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Ultrastructural Pathology in Congenital Defects of the Urea Cycle: Ornithine Transcarbamylase and Carbamylphosphate Synthetase Deficiency

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Summary. Inborn defects of urea synthesis, leading to hyperammonemia, are complex inherited disorders, whose structural sequelae in different tissues and organs have not yet been studied in detail. Ultrastructural investigations have been performed on two cases of deficiencies of two consecutive enzymes of the urea cycle, carbamylphosphate synthetase and ornithine transcarbamylase, and the findings are compared with previously reported results. With regard to liver pathology it appeared that 1). Hepatocytes in CPS deficiency mainly exhibited changes of SER and mitochondrial compartments, whereas 2). OTC deficiency was characterized by regressive liver cell change, with abnormal configuration of the RER, formation of telolysosomes and peribiliary vesiculation. It is suggested that the mitochondrial disorder in the CPS defect is directly related to the lack of a major enzyme protein in this organelle, resulting in structural damage. The leading renal change in CPS deficiency is foot process fusion of glomerular podocytes. Brain alteration in this disorder is similar to that reported for other hyperammonaemic urea cycle defects.

Key words: Urea cycle defects – OTC deficiency – CPS deficiency – Ultrastructural pathology

Introduction

Inborn defects of urea synthesis are complex disorders which are of theoretical as well as practical interest. The study of structural changes associated with an enzyme deficiency per se and/or increased concentrations of ammonia in the cellular compartment may have important implications for a better understanding of the pathogenesis of these rare diseases. Of the known primary defects of urea cycle enzymes (Colombo 1971), ornithine transcarbamylase (OTC) deficiency is probably the most frequently reported defect, while only

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a few cases of carbamylphosphate synthetase (CPS) deficiency have been described. These two defects of consecutive enzymes of urea synthesis differ by the fact that, in OTC deficiency, there is an accumulation of carbamylphosphate, while in CPS deficiency this compound is not formed. However, both diseases lead to hyperammonaemia. Histopathological studies have been performed in few of the cases reported to date, and therefore the structural sequelae of these inborn disorders in different organs are far from being clear.

This communication aims at further elucidating the structural changes to be found in OTC and CPS deficiency.

Material and Methods

Tissue samples (tissue obtained at autopsy and liver biopsy material) were either fixed in 4% buffered aqueous formaldehyde or in 2.5% glutaraldehyde in sodium cacodylate buffer. For light microscopy, tissue was dehydrated in graded alcohols, embedded in paraffin, processed to 4 to 6 μ thick sections and stained with haematoxylineosin, periodic acid Schiff (PAS), van Gieson, Gomori's silver and Turnbull's reagents. For electron microscopy, samples were embedded in Epon or Spurr's low-viscosity medium and stained with uranyl acetate and lead citrate. Preparations were examined in a Zeiss EM 10 transmission electron microscope.

Results

Case 1 (CPS Deficiency; G.D., Male)

Clinical Data. Two siblings had died in the neonatal period of unknown causes. After a normal pregnancy and birth, the first symptoms were observed in the proband at the end of the first postnatal day (hyper-reflexia). Afterwards, respiratory distress and muscular hypertonicity occurred. On the third day, the boy had respiratory insufficiency, and hepatomegaly was noted. Plasma ammonia concentration was increased to 1830 μmol/l, concentrations of urea and creatinine being 2.5 mmol/l and 141 μmol/l, respectively. Transaminase activities were normal. There was no glucosuria, but proteinuria was noted. Despite intensive treatment, including blood exchange transfusions, the neonate died on the fifth day of life. More detailled analyses of blood plasma revealed increased concentrations of glutamine, glutamic acid and alanine (3.4, 0.41, and 0.91 mmol/l, respectively), while in the urine a gross generalized hyperaminaciduria was found. The concentration of orotic acid was not increased (13 μmol/mg creatinine). There was no organic aciduria. Enzymatic analysis of liver tissue obtained at autopsy showed a normal OTC activity, whereas CPS activity was decreased to 10% normal (0.19 $\mu mol\ h^{-1}$ mg $protein^{-1}$). Time lapse between autopsy and preparation of samples was 4–5 h.

