MORPHOLOGY AND PATHOMORPHOLOGY

Ultrastructural Analysis of Liver Biopsy Specimens in Chronic Hepatitis and Hepatopathy

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Three types of morphogenesis of a chronic general pathological process in the liver are distinguished: active hepatitis, hepatitis attended by a pronounced and early tendency toward sclerosis, and hepatopathy, a morphological phenotype of parenchymal dystrophy in the absence of a stromal inflammatory component, which is defined as a syndrome of regenerative-plastic insufficiency.

Key Words: *liver; electron microscopy; regenerative-plastic* insufficiency

Studies describing relationships between the destructive and regenerative reactions in the liver and presenting an in-depth structural-functional analysis are numerous and of fundamental importance [5,9-11,13]. The clinical ultrastructural pathology of the liver, however, has received far less attention [1-4], even though the importance of such studies under modern ecological conditions is obvious.

The aim of the present study was to perform a complex morphological analysis of liver biopsy specimens in cases of chronic general pathological processes.

MATERIALS AND METHODS

Morphological investigation of 50 liver tissue specimens obtained during laparoscopy (42 bioptates) and by transcutaneous puncture biopsy (8 bioptates) was performed. The larger part of each specimen was used for preparing paraffin sections (fixing in 10% neutral Formalin and hematoxylin-eosin staining in combination with Perls reaction, Van-Gieson staining, and Schiff reagent). The smaller part of each specimen was fixed in 4% paraformaldehyde and postfixed in 1% osmium tetroxide; after dehydration the specimens were embedded in Epon-Araldite.

Semithin sections were stained with azure II and Schiff reagent; ultrathin sections obtained on an LKB III ultratome were double-contrasted and examined in a JEM-100B electron microscope at an accelerating voltage 60 kV.

Liver biopsy was performed in patients who had undergone the requisite battery of clinical and laboratory tests, which yielded the diagnosis of active chronic hepatitis [15-17]. The pattern of the process was morphologically assessed from the degree of damage to hepatocytes, as well as the degree and nature of inflammatory cellular infiltration.

RESULTS

Morphological investigation helped identify three different types of structural changes in the liver tissues. In the majority of biopsy specimens the

