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Ultrastructure of the Liver in a Case of Childhood Cystinosis

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Summary. Ultrastructural findings in the liver in a case of childhood cystinosis are reported. Crystalline structures were found mainly in Kupffer cells. The presence of dark cells, with or without crystals, was the most striking feature observed. Such cells have already been noted within the kidney on one occasion when it was shown that the dark substance was L-cystine (Spear et al., 1971). In this case identical dark material was also found extracellularly.

The data shows that free cystine can fill cell cytoplasm and extracellular spaces and the possibility that cystine overproduction may take place in the hyaloplasm should be considered. Extracellular location of cystine in the tubules might account for an increase in epithelial permeability and thus for the Fanconi syndrome.

Key words: Childhood cystinosis – Liver – Electron microscopy – Dark cells – Tight junctions.

Introduction

Childhood cystinosis is an autosomal recessive disorder of metabolism characterized by renal tubular abnormalities which constitute the Fanconi syndrome and which are followed by rickets and retarded growth. Various ocular troubles with a characteristic retinopathy permit early diagnosis. The course of the disease which is marked by severe glomerular damage, is relentless with death before puberty. Biochemistry and histology reveal high intracellular concentrations of L-cystine with the deposition of crystals in many tissues.

Our report concerns the subcellular features noted in the liver of a typical case of childhood cystinosis.

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Patient

At the time of the liver biopsy the female patient was ten years old. Her disease was diagnosed during the first year of life. Renal damage was extensive enough to need dialysis from the age of six. Splenectomy and bilateral nephrectomy were later carried out in view of the development of hemolytic anemia and systemic hypertension.

In preparation for renal homotransplantation, various liver tests, including a liver biopsy, were performed. These tests were of special importance since the patient had had an acute type B viral hepatitis one year previously.

Material and Methods

Liver tissue was fixed for 1 h in 2.5% glutaraldehyde in phosphate buffer 0.1 M at pH 7.2, postfixed for 1 h in buffered 1% osmium tetroxide, dehydrated in a graded series of alcohols and embedded in epoxy resin.

Ultrathin sections were stained with uranyl acetate and lead citrate as usual.

Results

Within the hepatocytes (Fig. 1) widespread but mild vesiculation of the smooth endoplasmic reticulum was commonly observed. The rough endoplasmic reticulum was less frequently dilated. Steatosis was occasionally encountered. Cytoplasmic heterogeneous dense bodies, membranous at times, were found in some cells. No crystal-shaped structures were found in hepatocytes but were often seen within Kupffer cells or other phagocytic cells located in portal areas.

These crystalline structures were frequently triangular, rectangular or hexagonal in form and thus corresponded to various sections of hexagonal prisms. Some crystals were simply limited by a single membrane, some others were situated within distinguishable lysosomes (Fig. 2). Their contents had more or less completely escaped, leaving vacant spaces; that which remained was homogeneous with a density ranging between light grey and jet-black (Fig. 3a and b).

Material of the same nature was also found within more irregular and larger cytoplasmic inclusions. These inclusions were often partly, but never completely, emptied; they contained loose material and occasionally, perfectly round dark bodies (Fig. 3c). Occasionally the same material was found in some of the lysosomes (Figs. 2 and 3d).

Two categories of macrophagic cells were encountered. The first one was composed of the usual clear cells (Fig. 2), the second one of dark cells. These latter cells were seen both on stained and unstained ultrathin sections, the darkening being caused by fine granular material entirely filling the cytoplasm and nucleus, sparing only the mitochondrial matrix and some of the cisternae of the endoplasmic reticulum (Fig. 4). In some areas dark cellular pseudopods intermigled with neighbouring cells (Fig. 3b). Some circulating monocytes, within the sinusoids, were also dark and contained crystals.

In some regions the dark substance was extracellular and occupied the space of Disse between dark cells and hepatocytes. Sometimes it extended into the intercellular spaces between hepatocytes and reached the canalicular lumen, after impregnation of the intermediary sheet of the tight junctions (Fig. 1).

