradually drowsy, hypotonic, and mildly dehydrated. The liver enlarged to 4 cm. below the costal margin. Blood pH 7·425, urinary pH 9, and the plasma standard bicarbonate 23·5 mEq/l. 12 hours later she collapsed and became deeply comatose. Reflexes were exaggerated and fundi clear. CSF was normal. Plasma Na+, K, and Cl levels were raised, and the blood urea was 25 mg./100 ml. Glucose saline solutions were given intravenously and an initial dose of 100 mg. hydrocortisone. She remained comatose for 2 days, after which twitchings of the face and gustatory movements with clonic convulsions of the limbs began, with phases of apnoea. Though the mental state improved thereafter, twitching increased in severity and frequency, developing into a state of continuous myoclonic jerks involving the whole body. EEG showed multiple focal abnormalities suggestive of widespread disturbances, most prominent in the right posterior frontal region. The convulsions were controlled with paraldehyde and primidone over the next
Hyperammonaemia

PATHWAY OF UREA FORMATION

Ammonia + Bicarbonate + 2ATP \rightarrow \text{Carbamyl phosphate synthetase} \rightarrow \text{Carbamyl phosphate} + 2ADP + Pi

\text{Carbamyl phosphate + Ornithine} \rightarrow \text{Ornithine - Transcarbamylase} \rightarrow \text{Citryllic} + Pi

\text{Citryllic + Aspartate + ATP} \rightarrow \text{ASA synthetase} \rightarrow \text{Arginosuccinic acid} + AMP + PP

\text{Arginosuccinic acid} \rightarrow \text{Argininosuccinase} \rightarrow \text{Arginine + fumaric acid}

\text{Arginine + water} \rightarrow \text{Arginase} \rightarrow \text{Urea + Ornithine}

\text{Pi} \rightarrow \text{Inorganic phosphate}

\text{PP} \rightarrow \text{Pyrophosphate}

Fig. 1.—Pathway of urea formation.

three days, and the liver decreased in size. Milk feeds were gradually reintroduced, but after 7 days when a full milk complement was achieved, a similar episode of drowsiness and hypotonia, followed by unconsciousness with convulsions, occurred. Again the liver enlarged. A second EEG showed a marked deterioration with generalized flattening indicating gross diminution in cerebral activity. Milk feeds were accordingly stopped and she was again treated with parenteral fluids over the next 3 days.

The sequence of unconsciousness and coma after administration of ammonium chloride or of full protein intake suggested an impairment of ammonia metabolism. This was confirmed by the high levels of plasma ammonia, both fasting (865 μg.NH₃-N/100 ml.), and after oral ingestion of 5 g. protein as a milk feed (1240 μg.NH₃-N/100 ml.). The level in CSF taken at the same time was also high (435 μg.NH₃-N/100 ml.).

The patient, then aged 5 months, was transferred to Queen Elizabeth Hospital for Children for further investigation. She was spastic, and lying in mid opisthotonos, with clenched fists. The pupils reacted sluggishly to light, but the fundi were clear. Both plantar reactions were extensor. Vision and hearing were both impaired. EEG showed an even more marked lack of cerebral activity over almost all areas than before. Glucose electrolyte therapy was continued by oral administration of the fluid through a gastric tube. Plasma ammonia fell to nearly normal levels over the next 14 days, with some clinical improvement and decrease in spasticity. A biopsy of the liver was taken by open operation. Histologically, the liver appeared normal. Enzyme assays confirmed the diagnosis of liver ornithine transcarbamylase deficiency. Protein as milk feeds was reintroduced initially to give a daily dose of 2 g., divided into 2-hourly feeds. As there was no rise in fasting plasma ammonia the protein intake was increased by stages to 6 g. daily, on which the fasting level was about 60 μg.NH₃-N/100 ml., i.e. slightly above the normal. Citric acid was found to be effective in reducing the post-prandial rise, and she was thereafter stabilized on 6 g. protein and 3 g. citric acid daily, divided into 6 doses given just before the feed (Fig. 3). Her weight which had previously fallen now began to increase (Fig. 2).

