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Structural Reorganization of the Rat Liver under Cytotoxic Effect of Doxorubicin

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We studied structural reorganization of the liver after toxic injury caused by a single injection of doxorubicin in a sublethal dose (10 mg/kg). The morphogenesis of doxorubicin injury to the liver is determined by two main pathogenetic factors: cytotoxic effect of doxorubicin and its metabolites on liver cell populations (primarily hepatocytes) and pronounced hemodynamic disorders in the greater circulation resuling from the development of chronic cardiac insufficiency. Changes in hepatocyte caused by doxorubicin manifest by fatty degeneration in the periportal zones and by pronounced lytic changes in the pericentral zones, most pronounced by day 30 of the experiment. Doxorubicin in the studied dose exhibited no cytostatic effect on the hepatocyte population. Hepatocyte proliferation, observed during the entire experiment, leads to an appreciable increase in their count and liver weight.

Key Words: *liver; doxorubicin; hepatotoxicity; hepatocyte count*

Liver involvement resultant from the effects of hepatotoxic drugs remains an unsolved clinical problem [6,13]. The spectrum of liver diseases caused by drugs includes intrahepatic cholestasis, granulomatous hepatitis, cirrhosis, and various tumors [8]. The pathogenesis of drug-induced liver injuries is explained mainly by the unique metabolic characteristics of the liver, with a system of microsomal monooxygenases neutralizing toxicants entering the body, specifically, drugs, in its parenchymatous cells [7].

An important aspect in the problem of drug-induced injuries of the liver is pronounced hepatotoxicity of drugs used in oncology, such as anthracycline antibiotics, metabolized mainly in the liver and eliminated with the bile [12]. One of the most effective drugs of this group is doxorubicin, characterized by pronounced cytostatic and cytotoxic effects [4].

Our knowledge of the structural and functional reorganization of the liver caused by anthracycline antibiotics is insufficient, which impedes the development of effective cytoprotective drugs. Experimental simulation of chronic anthracycline effect helps to evaluate the spectrum of structural and functional injuries to hepatocytes and other cell populations of the liver, the type and degree of their death, and the regeneratory potential of hepatocytes.

We studied the pattern of tissue reorganization of the liver, types of hepatocyte injuries and death, and quantitatively evaluated their population after toxic injury of this organ caused by a single injection of doxorubicin.

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MATERIALS AND METHODS

Doxorubicin hydrochloride (Ferane) in a single dose of 10 mg/kg was injected intraperitoneally to 25 rats, which were decapitated after 1, 3, 5, 7, 14, 21, and 30 days. Control group consisted of 10 rats intraperitoneally injected with saline.

The liver was separated from adjacent tissues and rapidly weighed. Tissue specimens were fixed in 10% neutral formalin and in 4% paraformaldehyde solution. Paraffin sections were stained with hematoxylin and eosin with Perls' reaction after Van-Gieson with poststaining of elastic fibers with Weigert's resorcin-fuchsin; PAS reaction was carried out. Semithin sections were stained with 1% Azur II solution. The preparations were examined under an Axioplan 2 imaging universal microscope (Carl Zeiss).

The method of alkaline dissociation of fixed tissues was used for quantitative evaluation of total population of the liver parenchymatous cells [4]. The results were statistically processed using Student's *t* test.

RESULTS

The mortality of experimental rats within 30 days after injection of a sublethal dose of doxorubicin was 20%. Congestive cardiac insufficiency manifestations were observed in all animals: hydrothorax (24%), ascites (60%), liver congestion (50), anasarca (60%). Hemorrhagic ascites was detected starting from day 14 in all rats; the content of transudate in the abdominal cavity reached 45 ml.

Animal body and liver weights during the first 14 days of the experiment were lower than in the control; only by the end of the experiment (day 21) they increased by 20 and 34%, respectively ($p<0.05$; Table 1). The percentage of liver weight increased negligibly during the entire experiment and remained virtually normal. Body weight of experimental animals increased as a result of accumulation of transudate in the abdominal and thoracic cavities.