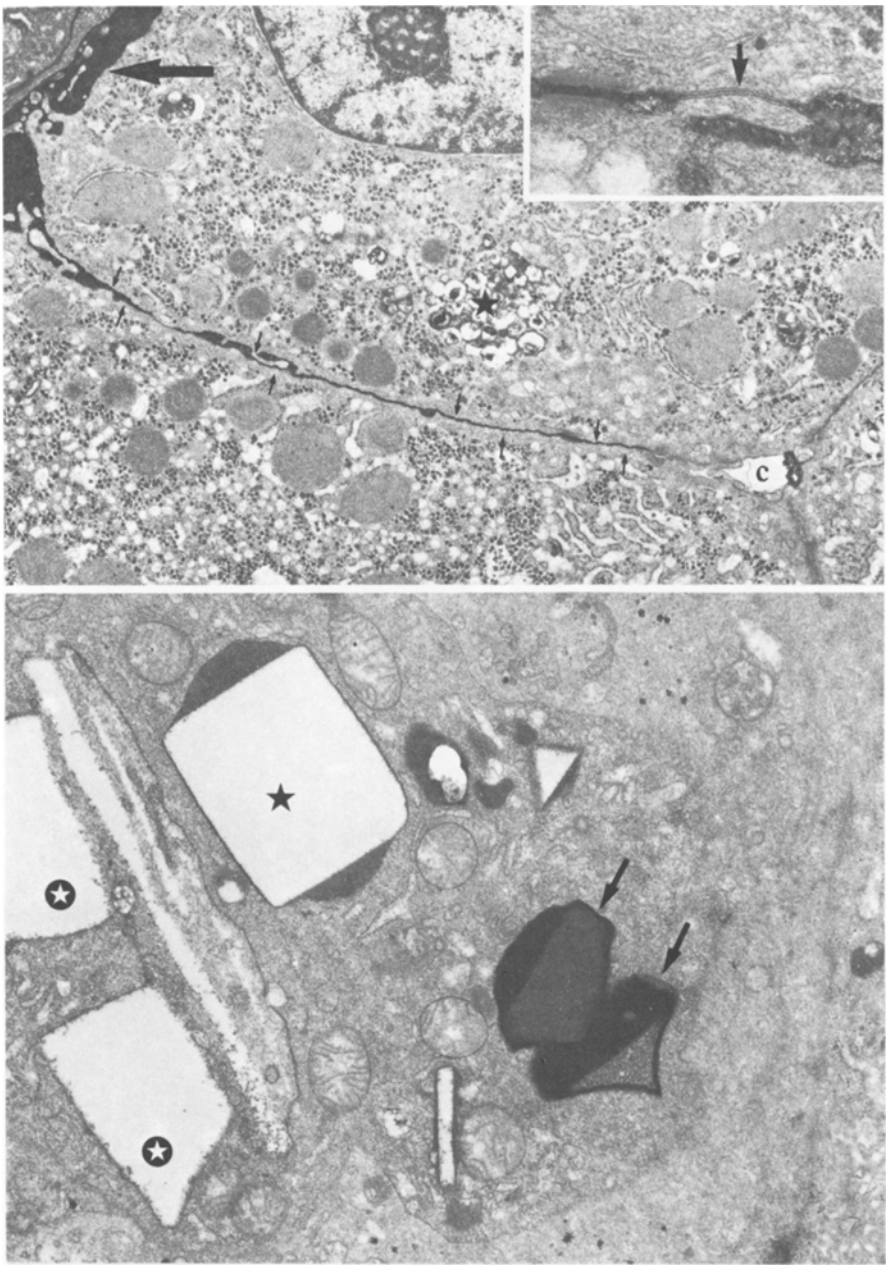

Fig. 1. Hepatocytes showing moderate dilatation of ER (SER and RER) and some heterogeneous, more or less membranous, dense bodies (★). The dark substance gives the impression of extending from the space of Disse (*large arrows*) and reaching the canalicular lumen (*c*). Inset: dark substance impregnating the intermediary sheet of the tight junction (*arrow*) (×8,500 inset, ×47,600)