At the age of 8 months she showed some clinical improvement; she could follow light and cried for food. However, she still had some spasticity of the lower limbs, and lay in a mild opisthotonic position with clenched hands. There was EEG evidence of gross brain damage. At operation for a biopsy, the brain was seen to be greatly reduced in size. The biopsy was reported by Dr. L. Crome to show, 'virtual disintegration of the cerebral cortex, with proliferation of astrocytes and lipophages' (Fig. 4). Growth was
Fig. 3.—Case 1. Effect of variation of daily protein intake, of protein load, and of oral citric acid on plasma ammonia. Note decrease in level with decreased protein intake; increase after a protein load; and reduction of the post-prandial rise of plasma ammonia by oral citric acid.

Fig. 4.—Case 1. Cerebral cortex. Note proliferation of astrocytes and lipophages. (H. and E.)
still retarded and there had been little or no increase in height or head circumference since the 5th month of age, despite some weight gain. X-rays showed no carpal centres of ossification.

**Case 2.** The mother of Case 1, age 23 years, was normal at birth, weighed 2-9 kg., and was completely breast-fed till 10 months of age, after which she was gradually weaned on to solid foods, mainly potatoes and other vegetables. She was finally completely weaned at nearly 3 years of age. This unusually prolonged period of breast-feeding was due to a refusal to take cows' milk and protein foods, and an aversion to a normal protein diet has continued up to the present. Her diet now contains less than 0·5 g./kg. body weight per day. She dislikes meat, milk, and white of egg, but takes fish in small amounts. The ingestion of foods of high protein makes her feel lethargic and sometimes induces vomiting. She appears normal both mentally and physically, weighs 50-8 kg., and is 154 cm. in height. There is no liver enlargement or abnormality of the central nervous system.

The plasma ammonia levels, both fasting and after ingestion of protein, were greatly above normal. The diagnosis of hyperammonaemia was confirmed by assay of the enzymes of the urea cycle in a biopsy specimen of liver taken by laparoscope. She was given no treatment other than continuance of her low protein diet.

**Family history.** The maternal grandparents of Case 1 are alive and well. The older brother and sister of Case 2 are both alive and well, but one brother died at 3 days old, and was said to have had jaundice. The paternal grandfather of Case 1 suffers from migraine and a paternal uncle died at 10 days of age, cause unknown.

**Laboratory Investigations**

Plasma ammonia nitrogen levels were estimated by the method of Fenton (1962) adapted to a micro scale suitable for capillary blood, the normal range obtained being 10-45 μg.NH₃-N/100 ml. Free amino acids in the plasma were determined on picric acid filtrates (Stein and Moore, 1954) using a Technicon automatic amino acid analyser. The usual Technicon procedure, however, was modified so as to give reliable estimates of glutamine levels (Technicon, 1968). The urea cycle enzymes were determined by the procedures of Brown and Cohen (1959). Lysine NAD-oxido-reductase was estimated by a modification of the method of Bürgi, Richterich, and Colombo (1966).

Orotic acid, uridine and uracil were detected and estimated in the urine by an application of the technique of Rinderknecht and Rinderknecht (1965) for urinary pseudouridine.

**Results**

**Biochemical investigations.**

**Case 1.** Plasma Na, K, and Cl were usually normal. The plasma standard bicarbonate was also normal, except a slightly low initial level and one which was high (31 mEq/L) because of alkali therapy. Blood pH was always near the upper limit of normal, and was on one occasion 7-59, i.e. grossly alkaline. The blood urea ranged between 20 and 39 mg./100 ml., when the infant was on a normal diet, but fell to less than 10 mg./100 ml., on a low protein intake.

Serum Ca, Mg, P, alkaline phosphatase, cholesterol, β-lipoprotein, protein-bound iodine, and glucose were all normal. Serum protein, albumin, globulin, zinc sulphate turbidity, γ-globulin turbidity, and serum bilirubin were also normal. Serum GOT, GPT, and LDH were markedly raised, but they decreased considerably when she was on a low protein diet.

