DISTRIBUTION OF ACID PHOSPHATASE IN THE LIVER IN CIRRHOSIS

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Lysosomal activity of hepatocytes and connective-tissue cells of the liver in cirrhosis and also during its involution has been studied largely biochemically [5-8, 11-14]. There have been only a few histochemical studies of liver function in this pathology [1-4, 15]. Lysosomal activity in the liver during cirrhosis has not been studied at all at the ultrastructural level.

Accordingly, to obtain a more complete idea of the functional activity of the different types of cells, and also to discover the connection between their lysosomal activity and the process of collagen resorption, it was decided to undertake a histochemical and electron-histochemical investigation of acid phosphatase (AP) activity in the liver during cirrhosis and during its regression.

EXPERIMENTAL METHOD

Cirrhosis of the liver was induced by subcutaneous injection of 0.2 ml of a 40% solution of CCl_4 in olive oil into noninbred male albino mice once a week for 5 months. To stimulate regeneration, 1 week after the last injection of CCl_4 the left lobe of the liver was resected in all the animals. Material for investigation was taken during resection and again 10 days thereafter, and was analyzed for the presence of AP by a histochemical method of simultanious combination with naphthol AS-BS phosphate [9] and electron-histochemically [10]. Incubation with the addition of 0.01M sodium fluoride, an inhibitor of AP, and incubation in medium without the substrate for AP (sodium β-glycerophosphate), were used as controls of the reaction. Ultrathin sections were examined in the EVM-100L electron microscope.

EXPERIMENTAL RESULTS

The study of histochemical preparations of the liver in cirrhosis revealed a marked difference in AP activity between the hepatocytes and connective-tissue cells of the fibrous band. Whereas the latter were intensely stained and the reaction product in them appeared in the form of concentration of granular character, the cytoplasm of the hepatocytes was almost unstained, and only a few granules could be seen in the cells (Fig. 1a).

The picture changed sharply 10 days after resection. AP activity in the hepatocytes was considerably increased. An increase in enzyme activity also was observed in the connective-tissue cells of the fibrous bands. The cytoplasm of the hepatocytes stained diffusely and very intensely, evidence of the high lysosomal activity in them. Reaction product was distributed in cells of the fibrous bands in the same way as in the resected material, but the clumps of precipitate were larger and more deeply stained (Fig. 1b).

Electron-histochemical investigation of AP in the liver during cirrhosis revealed reaction product in some hepatocytes in both primary and secondary lysosomes. These structures containing reaction product were located in different parts of the cytoplasm of the hepatocytes. Primary lysosomes were found quite frequently in the perinuclear zone. Just as in the hepatocytes, reaction product was found in by no means every connective-tissue cell located among well-developed bundles of collagen. AP activity in these cells was observed in primary lysosomes (more often in fibroblasts and in Itoh's cells), and also in various kinds of vacuoles and in phagosomes (more often in macrophages).

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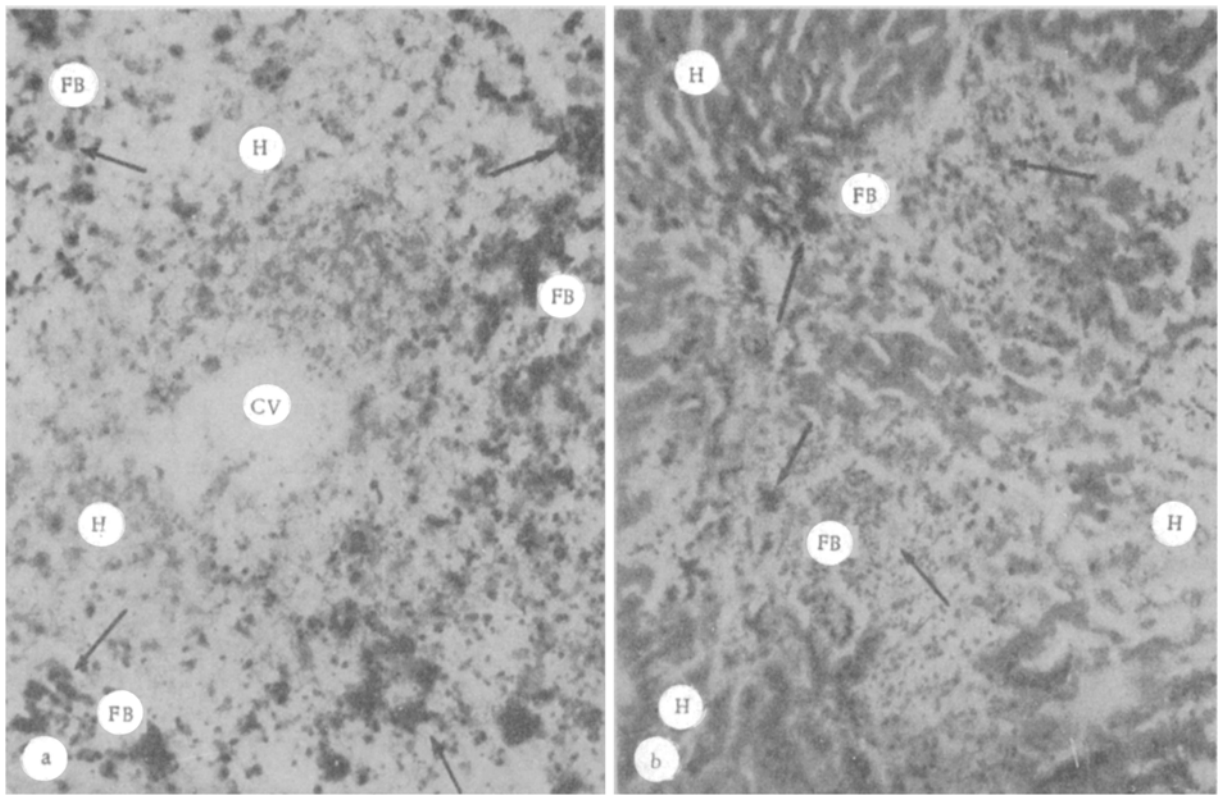

