Citrullinaemia with Rapidly Fatal Neonatal Course

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Van der Zee, S. P. M., Trijbels, J. M. F., Monnens, L. A. H., Hommes, F. A., and Schretlen, E. D. A. M. (1971). *Archives of Disease in Childhood*, **46**, 847. **Citrullinaemia with rapidly fatal neonatal course.** An infant with a deficiency of argininosuccinate synthetase in liver and brain developed rapidly increasing apathy, respiratory insufficiency, and convulsions from the fourth day of life, and died on the seventh day. There was a profound derangement of amino acid concentrations in blood, CSF, and urine, with very high citrulline levels. This patient differs from other cases reported previously, both in her fulminant and fatal course in the neonatal period and in the greater biochemical disturbance.

Citrullinaemia is a rare inborn error of metabolism of the urea cycle. Only 3 cases have been reported previously (McMurray *et al.*, 1962; 1963; McMurray *et al.*, 1964; Mohyuddin, Rathbun, and McMurray, 1967; Morrow, Barness, and Efron, 1967; Morrow, 1967; Wick, Brechbühler, and Girard, 1970). The normal synthesis of argininosuccinic acid is blocked in this disease due to a deficiency of argininosuccinate synthetase, which has been demonstrated in liver cells and fibroblasts (McMurray *et al.*, 1964; Tedesco and Mellman, 1967). The clinical symptoms are periodic vomiting and irritability, convulsions, and mental retardation. They are thought to be due to a chronic ammonia intoxication.

Our observation refers to a newborn infant with clinical features different from those of the other cases reported. Profound abnormalities in the pattern of amino acids of serum, CSF, and urine with very high levels of citrulline were found. Liver and cerebral cells were deficient in argininosuccinate synthetase.

Case Report

A girl, born 20 July 1970, was the second child of non-consanguineous healthy parents. The pregnancy was uneventful. The baby was delivered by caesarean section at 38 weeks, because of a slightly narrowed maternal pelvis. The infant was normal at birth,

birthweight 2600 g. One minute after birth the Apgar score was 10. She was breast-fed at first. During the first three days of life, the infant seemed to develop normally, sucking well. She became less alert on the fourth day and sucking became poor. Breast-feeding was changed to bottle-feeding of humanized milk. She became drowsy and hypotonic on the fifth day and breathing was depressed, and she was admitted to hospital, comatose. The Moro and sucking reflexes were absent. She was hypertonic and had frequent convulsions. The respiration was depressed. There was no cyanosis.

The blood pH was 7.27, Pco_2 6.65 kN/m², blood base excess -4.5 mmol/l., and serum bicarbonate 22.3 mmol/l. The serum Na^+ was 144 mmol/l., Cl^- 107 mmol/l., urea 2.54 mmol/100 ml., Ca^{++} 1.27 mmol/l., and blood sugar 1.11 mmol/l.

Glucose solution (10 g/100 ml) was administered intravenously (5 ml/hr). Calcium gluconate solution (10 g/100 ml) was injected intravenously (15 ml). During the next few hours the convulsions stopped, but she became more hypotonic. Protein-free food was given by stomach tube. She remained comatose; the respiration was still inadequate with periods of apnoea. A respiratory arrest necessitated intubation and artificial ventilation. However, the general condition deteriorated gradually and the infant died on the seventh day of life.

Family History

The parents were unrelated. The mother's first pregnancy ended in an abortion. After a second uneventful pregnancy a boy was born by vacuum extrac-

FIG.—High voltage electrophoresis of amino acids in patient's urine.

tion. This boy, the only sib of the proband, was normal at birth. He became lethargic on the fourth day of life and died suddenly. Necropsy revealed only a subdural haemorrhage.

Methods

Qualitative amino acid analysis of the patient's urine was performed by high voltage electrophoresis (CAMAG, Switzerland) on Whatman 3 MM filter-paper, 40 × 20 cm, for 20 min at 100 V/cm in formic acid : acetic acid : water (50 : 150 : 850, by volume), pH 2.0. Quantitative amino acid analyses of patient's serum, CSF, and urine were performed by elution chromatography with a Beckmann/Spinco Unichrom amino acid analyser. Deproteinization of the samples was carried out with sulphosalicylic acid. Enzymes of the urea cycle were assayed according to the methods

described by Brown and Cohen (1959). Specimens of tissue were homogenized with a Potter-Elvehjem homogenizer with ice-cold 0.25 M sucrose and 10 mM triethanolamine, pH 7.4.