Fig. 2. Crystalline structures within a clear cell. One of them is located within a recognizable lysosome (★), others are limited by a single membrane (✪). Two lysosome-like structures contain a foreign homogenous material (arrows) (×12,750)

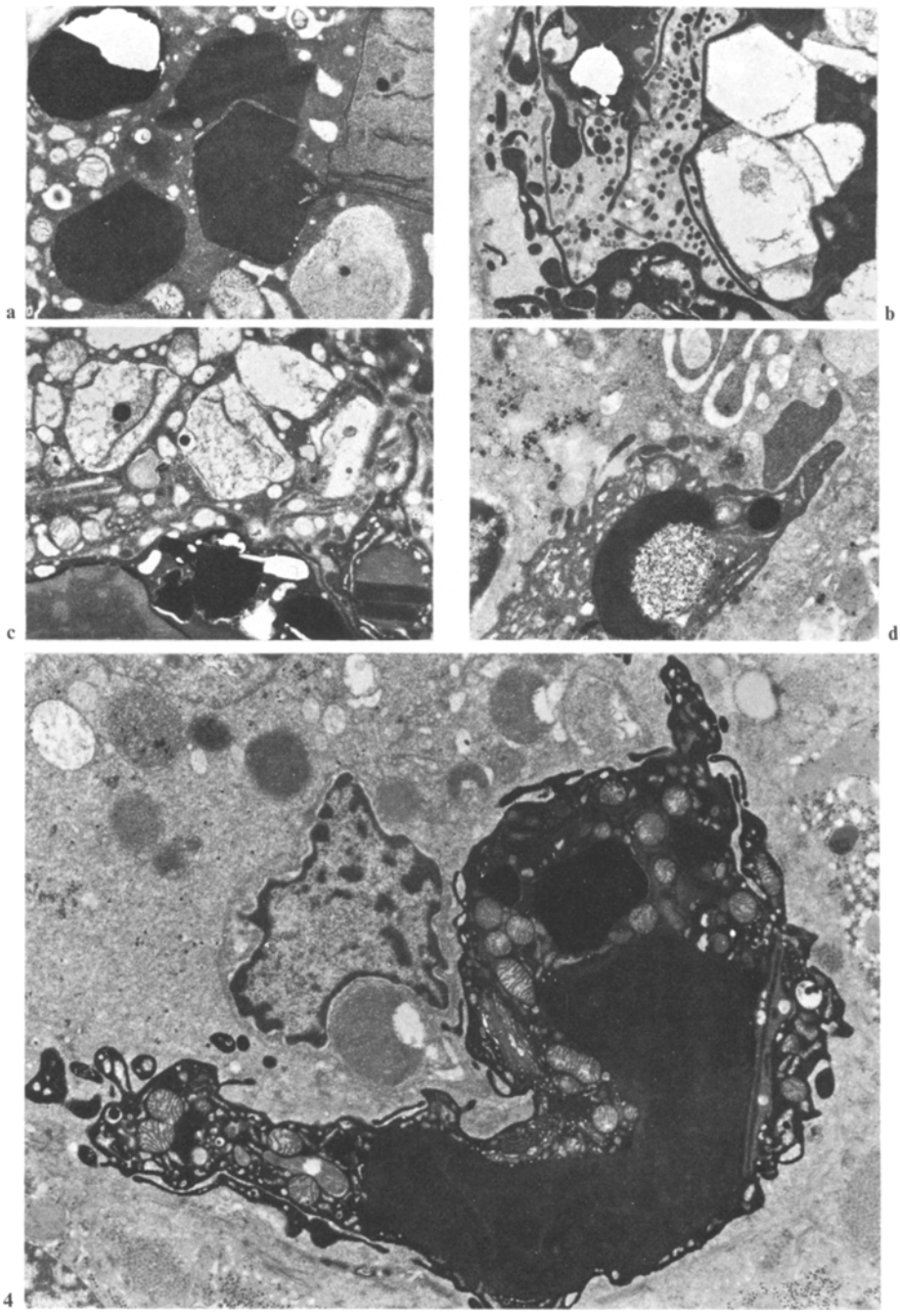

Fig. 3a–d. Miscellaneous cytoplasmic bodies with or without crystals. Some crystals are clearly hexagonal (**a** and **b**). The content of the inclusions is variable in density (**a**). The emptied inclusions still contain loose material with occasional round dark bodies (**c**). Some lysosomes contain foreign homogeneous material (**d**). Note the importance of black pseudopods at the periphery of a dark cell (**b**) (**a** and **c**: unstained sections) (× 7,650)

Fig. 4. Dark Kupffer cells. Black material is present both in the nucleus and the cytoplasm with the exception of the mitochondrial matrix and some of the occasional cisternae of the ER (× 6,800)

Discussion

A wide variety of tissues has already been studied by electron microscopy in cases of cystinosis (Brubaker et al., 1970; Hummeler et al., 1970; Kenyon and Sensenbrenner, 1974; Morecki et al., 1968; Schulman et al., 1970; Spear, 1974; Witzleben et al., 1972; Wong et al., 1970). On four occasions liver tissue was included in these studies (Bruck et al., 1976; Feist et al., 1972; Gould et al., 1964; Jackson et al., 1962).

Dark cells were reported only in one renal specimen (Spear et al., 1971) and the features of these dark cells were nearly identical to those we observed. Extracellular dark material has not, however, been reported until now. The localization does not seem to be an artifact, occurring during fixation or inclusion, and indeed the presence of dark material was far from constant in the neighbourhood of dark cells. It was never found on the sinusoidal side of these cells. Spear et al. (1971) have shown that the dark substance is L-cystine and that the darkening is the result of osmium tetroxide action during fixation.