Initially there was gross aminoaciduria, the amino acid nitrogen being 17·3% of the total nitrogen, but this later diminished to 7%. Paper chromatography gave a prominent glutamine band. **Case 2.** Serum electrolytes, Ca, P, alkaline phosphatase, uric acid, glucose, bicarbonate, total protein, urea, and creatinine were within normal limits. There was no hyperaminoaciduria, but an increased glutamine band was seen on paper chromatography.

**Protein intake and ammonia levels in plasma and CSF.**

**Case 1.** The fasting plasma ammonia estimated when she was on a normal protein intake for an infant was 865 μg., the level in CSF being 435 μg.NH₃-N/100 ml. The plasma level rose to 1240 μg.NH₃-N/100 ml. 3 hours after a meal containing 5 g. protein (Table 1). Exclusion of protein from the diet over the next two weeks led to a reduction of fasting plasma ammonia almost to normal levels. The plasma ammonia was measured both when she was fasting and after a protein load on increasing levels of protein intake per day. The levels when fasting and those 3 hours after a protein meal both rose with increasing daily protein intake (Fig. 3).

The urinary excretion of ammonia, urea, and total nitrogen after a protein meal are shown in Fig. 5. The rate of excretion of ammonia and urea were both increased, up to a maximum in the 4th hour. The increase in urea formation with higher protein ingestion suggests that the capacity to synthesize urea is not fully utilized at a low protein intake. The synthesis of urea is therefore capable of expansion with increased stress, but it is not sufficient to prevent a proportionally greater increase in blood ammonia.
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limit of normal, and the level rose to a maximum of 209 μg.NH₃-N/100 ml. 2 hours later. The blood urea rose, though only slightly. She became sick and vomited 2½ hours after eating the meal.

**Plasma ammonia and citric acid.** In *Case 1* the effect of oral ingestion of citric acid, 1 g., given just before the meal, on the rise in plasma ammonia occurring after a protein meal, was determined on several occasions (Fig. 3 and Table I). When citric acid was given there was little or no increase in plasma ammonia 3 hours after the meal, the level on one occasion being lower than the fasting one.

**Plasma amino acids.**

*Case 1.* The fasting levels of these acids were estimated when she was on a normal, low, and zero protein intake. The levels of some of them are shown in Table II, together with those of normal fasting adults. On a normal protein intake both plasma glutamine and glutamic acid were greatly increased, diminishing to nearly normal values when protein was excluded from the diet. As might be expected, there were significant changes in the levels of those amino acids which are inter-

**TABLE I**

<table>
<thead>
<tr>
<th>Protein Load</th>
<th>Plasma Ammonia (μg.NH₃-nitrogen/100 ml.)</th>
<th>Blood Urea (mg./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting</td>
<td>1 hr.</td>
</tr>
<tr>
<td>Case 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 g.</td>
<td>865</td>
<td>—</td>
</tr>
<tr>
<td>2½ g.</td>
<td>61</td>
<td>—</td>
</tr>
<tr>
<td>2½ g.+1 g.</td>
<td>62</td>
<td>—</td>
</tr>
<tr>
<td>citric acid</td>
<td>30 g.</td>
<td>116</td>
</tr>
</tbody>
</table>

**TABLE II**

<table>
<thead>
<tr>
<th>Plasma Amino Acid</th>
<th>Case 1 Protein Intake</th>
<th>Case 2 Protein Intake</th>
<th>15 Normal Adults Mean and SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Low</td>
<td>Nil</td>
</tr>
<tr>
<td>Glutamine</td>
<td>25·5</td>
<td>17·0</td>
<td>13·8</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>1·5</td>
<td>1·4</td>
<td>0·73</td>
</tr>
<tr>
<td>Citrulline</td>
<td>0·15</td>
<td>0·04</td>
<td>Nil</td>
</tr>
<tr>
<td>Ornithine</td>
<td>0·50</td>
<td>0·78</td>
<td>0·26</td>
</tr>
<tr>
<td>Arginine</td>
<td>0·46</td>
<td>0·49</td>
<td>0·31</td>
</tr>
<tr>
<td>Glycine</td>
<td>1·1</td>
<td>2·2</td>
<td>1·8</td>
</tr>
<tr>
<td>Alanine</td>
<td>3·9</td>
<td>4·6</td>
<td>2·8</td>
</tr>
<tr>
<td>Leucine</td>
<td>1·3</td>
<td>0·83</td>
<td>0·29</td>
</tr>
<tr>
<td>Plasma ammonia</td>
<td>865</td>
<td>62</td>
<td>66</td>
</tr>
</tbody>
</table>

