ULTRASTRUCTURAL CHANGES IN HEPATOCYTES DURING THE DEVELOPMENT OF EXPERIMENTAL CIRRHOSIS OF THE LIVER IN RATS

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During the development of toxic cirrhosis of the liver, necrosis and fibrosis are accompanied by intracellular reparative regeneration of the damaged cells and compensatory hypertrophy of the intact cells, aimed at maintaining the specific function of the liver. It is postulated that the outcome of cirrhosis is determined by whichever is the predominant process – death, regeneration, or hypertorphy of the cells, and its progression is connected with overstrain and increasing exhaustion of the intracellular compensatory-adaptive mechanisms.

KEY WORDS: rat liver; cirrhosis; ultrastructure; parenchyma.

One of the principal problems in the investigation of the pathogenesis of sclerotic changes in the liver is to establish the causes of and principles governing the conversion from acute and chronic hepatitis to cirrhosis.

The object of this investigation was to study the ultrastructure of hepatocytes at different stages of cirrhosis.

EXPERIMENTAL METHOD

Cirrhosis of the liver was produced in male Wistar rats weighing 130-180 g by inhalation of CCl_4 (for 4 h, twice a week) [10]. Material for investigation* was taken 1.5, 3, and 6 weeks from the beginning of the first inhalation and two days after the last. The tissue was fixed in buffered solutions of 3% glutaraldehyde, and then in 1% OsO_4, dehydrated in alcohols, and embedded in Araldite. Sections were cut on the LKB-8800 ultratome, stained with lead salts, and studied in the IEM-7A electron microscope.

EXPERIMENTAL RESULTS

Examination of the liver of the rats after three inhalations of CCl_4 under the light microscope showed changes of subacute hepatitis. Electron-microscopic examination showed moderate fragmentation of the tubules of the granular endoplasmic reticulum in the hepatocytes, a decrease in the number of ribosomes on the membranes and free ribosomes, and a reduction in the density of the material of the cytoplasm.

In the zones of the Golgi complex (GG) of many of the hepatocytes, collections of tubules of the agranular endoplasmic reticulum, which always communicated with its dictyosomes, were observed (Fig. 1A). In the writers' opinion this must be regarded as an expression of the compensatory plastic function of GC (acting as a membrane "depot" [9] or "conveyor" [11]), aimed at CCl_4 detoxication. It has been shown, for example, that the increase in volume of the agranular endoplasmic reticulum in the hepatocytes of rats under the influence of phenobarbital is accompanied by activation of the microsomal enzymes responsible for destroying it [13]. An increase in the length of the GC dictyosomes, the appearance of multivesicular bodies

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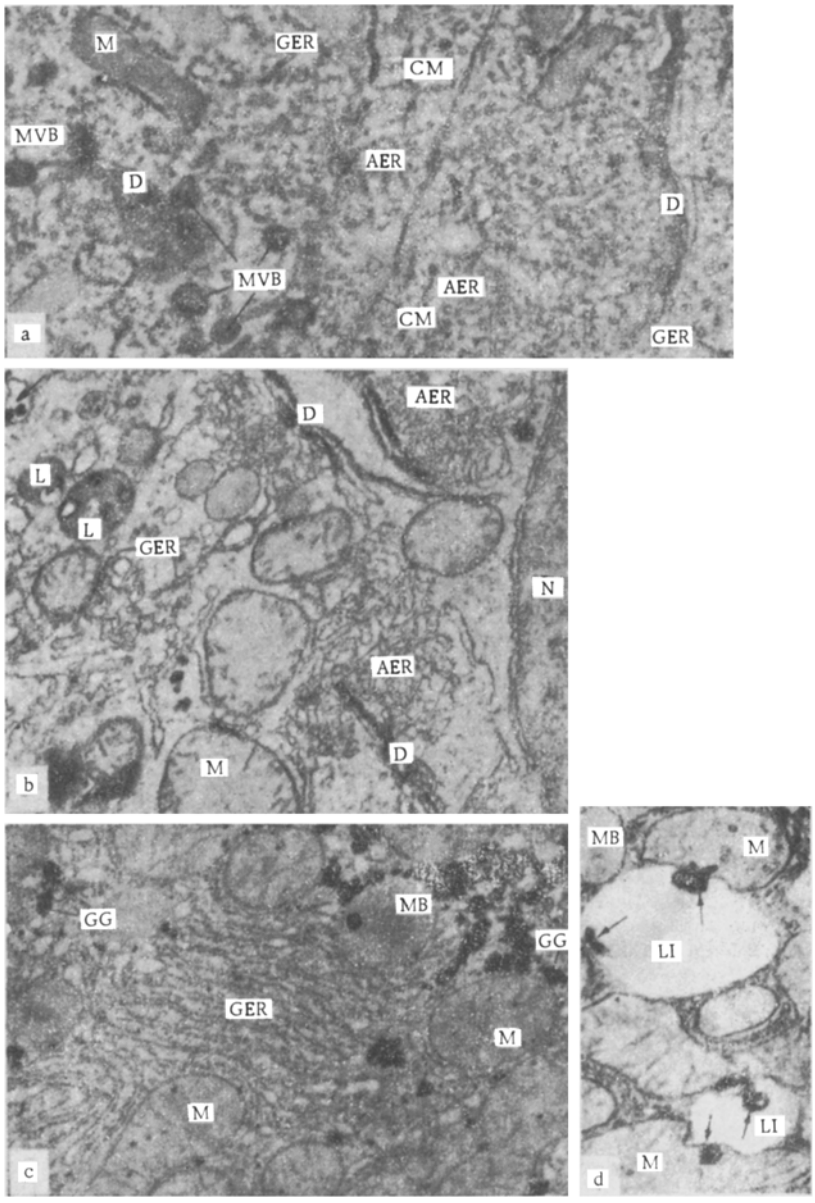