Light Microscopic Findings. The liver showed a regular structure, without portal fibrosis, cellular infiltrates or cholestasis. Focal monocellular necrosis and slight fatty change were noted. The changes observed in the kidney were minimal, chiefly consisting of a vacuolar change in tubular epithelial cells and in an accumulation of small lipid droplets in glomerular podocytes. Brain sections (precentral and frontal regions) revealed a patchy perivascular spongiosis and

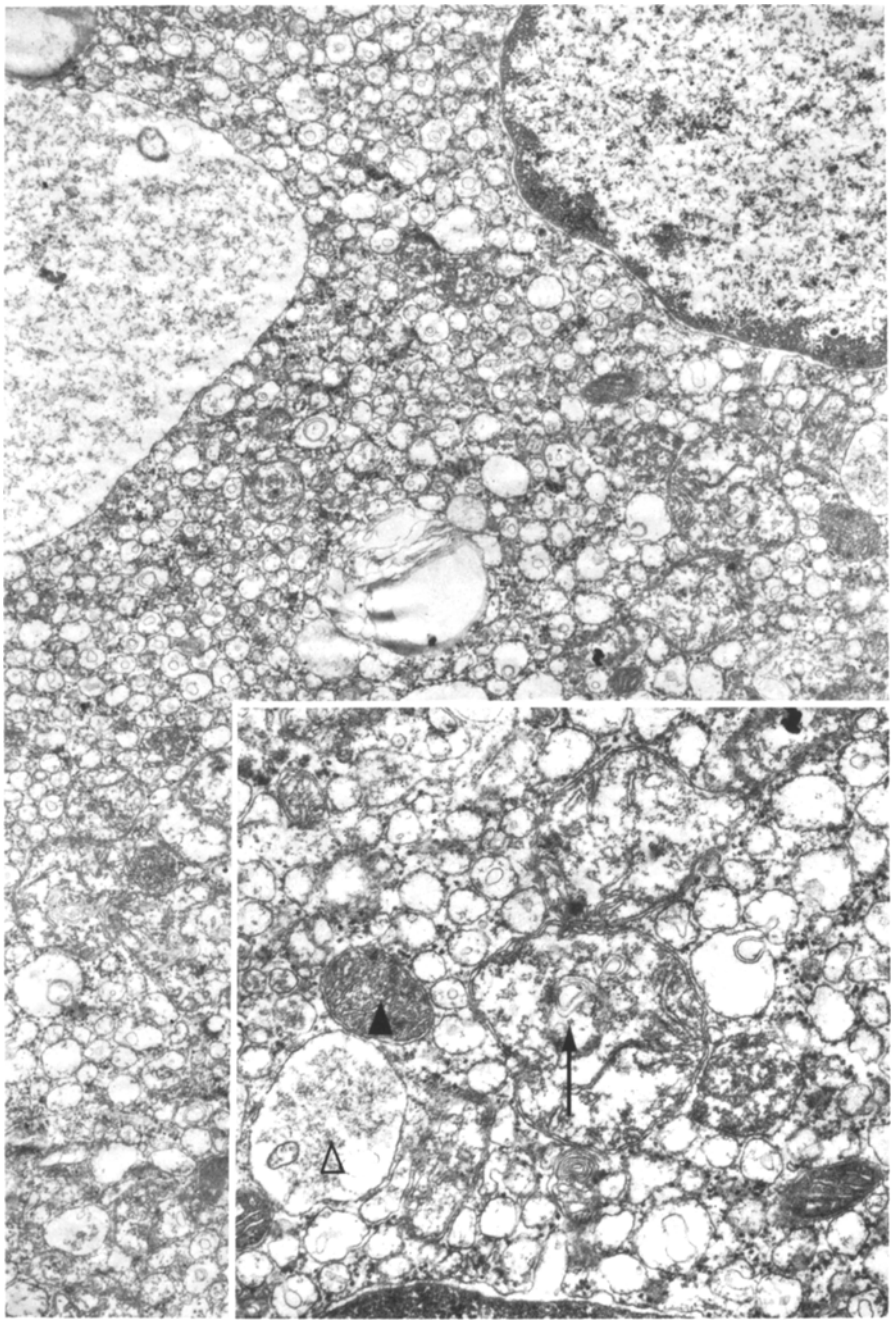

Fig. 1. Hepatocyte with strongly increased vesiculation of SER, vacuolation and disordered structure of mitochondria. Insert: Mitochondria with cristolysis (△), disarrangement of cristae (—►) and increased matrix density (▲). 15000:1