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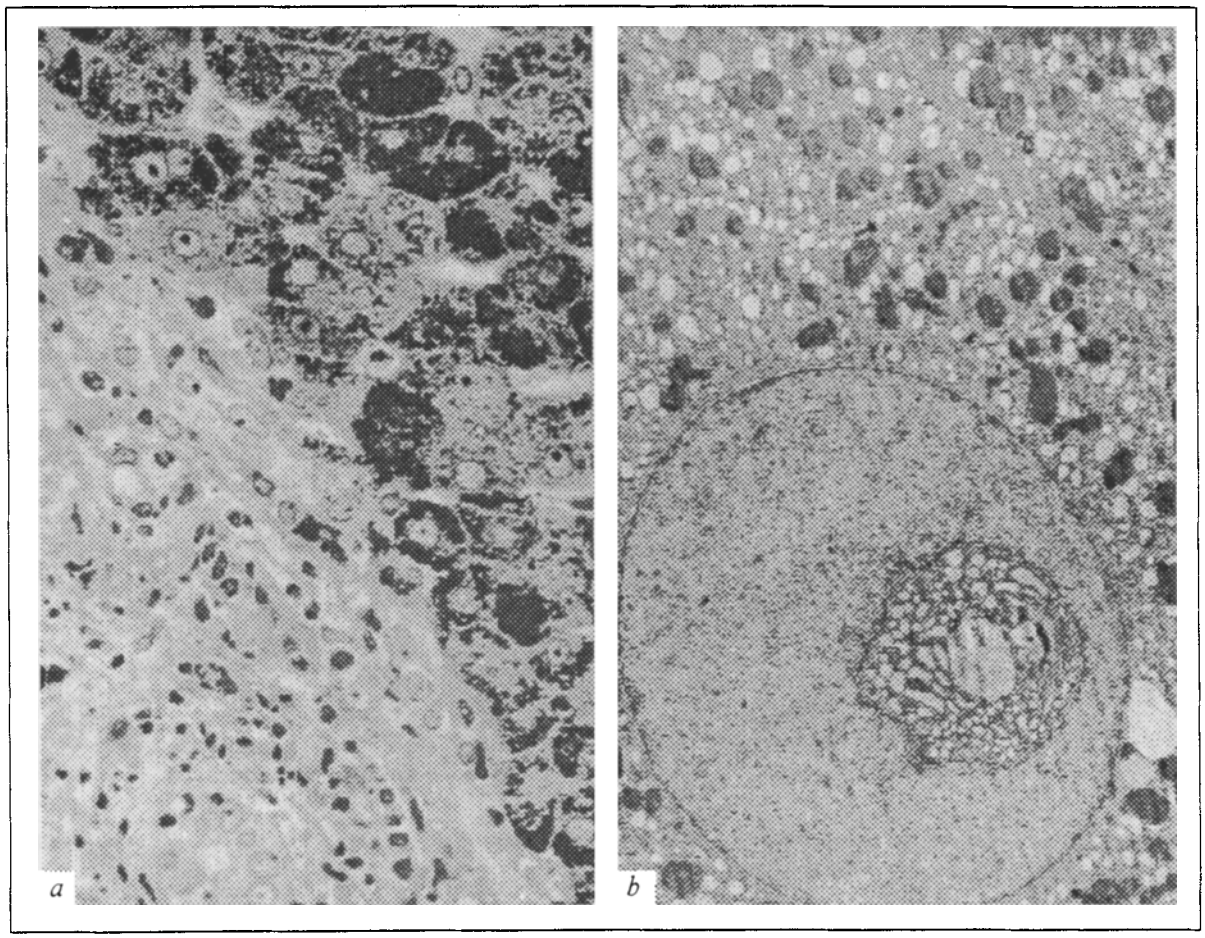

Fig. 1. Morphological changes in liver biopsy specimens in chronic hepatitis. *a*) polymorphous cellular infiltration and stromal sclerosis; hepatocytes are heterogeneous with respect to the content of glycogen granules; semithin section; Schiff–iodine acid (SIA) reaction; ×400. *b*) fragment of hepatocyte; invagination of cytoplasmic area into the nucleus; hyperplasia of the smooth cytoplasmic reticulum; ×2600.

morphological picture was typical of active chronic hepatitis (the first type of changes). In these cases hepatocyte dystrophy, which was sometimes pronounced, was attended by the development of necrobiosis and cell necrosis and was associated with intensive polymorphous cellular infiltration of the inter- and intralobular stroma. Signs of perihepatocellular, interlobular, septal, focal, and portal sclerosis were also noted (Fig. 1, *a*).

The second type was characterized by more marked sclerotic changes, attended by a focal deformation of the organ's structure and an abnormal reconstruction of the lobular architecture. Preserved hepatocytes predominantly exhibited a morphological phenotype of dystrophied cells; inflammatory infiltration was massive, penetrating deep into the parenchyma and accompanied by a pronounced sclerosis (Fig. 2, *a-c*).

In the third type of structural changes hepatocyte dystrophy in the absence of inflammatory infiltration was the chief component. During light-optic examination this manifested itself in alterations of the nuclei, total "depletion" of the cytoplasm and disappearance of glycogen, and the structural breakdown of the cytoplasmic membranes (Fig. 3, *a* and *b*).

Despite marked differences in the light-optic image, electron-microscopic analysis of preserved hepatocytes showed, on the whole, a stereotypic pattern of subcellular changes in the biopsy specimens of the first two morphogenetically different processes; they differed only with respect to the degree of their intensity.