* The usual protein intake of this patient was less than 0·5 g./kg. per day.
mediate metabolites of the urea cycle. Plasma citrulline and arginine were very low, the citrulline disappearing completely on the zero protein intake; on the other hand, ornithine, the precursor to the site of the block, was within normal limits.* The only other significant change was the moderately raised level of alanine. These changes were relatively small compared with the proportionally much greater changes in the plasma ammonia level. Thus, the decrease in plasma glutamine from 25.5 mg. to 17.0 mg./100 ml. coincided with a reduction of plasma ammonia from 865 µg. to 62 µg.NH₃-N/100 ml.

Case 2. The levels of plasma amino acids (Table II) when she was fasting were similar to those of Case 1. Two hours after ingestion of 30 g. protein, there was a moderate rise in plasma glutamine, coincident with the maximum level of plasma ammonia. There were only slight changes in the other amino acids.

Amino acids in CSF. The levels were estimated only once in Case 1 when she was 5 months old, after an overnight fast, but when she had been receiving the normal protein intake for her age. Table III shows the levels of some of the acids. As might be expected, a very high level of glutamine, over 10 times the normal, accompanied the much raised ammonia level. The level of arginine was lower and that of leucine and of a number of other amino acids not recorded in Table III were higher than normal; but the significance of these changes is doubtful, in view of the low levels of amino acids found in normal CSF.

Excretion of some metabolites of pyrimidine synthesis and breakdown. In Case 1 the excretion of orotic acid, uracil, and uridine, which are intermediates in the pyrimidine pathway (Fig. 6), was extremely high when she was on the normal protein intake, the concentrations being 240 mg., 40 mg., and 140 mg./100 ml., respectively. No orotic acid and only very small amounts of the other two substances were found when the protein intake was drastically reduced. In Case 2, the amount of orotic acid and uracil excreted was much less, 21.0 mg. and 4.8 mg./100 ml., respectively, and no uridine was found. This was probably due to her low protein intake.

Urea cycle enzymes in liver. These were assayed in the livers of both parents as well as of the infant, and the results are presented in Table IV together with some normal results for comparison. Ornithine transcarbamylase activity determined in tris buffer at pH 7.0 was severely reduced, to 8% of the mean normal value, in both infant and mother. The activity measured in glycy-glycine buffer at pH 8.0 was also decreased, but the reduction was not so great. The activity of the other enzymes appeared to be normal or nearly so. A defect in lysine metabolism due to a deficiency of lysine NAD oxide-reductase has been claimed to be the cause of impaired urea biosynthesis in a case of periodic ammonia intoxication (Colombo et al., 1967). In Case 1 the activity of this enzyme in the liver was, however, normal. In the father, all the urea cycle enzymes were normal.

### Table III

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Case 1 (mg./100 ml.)</th>
<th>(12) Normal Infants 3 dy. to 6 mth. (mg./100 ml.)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamine</td>
<td>0.06</td>
<td>10.9±2.1 (0.14)</td>
<td></td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.09</td>
<td>0.05±0 (0.14)</td>
<td></td>
</tr>
<tr>
<td>Citrulline</td>
<td>0.06</td>
<td>0.04±0 (0.06)</td>
<td></td>
</tr>
<tr>
<td>Ornithine</td>
<td>0.03</td>
<td>0.09±0.04</td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>0.10</td>
<td>0.32±0.09</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>0.06</td>
<td>0.048±0.014</td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>0.06</td>
<td>0.35±0.16</td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>0.47</td>
<td>0.24±0.09</td>
<td></td>
</tr>
<tr>
<td>Ammonia (µg.NH₃-N/100 ml.)</td>
<td>435</td>
<td>4.20 (range)</td>
<td></td>
</tr>
</tbody>
</table>