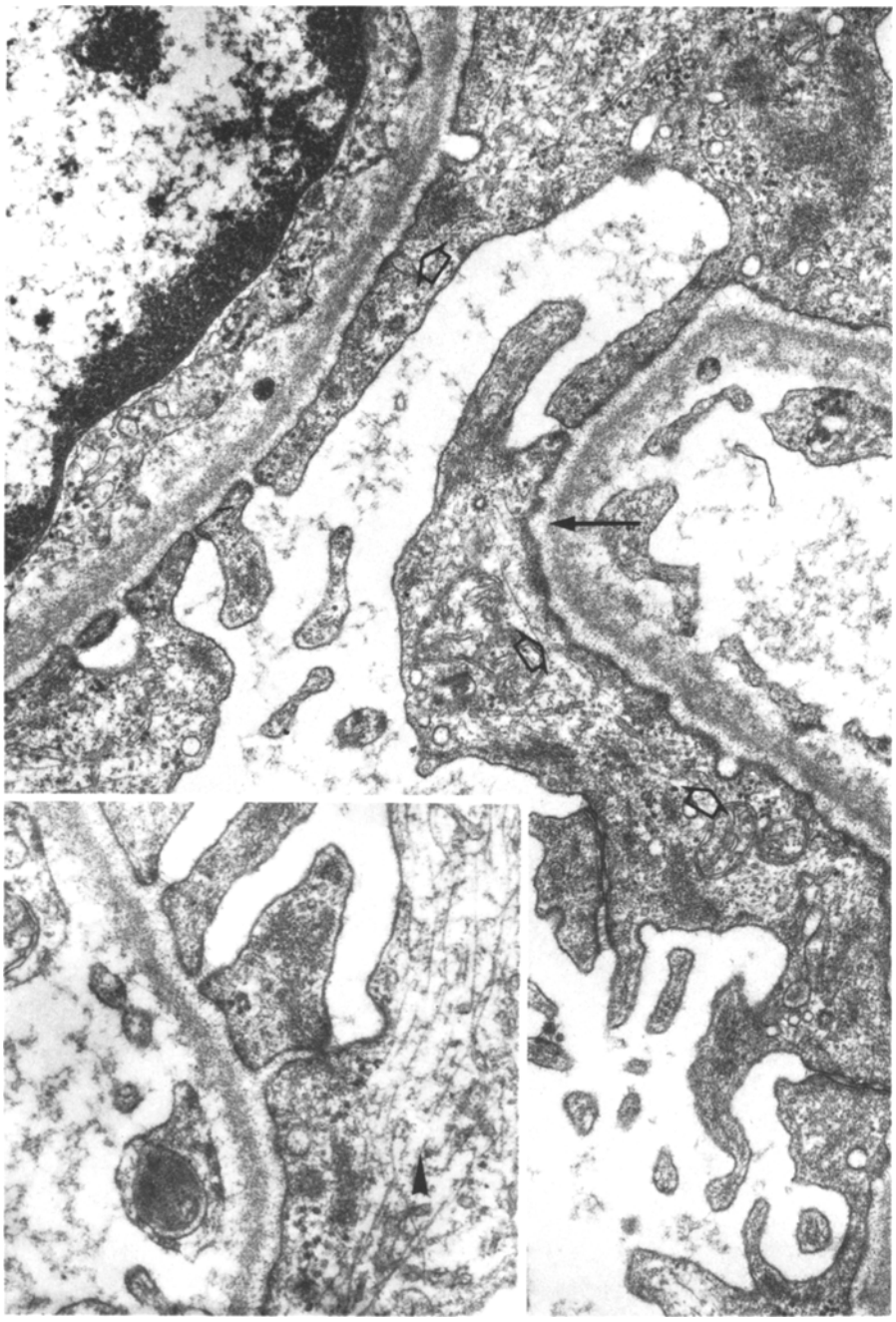

Fig. 2. Capillary loop of a glomerulus. There is foot process fusion (⇳), and the basement membrane exhibits rarefaction of its external lamina (⟶). Insert: Focal oedema of podocyte pedicle (▲). 15,000:1

