ROLE OF LYSOSOMES IN COLLAGEN RESORPTION BY HEPATOCYTES DURING REGRESSION OF CIRRHOSIS OF THE LIVER

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Resorption of fibrous tissue is a key process in the reversibility of chronic sclerotic changes in organs [2]. Breakdown of collagen in the cirrhotic liver has been the subject of many investigations [4-11], but they have only indirectly reflected its possible mechanisms. We know that hepatocytes are concerned in collagen resorption. Fragments of collagen fibers, in different stages of destruction [1], are present in their cytoplasm. In this connection it is interesting to study intracellular mechanisms of collagen destruction by hepatocytes and, in particular, to determine which organelles are involved in this process. Participation of the lysosomal apparatus of hepatocytes in collagen resorption during regression of cirrhosis of the liver was studied in the investigation described below by methods of electron microscopy and electron histochemistry.

EXPERIMENTAL METHOD

Noninbred male albino mice were used. Cirrhosis of the liver was induced by subcutaneous injection of 0.2 ml of a 40% solution of CCl_4 in olive oil once a week for 5 months. To stimulate regeneration, the left lobe of the liver was resected in all animals 10 days after the last injection of CCl_4. Material for investigation was taken during resection and 15, 20, and 30 days later. Material for histological investigation was fixed in 10% formalin, and for electron microscopy, in glutaraldehyde and postfixed with OsO_4, then dehydrated and embedded in Epon. Acid phosphatase (AP) activity was studied histochemically in part of the material [3]. Ultrathin sections were examined in the ÉVM-100L electron microscope.

EXPERIMENTAL RESULTS

Annular foci of connective tissue proliferation with the formation of pseudolobules were observed in histological sections of pieces of tissue removed at resection. Electron-microscopic investigation revealed many lipid inclusions in the cytoplasm of most hepatocytes. Bundles of collagen fibers of varied thickness were located in Disse's spaces and between hepatocytes.

During regeneration of the organ the number of lipid inclusions in the hepatocytes and the number of fibroblasts surrounded by newly synthesized collagen decreased. The bands of collagen 1 month after resection consisted largely of fibers which were losing or had lost their cross-striation. A well-developed rough endoplasmic reticulum and lamellar complex, many lysosomes and peroxisomes, and much glycogen were observed in the hepatocytes.

At all stages of regression of cirrhosis of the liver studied vacuoles containing collagen in the form of bundles or separate fibers could be seen in the cytoplasm of some hepatocytes (Fig. 1). Most of these collagen fibers were in various stages of lysis, fragmented and without cross-striation.

Electron-histochemical investigation of AP revealed concentration of reaction product, evidence of enzyme activity, in the vacuoles containing collagen described above in hepato-

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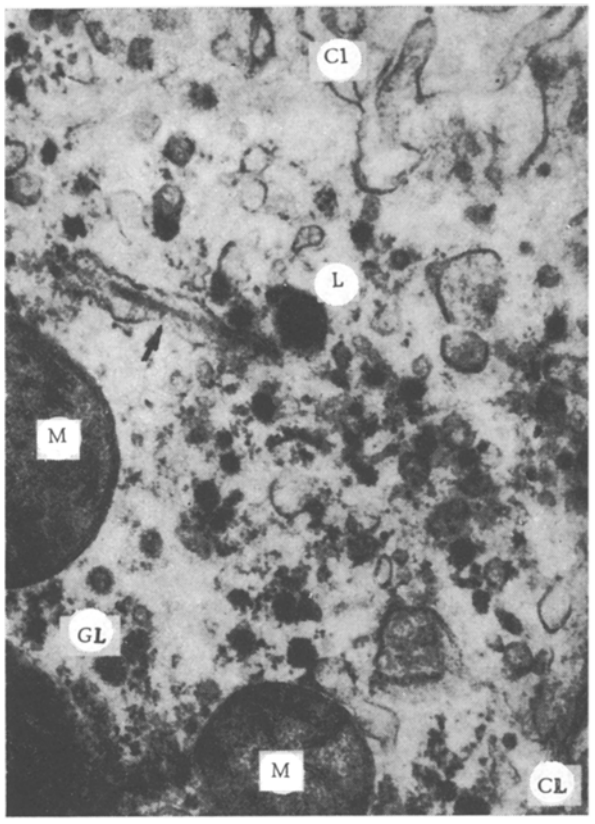