*These conclusions are unaltered even if comparison is made with the levels of plasma amino acids obtained in our small series of control children despite variation with age.
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TABLE IV
Levels of Enzymes of Urea Cycle in Liver

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Case 1 (units)</th>
<th>Case 2 (units)</th>
<th>The Father (units)</th>
<th>Normal (mean and range) (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamyl phosphate synthetase</td>
<td>152</td>
<td>153</td>
<td>182</td>
<td>320 (182-615)</td>
</tr>
<tr>
<td>Ornithine transcarbamylase at pH 7-0</td>
<td>432</td>
<td>415</td>
<td>5408</td>
<td>5183 (3950-6658)</td>
</tr>
<tr>
<td>at pH 8-0</td>
<td>1051</td>
<td>2288</td>
<td>5690</td>
<td>5787 (3900-9090)</td>
</tr>
<tr>
<td>ASA synthetase</td>
<td>37</td>
<td>41</td>
<td>15</td>
<td>37</td>
</tr>
<tr>
<td>ASA cleavage enzyme</td>
<td>122</td>
<td>190</td>
<td>130</td>
<td>177</td>
</tr>
<tr>
<td>Arginase</td>
<td>45,100</td>
<td>56,300</td>
<td>52,700</td>
<td>38,420 (24,600-56,300)</td>
</tr>
<tr>
<td>Lysine dehydrogenase</td>
<td>87</td>
<td></td>
<td></td>
<td>86</td>
</tr>
</tbody>
</table>

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

Discussion

The characteristic episodes of drowsiness, lethargy, and coma after protein or ammonium chloride ingestion in the baby (Case 1) led to a suspicion of hyperammonaemia. This diagnosis was supported by the extremely high levels of plasma ammonia found, both in the fasting state and after protein feeds. The characteristic urinary and plasma amino acid pattern, and the excretion of orotic acid and other metabolites of pyrimidine synthesis were further proofs of the diagnosis. It was confirmed by the gross deficiency of ornithine transcarbamylase activity of the liver.

The dietary history of the mother (Case 2), especially the aversion to protein foods, suggested the possibility of hyperammonaemia in her case also. Her plasma ammonia levels were also high though never as high as in the infant, nor was the rise after protein ingestion so great. The other biochemical findings were similar to those of her child, and she had a similar severe defect of liver ornithine transcarbamylase activity.

The two cases illustrate the variable clinical expression of hyperammonaemia which has been shown to occur in previous reported instances of this condition (Russell et al., 1962; Levin et al., 1969). In the infant, symptoms were manifest from the first few weeks of life, and by the 4th month there was clinical and EEG evidence of severe brain damage. On the other hand, her mother made no complaint of symptoms immediately referable to hyperammonaemia, and the syndrome would not have been suspected if she had not been investigated because of her child. Hyperammonaemia has not till now been conclusively proved in the mother of an affected infant.

The clinical features of hyperammonaemia show a marked resemblance to those of the other two congenital metabolic disorders of the urea cycle, argininosuccinic aciduria and citrullinuria. In all three syndromes the infant is apparently normal for the first few months of life, after which mental and physical retardation set in. Vomiting, for which there is no apparent cause, is also frequently the earliest sign. In all three, apart from mental retardation, there are other neurological manifestations, such as convulsions and ataxia. Lastly, in hyperammonaemia and argininosuccinic aciduria, the age at which the disease becomes apparent is variable. Hyperammonaemia differs from the others in that neurological manifestations are more severe, with episodic stupor and coma; and the abnormal hair and skin of argininosuccinic aciduria are not found in hyperammonaemia or in citrullinuria. It is plausible to suggest that in all three syndromes the neurological disorder is due to the raised blood ammonia. This rise seems to be more consistent in hyperammonaemia, possibly because the metabolic defect directly affects the

![Fig. 7.—Pathways of ammonia uptake, including urea cycle. Site of metabolic block is indicated.](http://adc.bmj.com/content/44/234/152)
uptake of ammonia by the urea cycle (Fig. 7), whereas in the other two defects, ammonia may still be utilized to form citrulline or argininosuccinic acid.

