MITOCHONDRIAL ULTRASTRUCTURE AND SUCCINATE DEHYDROGENASE ACTIVITY IN HEPATOCYTES DURING CHOLESTASIS

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Function tests have revealed a disturbance of oxidative phosphorylation in hepatocyte mitochondria in the early period of cholestasis [2, 8]. Morphological studies have shown that cholestasis, if it lasts 10-12 days, can lead to fibrosis, and if it lasts 18-20 days, can lead to secondary biliary cirrhosis of the liver [3]. However, the structural and functional bases of metabolic disturbances in the hepatocytes themselves in cholestasis are still unexplained.

The aim of this investigation was to study the ultrastructure of hepatocyte mitochondria and to undertake a parallel cytochemical study of the activity of a key enzyme of tissue respiration, namely succinate dehydrogenase (SDH), in them, so that it will be possible to judge the state and degree of disturbance of energy formation in hepatocytes of human patients and experimental animals in the course of cholestasis.

EXPERIMENTAL METHOD

Liver biopsy material was investigated from 40 patients with obstructive iaundice of varied duration: under 10 days — 8 patients, 10-20 days — 17 patients, and over 20 days — 15 patients.

The experimental investigations were conducted on 20 albino rats (5 served as controls). Obstructive jaundice was induced by ligation of the common bile duct with division between two ligatures. The animals were killed after 10, 20, and 30 days of cholestasis by decapitation.

The clinical and experimental material was subjected to electron-microscopic investigation by the usual method.

For electron-cytochemical detection of SDH activity by the ferrocyanide method pieces of liver tissue, immediately after excision, were washed in 0.25 M sucrose solution with 3 mM magnesium acetate. Frozen sections 30-40 μm thick were cut from them and placed in freshly prepared incubation medium for the cytochemical reaction by the method of Kerpel–Fronius and Hajos [1]. Ultrathin sections, both of the ordinary kind and after the cytochemical reaction, were stained with uranyl acetate and lead citrate and examined in the IEM-100B and 100S electron microscope.

EXPERIMENTAL RESULTS

In patients with obstructive jaundtce lasting under 10 days most hepatocyte mitochondria had an intact ultrastructure (Fig. 1a). A positive reaction for SDH was found on the inner and outer membranes and cristae of most mitochondria. Only a few mitochondria did not contain the reaction product.

Hepatocytes of patients with jaundice of 20 days contained not only round and oval mitochondria, but also mitochondria of angular shape with a translucent matrix and with destruction of the cristae. The reaction for SDH in mitochondria of this group of patients was weakly positive, and in some mitochondria it was absent altogether (Fig. 1b).