Up to day 14 of the experiment the liver looked unchanged, dark-cherry colored, homogenous on section. Starting from day 21 and to the end of the experiment the shape of the organ changed: the liver acquired a spherical shape and light-brown color, its lobes sticking to each other in virtually all animals. The capsule thickened significantly, with sites of compact whitish deposit emerging on its surface.

Tissue reorganization of the liver was determined by two main pathogenetic factors: cytotoxic effect of doxorubicin and its metabolites on the cell populations of this organ, primarily hepatocytes, and pronounced hemodynamic disorders in the greater circulation, resultant from development of chronic cardiac insufficiency. The cardiotoxic effect of doxorubicin in this model manifested by the development of regeneratory plastic cardiac insufficiency with heart remodeling by the dilatation variant [3].

Structural changes of predominantly toxic nature were observed during the early (up to day 7) period of the experiment. One day after injection the cytopathic effects manifested by vesiculation and small-droplet lipid infiltration of the hepatocyte cytoplasm. This was paralleled by the appearance of hepatocytes with focal and total lysis of the cytoplasm ("devastated" hepatocytes; Fig. 1, *a*) in the periportal zone. PAS reaction showed glycogen depositions virtually in all parenchymatous cells; polysaccharide presented mainly as dark-violet granules collected in rosettes. Apoptotic hepatocytes and cells in a state of mitosis (Fig. 1, *b*) were ob-

TABLE 1. Body and Liver Weights and Quantitative Characteristics of Hepatocyte Populations in Wistar Rats after Single Injection of Doxorubicin (*M*±*m*)

Group; day of observation		Body weight, g	Liver weight, g	Percentage of liver weight	Absolute count of hepatocytes in liver, $\times 10^6$
Control		179.8±2.3	7.6±0.6	42.3±2.7	169.7±8.6
Experimental	1	182.9±3.8	8.8±0.2	47.8±0.5	175.2±15.2
	3	166.3±4.7	7.2±0.2	43.9±1.5	184.1±29.4
	5	152.7±5.6*	6.9±0.6	51.5±1.9	215.4±32.1
	7	162.4±6.3	6.5±0.4	47.9±2.4	201.9±27.8
	14	204.9±10.1	8.70±1.1	46.1±2.6	257.8±44.9
	21	216.3±11.5*	10.2±0.9*	44.1±3.6	236.6±38.5
	30	203.3±17.2	10.1±2.2	50.7±14.9	267.8±35.3*

Note. *$p<0.05$.

served during the same period of the experiment. Liver stroma along the portal tracts was moderately infiltrated by mononuclears. Kupffer' cells containing hemosiderin appeared in sinusoids and extracellular spaces.

The progress of hepatocyte lysis (particularly in the pericentral zone) was paralleled by the appearance (starting from day 3 of the experiment) of small necrotic foci moderately infiltrated by mononuclear cells in the parenchyma. Hemodynamic disorders (plethora, hemostasis, small plasma- and hemorrhagic foci) appeared during the same period. Erythrocyte slugging, accumulation of mononuclears with admixture of neutrophils were seen in central and portal veins.

Pronounced hemodynamic disorders were observed from day 5 of the experiment: appreciable dilatation of sinusoids, central and portal veins, their uneven plethora, this rendering the liver parenchyma a "honeycomb" appearance (Fig. 1, *c*). Disorders in close contacts between hepatocytes were observed in the periportal zone, while the common radial structure of the liver was retained. The count of Kupffer's cells in the sinusoidal lumens and their significant hypertrophy were observed from day 7 to the end of the experiment (Fig. 1, *d*). Pronounced polymorphism of Kupffer's cells with predominating activated cells was observed.

During later periods (starting from day 14) structural reorganization of the liver was largely determined by hemodynamic disorders (venous congestion). By days 14-21 small foci of necrosis in the parenchyma, infiltrated by mononuclear, predominantly lymphoid, cells were seen in the pericentral and periportal zones (Fig. 2, *a*). Fatty degeneration of hepatocytes progressed in the periportal zone of the hepatic lobes: lipid droplets were larger and more numerous (Fig. 2, *b*). The number of hepatocytes with "devastated" cytoplasm increased significantly in the pericentral zone of the lobules and in the subcapsular area. On the other hand, hepatocyte mitoses were observed to the end of the experiment. Hypertrophic Kupffer's cells (siderophages) were present in sinusoids.

