gastric ulcer, glycosaminoglycans, proteins, and metabolites, promoting autologous rosette formation, accumulate in the interstitial substance of the connective tissue. The process of autologous rosette formation reveals the special features of the rosette-forming cells: the presence of immature T lymphocytes [9], widening of the range of function of polymorphonuclear leukocytes [3]. Meanwhile autologous rosette formation exerts its influence on processes taking place in the tissue of the ulcer itself. For instance, interaction of T lymphocytes with autologous erythrocytes is accompanied by release of a factor stimulating cell proliferation [1], which is connected with regeneration of the mucosa. Thus autologous rosette formation in the tissue of a chronic gastric ulcer reflects profound changes in local metabolism and probably depends on those changes.

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INTRACELLULAR AND EXTRACELLULAR CATHEPSIN D ACTIVITY IN THE LIVER DURING CIRRHOSIS AND INVOLUTION

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Acid endopeptidase or cathepsin D, the principal lysosomal enzyme, reflects the ability of cells to hydrolyze protein and plays an important role in the process of intracellular proteolysis [3, 11]. Cathepsin D can also produce lysis of collagen and other components of the intercellular matrix [5-7, 9, 12]. The enzyme has also been found extracellularly in connective tissue in vitro [8], although its involvement in extracellular catabolism has not been proved [4].

To discover the role of cathepsin D in the resorption of fibrous tissue we investigated the distribution of the enzyme in the liver at the ultrastructural level in cirrhosis and during the first 3 weeks of its involution.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 150 g. Cirrhosis of the liver was induced by subcutaneous injection of 50% CCl_4 in olive oil in a dose of 0.3 ml/100 g body weight twice a week for 15 weeks. Samples of liver for study were

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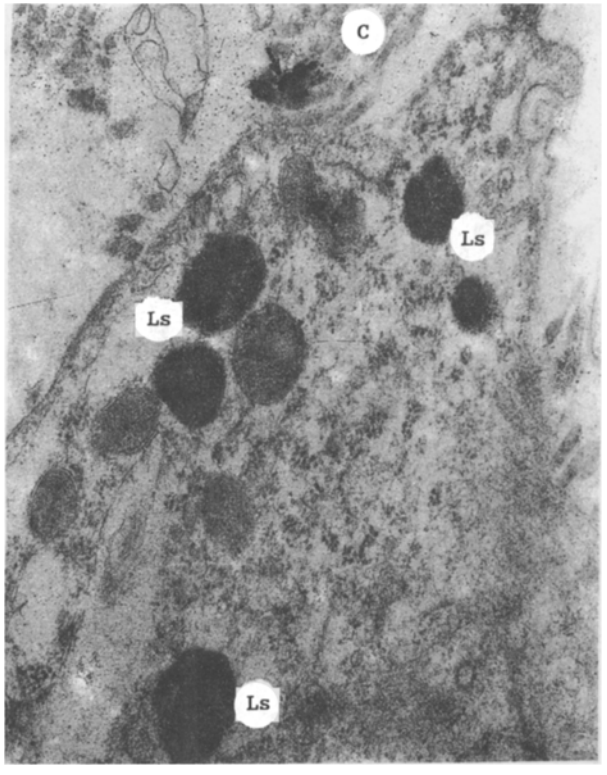

Fig. 1. Determination of localization of cathepsin D. 20,000×. a) Reaction product for cathepsin D in lysosome (Ls) of hepatocyte (H). BT) Biliary tubule; b) intensive reaction in one lysosome (Ls) of an endothelial cell (EC). Extracellular reaction for cathepsin D (arrows) on microvilli of a hepatocyte (H). E) Erythrocyte. [There are substantial discrepancies between the figure and caption; they are reproduced here as in the Russian original — Publisher.]

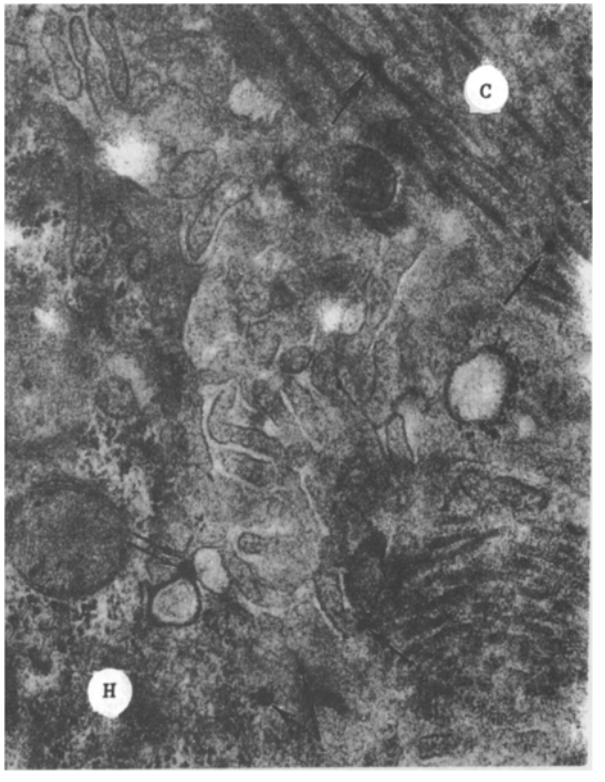

Fig. 2. Extra- and intracellular localization of cathepsin D. a) Unevenly distributed reaction product in lysosome (Ls) of Kupffer cell (KC) with intensive reaction at one pole of Ls, and also reaction product (arrow) in extracellular space on cytolemma of KC. H) Hepatocytes. 20,000×; b) Intensive reaction for cathepsin D in two lysosomes (Ls) of a fibroblast (F). Granules of reaction product (arrows) seen extracellularly in immediate vicinity of F. C) Collagen. 10,000×. [There are substantial discrepancies between the figure and caption; they are reproduced here as in the Russian original — Publisher.]