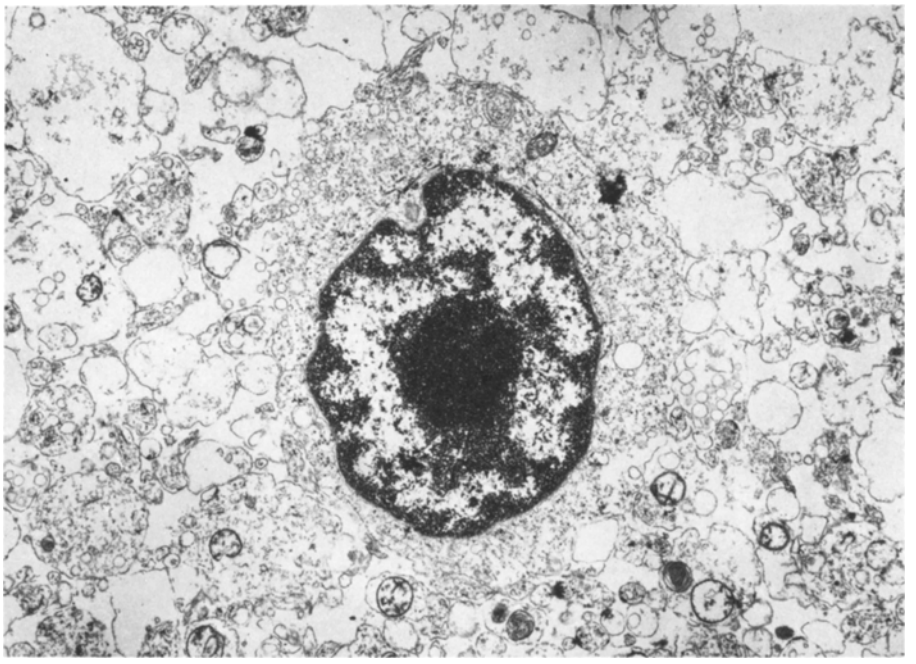

Fig. 3. Gliocyte with finely vesicular cytoplasm. Note the irregularly arranged, oedematous processes. 7,000:1

a focal increase of glial cellularity (gliosis), with some astrocytes showing large and translucent nuclei (in part so-called Alzheimer II type gliocytes).

Electron Microscopic Findings. The cytoplasm of hepatocytes exhibited a markedly increased density of vesicular type I SER (Fig. 1). About a third of all vesicular profiles presented pocket-like invaginations and contained a floccular or laminar material. Small lamellar structures reminiscent of myelin figures were seen in a paranuclear position. The profiles of the RER partly showed a concentric arrangement. Mitochondria of hepatocytes were heterogeneous in size, shape and internal structure. They revealed increased matrix density, deposition of dense material in the intercristal space, and fragmentation or forking of cristae. In about 10% of the cells, cristae were very short and projected less than one-half the usual distance towards the mitochondrial center. Some mitochondria contained membrane whorls. Mitochondrial tubular structures were not seen. In the kidney, glomerular podocytes showed extensive foot process fusion, pedicle oedema and irregular zones of increased cytoplasmic density (Fig. 2). The glomerular basement membrane exhibited, on the external face of the lamina rara externa, defects faced by pits in adjacent podocytes. Endothelia and tubular epithelia presented a moderate vesicular cytoplasmic change.

In the brain, the structural changes are mainly in glial cells. Astrocyte processes are swollen and appear oedematous. They contain increased amounts of vesicular profiles (Fig. 3), and sometimes membrane-enclosed spheroid bodies

