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Liver pathology in transient neonatal hyperammonemia

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Summary. Ultrastructural investigations have been performed on two cases of transient neonatal hyperammonaemia (TNH). This newly recognized metabolic disorder is chiefly characterized by severe hyperammonaemia in the postnatal period, a comatous state, absence of abnormal organic aciduria, normal activity of urea cycle enzymes and, usually, complete recovery. The aetiology is presently unknown.

Electron microscopy uncovered rather congruent alterations of hepatocyte structure, with a wide spectrum of mitochondrial lesions, an increase of autophagous bodies with organelle remnants, and changes in the excretory apparatus. Thus, in contrast to some of the hereditary disorders of the urea cycle, no specific structural changes could be found in TNH. This finding is in line with the observation of normal activities of main urea enzymes in these cases, and indicates that a different biochemical system may be pathogenetically involved in TNH.

Key words: Urea cycle defects – Transient neonatal hyperammonaemia – Ultrastructural pathology

Neonatal hyperammonaemia, which may result in fatal illness, is the biochemical consequence of a whole spectrum of complex disorders of urea synthesis. Most common causes of this dangerous metabolic disease of the neonatal period are X-linked ornithine transcarbamylase deficiency and citrullinemia (for reviews, see Colombo 1971; Bachmann 1974; Bachmann and Colombo 1982), whereas less frequent disorders leading to symptomatic hyperammonaemia include complex defects in the metabolism of lysine such as congenital lysine intolerance (Colombo et al. 1967) or lysinuric protein

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intolerance (Kekomäki et al. 1967), some organic acidemias (Mahoney 1976), including the one following valproate therapy (Murphy and Marquardt 1982), and familial hyperornithinaemia with hyperammonaemia and homocitrullinuria (Shih et al. 1969; Fell et al. 1974; Gatfield et al. 1975). In some instances, neonatal hyperammonaemia was found to be associated with liver failure of varying aetiology, or with high parenteral intake of protein hydrolysates.

Recently, a new type of neonatal hyperammonaemic disorder has been observed in premature infants and reported under the term, transient hyperammonaemia of the preterm infant (Ballard et al. 1978). This metabolic disturbance is characterized chiefly by an overwhelming illness developing within the first 48 h of extrauterine life, deep coma due to severe hyperammonaemia, absence of abnormal organic aciduria, normal activity of the urea cycle enzymes and, with one exception, complete recovery after aggressive therapy. Interestingly, transient neonatal hyperammonaemia (TNH) is seen almost exclusively in preterm infants and was, at least in the cases reported to date, always preceded by respiratory distress. Apparently, TNH may be at least as frequent as hereditary urea cycle defects, and presents a male preponderance (Jaeken et al. 1982a and b; see also Le Guennec et al. 1980; Tollefsen et al. 1980; Goldberg et al. 1978). The aetiology of this disorder has not yet been elucidated.

As for most inborn defects of urea synthesis, detailed pathological studies have not been carried out so far on cases of TNH. For deficiency states of ornithine transcarbamylase (OTC^-) and of carbamylphosphate synthetase (CPS^-) as well as for citrullinaemia, ultrastructural examination of tissues have provided information allowing for a better understanding of pathogenetic mechanisms involved (Mihatsch et al. 1974; Zimmermann et al. 1981). In this communication we present structural changes observed in liver tissue of two patients with TNH. The findings are compared with hepatocyte alterations reported for hereditary disorders of urea synthesis.

Material and methods

Liver tissue, samples of which had also been used for analysis of urea cycle enzyme activites, was fixed in 2.5% glutaraldehyde in sodium cacodylate buffer and postfixed with OsO_4. For electron microscopy, tissue fragments were embedded in Spurr's low viscosity medium after appropriate dehydration. Ultrathin sections were stained with uranyl acetate and lead citrate, and these preparations were examined in a Zeiss EM 10 transmission electron microscope.