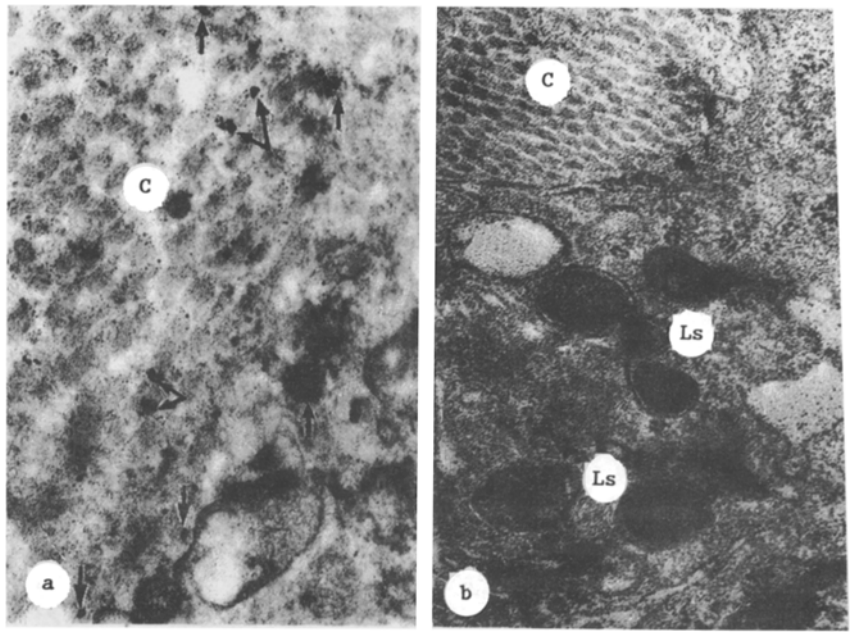

Fig. 3. Localization of reaction product. a) Extracellular localization of cathepsin D (arrows) on collagen fibers (C) in Disse's space. H) Hepatocyte. 15,000×; b) Control for reaction for cathepsin D. No reaction product can be seen either intra- or extracellularly. Ls) Lysosomes, C) collagen. 30,000×.

taken at the height of development of cirrhosis (24 h after the last injection), and again 10 and 20 days later. The material was subjected to histochemical treatment to demonstrate cathepsin D at the ultrastructural level by the method in [10]. Pieces of tissue measuring 0.5 × 0.5 × 1 mm were fixed for 3 h with 1.5% glutaraldehyde in cacodylate buffer, pH 7.2. For the next few days the material was washed with cacodylate buffer, pH 7.2, containing 7% sucrose. Incubation lasted 30 min at 37°C in medium containing the substrate 24 mg of BL-Arg-Gly-Phe-Phe-Pro-MBNA, dissolved in 1 ml of dimethylformamide, with the addition of 25 mg of glycine buffer, pH 3.1. The reaction was stopped by the addition of 10% KOH to the medium, followed by washing with HEPES buffer, pH 7.0. Next followed incubation in medium containing 10 μg of dipeptidylaminopeptidase II in cacodylate buffer, pH 5.4, with hexazotized pararosaniline (1 ml to 20 ml of medium) at 37°C for 15 min. After fixation in OsO_4 the material was dehydrated and embedded in Epon. As the control reaction incubation was carried out in medium without the substrate. Some section were stained with uranyl acetate and lead citrate, others with uranyl acetate alone. The preparations were examined in the JEM-100S electron microscope.

EXPERIMENTAL RESULTS

Electron-histochemical investigation of the liver at the height of development of cirrhosis, and also 10 and 20 days after the ending of CCl_4 injections revealed the reaction product for cathepsin D in lysosomes of hepatocytes, macrophages (Fig. 1), and fibroblasts. The considerable heterogeneity in the distribution of the reaction product will be noted, both among different types of cells and among lysosomes in each cell containing the reaction product, and even in many individual lysosomes. The highest activity at all times was observed in macrophages and fibroblasts. At the height of development of cirrhosis the product of the reaction for cathepsin D was observed on collagen fibers, which appeared to penetrate into destroyed hepatocytes. Sometimes the reaction for the enzyme also was observed in lysosomes and vesicles released into the extracellular space from destroyed cells. After 10 days of involution of cirrhosis we often observed cathepsin D activity in myelin-like structures and in autophagic vacuoles of hepatocytes.

The most important aspect of the present investigation can be regarded as the discovery of extracellular cathepsin D activity, which was observed to a varied degree at all times. The reaction product was localized most frequently on collagen fibers alongside connective-tissue cells (Fig. 1) and hepatocytes (Fig. 2), and also on microvilli of hepatocytes and on the outer side of the cell membrane of connective-tissue cells (macrophages, fibroblasts, Ito cells). Consequently, the sources of extracellular

cathepsin D in the liver are not only parenchymatous, but also nonparenchymatous cells. The highest extracellular activity of the enzyme was observed after 20 days of involution of cirrhosis (Fig. 3a). Meanwhile, activity in the hepatocytes was low. In the control preparations there was no reaction for cathepsin D (Fig. 3b).

The results of this investigation are in full agreement with the writers' previous observations, made by electron-histochemical demonstration of acid phosphatase, showing involvement of lysosomal enzymes in the extracellular catabolism of connective tissue in the process of reversibility of cirrhosis [2], and they are evidence that cathepsin D, besides its role in intracellular proteolysis, is secreted by the hepatocytes and connective-tissue cells of the liver into the intercellular space in cirrhosis and during its evolution, and that it is involved in the extracellular resorption of fibrous tissue.

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