Fig. 1

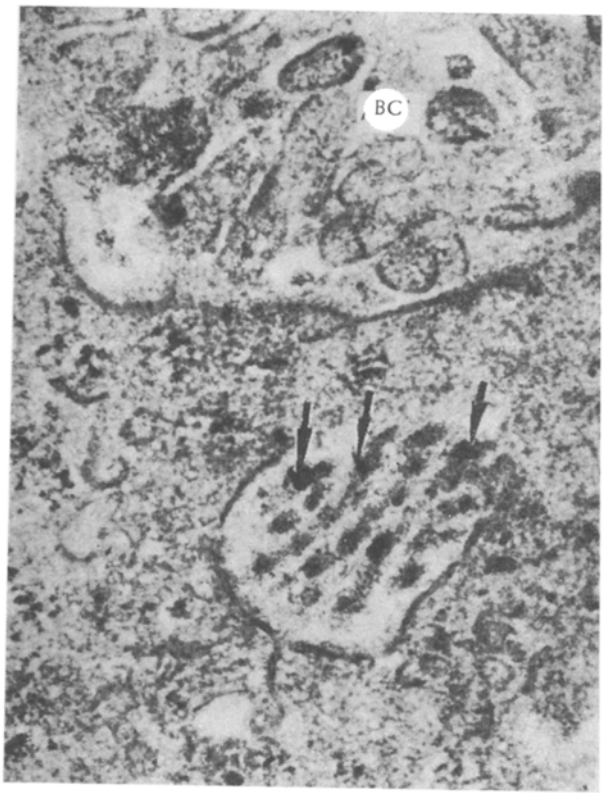

Fig. 2

Fig. 1. Area of cytoplasm of hepatocyte 15 days after resection. Arrow indicates vacuole containing collagen fiber with preserved characteristic cross-striation. Cl) Cytolemma; L) lysosome, M) mitochondrion, Gl) glycogen. 30,000 ×.

Fig. 2. Vacuole with bundle of obliquely transected collagen fibers in cytoplasm of hepatocyte 20 days after resection. Arrows indicate AP reaction product. BC) Bile capillary. 90,000 ×.

cytes. The reaction product was found both in vacuoles containing single collagen fibers and in vacuoles containing bundles of collagen fibers (Fig. 2); it was also found in lysosome-like bodies.

The presence of the lysosomal enzyme (AP) in collagen-containing vacuoles of hepatocytes is additional to the morphological data indicating intracellular destruction of collagen by hepatocytes. The presence of AP in the vacuoles is evidence that hepatocytes undertake lysis of collagen by a process resembling phagocytosis, i.e., by ingestion of fragments of collagen bundles and fibers, by their isolation in vacuoles, and by the release of hydrolytic lysosomal enzymes capable of degrading collagen into these vacuoles.

The presence of collagen fibers in vacuoles in hepatocytes during regression of cirrhosis of the liver thus indicates the possibility of intracellular resorption of collagen by hepatocytes through phagocytosis. Intracellular breakdown of collagen in hepatocytes takes place with the active participation of lysosomal enzymes.

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IMMUNOLOGIC CHANGES IN EPITHELIUM OF MOUSE THYMUS DURING ACCIDENTAL INVOLUTION

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The epithelial tissue of the thymus plays a determinant role in the formation and function of the immune system of the body. Its cells synthesize many hormones and biologically active substances [5, 9, 10, 11] and also secrete antigens of various tissues, including tissues of highly specialized organs, into the internal medium of the gland [4, 12]. By means of the ultrastructures of the cytoplasmic membranes of the epithelial cells of the thymus, lymphocytes are "familiarized" with the individual's histocompatibility antigens [13, 15]. It was shown previously that cells of the epithelial reticulum contain in their cytoplasm and processes antigens common with those of cells of the basal layer of stratified epithelium [1, 2, 7, 8]. With the aid of serum containing antibodies against basal-cell antigens, the epithelial reticulum of the thymus, unscreened by lymphocytes [3], can be electively demonstrated, so that the degree of its integrity and unmasking can be estimated. The complexity and the uninformativeness of the ordinary methods of assessing the action of immunodepressive preparations motivated an immunomorphologic study, which this paper describes, of the response of the epithelial tissue of the mouse thymus to prednisolone and azathioprine — drugs widely used in medical practice.

EXPERIMENTAL METHOD

Noninbred SHK albino mice and BALB/c mice weighing 17-20 g were used. Prednisolone, after suitable dilution, was injected intramuscularly into 28 mice in doses from 10 to 40 mg/kg over a period of 3-12 days. Azathioprine, in doses of 100 to 200 mg/kg, also was injected daily from one to nine times and with an interval of 3-7 days. When both drugs were given, a single dose of azathioprine of 100 to 250 mg/kg was given to 16 mice at the beginning of the course, and this was followed by injections of 10-40 mg/kg of prednisolone three times a day. The animals were killed by cervical dislocation 1-16 days after the last injection. The control consisted of 18 mice which received no treatment of any kind. Rabbit serum (B-1) containing high titers (1:128) of natural antibodies against basal-cell antigens, were used as the source of these antibodies. Pure antibodies against rabbit immunoglobulins, labeled with fluorescein isothiocyanate, or commercial luminescent serum against rabbit IgG, prepared by the Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, were used in the indirect immunofluorescence test. Pieces of thymus tissue were frozen to −85°C (with a mixture of dry ice and acetone). Sections 5 μ thick were cut in a cryostat (−20°C) and used unfixed. The sections were treated by the method described previously [6] and mounted in 60% neutral glycerin under a coverslip. They were examined under the LYUMAM-2 luminescence microscope. The intensity of the reaction was assessed in crosses (from 1 to 4). A very weak reaction was assessed as negative. Sections fixed in ethanol were stained with hematoxylin and eosin for use as the control.

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