REVIEWS

Ultrastructural Characteristic of Collagen Resorption in Cirrhotic Liver

M. M Kalashnikova

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Partial hepatectomy of mouse cirrhotic liver leads to rapid degeneration of hypertrophied connective tissue. Enlarged cisterns of the smooth endoplasmic reticulum lie close to the plasma membrane on the sinusoidal surface of hepatocyte. Golgi apparatus of Kupffer cells is hypertrophied, swollen collagen fibrils lose their specific striation. Microvilli of hepatocyte plasmalemma separate swollen collagen fibrils and draw them into the cytoplasm. Lysosomes, peroxisomes, and hypertrophied Golgi apparatus are spread from the sinusoidal surface to bile capillaries, which indicates that these structures are involved in collagen lysis and release of metabolites into the bile capillary. After partial hepatectomy of cirrhotic liver, hepatocytes, Kupffer cells, and neutrophils play the major role in collagen lysis. Kupffer cells and neutrophils are involved in the lysis of collagen fibrils during CCl_4-induced liver cirrhosis.

Key Words: *hepatocyte; CCl_4, collagen degradation*

A large body of data suggests reversibility of sclerotic changes in organs. It was shown that under certain conditions, hypertrophied fibrous tissues in the liver undergo degeneration [27,34]. Light microscopy demonstrated regeneration of cirrhotic liver after termination of CCl_4 poisoning [18], and partial hepatectomy accelerates this process [22].

Changes in collagen fibrils, hepatocytes, and Kupffer cells (KC) in cirrhotic liver, formation of regeneration hepatomas, and regeneration of sclerotic liver after termination of CCl_4 poisoning and partial hepatectomy were studied by electron microscopy. Liver cirrhosis in mice was induced by administration of 0.2 ml 40% CCl_4 in peach oil 2 times a week for 13 months. The left hepatic lobe was removed 5 months after the start of CCl_4 poisoning to study collagen resorption and to identify cells involved in this process. Some animals were sacrificed 1 day or 1, 2, 3, 4, and 5 weeks postoperation [3], while others were killed 7, 8, 10, 11, 12, and 13 months after the start of CCl_4 poisoning to examine the ultrastructure of hepatoma cells [4].

Histological examination showed that liver fibrosis in mice was followed by the development of cirrhosis, hypertrophy of coarse fibrous tissue, changes in the parenchyma, and formation of regeneration hepatomas. Electron microscopy 5 months after the beginning of CCl_4 administration revealed striated collagen bundles between hepatocytes and in the Disse spaces (DS).

One day after partial hepatectomy, light and swollen collagen fibrils in DS lost their striation (Fig. 1, *a*) and were often seen in invaginations of hepatocyte membrane. The number of smooth endoplasmic reticulum (SER) cisterns markedly increased, and some enlarged cisterns and numerous vesicles were localized along the hepatocyte sinusoidal surface (Fig. 1, *a*). The cytoplasm of KC included a great number of SER channels, some of these channels were in close con-

Laboratory of Evolution Histology, A. N. Severtsov Institute of Ecological and Evolution Problems, Russian Academy of Sciences, Moscow