Results

Case 1 (P., male)

Clinical and biochemical data. This boy is the third of 3 sibs; the two other children are well. The family history is not significant. The proband was delivered by caesarian section due to breech presentation and primary uterine contraction insufficiency. Birth data: weight, 3,100 grams; body size, 51 cm; maturity signs corresponding to the 38th week of gestation; Apgar

Table 1. Enzyme activities measured in liver tissue of two patients with TNH

		Patient P	Patient R	Reference range
Carbamylphosphate synthetase (CPS)	U/g	2.46	1.94	1.83– 5.94
	U/g protein	16.8	15.7	11.0 – 34.5
Ornithinetrans-carbamylase (OTC)	U/g	51	29	42 – 95
	U/g protein	365	235	158 – 555
Arginase	U/g	341	196	90 – 243
	U/g protein	2,443	1,350	589 –1,652
N-Acetylglutamate synthetase	mU/g	2.24	not done	5.6 – 33
	mU/g protein	15	not done	34 – 203
With arginine 1 mmole/liter	mU/g	4.2	not done	22.5 – 52
	mU/g protein	28	not done	144 – 320

score, 8/9/10. Due to respiration problems and acrocyanosis intensive care had to be initiated at 6 h postpartum. Hyperreflexia and muscular hypotonia developed. One day after birth, loss of spontaneous respiration called for tracheal intubation. Generalized tonic and clonic seizure attacks were successfully treated with Valium. However, the general condition deteriorated, further ending up with areflexia and coma. Laboratory results were as follows: plasma ammonia concentration, 2,000 µmol/l (normal up to 80 µmol/l); normal values in plasma for urea, uric acid, creatinin, T 3, T 4 and iron; normal results of amino acid Guthrie screening test; no abnormality of coagulation factors; titers for toxoplasmosis, listeriosis and rubella normal. Electro-encephalography revealed a multifocal decrease of convulsion threshold. This isolated hyperammonaemic disorder was treated with peritoneal dialysis, repeated blood exchanges, and parenteral alimentation. Within 5 days, this combined therapy resulted in a decrease of blood ammonia concentration to 170 µmol/l. A liver biopsy was performed at 17 days of age. A test exposure to amino acids did not lead to an increase of ammonia concentration, so that amino acid substitution could be installed, followed by a gradual replacement of parenteral alimentation by food. At the age of 8 weeks, the boy was dismissed in good health. The enzymatic findings are shown in Table 1.

Light microscopic findings. In small samples of liver tissue stained with the PAS method, a normal hepatic structure was noted. Hepatocytes exhibited a slightly increased cytoplasmic granularity, and a few of these cells appeared to be damaged or even necrotic. Empty droplets indicative of fatty change, cholestasis, cellular infiltrates and portal fibrosis were absent.

Electron microscopic findings. In most of the hepatocytes, mitochondria revealed an increased heterogeneity of size, shape and structure. Some of these organelles appeared to undergo disintegration; however, at least part of these changes may have been influenced by incipient autolysis. Many mitochondria showed an increase of matrix density and a reduction of cristal