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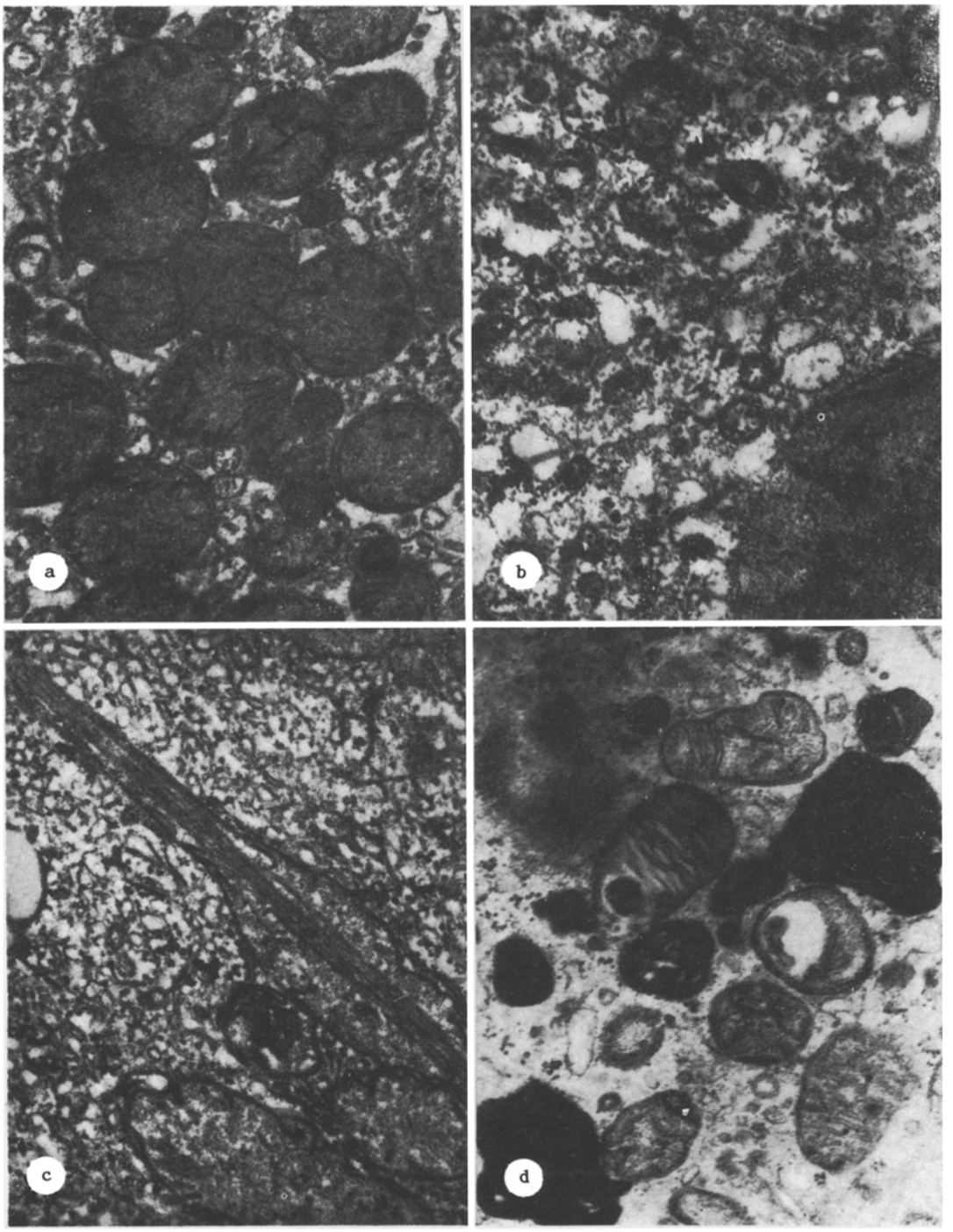

Fig. 1. Changes in ultrastructure and succinate dehydrogenase activity of hepatocyte mitochondria on 10th (a) and 20th (b) days and after more than 20 days (c, d) of cholestasis: a) translucency of matrix and partial reduction of cristae of hepatocyte mitochondria. 10,000×; b) reduction and absence of SDH activity in mitochondria of liver cells. 5600×; c) recurrence of disease, intramitochondrial inclusions in longitudinal and transverse sections. 18,000×; d) different degree of destruction of mitochondria of hepatocytes containing clots of bile in their cytoplasm. 12,000.

In patients with obstructive jaundice for more than 20 days marked polymorphism of the mitochondria was observed. In some patients, especially those with frequent recurrence of the disease, large, irregularly shaped mitochondria containing crystalloid inclusions in their matrix were found. These inclusions lay freely in the matrix of the mitochondria and under high power, the packets of parallel filaments forming them were clearly visible (Fig. 1c).