The real congenital defect in cystinosis is not known. It is certain that the stored cystine does not come from the blood, and that the tissue excess of this amino acid does not originate from the metabolism of methionine or cystathionine. It is more likely that it is derived either from a faster turn-over of glutathione or from lysosomal protein degradation (Crawhall et al., 1977).

Experimental studies have concluded that cystine is primarily enclosed within membrane-limited subcellular organelles (Schneider et al., 1967; Seegmiller et al., 1968). Several morphological and biochemical arguments for the lysosomal nature of these organelles have been advanced (Patrick and Lake, 1968; Schulman and Bradley, 1970). Continued accumulation of cystine could bring about crystallization, and large crystals could then rupture lysosomal membranes, with cell death following this charge (Schulman, 1973).

The presence of dark cells might lead us to consider that the primary site of cystine over-production could be the hyaloplasm. Initially cystine excess might be efficiently captured by lysosomes and the cell cytoplasm remain clear. Later, lysosomal capacity would be overwhelmed and cystine would accumulate, occasionally crystallizing in other parts of the cell. Osmium fixation of these cells would then account for their darkening.

The only membranes which seem to be impervious to the diffusion of cystine are the internal mitochondrial membrane and some membranous portions of the endoplasmic reticulum. The plasma membrane appears permeable and extracellular cystine can flow into parenchymal intercellular spaces. By crossing tight junctions it can reach lumena. In the kidney, such a process might play a role in the pathogenesis of the tubular impairment seen in the Fanconi syndrome. It has been shown experimentally that hypertonic solutions of lysine can weaken the tran-epithelial resistance of the frog urinary bladder (Martinez-Palomo and Erlig, 1975). By analogy we could postulate that a focal increase of cystine concentration in extra-cellular spaces may act on renal epithelia in the same way. The resulting increase in epithelial permeability may either increase glomerular filtration or impede tubular reabsorption, or both.

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