EARLY POST-TOXIC DEFECTS REVEALED ELECTRON-MICROSCOPICALLY IN HEPATOCYTE MEMBRANES BY MEANS OF A LANTHANUM TRANSMEMBRANE TRACER

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An important role in the genesis of reversible and irreversible ultrastructural disturbances of different types of cells during the development of pathological changes in them is attributed at the present time to pore formation in membranes under the influence of lipid peroxidation and accumulation of calcium ions by the cells [3]. Since the pathogenesis of the liver damage induced by carbon tetrachloride (CCl_4) is based on both these phenomena, which can be recorded biochemically [5, 10], the aim of this investigation was to discover early injuries in hepatocyte membranes electron-microscopically, with the aid of colloidal lanthanum (particle diameter 2 nm), which can penetrate into intravitally formed cell membrane defects [3, 9].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g. The rats were given an intraperitoneal injection of CCl_4 in a dose of 0.15 ml/100 g body weight. Samples of liver were taken after decapitation of the rats, 30 and 60 min after injection of CCl_4, and also from intact control rats. The solution of colloidal lanthanum was prepared from lanthanum nitrate by a modified method of Revel and Karnovsky [4] and was added to 3.5% glutaraldehyde and 1% osmium fixatives, made up in cacodylate buffer in accordance with this technique. After dehydration the specimens were embedded in Epon-812. Ultrathin sections were not stained, to facilitate identification of the tracer particles. The sections were contrasted only by the osmium in the second fixative, and also by choice of the aperture diaphragm on the JEM-7A electron microscope. The study of ultrathin sections was preceded by the study of semithin sections, stained with toluidine blue, so that ultramicrotomy could be targeted on the centrilobular hepatocytes and, in particular, those damaged by CCl_4 [8].

EXPERIMENTAL RESULTS

Colloidal lanthanum particles did not penetrate into the cytoplasm of hepatocytes of the control rats but were found in the intercellular spaces, in the form of an electron-dense residue, evidence of integrity of the plasmalemmas [9].

Concentrations of particles of the tracer could be seen 30 min after injection of CCl_4 into the rats in the cytoplasm of the centrilobular hepatocytes, lying close to the central veins (Fig. 1a), evidence of the presence of defects in the plasmalemmas.

Concentrations of colloidal lanthanum particles were discovered both on the outer membranes of the mitochondria and in the matrix of these organoids (Fig. 1a, b). Concentrations of calcium phosphate and a large quantity of tracer particles were observed in the matrix of some mitochondria (Fig. 1a), possibly indicating multiple defects in the membranes, and confirming the role of calcium in their formation [3]. The calcium ion concentration in the extracellular medium is known to be 1000 or more times greater than that in the cytosol, depending on the type of cell, and for that reason pore formation in the plasmalemmas quickly leads to intracellular accumulation of calcium, which plays a definite role in death of the cells, and injury to the mitochondria is the main factor involved in this case [11]. Besides concen-

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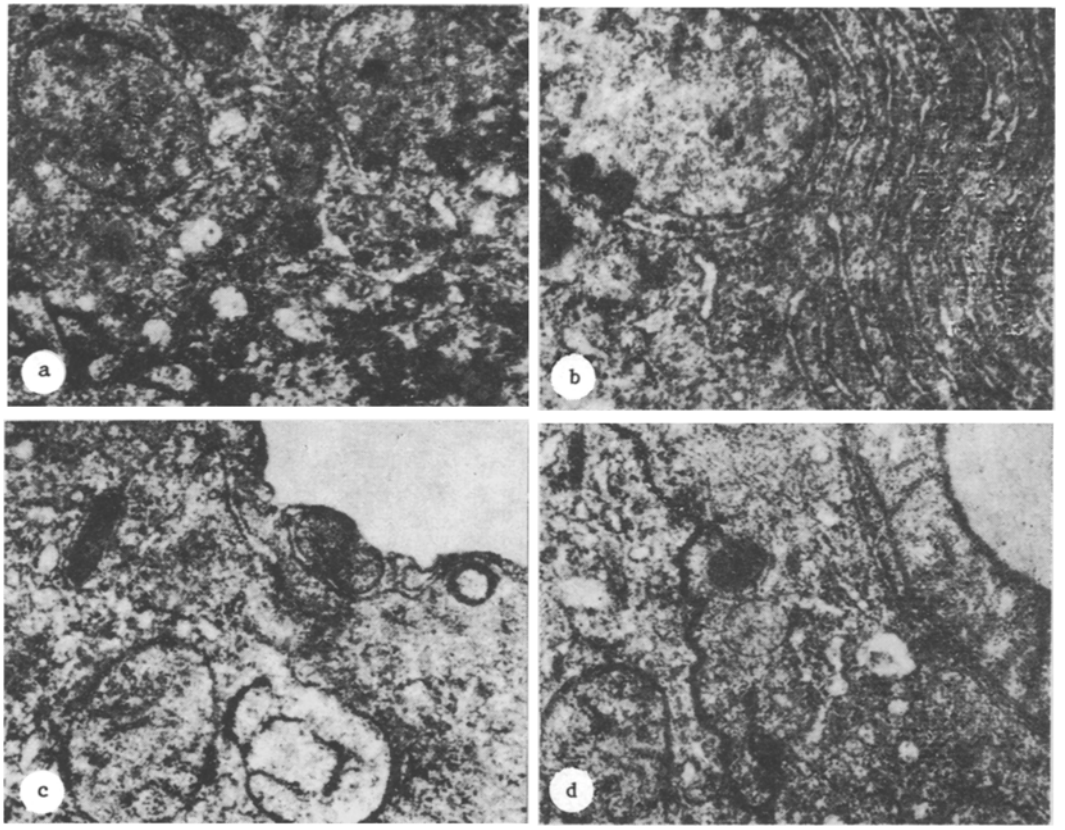