Hemodynamic disorders persisted and progressed. Congestive phenomena in the hepatic vein system in many cases caused necrotic and necrobiotic changes of hepatocytes in the central lobular zone. This was associated with melting of the central vein walls with the development of lymphohistiocytic infiltration and emergence of clot-like formations (Fig. 2, *c*). Portal veins were plethoric. The arteries were in a state of spasm or secondary paresis, their walls were thickened, their myoelastofibrosis was observed.

By the end of the experiment the count of stellate cells (Ito's cells), containing numerous lipid inclusions, increased in the stroma of the organ (Fig. 2, *d*), which was paralleled by thickening of cell-cell, perisinusoidal and perivascular connective tissue, resultant from their fibrosis. Stellate cells are considered to be involved not only in activation of fibroplastic processes [5,10], but also in regulation of microcirculation in the liver and development of portal hypertension [11]. Sclerotic processes were most pronounced in the portal system.

Bile ducts were dilated, sometimes filled with compact contents, with vacuolated cells of their epithelial lining. Choledochal hyperplasia was observed during later periods of the experiment.

Significant changes in the liver capsule were observed during the entire experiment. It gradually thickened (Fig. 3, *a*), which was particularly pronounced by day 30 of the experiment (Fig. 3, *b*) and was paralleled by connective tissue growth into the parenchyma, vascularization and focal necrosis of the capsule, deposition of hemosiderin. These changes were caused by local toxic effect of doxorubicin injected intraperitoneally (necrotic changes in the peritoneal visceral leaflets and development of aseptic inflammation) and its hepatotoxic and cardiotoxic effects. Plethoric vessels and connective tissue edema, causing pressure elevation in the liver, caused thickening of the capsule.

Quantitative evaluation of hepatocyte population in the course of the experiment showed an increase in its absolute count, most significant by day 30 (by 58%, $p<0.05$). This increase in total hepatocyte count in comparison with control animals, reflecting intensification of the proliferative activity, indicates retained regeneratory potential of the liver after a single injection of doxorubicin, characterized by a pronounced cytostatic effect. Increase in the total count of hepatocytes after doxorubicin injection can be regarded as a compensatory adaptive reaction of the liver, aimed at maintenance of the population of actively proliferating parenchymatous cells under conditions of alteration and reduction of functional activity of an appreciable number of cells. On the other hand, a significant increase in the hepatocyte proliferative activity, surpassing their death, is the main factor determining liver remodeling, increase of its weight and deformation.

Hence, the morphogenesis of chronic doxorubicin injury to the liver in experimental animals is determined by toxic and ischemic injuries of hepatocytes and vascular endotheliocytes. Ischemic hepatopathy and fulminant hepatic insufficiency are observed in patients with congestive cardiac