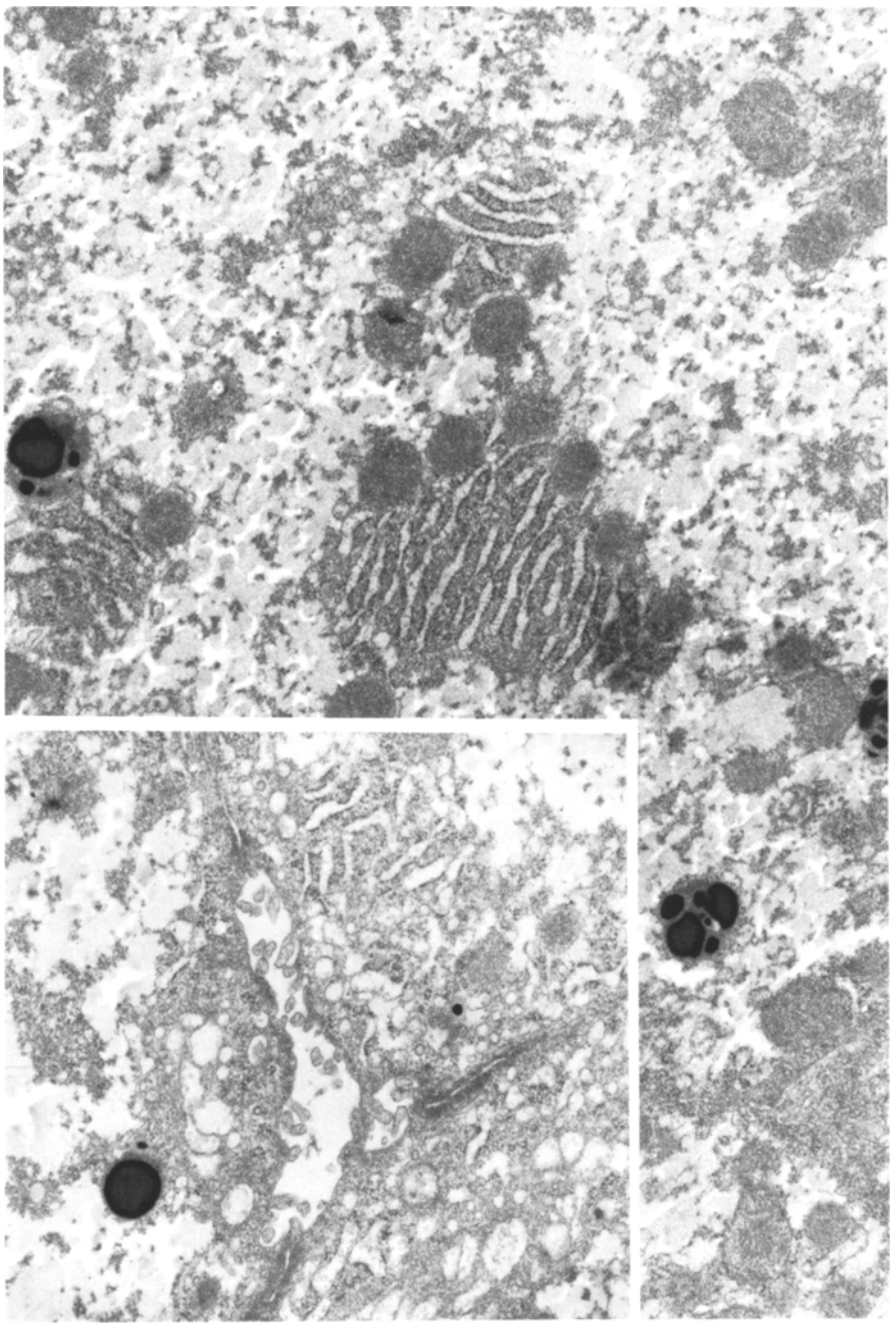

Fig. 4. Hepatocyte with dense cytoplasm, irregular membrane stacks, and small lipid droplets. Insert: Pericanalicular vesiculation of cytoplasm. 15,000:1

with a finely granular content. Mitochondria are partly disintegrated. Occasionally astrocyte nuclei are large and present a pale center with low chromatin density (compatible with so-called Alzheimer type II change, "liver glia").

Case 2 (OTC Deficiency; S.B., Female)

Clinical Data. The family and perinatal histories are uneventful. First symptoms of the disease appeared at age 20 months as repeated episodes of vomiting of 3 days' duration and markedly increased irritability. At 4 years apathy and incoherent language were noted. During a hospitalization due to an ulnar deformity hyperammonoemia of 174 µmol/l was found. This increased to 360 µmol/l on a higher protein diet, and led to ataxia, vomiting, lethargy and hyper-reflexia. Tonic seizures occurred once. In liver tissue obtained by biopsy, OTC activity was reduced to one third of normal. The child was put on a free diet, but received 0.3 mol HCl/kg body weight (given as a 0.1 N solution) to increase ammonia excretion. Liver biopsy was repeated at 12 years of age and used for the studies reported below. OTC deficiency was confirmed in this sample. The patient suddenly died at age 14 years. No autopsy was done.

Light Microscopic Findings. Liver tissue, obtained through biopsy, exhibited a slight fatty change. Part of the hepatocytes showed regressive changes and signs of necrosis. Portal fibrosis, cellular infiltrates or cholestasis were absent.