Fig. 1. Cytotoxic defects in hepatocyte membranes revealed electron-microscopically.

trations of tracer, in some mitochondria in hepatocytes small lipid droplets were found, associated with the membranes (Fig. 1b), and this could be linked with damage to them and their participation in the development of fatty degeneration of the hepatocytes, characteristic of the effect of CCl_4 on the liver [7]. Accumulation of calcium in the mitochondria, connected with disturbances of its elimination, has been shown to lead to uncoupling of oxidative phosphorylation, to a change in pH of the cell, and to release of fatty acids, which aggravate its uncoupling even more [6].

The considerable disturbance of the ultrastructure of the mitochondria, in which nearly all the cristae had disappeared and the matrix had become irregular in density, was noted, whereas the rough endoplasmic reticulum (RER) remained almost unchanged in its structural features (Fig. 1a, b), with the exception of slight widening of the channels and loss of isolated ribosomes by the membranes. In the channels of RER the tracer was observed only in hepatocytes near the central veins, and as the distance from the latter increased they were seen less and less frequently, indicating differences in the vulnerability of membranes of RER in hepatocytes in different zones of the hepatic lobules, or differences in ability of the cells to correct defects of the plasmalemma by the phenomenon known as "darning" [1]. The degree of disintegration of RER exhibited the same dependence. Moreover, in hepatocytes located nearer to the central zones of the lobules the channels of RER contained no tracer and they were grouped in the form of extensive fields (Fig. 1b). This reflects hyperplasia of the RER, evidently arising as an adaptive subcellular manifestation of compensation for insufficiency of the protein-synthesizing function of the injured centrilobular hepatocytes.

The tracer was detected much less frequently in mitochondria of the centrilobular hepatocytes 60 min after injection of CCl_4 into the rats, and disturbances of their ultrastructure were less marked (Fig. 1c, d); this may reflect replacement of the mitochondrial population or restoration of the structure of these organoids. Nevertheless, signs of fatty acid release were still present, for large lipid drops were frequently seen in close contact with the mitochondria (Fig. 1d), as was also observed during repeated exposure of the hepatocytes to CCl_4, accompanied by restoration of their ultrastructure [2].

The tracer was observed to accumulate in the channels of RER, which were widely dilated, fragmented, and deprived of ribosomes (Fig. 1c). These phenomena of RER disintegration in the hepatocytes spread to the central zones of the lobules, and hepatocytes with RER fields could no longer be observed; this rules out any possibility of compensation of protein synthesis by some hepatocytes 60 min after injection of CCl_4 into the rats.

As a rule the tracer was found in lysosomes of the hepatocytes (Fig. 1 c, d), evidently in connection with intravital injury to their membranes. Pore formation in the lysosomal membranes opens the door for their enzymes to emerge into the cytosol of the hepatocytes, and in the later stages after exposure to CCl_4 this evidently leads to irreversible changes and death of the centrilobular hepatocytes.

The formation of membrane defects thus precedes the development of ultrastructural changes in the hepatocytes characteristic of CCl_4 poisoning, and the latter process is largely the result of damage to the membranes.

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ANALYSIS OF BINDING AND UPTAKE OF NATIVE AND MODIFIED LOW-DENSITY LIPOPROTEINS BY HUMAN LIVER CELLS IN PRIMARY CULTURE

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The liver plays a key role in regulation of the blood level of circulating low-density lipoproteins (LDL). Not only are LDL precursors synthesized in the liver, but most lipoproteins of this class are broken down in it [6]. The role of the liver cells in LDL elimination is particularly important. Liver cells are known to bind and take up LDL through specific receptors located on their surface [3]. Investigations have shown that all liver cells, including parenchymatous (hepatocytes), have not only receptors for native LDL but also specific receptors for chemically changed or modified LDL [10, 14]. Interest in the latter is due to their possible role in the accumulation of intracellular lipids and the formation of foam cells [4]. It was reported comparatively recently [12] that rat hepatocytes have no receptors for modified LDL. This suggests that some degree of specialization of the cells exists in the

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