The contrast between the severe neurological manifestation of the acute infantile form and the apparent absence of symptoms in the mother is explicable if the cerebral damage is due to the raised ammonia levels (Levin et al., 1969). Since the protein intake of the artificially fed normal infant, calculated on body weight, is very much greater than that of the adult or older child, the level of ammonia will be much higher in an affected infant on normal diet than in an affected older person. It may be also that the infant’s brain is more susceptible to damage by the high ammonia level. Cerebral damage may therefore be avoided in infantile hyperammonaemia if the ammonia level does not rise excessively.

Babies fed on the breast after the first week of life get less than half the amount of protein that a baby obtains if given half-cream dried cows’ milk, and the older breast-fed infant has a diminishing protein intake per kg. body weight (Levin et al., 1959). In Case 2 there was a prolonged period of breast-feeding, consequent upon difficulties of weaning because of an aversion to protein foods, and tolerated by her mother because of the shortage of such foods in war-time England. This aversion has continued to the present day. It is plausible to suppose that brain damage did not occur because of the prolonged period of breast-feeding, and the consequent relatively low protein intake.

This second case also suggests that early diagnosis is likely to be important, as protein restriction in the first year of life, together with laboratory assessment of plasma ammonia to ensure this is not raised above about 100 μg.NH₃-N/100 ml. may be sufficient to avert serious consequences. Persistence with normal diet can lead to severe brain damage and death.

The diagnosis can be confirmed by relatively simple biochemical investigations. In argininosuccinic aciduria and citrullinuria, large amounts of the corresponding amino acids are found in the urine; in hyperammonaemia, there is usually only an increase in glutamine. Orotic acid, uracil, and uridine are detectable in the urine, but these are found both in argininosuccinic aciduria and in hyperammonaemia. The plasma aminogram will also differentiate the three disorders. Final confirmation can only be achieved by an assay of the urea cycle enzymes of the liver.

**Treatment.** The methods of treatment in these cases and the underlying reasons have been discussed elsewhere (Levin, 1967; Levin and Russell, 1967). In Case 1, restriction of protein was effective in reducing plasma ammonia levels nearly to normal. Citric acid given in association with protein restriction was also effective, but prolonged administration caused ulceration of the mouth so that the dosage had eventually to be reduced.

**Nature of enzyme defect.** The formation of citrulline from carbamyl-aspartate and ornithine in the urea cycle is mediated by the enzyme ornithine transcarbamylase (Fig. 7). The reduction to 8% or less of the normal activity of this enzyme in the liver, which is the main site of urea production in the body, results in a gross impairment of urea synthesis. Since ammonia is a major source of nitrogen for urea, it is not surprising that any limitation of this cycle leads to an increase in the blood ammonia. It is remarkable that the blood urea level was within normal limits in all cases, so that the ability to synthesize urea must have been present. Urea formation could actually be increased when dietary protein was increased, indicating that the diminished capacity to synthesize urea was not fully utilized on the usual protein intake. The reason for this is not fully understood. It is possible that the residual activity of the defective liver ornithine transcarbamylase is still higher than that of argininosuccinic acid synthetase, the rate-limiting enzyme; hence the urea cycle in hyperammonaemia may function at nearly its normal rate under stress. It has been pointed out, however, that this explanation is unsatisfactory (Levin, 1968). There is no experimental evidence so far to support the suggestion of an alternative pathway of urea synthesis (Levin, 1968).

As in the other patients with hyperammonaemia, the blood urea levels in both these cases were within normal limits when the protein intake was normal, showing that urea could still be formed as the major end product of nitrogen metabolism. This was confirmed by the increased rate of excretion in the infant after protein ingestion, which presumably reflected an increased formation of urea, but which was not enough to prevent a marked rise in plasma ammonia.