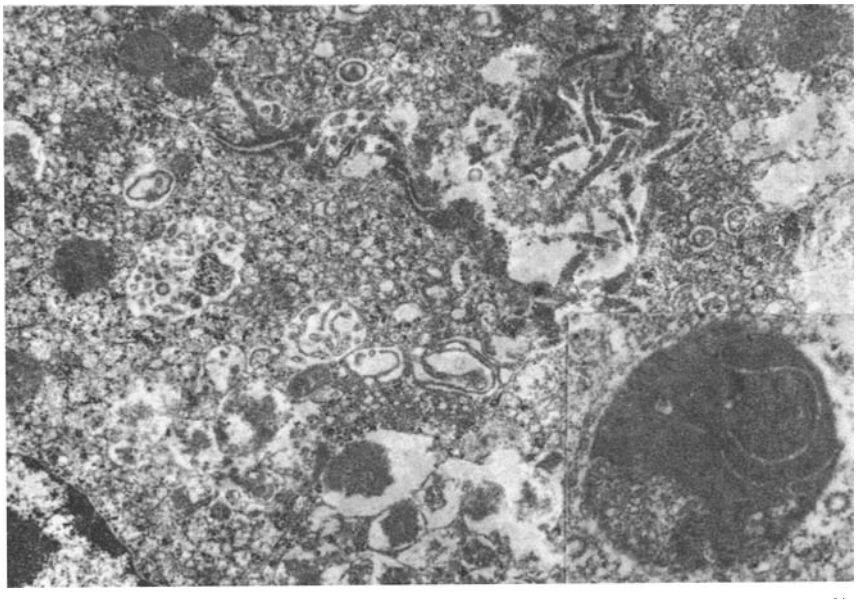

Fig. 1. Hepatocyte with signs of organelle disintegration (mainly mitochondrial damage). *Insert:* Residual body with organelle remnants. 15,000:1

profiles. The intercristal space contained dense granular structures reminiscent of aggregations of matrix granules.

The biliary canaliculi were of normal width, their microvilli were, however, focally diminished in numbers, and – here and there – appeared somewhat distorted. The canalicular lumina contained a fine, floccular material (Fig. 1). The density of peribiliary cytoplasm was increased. Zonae occludentes, zonulae adhaerentes and maculae adhaerentes were normal. A more prominent alteration consisted of increased numbers of residual bodies or telolysosomes, containing remnants of membranes or even organelles (probably including mitochondria; Figure 1, insert). The autophagous bodies were frequently associated with small lipid droplets. Rough (RER) and smooth (SER) endoplasmic reticulum and peroxisomes were structurally normal.

Case 2 (R., male)

Clinical and biochemical data. This boy is the first child of Afro-American parents. He was delivered 10 days after the calculated term by drug-induced labor. His mother had been suffering from infection in the 32nd week of gestation (most probably of viral origin). Birth data: weight, 2,250 g; body size, 47 cm; Apgar score, 8/8 after 1 and 5 minutes, respectively. Shortly after birth respiratory stridor started, followed by acrocyanosis, tachypnoea and tachycardia. Blood gas analysis revealed a pH of 7.19 and a pCO_2

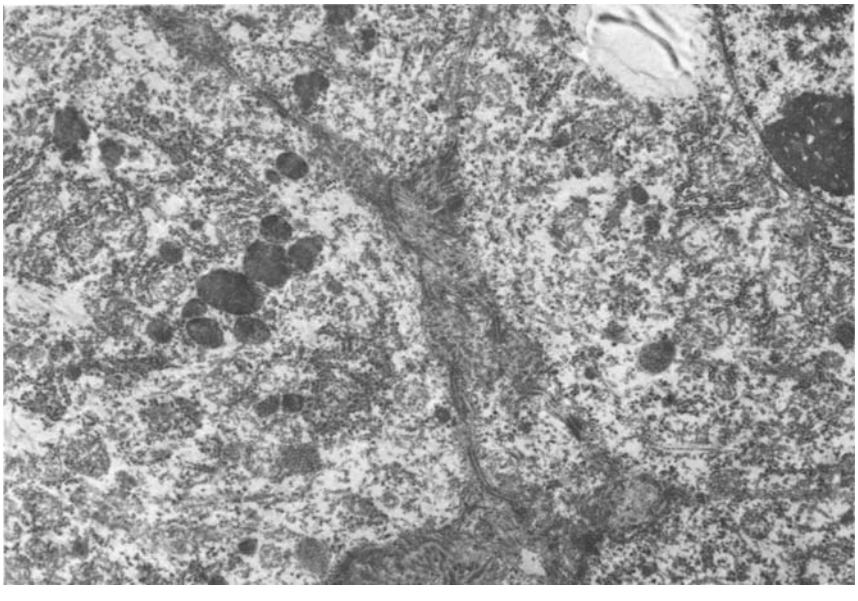

Fig. 2. Hepatocytes with increased density of cytoplasm, mitochondrial heterogeneity and clusters of residual bodies. 15,000:1