Electron Microscopic Findings. Preserved parenchymal cells revealed increased amounts of small lipid droplets, of microbodies and of lysosomes (in part telolysosomes). The SER was moderately vesiculated, mainly in pericanalicular parts of the cytoplasm. Ribosome-containing reticulum was arranged in peculiar clusters, sometimes to form zig-zag-like configurations, and some mitochondria showed an increased matrix density (Fig. 4). Some of the hepatocytes contained pyknotic nuclei and condensed cytoplasm with disintegration of organelles, indicative of severe regressive change, or necrosis.

Discussion

The morphological investigations performed on tissues of two patients with CPS and OTC deficiency, respectively, add new information on structural changes observed in this group of rare inherited disorders. Changes in the liver in cases of inborn errors of urea synthesis comprise a broad spectrum of visible alterations, the causal pathogenesis of which is not clear. Table 1 summarizes hepatic lesions reported in the literature. The table aims at providing the reader with the background information available on the changes observed in some urea cycle disorders to date. It is seen that light microscopy alone is not sufficient to elucidate the precise nature of the structural changes, as the alterations found by this approach, if one finds any, are largely nonspecific. As in other inborn errors of metabolism, excluding disorders where a specific product is stored (thesaurismoses), the interpretation of these findings with regard to a specific enzyme defect is difficult and often hampered by superim-

Table 1. Liver pathology in congenital disorders of the urea cycle

1. Light microscopic features	
No visible liver change	Levin et al. 1969 Campbell et al. 1973 Sandubray et al. 1973 Farriaux et al. 1974 Goldstein et al. 1974 Ricciuti et al. 1976
Increased glycogen deposition in hepatocytes	Hopkins et al. 1969 Bruton et al. 1970 Hug et al. 1978
Reduced glycogen content of hepatocytes	Sunshine et al. 1972
Fatty change of hepatocytes	Mihatsch et al. 1974 Hommes et al. 1969 Hopkins et al. 1969 Corbeel et al. 1969 Freeman et al. 1970 Vidailhet et al. 1971 Matsuda et al. 1971 Mihatsch et al. 1974 La Brecque et al. 1977 Latham et al. 1977 Leibowitz et al. 1978
Cholestasis	Mihatsch et al. 1974 Leibowitz et al. 1978
Increase of fibrous tissue	La Brecque et al. 1977 Shapiro et al. 1980
2. Ultrastructural features	
Normal mitochondria	Latham et al. 1977 Hug et al. 1978
Giant mitochondria	Mihatsch et al. 1974
Mitochondrial heterogeneity with matrix changes	Mihatsch et al. 1974
Mitochondrial tubules	Shapiro et al. 1980
Normal endoplasmic reticulum	Latham et al. 1977
Changes of SER	Mihatsch et al. 1974 Hug et al. 1978
Changes of RER	Mihatsch et al. 1974
Increase of Golgi elements	Hug et al. 1978
Increase of microbodies and telolysosomes	Mihatsch et al. 1974
Increase of peroxisomes	Latham et al. 1977

posed secondary changes. Moreover, structural alterations may be heavily influenced by processes such as postmortem autolytic change. Variations of hepatocytic glycogen content illustrate nothing more than the site of carbohydrate turnover, and fatty change reflects, amongst other factors, disordered production of apolipoproteins in a damaged hepatocyte. Cholestasis may also be a nonspecific reaction of diseased liver cell populations. However, taking these restrictions into account, a more detailed electron microscopic investigation may provide an increasing amount of information on the type of cellular structural change associated with a given metabolic disease. In CPS deficiency, as based on our observation, an abnormal configuration of the SER appears to be one of the leading structural lesions. The second organelle severely altered in CPS defect