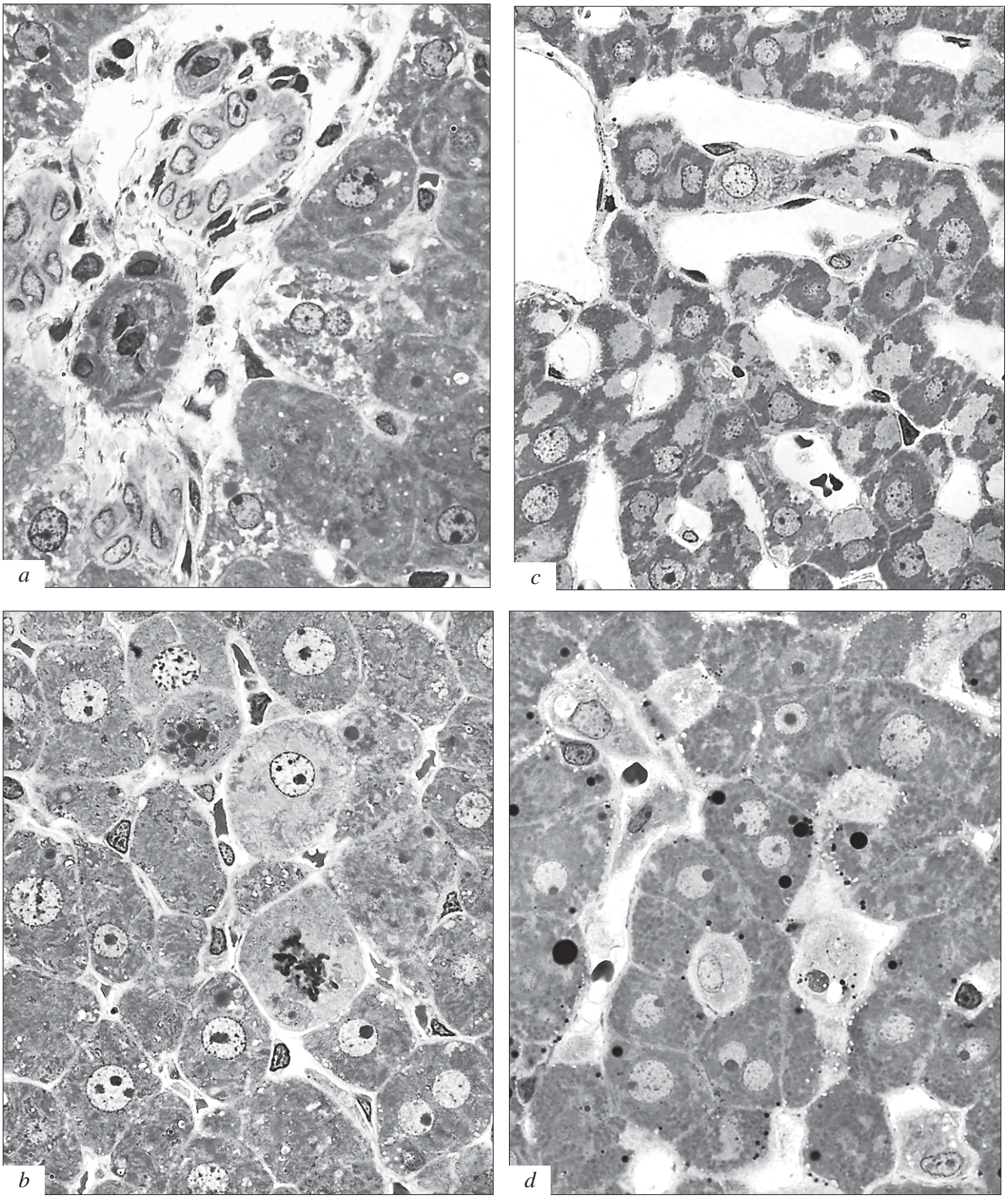

Fig. 1. Morphological changes in Wistar rat liver during early period after doxorubicin injection. Semithin sections, Azur II staining, ×1100. *a*) vesiculation and focal lysis of hepatocyte cytoplasm in periportal zone 1 day after drug injection; *b*) hepatocyte mitosis 1 day after injection; *c*) significant dilatation of pericentral sinusoids 5 days after drug injection; *d*) hypertrophic and hyperplastic Kupffer's cells 7 days after injection.