of 69 Torr. Chest X ray findings were compatible with respiratory distress syndrome stage II to III. Assisted respiration was installed. At the 2nd day postpartum progressive hypotonia was noted. Due to gastric reflux, parenteral alimentation was planned. Before infusion of amino acid solutions, free plasma amino acids and urinary amino acid and organic acid excretion were determined. In plasma, concentrations of glutamine, alanine, proline and citrulline were increased, and there was iminoglycinuria. Plasma ammonia concentration was 3,600 μmol/l (upper limit of normal for this age: 87 μmol/l). Plasma urea was normal. There was no orotic acid overexcretion, nor were methylmalonic and propionic acids increased. These findings led to an immediate blood exchange transfusion, resulting in a reduction of ammonia concentration to 2,270 μmol/l within 10 h. However, at day 3, the neonate presented without any adequate reaction and showed a fixed pupillary configuration. No signs of increased intracerebral pressure were noted. In an attempt at augmenting binding and excretion of nitrogen by stimulating the alternative pathway of hippuric acid synthesis, sodium benzoate was given at a dose of 200 mg per kg and per day. Furthermore, L-arginine was administered at a dose of 1 mmol per kg and per day, to supply this essential amino acid and to stimulate N-acetyl-glutamate synthesis. Notwithstanding these efforts, oliguria and oedema developed, and the boy expired on the 4th postpartual day.

As shown in Table 1, the activities of the main enzymes of the urea cycle were within the normal range. Because only a small liver sample was

obtained, the material was not sufficient for determining the activity of N-acetylglutamate synthetase.

Light microscopic findings. As in the first case, the liver presented an essentially normal light microscopic structure. In PAS-stained sections, small cytoplasmic droplets reminiscent of slight fatty change could be seen. As in case 1, frank necrosis or cellular infiltrates were lacking.

Electron microscopic findings. The mitochondrial population was rather heterogeneous as to size and shape of these organelles. There was a general increase of matrix density, while the number and distribution of matrix granules were apparently normal. A decrease of the density of mitochondrial cristae was found, with formation of semilunar cristal profiles. Some mitochondria exhibited bud-like projections.

An increased number of residual bodies with high electron density was noted (Fig. 2). Some of these autophagosomes contained membrane remnants or myelin-like profiles, reminiscent of partly desintegrated organelles. Clusters of myelin bodies were found in the cytoplasm of some cells with signs of incipient necrosis.

As in case 1, the biliary canaliculi showed an increased width and a rarification of microvilli. SER and RER were structurally normal.

In both cases, no relevant alterations were observed in cells other than hepatocytes.

Discussion

Transient neonatal hyperammonaemia (TNH) is a newly recognized and intriguing metabolic disorder which apparently is observed with increasing frequency since the late seventies. As the aetiology of this potentially life-threatening disease is, at present, not well understood, ultrastructural findings may contribute to pathogenetic considerations. In fact, it has been demonstrated with other defects of urea metabolism that structural investigations add to the elucidation of possible mechanisms involved (Mihatsch et al. 1974; Latham et al. 1977; Hug et al. 1978; Shapiro et al. 1980; Zimmermann et al. 1981). As regards TNH, Ballard and coworkers, in their original publication did not find any relevant pathological tissue changes in the 3 of their 5 patients on which such investigations had been performed (Ballard et al. 1978). The lack of light microscopic alterations in the liver of the 2 patients of our study, together with a corresponding observation in other reported cases of TNH and in cases of hereditary urea cycle disorders illustrates that light microscopy alone does not suffice to analyze the nature of structural sequelae of these metabolic disease states. The liver cell changes observed in our 2 patients with TNH are also of interest when compared with those found in defects of ornithine transcarbamylase (OTC^-), of carbamylphosphate synthetase (CPS^-), and in citrullinemia (CA), respectively. Liver tissue of both patients presented a well-preserved lobular architecture, and signs of inflammation, prominent fatty change,

