EXTRACELLULAR COLLAGEN DEGRADATION THROUGH THE ACTION OF LYSOSOMAL ENZYMES DURING INVOLUTION 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 the liver, but the mechanisms of this process are not known. In all probability collagen degradation in the liver is initiated and effected by collagenase [8-10, 13]. It has been suggested that, besides by collagenase, the collagenolytic function in the liver may also be performed by lysosomal enzymes [1, 7, 15] and, in particular, by peptidases [2, 5, 12] and glycosidases [8, 14], which produce lysis of collagen at acid pH values. It has been shown that enzyme activity in the liver rises by 45% in cirrhosis, and by 145% during its involution [3, 4]. Close correlation has been found between collagen resorption in the liver and increased activity of its lysosomal enzymes [8, 11, 14].

However, these results were obtained in vitro and require verification by further experimental study in vivo. Accordingly, in the investigation described below, participation of lysosomal enzymes in extracellular collagen degradation in cirrhosis of the liver and during its involution in mice was studied by methods of electron microscopy and electron-microscopic histochemistry.

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 in noninbred male mice once a week for 5 months. The injections of CCl_4 were then stopped and animals with developed cirrhosis were divided into two groups. To stimulate regeneration, the left lobe of the liver was resected in the animals of group 1 7-10 days after the last injection of CCl_4. No operation was done on the animals of group 2. Material for study was taken during resection (group) 1) and 3, 5, 7, 10, 15, 21, 30, and 45 days after resection in parallel from animals of both groups. Material for histological investigation was fixed in 10% neutral formalin and sections were stained with hematoxylin and eosin and by Van Gieson's method. Material for electron microscopy was fixed in 2.5% glutaraldehyde, and subsequently postfixed in OsO_4, dehydrated, and embedded in Epon. Some material was treated histochemically for acid phosphatase (AP) [6]. As a control test the material was incubated in medium without substrate, and incubated with the addition of 0.01 M sodium fluoride to the medium. Ultrathin sections were examined in the EMV-100L electron microscope.

EXPERIMENTAL RESULTS

Marked annular cirrhosis with the formation of pseudolobules and considerable lymphoid-cell infiltration in the bands of fibrous tissue were observed in histological preparations from pieces of liver taken at resection. The fibrous bands were much thinner 15-30 days after resection and the infiltration gradually disappeared. On electron-microscopic investigation of the resected material, powerful bundles of mature collagen fibers could be found in the Disse's spaces and between the hepatocytes. Many fibroblasts, surrounded by newly formed collagen, and many macrophages, Itoh's cells, lymphocytes, polymorphonuclear leukocytes, eosinophils, and plasma cells appeared in the Disse's spaces. Hepatocytes contained many lipid inclusions in their cytoplasm.

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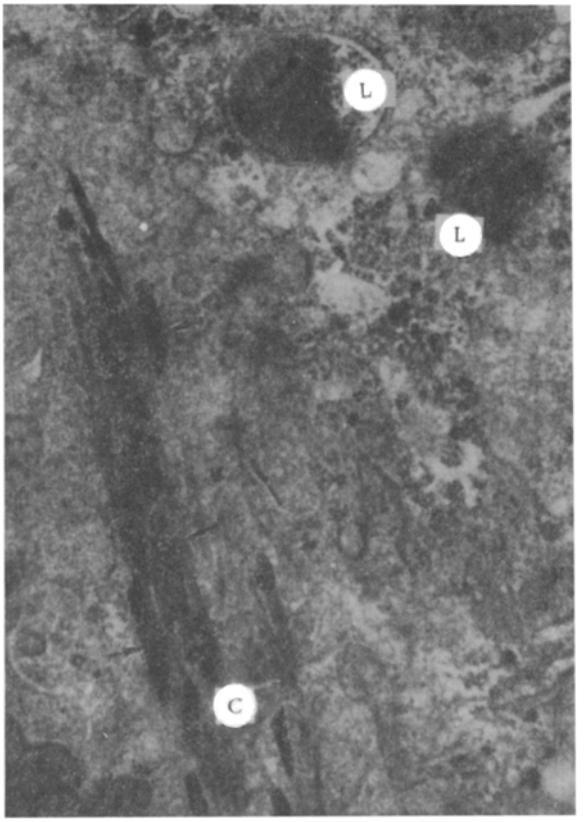

Fig. 1. Intensive reaction for AP in band of collagen located between hepatocytes (10 days after resection). Reaction product (arrows) directly on collagen fibers (C). L) Secondary lysosomes of a hepatocyte, also containing reaction product. 25,000×.

As regeneration of the liver tissue continued the ultrastructure of the fibrous bands changed. They became looser in texture, disintegrated, became pale (lost contrast), and the collagen fibers fragmented and lost their characteristic cross-striation. These changes indicate progressive degradation of the collagen fibers. Parallel with these changes, during involution of cirrhosis, besides absorption of collagen, the number of connective-tissue cells, which was considerable in the fibrous bands at the height of development of cirrhosis, gradually decreased also. In the course of involution of cirrhosis changes also developed in the hepatocytes. The number of lipid inclusions and peroxisomes in their cytoplasm decreased, glycogen appeared, marked hyperplasia of the rough endoplasmic reticulum was observed, the number of lysosomes increased, and numerous microvilli were formed on the sinusoidal surface of the cells. In animals not subjected to partial hepatectomy all these changes took place later and to a lesser degree than in the hepatectomized animals.