is the mitochondrion. We noted mitochondrial heterogeneity with focal membrane budding, and disarrangement, shortening or forking of cristae, with intercristal membrane whorls and high matrix density. The causal link between the enzyme defect, or its metabolic sequelae and the disorder of cell structure observed, is not known. The changes may not exclusively be due to toxic concentrations of ammonia, as other enzyme defects differ considerably in their ultrastructural pathology. As tissue was obtained through autopsy in the case of CPS deficiency, some cytoplasmic and/or organelle change may also be due to postmortem autolysis. However, at least the disorder of mitochondrial structure found in the liver may be directly related to the enzyme defect per se. Carbamylphosphate synthetase (E.C. 2.7.2.5.), which catalyzes the synthesis of carbamyl phosphate as the first step in the conversion of ammonia to urea, is a mitochondrial matrix enzyme. Its precursor is transported to mitochondria via a cytosolic route (Raymond and Shore 1979). Processing of this precursor to its final size probably takes place at some point after its initial interaction with the surface of the mitochondrion. As CPS is normally present in the hepatic mitochondrial matrix at very high concentrations (0.4 mM to 1.0 mM; Raijman and Jones 1976; Lusty 1978), constituting one-fifth to one-fourth of the total matrix proteins of liver mitochondria (Clarke 1976), a lack of this enzyme protein as such may influence the structural integrity of these organelles. Accumulation of lipofuscin bodies in CPS deficiency (Hug et al. 1976) may result from increased organelle breakdown, but has not been observed in our case. On the other hand, changes found in the brain and in the kidney may be related to the action of a metabolite, e.g. ammonia. The tissue alterations found in the kidney of the patient with CPS deficiency are – at least in part – nonspecific. Foot process fusion is seen in different renal diseases, e.g., minimum change nephropathy, and CPS deficiency can, therefore, be added to the increasing list of entities presenting with this lesion. As far as we know, foot process fusion has not been observed as a consequence of autolytic processes, being a vital reaction of metabolically altered podocytes. In our case, the change is in line with the clinical finding of proteinuria and, possibly, generalized hyperaminaciduria. Brain changes similar to those of our case 1 have been reported in former examples of urea cycle disorders (Baumgartner et al. 1968; Hopkins et al. 1969; Levin et al. 1969; Van der Zee et al. 1971; Ebels 1972; Campbell et al. 1973; Wick et al. 1973; Roerdink et al. 1973; Leibowitz et al. 1978). These reports stress the importance of regressive changes, of focal to diffuse necrosis, and to oedema and spongiosis. Acute necrosis in the central nervous system, being a more reliable morphologic variable than spongiosis, has chiefly been observed in citrullinemia (Wick et al. 1973; Leibowitz et al. 1978). Part or most of this lesion has been attributed to the toxic effects of high concentrations of ammonia, which may also be instrumental in the formation of Alzheimer II type gliocytes (so-called "liver glia"). This peculiar cell alteration was found in our case with CPS defect, and has been reported to occur in other disorders (Bruton et al. 1970; Colombo 1971; Campbell et al. 1973; Leibowitz et al. 1978). In rats with experimental portocaval shunts the degree of this astrocytic lesion showed a direct positive correlation with blood ammonia concentration (Cavanagh and Kyu 1971).

The findings in the patient with OTC deficiency were obtained from a liver

biopsy when the child was clinically well. Thus, the morphological results are not biased by secondary terminal alterations. This is important in the light of the regressive changes found in hepatocytes, with formation of telolysosomes. Some of the hepatocytes were even necrotic. In addition, there was increased vesiculation of the peribilary SER, and a peculiar conformation of RER profiles. These alterations are, in some respect, similar to those observed in the liver of patients with citrullinemia, where the RER also presents a disordered arrangement of profiles, e.g. concentrically formed and "zig-zag-like" structures (Mihatsch et al. 1974). Furthermore, in both OTC deficiency and citrullinemia hepatocytes exhibit an increase in the number of microbodies and telolysosomes. This suggests a similar pathogenetic mechanism in these more distal disorders, with regard to the position of the defect within the urea cycle. The presence of hepatocyte necrosis, together with signs of increased turnover of intracellular components, is in line with the assumption that, in these two diseases, more (or at least more vital) structures of the liver cell are damaged in comparison to CPS deficiency. In contrast to a former report on OTC defect (Shapiro et al. 1980) we did not find intramitochondrial tubular profiles. Our findings illustrate that, at least what regards CPS deficiency, special (and perhaps specific) ultrastructural changes can be found in the target tissue of a given defect of the urea cycle. The results further demonstrate that primary alterations may interfere with effects due to ammonia toxicity alone. The question as to how reactivity to toxic concentrations of ammonia is modified by other metabolites occurring in hyperammonemic hereditary enzymopathies remains to be further investigated, hopefully leading to a better understanding of pathogenetic mechanisms involved.

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