Fig. 1. Changes in ultrastructure of hepatocytes in toxic hepatitis: a) structural features of the Golgi complex in subacute toxic hepatitis (30,000 ×); b) part of a necrotically changed hepatocyte (20,000 ×); c) part of a hepatocyte with features of regeneration (30,000 ×); d) localization of myelin-like formation (30,000 ×). M) Mitochondrion; GER) granular endoplasmic reticulum; AER) agranular endoplasmic reticulum; N) nucleus; D) dictyosomes of Golgi complex; MVB) multivesicular body; CM) cell membrane; LI) lipid inclusion; L) lysosomes; GG) glycogen granules; MB) microbodies. Arrows: in a) widenings of intermembranous spaces of mitochondria, in b) long arrows indicate lipid and myelin-like inclusions in widened spaces of GER and perinuclear space, short arrows indicate dilated nuclear pores, in c and d) myelin-like inclusions.

budding from the dictyosomes (Fig. 1A), and a clear decrease in the number of lysosomes in the cells were observed. The view is held that the multivesicular bodies are prelysosomes [12]. The new generation of lysosomes was evidently formed through the "wearing out" of the preexisting lysosomes in the course of triple inhalation of CCl_4, and this must be regarded as a manifestation of ultra structural renewal of the hepatocytes.

The reduction in the glycogen content of the hepatocytes or, more frequently, its total disappearance (Fig. 1a), was probably associated with this phenomenon.

The observed behavior of the hepatocytes evidently reflects subcellular reconstructions with an adaptive or compensatory role during exposure to CCl_4.

A decrease in size and density of the mitochondria, widening of the intermembraneous spaces, and close contact between some mitochondria and lipid inclusions were found. These structural changes point to a change in the function of the mitochondria. Appearances of this type are known to be characteristic of the condensed configuration of the mitochondria [7], associated with its low-energy metabolic state both in vitro and, evidently, in situ also [3]. The presence of close contact between mitochondria and lipid droplets is evidently a reflection of liberation of fatty acids. The detection of osmiophilic myelin-like formations at the sites of contact could indicate changes in the phospholipid metabolism of the mitochondria, for such formations are known to consist of phospholipid–cholesterol complexes [14].

After six inhalations of CCl_4, evidence of chronic hepatitis with signs of fatty and acidophilic degeneration and with necrotic and necrobiotic changes was observed in the liver under the light microscope. In many hepatocytes the number of ribosomes, polysomes, and granular membranes was reduced, and fragmentation and vacuolation of the tubules of the granular endoplasmic reticulum, associated with a disturbance of protein synthesis and with structural changes in the membranes [8], were observed. This association is confirmed by biochemical data [15] obtained during a study of the liver microsomes from the rats used in the present experiments.

A well-developed agranular endoplasmic reticulum was observed in some of the hepatocytes. The dictyosomes of the GC, around which collections of agranular endoplasmic reticulum were observed, were converted into bundles of osmiophilic undulating membranes. The membranes of the dictyosomes in the necrotically changed hepatocytes had indistinct outlines (Fig. 1b), indicating their degradation. The cause of necrosis of the hepatocytes could have been a progressive disturbance of the compensatory function of GC as regards providing for the formation of a new agranular endoplasmic reticulum, responsible for destroying the poison. The disturbance of this function, in turn, could depend on injury to the phospholipid component of the hepatocyte membranes discovered biochemically [5].