In the majority of cells mitochondria were enlarged, had a moderately dense, sometimes lysed, matrix, and a few shortened and widened cristae. The granular cytoplasmic reticulum was partially degranulated and was mainly represented by vacuoles; only in some cells did it preserve its structure, forming cisternae containing floccular material. Vesicles of the smooth cytoplasmic reticulum were concentrated predominantly at the sinusoidal pole of hepatocytes and were filled with a floccular substance. In some hepatocytes we en-

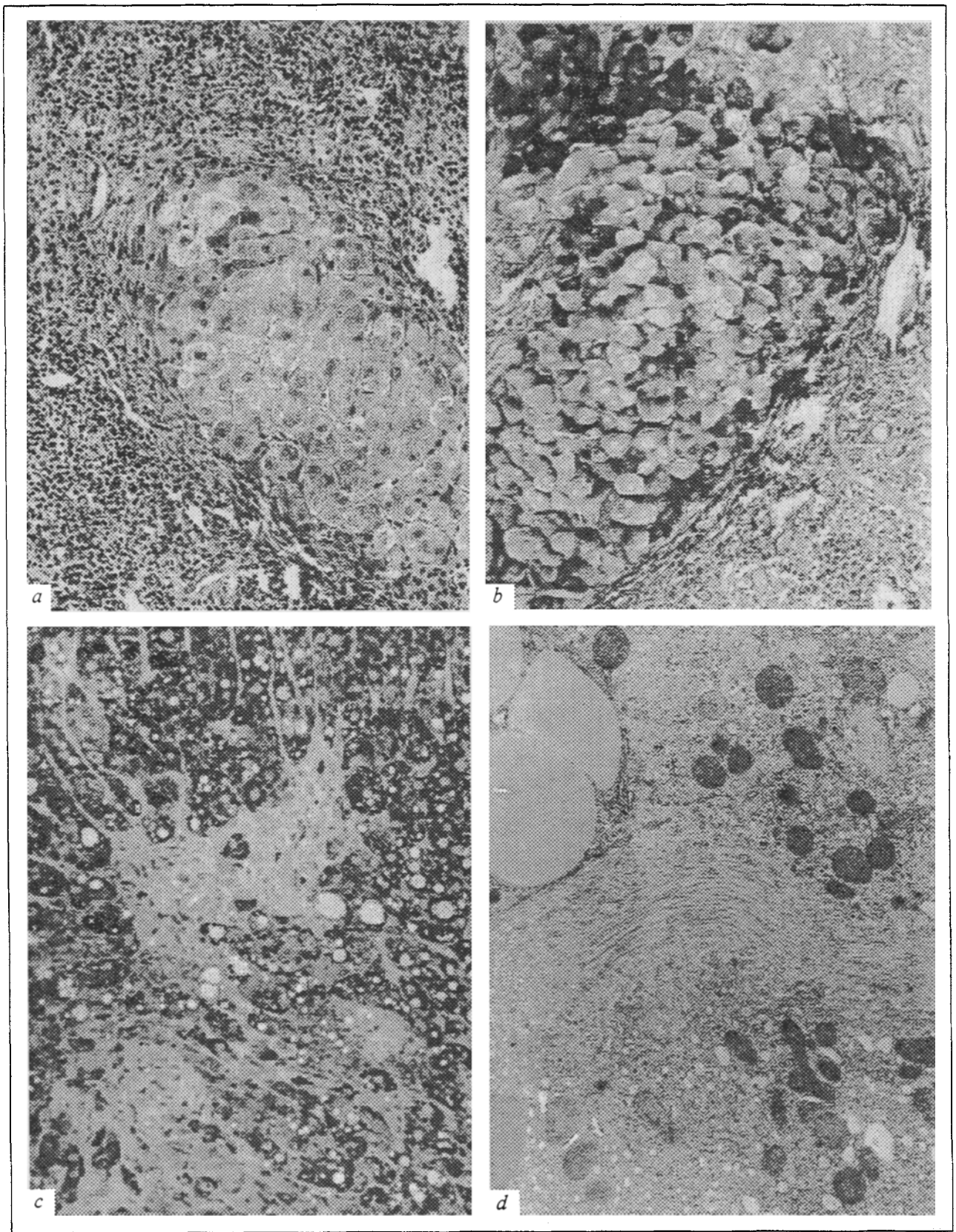

Fig. 2. Morphological changes in liver biopsy specimens in chronic sclerotizing hepatitis. *a*) isolated group of hepatocytes with cleared cytoplasm and polymorphous nuclei in inflammatory infiltrate; hematoxylin–eosin staining; ×312. *b*) the same cells; SIA reaction; ×340. *c*) thickening and sclerosis of interlobular septa; diffuse lipodystrophy of hepatocytes; semithin section; SIA reaction; ×400. *d*) bundles of collagen fibers in the intrahepatocellular space; ×2000.