Results

Routine screening of the patient's urine by high voltage electrophoresis showed the presence of high amounts of citrulline (Figure). The concentrations of amino acids in the patient's serum are presented in Table I. A remarkable increase in the citrulline level was found (about four-hundredfold as compared to normal 5-day-old infants). Moreover, a high level was noted for methionine and to a lesser degree for glutamine, alanine, glycine, proline and tyrosine. The concentrations of valine, isoleu-

TABLE I

Amino Acids in Serum of Patient with Citrullinaemia
($\mu\text{mol/l.}$)

Amino Acid	Patient		Controls*
	4th Day	6th Day	
Hydroxyproline	0	0	—§
Aspartic acid	trace	trace	—
Threonine	240	240	165 $\pm$ 62
Serine	330	460	181 $\pm$ 93
Asparagine† + glutamine	5500	8700	667 $\pm$ 19
Proline	1760	2170	287 $\pm$ 63
Glutamic acid‡	—	—	—
Citrulline	4260	4570	10.3 $\pm$ 22.3
Glycine	1490	2050	236 $\pm$ 115
Alanine	3000	5580	332 $\pm$ 108
Valine	430	—	224 $\pm$ 75
Methionine	470	900	10.1 $\pm$ 26.9
Isoleucine	140	—	69.4 $\pm$ 22.9
Leucine	350	390	137 $\pm$ 43
Tyrosine	1000	—	153 $\pm$ 50
Phenylalanine‡	—	—	69.6 $\pm$ 55.1
Ornithine	—	50	154 $\pm$ 30
Lysine	—	930	240 $\pm$ 79
Histidine	—	200	86 $\pm$ 64
Arginine	—	0	—

*Mean value for 6 infants on the 5th day of life (Ghadimi and Pecora, 1964) $\pm$ standard deviation.

†Calculated as glutamine.

‡Not calculated because of overlapping of peaks by other amino acids, present in increased amounts.

§Not determined.

TABLE II

Amino Acids in CSF Patient with Citrullinaemia
($\mu\text{mol/l.}$)

Amino Acid	Patient 4th Day	Controls*
Hydroxyproline	0	—§
Aspartic acid	Trace	2.9 $\pm$ 2.7
Threonine	120	26.6 $\pm$ 9.3
Serine	310	35.7 $\pm$ 9.6
Asparagine† + glutamine	8660	455 $\pm$ 121
Proline	0	Trace
Glutamic acid‡	—	14.7 $\pm$ 13.3
Citrulline	2560	2.1 $\pm$ 0.7
Glycine‡	—	8.5 $\pm$ 2.5
Alanine	550	27.9 $\pm$ 9.9
Valine	140	14.3 $\pm$ 4.0
Methionine	340	3.2 $\pm$ 1.0
Isoleucine	50	5.0 $\pm$ 0.9
Leucine	140	11.6 $\pm$ 2.4
Tyrosine	700	7.9 $\pm$ 2.3
Phenylalanine‡	—	7.5 $\pm$ 2.2
Ornithine	Trace	8.4 $\pm$ 2.3
Lysine	230	18.6 $\pm$ 6.4
Histidine	190	11.1 $\pm$ 2.9
Arginine	0	14.2 $\pm$ 7.4

*Mean value for 13 subjects aged 9 months to 65 years (Van Sande *et al.*, 1970) $\pm$ standard deviation.

†‡§See Table I.

cine leucine and lysine were increased two to three-fold only. Ornithine was the only amino acid present in a decreased concentration. Table II shows the amino acid concentration in the patient's

CSF. An exceptionally high citrulline level was again found. The concentrations of methionine and tyrosine were also markedly raised (about one-hundredfold). The amino acids alanine, glutamine histidine lysine, leucine, isoleucine, valine serine, and threonine were present in concentrations 5 to 20 times higher than in normal human subjects.

The urinary excretion of amino acids by the patient is shown in Table III. A very marked

TABLE III

Urinary Excretion of Amino Acids by Patient with Citrullinaemia (mg/mg creatinine)

Amino Acid	Patient 4th Day	Controls*
Hydroxyproline	Trace	0.089–0.138
Aspartic acid	—§	0.014–0.024
Threonine	—	0.006–0.133
Serine†	—	0.014–0.238
Asparagine† + glutamine	5.90	0.000–0.200
Proline	3.16	0.019–0.063
Glutamic acid	1.43	0.027–0.078
Citrulline	13.70	0.000–trace
Glycine	1.80	0.258–0.394
Alanine	3.40	0.033–0.146
Valine	0.07	0.010–0.044
Methionine	—	0.000–0.010
Isoleucine	—	0.000–trace
Leucine	—	0.000–0.010
Tyrosine	1.91	0.008–0.114
Phenylalanine‡	—	0.004–0.025
Ornithine	—	0.000–0.007
Lysine	—	0.008–0.075
Histidine	0.59	0.065–0.242
Arginine	0.00	0.000–trace

*Values for 5 infants on the 6th day of life (Baumgartner *et al.*, 1968).

†‡§See Table I.

increased in the citrulline excretion was found. The urinary excretions of glutamine, proline, glutamic acid, glycine, alanine, valine, tyrosine, and histidine were also increased.

The activities of the urea cycle enzymes were found to be somewhat reduced. The most striking finding, however, was the absence of argininosuccinate synthetase activity in the liver and the very low activity of this enzyme in brain (Table IV).