Fig. 1. Distribution of AP in hepatocytes (Burstone's reaction for AP): a) hepatocytes (H) arranged around central vein (CV) contain hardly any reaction products for AP. Connective-tissue cells of periportal fibrous bands (FB) contain large quantity of reaction product in the form of granules and clumps (arrows). 200 ×; b) Intensive reaction for AP in hepatocytes (H). Diffuse distribution of reaction product in them. Reaction product in the form of granules and clumps (arrows) can been seen in connective-tissue cells of fibrous bands (FB) 10 days after resection. 160 ×.

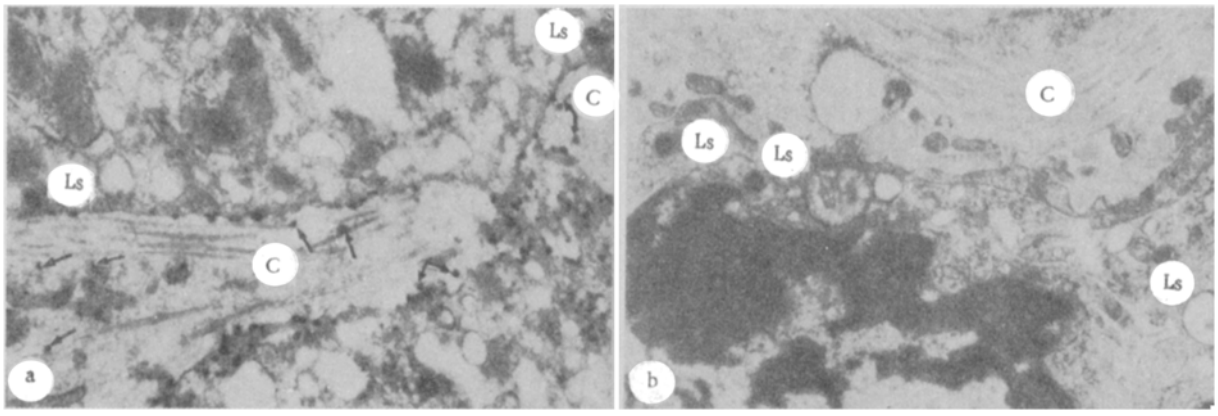

Fig. 2. Electron-histochemical detection of AP (10 days after resection): a) reaction product for AP in lysosomes (Ls) of hepatocytes, in villi, on cytolemma (intra- and extracellularly), and also on collagen (C) fibers (arrows). 16,000 ×; b) Control for reaction for AP with addition of enzyme inhibitor (sodium fluoride) to incubation medium. Reaction product not found in lysosomes (Ls) or in collagen (C). 7000 ×.

Sometimes the reaction for AP in cirrhosis was observed also in lysosomes and vacuoles which had escaped into the extracellular space from fragmented cells. Connective-tissue cells in which AP activity was observed were found 10 days after resection in far greater numbers than in the resected material. The location of the reaction product was the same as in cirrhosis. As regards hepatocytes at this stage of involution of cirrhosis, a reaction for AP was observed in most of them, evidence of very high lysosomal activity. Unlike in resected material, the majority of primary lysosomes containing reaction product were located near the cell membrane. The main distinguishing feature of this period was accumulation of reaction product either in all vesicles or directly in the cytoplasm, and arranged at the periphery near the cell membrane of the hepatocytes, in villi, and also in the intercellular space directly on the cytolemma and on nearby collagen fibers (Fig. 2a). Reaction product for AP also was observed on collagen fibers and alongside connective-tissue cells.

No traces of reaction product for AP could be found in control preparations either in lysosomes or extracellularly (Fig. 2b).

To sum up the results, the first point to note is that they provide structural and functional confirmation of existing biochemical data indicating that enzyme activity in the liver rises sharply during regression of cirrhosis [7, 8] and that a close connection exists between the increase in activity of lysosomal enzymes of the liver cells and collagen resorption, i.e., that lysosomal enzymes take part in lysis of collagen in the liver [6, 9, 11-15].

The data showing that reaction product may be located extracellularly (on collagen fibers) are evidence that during involution of cirrhosis of the liver lysosomal enzymes are released from the hepatocytes and connective-tissue cells of the fibrous bands by exocytosis, and that they participate in the lysis of collagen.

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