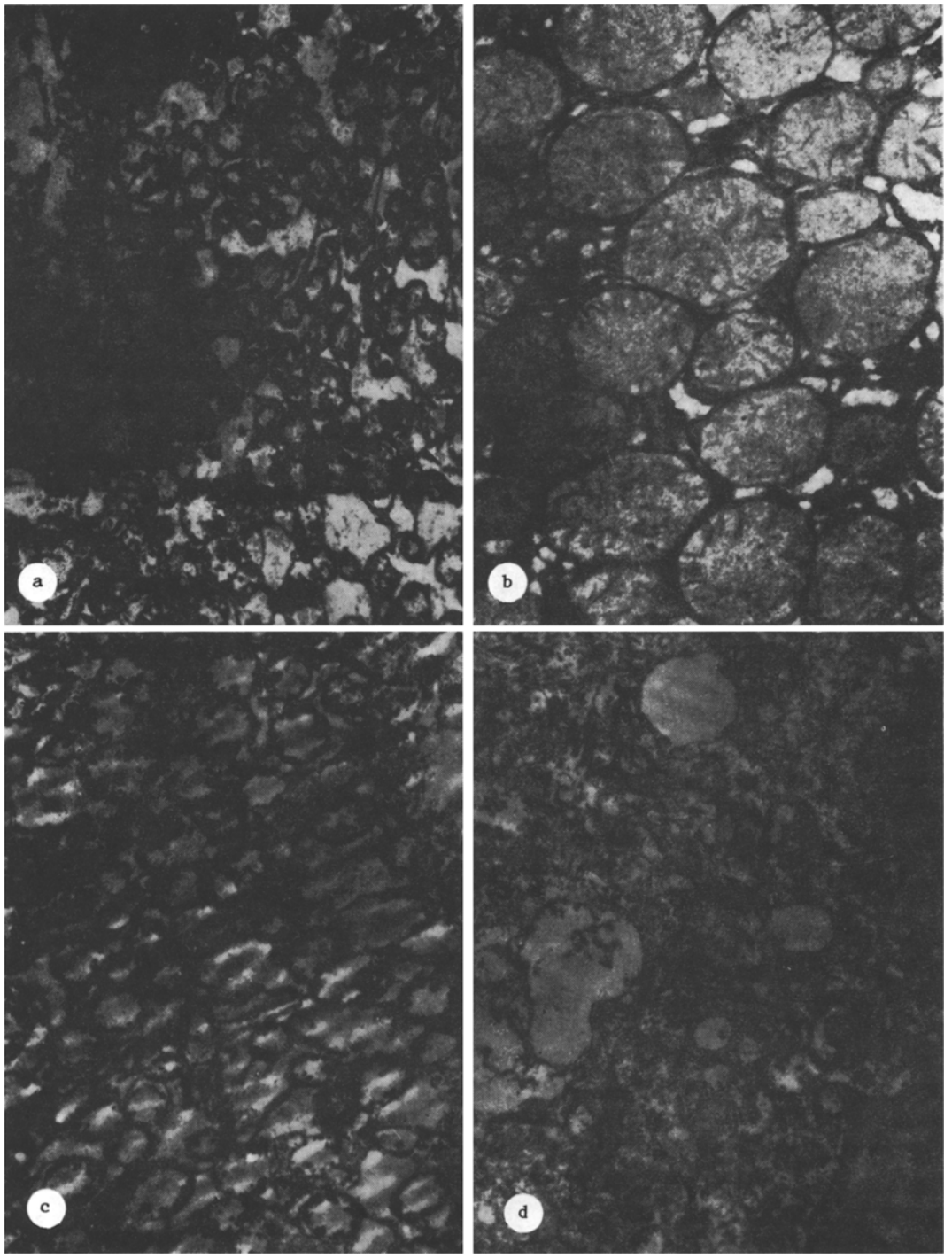

Fig. 2. Changes in ultrastructural and succinate dehydrogenase activity of mitochondria of rat hepatocyte in control (a) and on 10th (b, c) and 20th days of cholestasis (d): a) intensive reaction for SDH in hepatocyte mitochondria. 4200×; b) mitochondria greatly swollen, cristae disorganized or reduced. 12,000×; c) very small decrease in SDH activity in most mitochondria of hepatocyte. 5600×; d) SDH activity detectable only in single mitochondria of hepatocytes. 4200×.