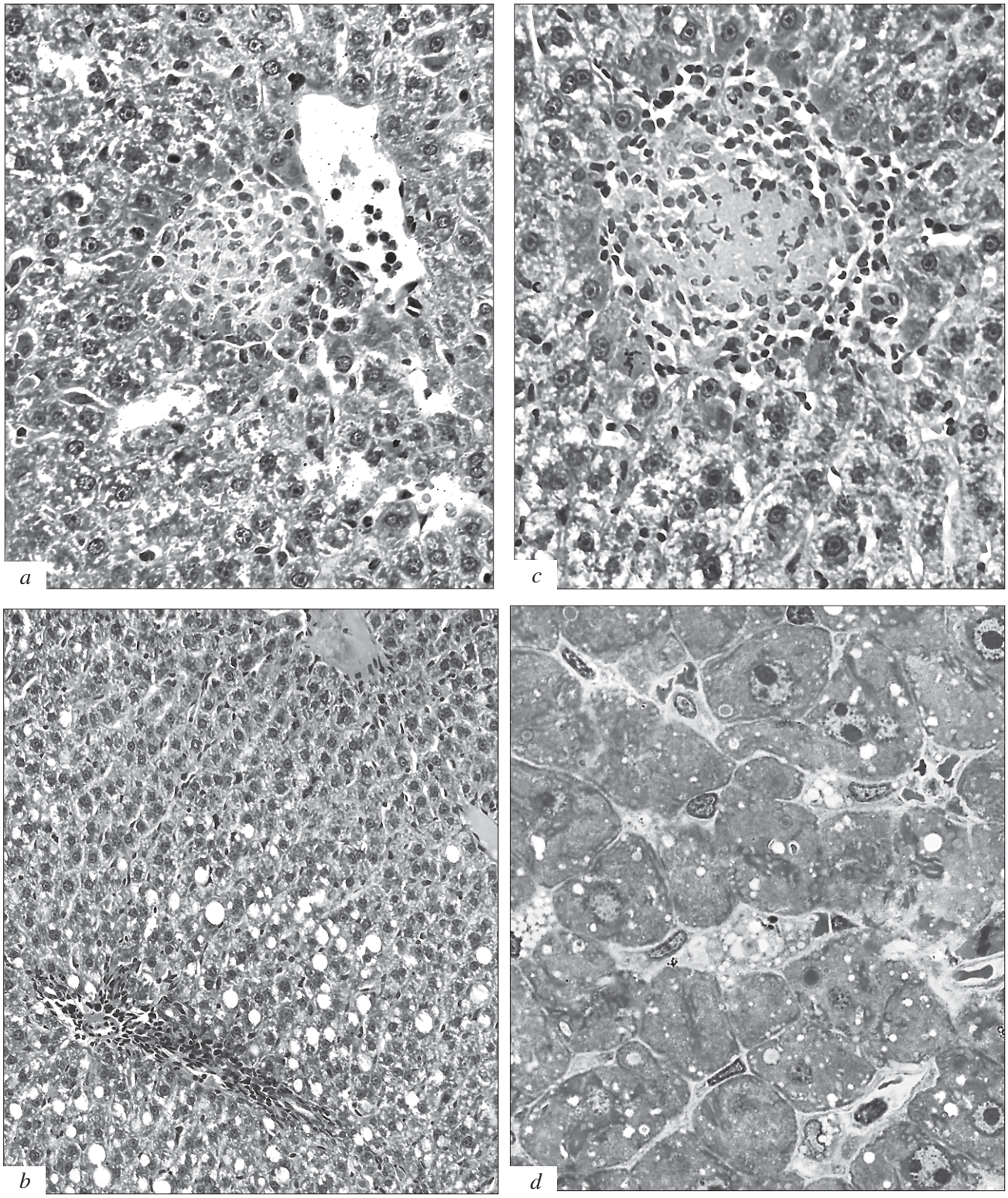

Fig. 2. Morphological changes in Wistar rat liver during late period after doxorubicin injection. *a*) lymphohistiocytic infiltration in hepatocyte necrosis focus in pericentral zone 14 days after drug injection; *b*) progress of lipid infiltration of hepatocytes in periportal zone 21 days after drug injection; *c*) melting of central vein wall and organization of a clot-like formation 21 days after drug injection; *d*) pronounced vesiculation of hepatocytes, hypertrophic stellate cells, and thickened connective tissue strata 30 days after injection. Semithin sections, hematoxylin and eosin staining, ×450 (*a*-*c*), Azur II staining, ×1100 (*d*).