**Plasma amino acids.** As would be expected, the plasma levels of citrulline and arginine are low, both being intermediate metabolites beyond the metabolic block. It is surprising, however, that ornithine is not raised but is within normal
limits. This is presumably partly because there is decreased formation of ornithine when the urea cycle is blocked, and partly because of its participation in other metabolic pathways. These findings, together with the raised glutamine and glutamic acid, form a recognizable pattern which appears to be characteristic of hyperammonaemia.

Regulation of blood ammonia levels. The mechanisms, other than the urea cycle available to regulate the blood ammonia levels, are the reversible conversion of α-ketoglutarate to glutamic acid and of glutamic acid to glutamine which will yield ammonia for renal excretion (Fig. 6), and the synthesis of pyrimidines for nucleic acids via carbamylaspartate. These mechanisms will be stressed when the urea cycle is not functioning normally and the blood ammonia is high. This leads to an increase in plasma glutamic acid and glutamine, especially the latter, which may be more than twice the normal level. The stress on pyrimidine synthesis leads to increased formation of the intermediates in this pathway, and accounts for the increased urinary excretion of orotic acid, uracil, and uridine. However, these mechanisms are not able to keep blood ammonia within normal limits at all times if the urea cycle is blocked, since there is probably a limit to the amount of available α-ketoglutarate, and the rate of synthesis of pyrimidines is governed independently by the body requirements for nucleic acid. The latter may in part account for the apparently normal development in the first few months of life, when the requirement of pyrimidines for growth would be very high.

Chronic ammonia intoxication. The differentiation of hyperammonaemia from other recently reported and probably congenital types of chronic ammonia intoxication, which are clinically similar, may be difficult, and an assay of the urea cycle enzymes of the liver may be necessary. The female infant with periodic ammonia intoxication described by Colombo et al. (1967) who had no apparent defect of the urea cycle enzymes in the liver was considered to have an impaired lysine metabolism due to a deficiency of liver L-lysine NAD oxido-reductase. A similar aetiology was excluded in our patients, since this enzyme was normal, whereas the liver ornithine transcarbamylase was deficient. Perheentupa and Visakorpi (1965) have recorded protein intolerance with hyperammonaemia in 10 children, including 3 pairs of sibs. The cause was postulated to be an inherited defect of the transport of the basic amino acids, including lysine, arginine, and ornithine. Again the urea cycle enzymes were normal.

Finally, in a survey of 6000 mentally retarded children, Rett (1966) found 21 girls with a cerebro-atrophic syndrome and hyperammonaemia, for which no cause was discovered.

Genetics. These instances of a mother and her female infant both with hyperammonaemia suggest that the condition is inherited as a dominant. That the father appears to be normal by all tests including liver enzyme studies is support for the likelihood that he is not heterozygote for the disease, though this cannot be completely excluded. It was unfortunately not possible adequately to investigate the other members of the family. Plasma ammonia levels in the fasting state and after a protein meal, in the grandparents of Case 1, and the brother and sister of Case 2 were considered normal, though some results were equivocal. Neither orotic acid nor uridine was detected in their urines. Unfortunately adequate supervision of the tests was not possible nor could they be repeated, and further studies would be necessary to exclude hyperammonaemia with certainty.

It may be significant that 5 of the 6 cases of hyperammonaemia are female. Since the only male infant with hyperammonaemia probably has a different gene mutation (Levin et al., 1969), it is possible that the condition occurring in these cases may be sex-limited.

Summary

Hyperammonaemia, due to liver ornithine transcarbamylase deficiency, is described in a female infant who was severely affected, and her mother who was apparently asymptomatic.

The infant began with vomiting and failure to thrive, and developed neurological manifestations due to cerebral damage. She improved on a severely restricted protein intake. Her mother had an aversion to a normal protein intake and had raised plasma ammonia levels. The father was normal.

The evidence suggests that hyperammonaemia may have a dominant mode of inheritance, and may be sex-limited.

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