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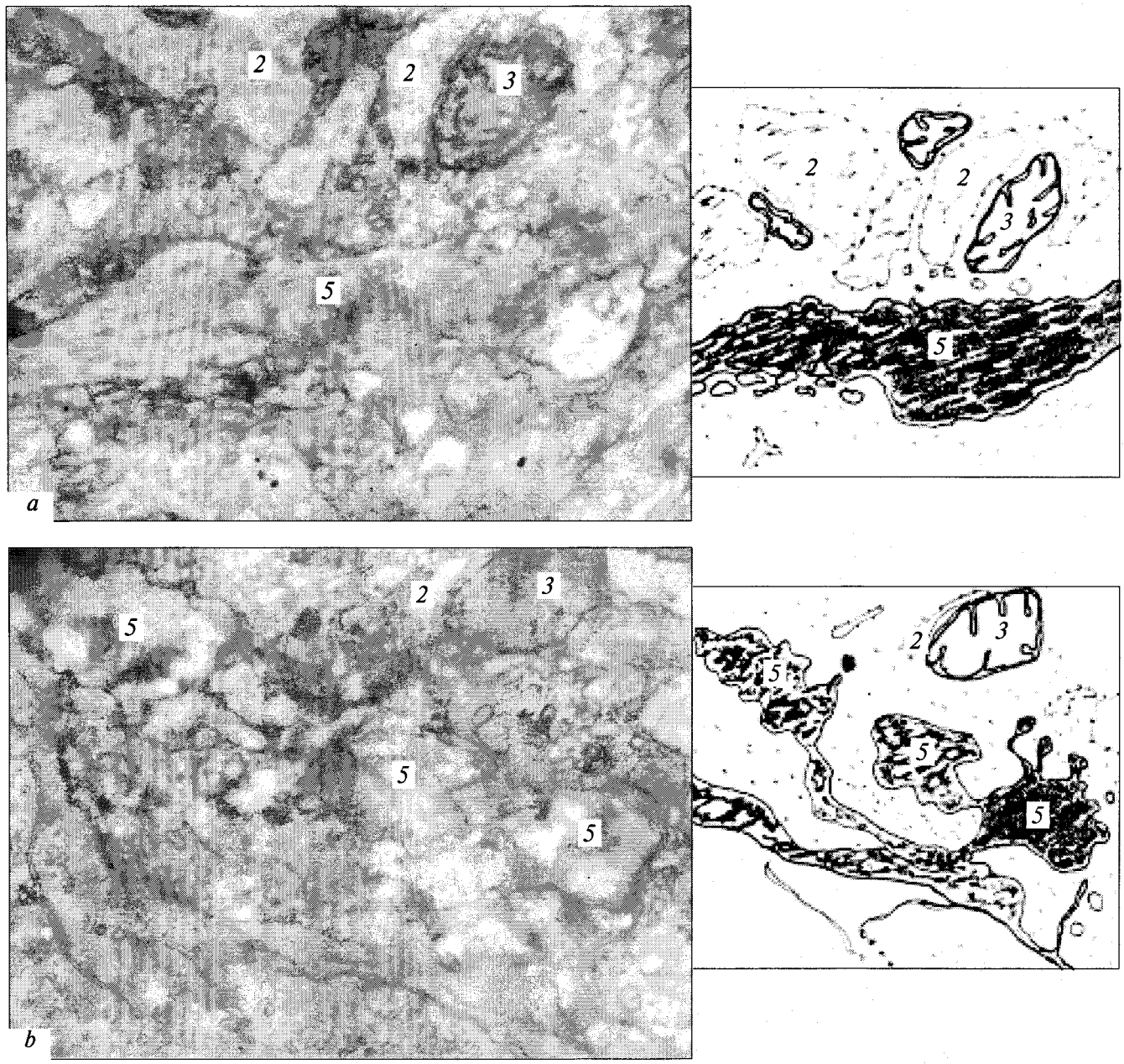
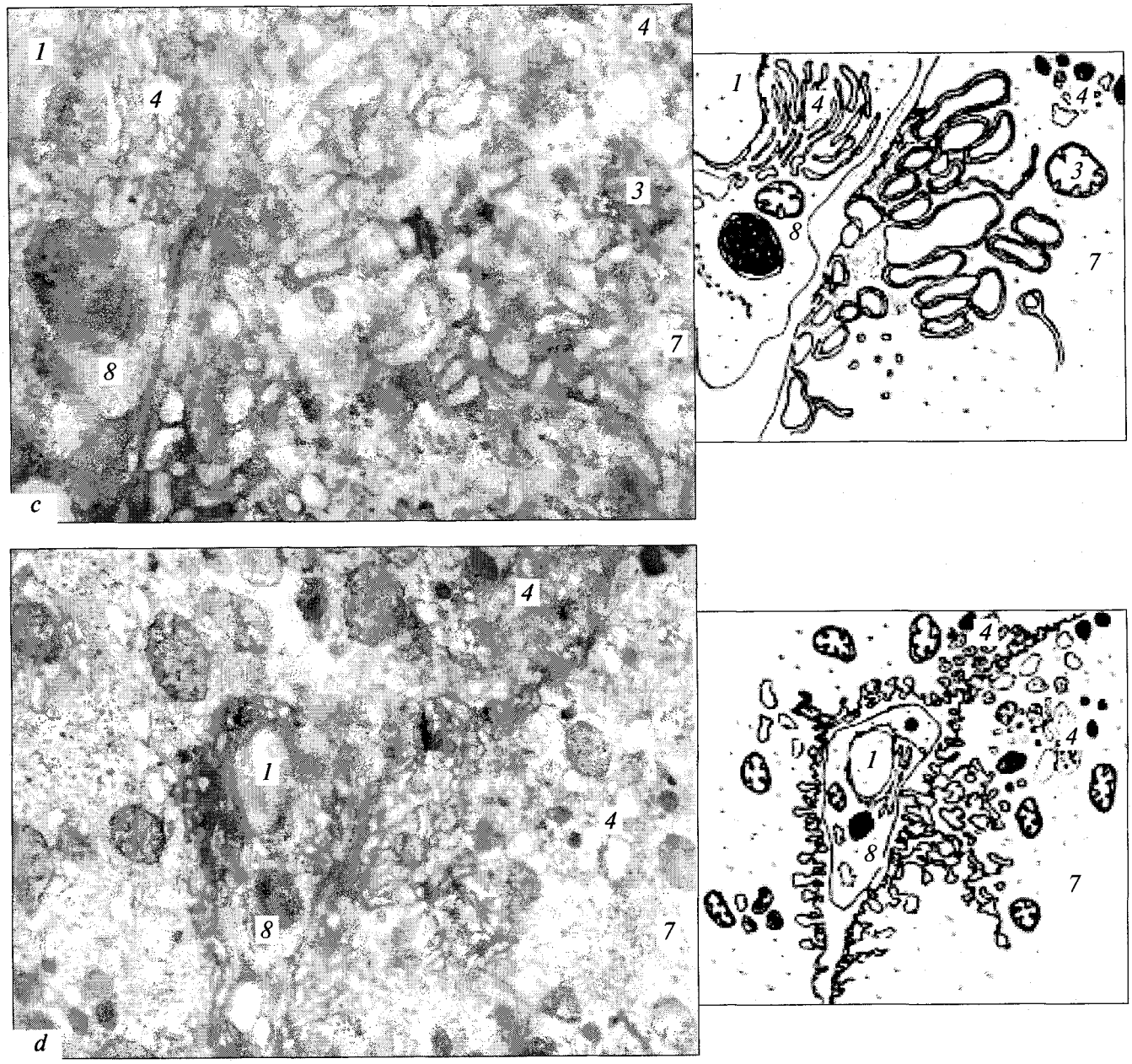