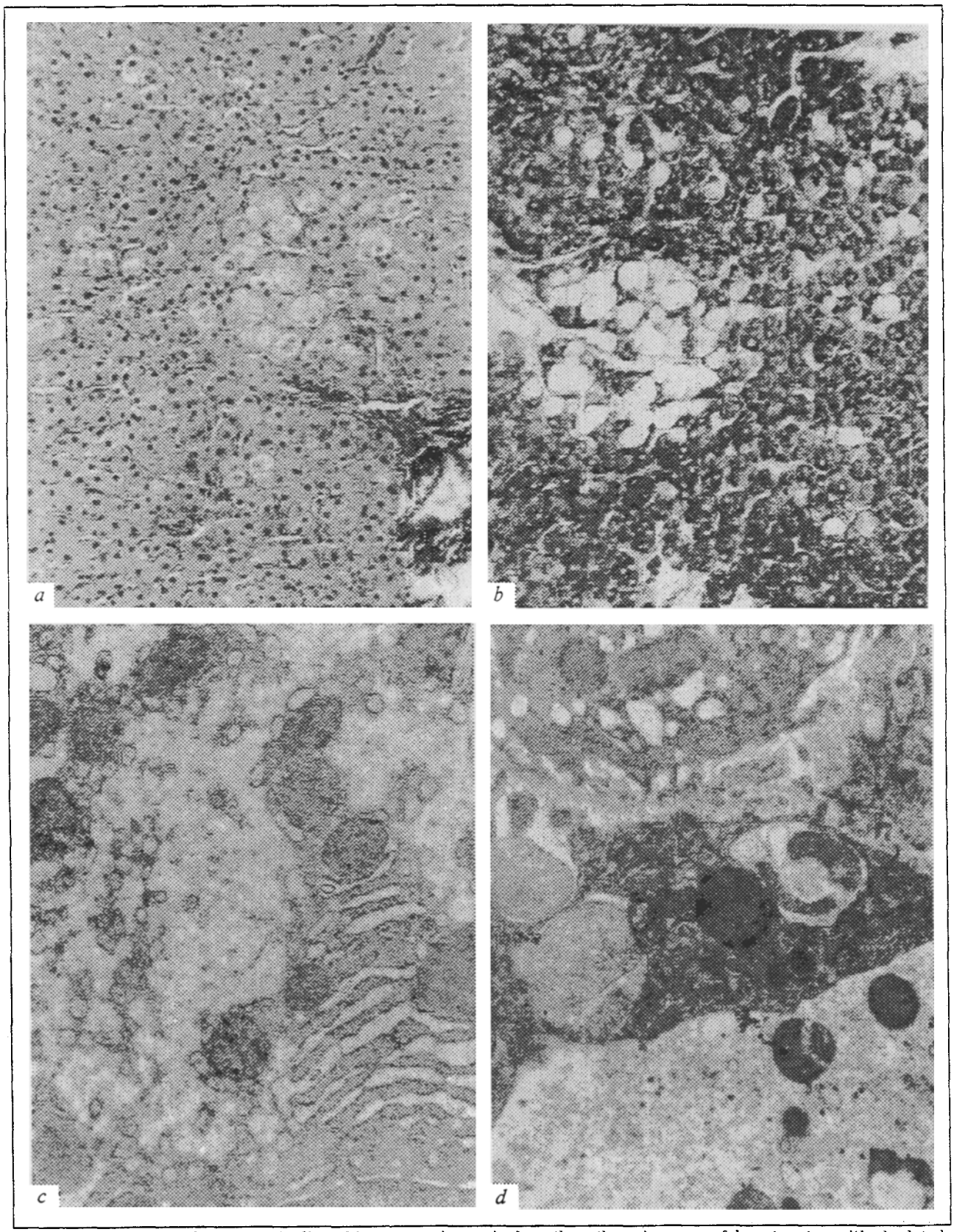

Fig. 3. Morphological changes in liver biopsy specimens in hepathopathy. *a*) group of hepatocytes with depleted cytoplasm; Van–Gieson staining; ×312. *b*) the same cells for Schiff reagent staining; absence of glycogen; ×340. *c*) fragment of hepatocyte; marked reduction of cytoplasmic organelles and depletion of cytoplasm; ×8300. *d*) Kupffer cell containing large phagosomes; ×6600.

countered a lamellar complex containing 4-5 cisternae and a few vesicles. The lysosomal apparatus was represented by a few secondary lysosomes, which were usually located at the biliary pole. Glycogen in the form of rosettes (γ-form) was uniformly distributed over the cell cytoplasm.

The lumens of the bile capillaries were slightly dilated and filled with abundant microvilli; bundles of collagen fibers were observed in the perisinusoidal spaces (Fig. 2, *d*). The hepatocyte nuclei were oval and predominantly contained decondensed chromatin. Large looped nucleoli were formed by the granular and fibrillar structures with a predominance of the latter; ring-shaped nuclei were encountered (Fig. 1, *b*).

In biopsy specimens of patients with chronic hepatitis multiple contacts between small lymphocytes and hepatocytes were discovered. Evidently, lymphocytes migrated from the lumen of sinusoids through the Disse space and penetrated into the parenchyma. The lymphocyte-hepatocyte contact was realized via its waveform outer membrane, frequently forming pseudopodium-like structures; the contacting membranes were destructured. The cytoplasmic hepatocyte membrane was ruptured, and lymphocyte invasion into the cytoplasm occurred. Two types of invasion have been described [14]: the first type is a direct invagination (the lymphocyte is surrounded by the hepatocyte membrane); the second type is a direct invasion of the lymphocyte into the necrotizing hepatocyte without being surrounded by the plasma membrane.