In areas of cholestasis many hepatocytes contain clots of bile in their cytoplasm. Under these circumstances the mitochondria of the cells frequently underwent destruction in the form of a marked decrease in the density of the matrix, fragmentation and disappearance of the cristae, rupture of the membranes, and the formation of myeloid structures. Often electron-dense particles could be seen in the matrix of the mitochondria (Fig. 1d).

On electron-cytochemical investigation of material from this group of patients, the reaction product for SDH was visible only in individual mitochondria. In some cases a strong reaction was found both on the cristae and on the inner membrane of the mitochondria.

Experimental cholestasis in the rats as a whole was characterized by changes in the hepatocyte mitochondria similar in type to those described above. After 10 days of cholestasis the submicroscopic organization of the rat liver mitochondria corresponded to that typical of patients with jaundice for 10 days. Most mitochondria under these circumstances preserved their intact structure (Fig. 2b). Their reaction for SDH was somewhat weaker, although the reaction product could be found in nearly all mitochondria (Fig. 2c).

In the control rats intensive deposition of the product for SDH was found in all mitochondria, in which it was localized mainly in the inner membrane and cristae (Fig. 2a).

During progression of the cholestasis (until 20 days after the operation) a picture of biliary cirrhosis became apparent in the liver. During this period the hepatocyte mitochondria showed marked polymorphism, as in the patients with cholestasis for a similar period. The mitochondrial matrix was composed of finely granular material and the cristae were reduced. Nevertheless, intramitochondrial inclusions could not be detected. In this period SDH activity was significantly reduced: in the overwhelming majority of hepatocyte mitochondria the reaction for SDH was absent: only in individual mitochondria was a positive reaction for SDH observed (Fig. 2d). On the 30th day of cholestasis, when progressive cirrhosis of the liver was present, SDH activity was virtually absent in the majority of mitochondria. Only isolated mitochondria in the hepatocytes contained a very small quantity of reaction products.

SDH is known to be a mitochondrial "marker" enzyme, whose activity is directly proportional to deposition of the electron-dense product on the membrane [6]. We found that with an increase in the duration of jaundice, both in patients and in experimental animals, SDH activity decreased in the hepatocyte mitochondria. Whereas in the patients SDH activity declined slowly, in animals a very slight decrease on the 10th day of cholestasis was followed by profound depression of SDH activity on the 20th day of the experiment, and by the 30th day SDH activity was virtually completely suppressed. Consequently, depression of SDH activity correlates with the formation of fibrosis, and of biliary cirrhosis of the liver. Some workers associate the disturbance of function of the mitochondria and depression of enzyme activity in them in obstructive jaundice with the effect of bile acids and their salts on mitochondrial structure [2, 4, 5, 8, 9]. Bile acids and their salts not only affect the structure and function of mitochondria, but they can also release some enzymes from hepatocyte membranes without disturbing the integrity of the cells [7, 10, 11].

These investigations confirm the harmful effect of components of the bile on mitochondria, as is shown by the signs of destruction and depression of SDH activity in most mitochondria. However, even in prolonged experimental cholestasis the accumulation of bile condensates in the cytoplasm of the hepatocytes is not observed, yet SDH activity in this situation is depressed. This state of affairs indicates that not only bile acids and their salts are involved in the depression of SDH activity, but also fibrotic and serotic processes developing in the liver itself and leading to a disturbance of the exchange of materials between the blood and hepatocytes.

In the early periods of cholestasis changes in hepatocyte mitochondria are thus stereotyped in character. Prolonged cholestasis, leading to accumulation of components of the bile, causes destructive changes in the mitochondria, accompanied by weakening of SDH activity in them, or even its complete suppression. These changes in the hepatocyte mitochondria evidently lie at the basis of the disturbances of liver function and of the development of hepatic failure associated with prolonged cholestasis.

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