Electron-histochemical testing for AP in the liver in cirrhosis revealed activity of the enzyme in some hepatocytes and connective-tissue cells of the fibrous bands. The reaction product was located in both primary and secondary lysosomes; primary lysosomes in hepatocytes were found most frequently in the perinuclear zone. At subsequent times of evolution of cirrhosis most primary lysosomes containing reaction product in hepatocytes were located near the cell membrane.

During regeneration of the liver AP activity in its cells increased, as was reflected in an increase in the number of lysosomes in which reaction product was found, and also in the number of hepatocytes and connective-tissue cells containing lysosomes of this sort. This applies to a greater degree to the hepatocytes. Highest activity in both hepatocytes and connective-tissue cells was observed 5-10 days after resection. Later, during involution of cirrhosis, together with a progressive decrease in the number of connective-tissue cells in the liver, their lysosomal activity also declined. In the hepatocytes, however, it fell much more slowly, and still remained fairly high even 45 days after resection.

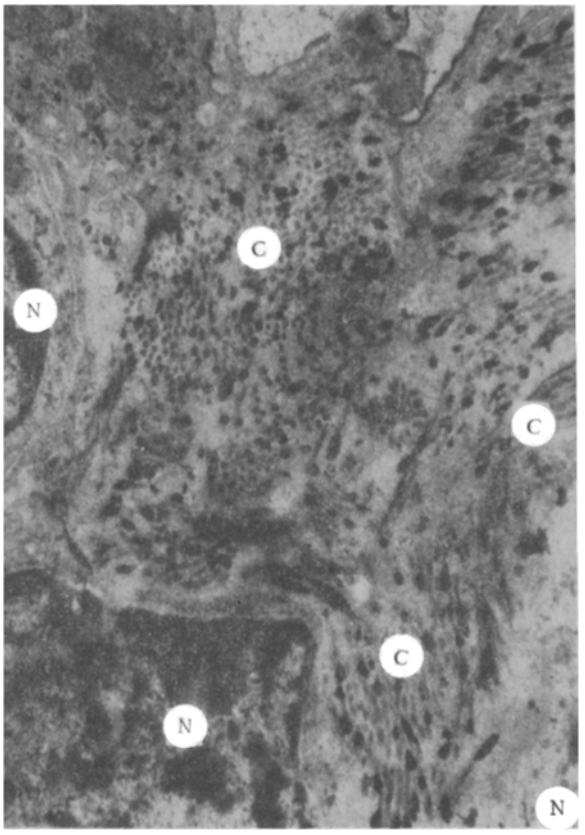

Fig. 2. Intensive reaction for AP in collagen bundle surrounded by connective-tissue cells (20 days after last injection of CCl_4, animals of group 2). Reaction product directly on collagen fibers (C). N) Connective-tissue cell nuclei. 13,000×.

The same tendency toward an increase, followed by a gradual decrease in lysosomal activity also were observed in animals not subjected to partial hepatectomy, but this process was delayed in these animals and its manifestations were not so clearly defined.

The main distinguishing feature discovered during electron-histochemical investigation of the different stages of involution of cirrhosis was liberation of lysosomal enzymes from hepatocytes and connective-tissue cells by means of exocytosis, as shown by concentrations of the AP reaction product at the periphery of the cytoplasm near the cell membrane, in the villi, and also in the intercellular space directly on the cytolemma and adjacent collagen fibers (Figs. 1 and 2).

The reaction for AP was absent in both lysosomes and fibrous bands in the control preparation (Fig. 3).

An extremely intensive reaction for AP was observed 10 days after resection in collagen bundles located in Disse's spaces between connective-tissue cells, and also in bands located between hepatocytes (Figs. 1 and 2). A similar picture was observed at different times of involution of cirrhosis, but it differed in degree. After resection, during involution of the cirrhotic changes, the outflow of lysosomal enzymes from the cells gradually increased and their most intensive release was observed 10 days after partial hepatectomy. In subsequent stages, besides the progressive decrease in the number of connective-tissue cells, mentioned above, the quantity of reaction product on the adjacent collagen fibers also decreased. Lysosomal activity of the hepatocytes fell much more slowly.