Pathological findings. Necropsy showed a diffuse bilateral pneumonia. Examination of the liver showed a diffuse necrosis in which the cells were affected in groups. In some necrotic parts the pre-existent cell structure was still visible. Glycogen could hardly be found in the liver. Microscopical examination of the brain revealed an intracellular oedema.

TABLE IV
Activities of Urea Cycle Enzymes in Liver and Brain
(μ mol/min $\times$ g wet weight of tissue)

	Liver of Patient*	Controls†	Brain of Patient*	Controls †
Carbamylphosphate synthetase	0.13	0.60–0.90	‡	0.40
Ornithine trans-carbamylase	2.90	9.0–12.0	0.29	3.45
Argininosuccinate-synthetase	Not detectable	0.5–1.5	0.06	—
Arginase	51.0	238–800	0.69	—

*Pieces of liver and brain, obtained one hour after death, were immediately frozen in liquid N₂ and preserved at -70°C .

†Normal control liver tissue was obtained surgically from traffic accident cases and analysed immediately.

‡Not determined.

Discussion

The extremely high level of citrulline in serum and CSF, coupled with the argininosuccinate synthetase deficiency in liver and brain verified that this infant was suffering from citrullinaemia. The clinical picture of this patient differs greatly from that of the patients reported previously (McMurray *et al.*, 1963; Morrow *et al.*, 1967), in whom the first symptoms of the disease appeared during the first year of life with mental retardation, and periods of irritability, vomiting, and semicoma, sometimes combined with convulsions. A low protein diet was of benefit in these patients.

The clinical course of our patient was fulminating with very early death at the seventh day. A protein-free diet, given from the fifth day of life, was of no benefit. The only sib of the patient also suddenly died after three days of apparently normal development. His clinical features strongly resembled those of the proband and it is likely that he suffered from the same disease. A similar case has recently been published by Wick *et al.* (1970). In another inborn error of metabolism of the urea cycle argininosuccinicaciduria the clinical features can be very similar to that observed in our patient (Baumgartner *et al.*, 1968; Carton *et al.*, 1969). Therefore, citrullinaemia should be added to the list of inborn errors of metabolism which may have a fulminating and often fatal neonatal course. These include maple syrup urine disease, non-ketotic hyperglycinaemia, argininosuccinic aciduria propionic acidemia methylmalonic acidemia, isovaleric acidemia, and galactosaemia.

The biochemical abnormalities of our case also differ from previously reported cases. The citrulline concentration in blood, CSF, and urine was considerably higher, and the derangement of the amino acid pattern of blood was much greater in

our patient. McMurray *et al.* (1963) and Morrow (1967) found, apart from the very high citrulline levels, only an increase of glutamic acid and methionine and a decrease of ornithine in the serum of their patients. In our patient, however, the concentration of most amino acids was greatly increased (Table I).

In a sample of liver obtained at necropsy, no argininosuccinate synthetase activity could be found. The value for this enzyme activity in a liver biopsy specimen in McMurray's case was between 4 and 8% of the activities found in two controls. The more fulminant clinical course and greater biochemical disturbance suggests an almost complete deficiency of argininosuccinate synthetase. This has indeed been found in liver.

The variation in the clinical and biochemical characteristics of our patient as compared to the cases of citrullinaemia reported previously could be another example of genetic heterogeneity. In an enzymic block of the urea cycle it would be expected that the patient would be unable to produce urea and arginine. We could not detect arginine in the serum of our patient. Tedesco and Mellman (1967) have shown a twenty-fivefold increase in the K_m for citrulline of argininosuccinate synthetase of a patient suffering from citrullinaemia. Mutations of the active centre of the enzyme, and particularly of the site responsible for the binding of citrulline, are therefore possible without a complete loss of enzyme activity. It is suggested that a similar mutation has taken place in this case. The very high citrulline levels observed here suggest an even further increased K_m for citrulline. The urea level in serum was normal, an observation that has also been made in other urea cycle disorders like ornithine transcarbamylase deficiency (Russell *et al.*, 1962) and argininosuccinase deficiency (Allan *et al.*, 1958). Unfortunately, we did not have the opportunity to determine the plasma ammonia concentration.

The hypoglycaemia found in the patient on admission to our hospital might be caused by a high insulin level brought about by the extreme hyperaminoacidaemia (Floyd *et al.*, 1966). Räihä and Suikonen (1968) have shown that urea cycle enzymes are fully developed in the liver of a newborn. Nevertheless, we found a decreased activity of all urea cycle enzymes in this patient. A possible explanation for this finding may be found in a decreased enzyme induction. Schimke (1962) and recently Szepesi and Freedland (1969) have shown that the urea cycle enzymes can be induced by a high protein intake. The patient received no protein for at least two days before death. This

lack of protein may be part of the explanation for the low activities of the enzymes of the urea cycle found.

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