Marked lipid infiltration of the cytoplasm is highly pathognomonic for many hepatocytes. Fatty infiltration is an important pathogenetic stage in the development of cirrhosis, and is essentially precirrhosis [10]. According to Leites [2], a decrease in phospholipid synthesis is also characteristic of hepatocytes damaged by CCl_4. This evidently has a discoordinating effect on the function of the hepatocyte organoids, thereby enabling fatty degeneration of the organ to develop and the cirrhosis to progress.

The mitochondria of hepatocytes with an agranular endoplasmic reticulum were increased in size and the matrix and granules were reduced in density; these changes could be connected with the active provision of energy for the detoxication of the CCl_4 and they can be regarded as a reflection of an adaptive reaction. This view is supported by an increase in the cytochrome content in the mitochondrial fractions of the experimental rats [5].

Changes in the nuclear ultrastructure of hepatocytes with a high content of agranular endoplasmic reticulum were noteworthy. The quantity of heterochromatin was appreciably reduced; the nuclear membranes often formed projections and invaginations with the result that the area of their contact with the cytoplasm was increased. An increase of the number of dilated nuclear pores was observed. These features are usually associated with an increase in the intensity of nucleo-cytoplasmic relations. They could have been due to intensification of the discharge of nuclear material into the cytoplasm in order to provide for the phenomena of subcellular compensation during repeated exposure to CCl_4.

After 12 inhalations of CCl_4 features of posttoxic cirrhosis were observed in the rats' liver. Besides marked degenerative changes and evidence of necrosis and fibrosis, foci of nodular regeneration of liver tissue were found. Necrotically changed hepatocytes and large aggregations of fibrils in the debris of the destroyed cells and the Desse's spaces were seen in the electron microscope. The number of hepatocytes with hyperplasia of the agranular endoplasmic reticulum was appreciably reduced. In some cells the degree of change in the granular endoplasmic reticulum was reduced and the number of enlarged mitochondria increased (Fig. 1c). These changes were evidently an indication of the development of intracellular reparative regeneration in the cells [4]. This conclusion is confirmed by the appearance of glycogen granules in the cells together with an clear decrease in the number of lipid inclusions. A biochemical study of the microsomal fractions of the experimental rats revealed a decrease in the degree of unsaturation of the fatty acids of the membrane phospholipids [5], which was interpreted as a manifestation of molecular adaptation of the membranes to the action of CCl_4. Intracellular regeneration was evidently possible under these experimental conditions only in hepatocytes that had become adapted to the chronic action of the poison.

In some mitochondria on the hepatocytes myelin-like, strongly osmiophilic inclusions, visible in the lipid drops adjacent to the mitochondria, were found (Fig. 1d). They were also found in the tubules of the granular endoplasmic reticulum and also outside the cells, possibly reflecting the path of elimination of the inclusions from the hepatocytes. The formation of inclusions evidently depends on increasing disturbance of the phospholipid metabolism of the mitochondria, depressing their function. This could possibly explain the compensatory increase in the number of unchanged mitochondria. The process of formation and liberation of inclusions from mitochondria is evidently of adaptive importance at the organoid level. The possibility cannot be ruled out that the further disturbance of metabolism of these mitochondria leads to exhaustion of their compensatory powers and is the cause of development of fatty degeneration in a certain number of hepatocytes. A role of this sort has been ascribed to the mitochondria by other workers during the development of chronic hepatitis and cirrhosis in man [1, 6]. Changes in the fatty-acid composition of the phospholipids also were found characteristically in the mitochondrial membranes of the rats in this series of experiments [5].

Hepatocytes of the foci of nodular regeneration are characterized by their large size, condensation of their cytoplasm, and an increase in the number of mitochondria and the amount of unchanged granular endoplasmic reticulum and ribosomes. The mitochondria differ from intact in their larger size and the lower density of their matrix, i.e., they have the orthodox configuration reflecting the high productivity of energy liberation [3]. Large accumulations of glycogen were found in the hepatocytes, with osmiophilic membranes of uncertain nature often visible inside them. The cell nuclei were usually enlarged and irregular in shape and their nucleoli also were enlarged. Autophagosomes were seen quite frequently, pointing to a high level of the metabolic reactions in the cells. These features are evidence of activation of hyperplastic processes in the cells aimed at compensating for the functions of the injured and irreversibly changed hepatocytes.

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