The third type of morphological changes in liver biopsy specimens, characterized by hepatocyte dystrophy in the absence of inflammatory cellular infiltration, is of special interest. This type of change in the liver may be regarded as hepathopathy.

Ultrastructural analysis demonstrated that reduction of the granular cytoplasmic reticulum and of the protein-synthesizing compartment as a whole (Fig. 3, *c*), a decrease in the glycogen content and its molecular transformation (the predominance of the α-form), as well as the accumulation of aggregated products of metabolism (such as lipofuchsin) in the hepatocyte cytoplasm formed the basis of the dystrophic process. The ultrastructural changes were most pronounced in the nucleoli, manifesting themselves in segregation of the granular and fibrillar components, reduction of the granular material, and, sometimes, ring-shaped nucleoli.

Reduced synthesis of structural proteins led to depletion of the cells; the process started in the perinuclear zone and then extended to other areas. As a result, a considerable number of cells proved to be damaged to varying extents. In several cases hyperplasia of the agranular cytoplasmic reticulum was revealed, which was indicative of functional loading of the system of metabolism of xenobiotics (the microsomal system). The dystrophic process was not attended by an inflammatory reaction, but perihepatocellular sclerosis, going hand in hand with the accumulation of Ito cells, was noted. Sometimes, clusters formed by several lymphocytes showing no signs of contacts with hepatocytes and Kupffer's cells (Fig. 3, *d*) were encountered in the stroma or in the perisinusoidal space.

Thus, we have described a fundamentally new type of structural changes in the liver with the predominance of hepatocyte involution in its morphogenesis, which may be regarded as a manifestation of the syndrome of regenerative-plastic insufficiency [6-8,12].

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