Throughout the period investigated the time course of lysosomal activity of connective-tissue cells in the animals of group 2 (not undergoing partial hepatectomy), expressed as the intensity of the reaction for AP in bundles of collagen located between these cells, corresponded on the whole to that in the animals of group 1 (Fig. 2), although it remained at a high level for a longer time. In all probability this was connected with the slower resorption of

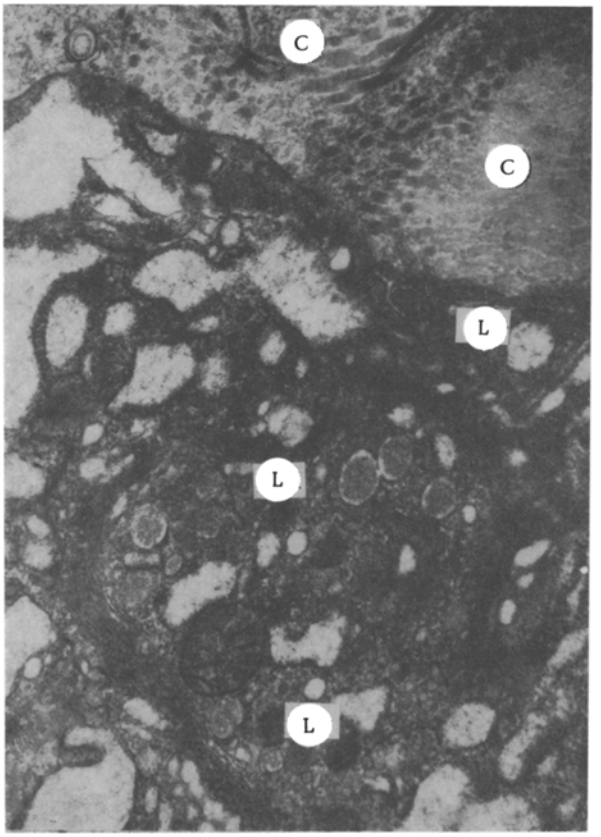

Fig. 3. Control for reaction for AP with addition of enzyme inhibitor, sodium fluoride, to incubation medium (10 days after resection). No reaction product found in lysosomes (L) of connective-tissue cell or in collagen (C). 17,000×.

collagen during involution of cirrhosis than in the hepatectomized animals. As regards hepatocytes, the increase in their lysosomal activity was exhibited later and was less marked than in the animals of group 1.

The data described above are evidence of the stimulating effect of partial resection on lysosomal activity of cells of the cirrhotically changed liver. Direct correlation is known to exist between AP activity and the intensity of catabolic processes. High AP activity revealed by these experiments in the fibrous bands, with the reaction product located directly on the collagen fibers (Figs. 1 and 2) is evidence in this case of the collagenolytic function of the lysosomal enzymes. AP, which attacks the substrate at its monophosphoric acid residues, cannot participate in the hydrolysis of collagen, which does not contain bonds of this type. While behaving as a marker, AP is most probably only a satellite of other lysosomal enzymes, including the proteinases which degrade collagen.

The time course of AP distribution during cirrhosis and its involution thus indicates that the lysosomal enzymes of hepatocytes and of connective-tissue cells of the liver not only effect intracellular degradation of various substrates, but may also be secreted into the extracellular space. This investigation has shown that partial hepatectomy during cirrhosis stimulates AP activity in the liver cells considerably. Lysosomal enzymes, released from hepatocytes and connective-tissue cells by exocytosis, play an active part in the extracellular degradation of collagen in vivo during involution of cirrhosis of the liver. In the initial stages of involution of cirrhosis extracellular degradation of collagen in the liver takes place on account of the lysosomal enzymes of the hepatocytes and connective tissue cells. Later, during involution of cirrhosis, the leading role in the lysis of collagen gradually switches to lysosomal enzymes of the hepatocytes.

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ACTIVITY OF NUCLEOLAR ORGANIZERS OF EPITHELIAL TUMOR CELLS OF THE HUMAN LARGE INTESTINE

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The introduction of the method of silver impregnation of nucleoli into cytology has provided the investigator with a unique opportunity of assessing activity of ribosomal cistrons in single cells actually in cytologic preparations; moreover, the number of silver granules in the nucleoli corresponds approximately to the number of actively working ribosomal cistrons (rC) or of RNA-polymerases-1 in the cells analyzed [11-14].

High activity of rC of human tumor cells, discovered recently with the aid of silver nitrate [3, 6], may be linked with their low differentiation, their relatively high proliferative activity, and also a possible increase in the number of acrocentric chromosomes in the cells with actively functioning nucleolar organizers (NO).

To test these hypotheses, it was decided to compare the character of silver staining of NO and mitotic activity of cells of epithelial tumors of the human large intestine, characterized by different degrees of disturbance of cell differentiation.

EXPERIMENTAL METHOD

Material for investigation consisted of preparations of 48 adenomas of different histological types (14 tubular, 34 villous and tubulo-villous), and 13 adenocarcinomas, obtained as a result of local and radical operations for tumors of the large intestine, and also biopsies performed in the Leningrad City Oncologic Dispensary. Regions of macroscopically unchanged mucosa from segments of large intestine, resected mainly for cancer, taken as far away as possible from the tumor, were used for the control. Pieces of tumor were placed on defatted slides and ground to a paste. The smears were fixed, treated with formic acid, and stained with silver by the method described previously [5, 6, 12]. From 50 to 100 cells were analyzed in the smears and the number of nucleoli and argentophilic granules in them determined. The mean number of nucleoli and argentophilic granules in all the cells studied was then calculated. Ability of the cells to start mitosis was studied in paraffin sections stained with hematoxylin and eosin, using Alov's classification to evaluate pathology of mitoses [1]. In each

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