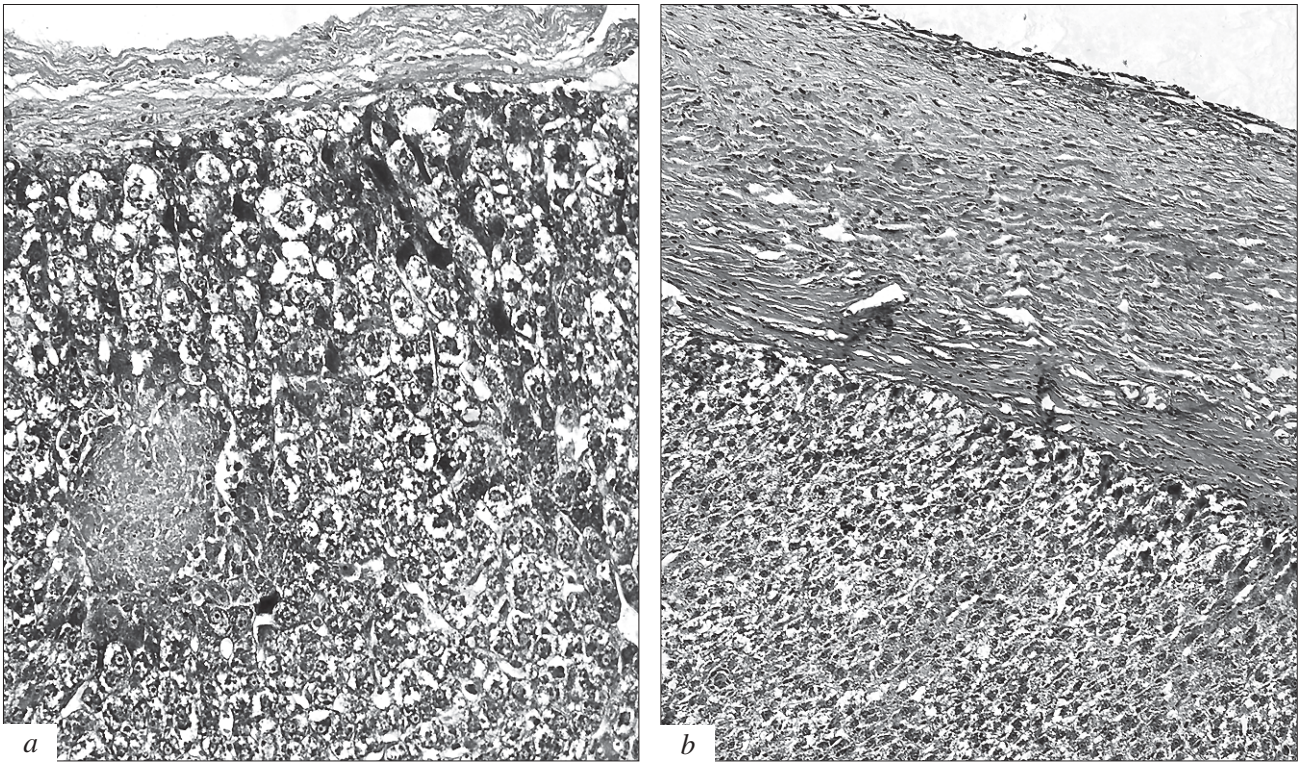

Fig. 3. Morphological changes in Wistar rat liver capsule after injection of doxorubicin, ×450. *a*) thickening and loosening of liver capsule, hepatocyte necrobiosis 14 days after injection. PAS reaction; *b*) vascularization of thickened hepatic capsule 30 days after injection. Hematoxylin and eosin staining.

insufficiency and low ejection syndrome [1,2] and are caused by the development of hypoxia because of reduced venous outflow [9]. Injuries to the vascular wall, particularly to the sinusoidal endothelial lining, play an important role in the development of degenerative and necrobiotic changes in hepatocytes. Among the specific features of doxorubicin hepatotoxic effect are its pronounced cytostatic effect on the hepatocyte population, promoting not only restoration of the total count of parenchymatous cells, but its appreciable increase with time. This proliferative activity of hepatocytes not related to cell death can lead to rapid exhaustion of proliferative reserve and to atypical variants of liver remodeling.

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