or cholestasis were lacking. On the other hand, electron microscopy uncovered rather congruent alterations of hepatocyte structure, being mainly characterized by a wide spectrum of mitochondrial lesions, an increase of autophagous bodies containing fragments of organelles, and more discrete changes of the structure of the excretory apparatus. Notwithstanding the fact that fresh tissue had been processed, one has to keep in mind that liver tissue is prone to secondary changes induced by autolytic processes. Furthermore, child R was under severe metabolic stress which may have added to changes of the liver as a whole. Thus, the interpretation of the ultrastructural findings is hampered by these superimposed factors. However, it could be demonstrated in CPS^-, that characteristic structural alterations can be expected in the presence of a specific enzyme defect (Zimmermann et al. 1981), even if comparable secondary phenomena modify its expression. In the latter disorder, an abnormal configuration of the SER appeared to be a leading lesion, together with a peculiar change of mitochondria. In OTC^-, on the other hand, liver alterations were dominated by regressive changes with formation of telolysosomes, vesiculation of the peribiliary SER, and an abnormal configuration of RER profiles. These changes were similar to those reported for CA (Mihatsch et al. 1974). Comparing these findings with those obtained in the two patients with TNH, one has to notice that specific alterations of hepatocyte structure cannot be discerned, although both cases expressed similar changes. They are in fact dominated by signs of widespread liver cell damage, with a broad spectrum of mitochondrial alterations and sequelae of organelle breakdown. Apparently, structural integrity of hepatocytes in TNH is changed as a whole. This finding is well in line with the biochemical observation that activites of main urea cycle enzymes were within the normal range. This holds, however, not exactly true for the regulatory enzyme, N-acetylglutamate synthetase, measured in patient P only, in which the activity both without added arginine and stimulated in vitro by arginine was lower than the controls, but not absent. This could support the hypothesis that, in TNH, a reduced N-acetylglutamate synthetase activity limits the capacity for ammonia detoxication (Bachmann et al. 1983). In other patients with TNH, where liver tissue had been obtained after recovery as in the patient P, this phenomenon was not observed (Nyhan et al. 1982).

Nonspecific organelle damage comparable to the one seen in TNH has been found in other disorders of hitherto unknown aetiology, e.g. Reye's syndrome. This disorder is of some interest insofar as deficiencies of mitochondrial enzymes of the urea cycle have been reported (Sinatra et al. 1975; Brown et al. 1976; Snodgrass and DeLong 1976). These data are difficult to interpret, as the severe fatty change of the liver in Reye's syndrome contributes extensively to the sample weight to which the enzyme activity measurements are related.

The possible cause(s) of TNH are still a matter of speculation. It cannot be excluded that there might have been a transient anomaly of one or more of the urea cycle enzymes in the immediate neonatal period. It has furthermore been theorized that an inhibitor of urea cycle enzyme function

resulting from delayed development of an enzyme outside the urea cycle might be involved, or that transient portal-systemic shunting of blood could result in TNH (Ballard et al. 1978). Thus, the question as to the mechanisms involved in the pathogenesis of liver damage in TNH must remain open.

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References

Bachmann C (1974) Urea cycle. In: Nyhan WL (ed) Heritable disorders of amino acid metabolism. Wyley, New York, p 361

Bachmann C, Colombo JP (1982) Hyperammonämie: Ein Vorschlag für das diagnostische und therapeutische Vorgehen. Pädiatrie und Pädologie 17:141–148

Bachmann C, Colombo JP, Jaggi K (1983) N-acetylglutamate synthetase (NAGS) deficiency: diagnosis, clinical observations and treatment. In: Lowenthal A, Mori A, Marescau B (eds) Urea cycle diseases. Plenum Publication Corporation, p 39

Ballard RA, Vinocur B, Reynolds JW, Wennberg RP, Merritt A, Sweetman L, Nyhan WL (1978) Transient hyperammonemia of the preterm infant. N Engl J Med 299:920–925

Brown T, Hug G, Lansky L, Bove K, Scheve A, Ryan M, Brown H, Schubert WK, Partin JC, Lloyd-Still J (1976) Transiently reduced activity of carbamylphosphate synthetase and ornithine transcarbamylase in liver of children with Reye's syndrome. N Engl J Med 294:861–867

Colombo JP (1971) Congenital disorders of the urea cycle and ammonia detoxification. Monogr Paediatr 1, Karger, Basel

Colombo JP, Bürgi W, Richterich R (1967) Congenital lysine intolerance with periodic ammonia intoxication: a defect in L-lysine degradation. Metabolism 16:910–925

Fell V, Pollitt RJ, Sampson GA, Wright T (1974) Ornithinemia, hyperammonemia, and homocitrullinuria: a disease associated with mental retardation and possibly caused by defective mitochondrial transport. Am J Dis Child 127:752–756

Gatfield PD, Taller E, Wolfe DM (1975) Hyperornithinemia, hyperammonemia, and homocitrullinuria associated with decreased carbamylphosphate synthetase I activity. Pediatr Res 9:488–497

Goldberg RN, Cabal LA, Sinatra FR, Plajstek CE, Hodgman JE (1978) Hyperammonemia associated with perinatal asphyxia. Clin Res 26:198A

Hug G, Kline J, Schubert W (1978) Liver ultrastructure: dissimilarity between Reye's syndrome and heritable defects of carbamyl phosphate synthetase or ornithine transcarbamylase. Pediatr Res 12:437

Jaeken J, Devlieger H, Casaer P, Eggermont E (1982a) Transient neonatal hyperammonemia and herpes simplex infection (letter). Lancet II:272

Jaeken J, Devlieger H, Casaer P, Eggermont E (1982b) Transient hyperammonemia in the preterm infant (letter to the editor). Neurology 32:1070–1071

Kekomäki M, Visakorpi JK, Perheentupa J, Saxén L (1967) Familial protein intolerance with deficient transport of basic amino acids: an analysis of 10 patients. Acta Paediatr Scand 56:617–630

Latham PS, La Brecque DR, Kashgarian L, Klatskin G (1977) A comparative ultrastructural study of inherited urea cycle enzyme deficiency states and Reye's syndrome. Gastroenterology 73:1230

Le Guennec JC, Qureshi IA, Bard H, Siriez JY, Letarte J (1980) Transient hyperammonemia in an early preterm infant. J Pediatr 96:470–472

Mahoney MJ (1976) Organic acidemias. Clin Perinatol 3:61–78

Mihatsch MJ, Riede UN, Ohnacker H, Wick H, Bachmann C (1974) Liver morphology in a case of citrullinemia (a light and electron microscopic study). Beitr Pathol 151:200–207

Murphy JV, Marquardt K (1982) Asymptomatic hyperammonemia in patients receiving valproic acid. Arch Neurol 39:591–592

Nyhan WL, Rubio V, Jordá A, Grisolia S, Gutierez F, Canosa C (1982) Transient hyperammonemia in infants with and without organic acidemia. Adv Exp Med Biol 153:331–338

Shapiro JM, Schaffner F, Tallan HH, Gaull GE (1980) Mitochondrial abnormalities of liver in primary ornithine transcarbamylase deficiency. Pediatr Res 14:735–739

Shih VE, Efron ML, Moser HW (1969) Hyperornithinemia, hyperammonemia, and homocitrullinuria: a new disorder of amino acid metabolism associated with myoclonic seizures and mental retardation. Am J Dis Child 117:83–92

Sinatra F, Yoshida T, Applebaum M, Mason W, Hoogenraad NJ, Sunshine P (1975) Abnormalities of carbamylphosphate synthetase and ornithine transcarbamylase in liver of patients with Reye's syndrome. Pediatr Res 9:829–833

Snodgrass PJ, De Long GR (1976) Urea-cycle enzyme deficiencies and an increased nitrogen load producing hyperammonemia in Reye's syndrome. N Engl J Med 294:855–860

Tollefsen SE, McCabe ERB, Goodman SI (1980) Neonatal hyperammonemia. Pediatrics 65:1197–1198

Zimmermann A, Bachmann C, Colombo JP (1981) Ultrastructural pathology in congenital defects of the urea cycle: ornithine transcarbamylase and carbamylphosphate synthetase deficiency. Virchows Arch [Pathol Anat] 393:321–331

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