Fig. 1. Resorption of collagen fibrils by hepatocytes after partial hepatectomy of cirrhotic liver. Here and in Figs. 2 and 3: nucleus(*1*), smooth endoplasmic reticulum cisternae (*2*), mitochondrion (*3*), Golgi apparatus (*4*), collagen mass (*5*), collagen fibrils (*6*), hepatocyte (*7*), and sinusoidal cell (*8*). Swelling of collagen fibrils in Disse space and loss of striation; enlarged cisterns of smooth endoplasmic reticulum near collagen lumps 1 day postoperation (*a*, ×24,750). Transport of collagen lumps into hepatocyte cytoplasm 1 week postoperation (*b*, ×24,750). Large Golgi apparatus in Kupffer cell; fragmentation of collagen lumps by numerous invaginations of hepatocyte membrane 2-3 weeks postoperation (*c*,×16,500). Membrane of hepatocyte similar to that of osteoclast; large Golgi apparatus near collagen mass 3-4 weeks postoperation (*d*, ×15,000).

tact with plasma membrane. These data suggest activation of protein synthesis in hepatocytes and KC enhanced production of hydrolytic enzymes released into DS. Vesicles with various electron density were found in KC cytoplasm. Plasma cells and neutrophils were often seen in DS.

One week after resection, swollen collagen fibrils were transported into hepatocyte cytoplasm (Fig. 1, *b*), but hepatocyte membranes were undamaged. The number of microvilli on the hepatocyte sinusoidal surface considerably increased. They penetrated loose and amorphous collagen and divided it into fragments, which were then transported into the hepatocyte cytoplasm. This was confirmed by the presence of numerous vesicles and vacuoles along the hepatocyte sinusoidal surface containing collagen lumps absorbed



from DS. Hypertrophied hepatocytes had large nuclei with several nucleoli, their cytoplasm included many mitochondria and SER cisterns, and the Golgi apparatus was enlarged. Glycogen was practically absent in the cytoplasm, and the number of lipid droplets was sharply reduced compared to the state before partial hepatectomy. The sinusoidal surface of large dark hepatocytes contained a great number of vesicles. Plasma cells, neutrophils, and lymphocytes were found in DS. The appearance of numerous plasma cells [23] is probably related to autoimmune processes induced by depolymerized collagen in the serum. It was shown that collagen resorption elevates the contents of soluble collagen, peptides, and free amino acids in the serum [41].

Retraction of swollen collagen fibrils was also observed 2-3 weeks postoperation. A specific feature was the presence of lysosomes, peroxisomes, and hypertrophied Golgi complex scattered from sinusoids to bile capillaries (Fig. 1, *c*), which indicated that these organelles are involved in collagen lysis and release of metabolites into bile capillaries.

Four-five weeks after hepatectomy these changes were less pronounced, because collagen resorption was practically completed. The presence of collagen fibrils in DS was associated with concentration of numerous vesicles with electron light content on the sinusoidal surface of hepatocyte cytoplasm. The number of lysosomes in the hepatocyte cytoplasm sharply increased.

At the early stages, the sinusoidal surface of hepatocyte membranes adjacent to collagen fibrils did not form microvilli. Swelling of collagen fibrils was accompanied by an increase in the number of microvilli penetrating the collagen lumps and acceleration of collagen transport into hepatocytes (Figs. 1, *c* and *d*). At the late stages of collagen resorption, DS contained numerous microvilli, whose number was much greater than in the control. The sinusoidal surface of hepatocytes was similar to that of osteoclasts (Fig. 1, *d*). The cytoplasm of KC contained many pinocytotic vesicles which formed chains from deep and narrow cytoplasmic invaginations.

These morphological signs of collagen resorption [3] were confirmed by experiments showing that hepatocytes and KC are involved in collagen resorption [1,8-15]. Hepatic lysosomal fraction contains collagenase-like enzymes [30]. Collagenase activity was detected on the sinusoidal surface of hepatocytes, which indicated that this enzyme is activated after the release from hepatocytes [16]. Cathepsin D involved in the metabolism of partially depolymerized collagen was found in hepatocyte lysosomes and microvilli [13], in lysosomes of endothelial cells and KC, on the cytolemma of macrophages, and on collagen fibrils lying close to hepatocytes and connective tissue cells. The data suggest that cathepsin D involved in intracellular proteolysis is secreted by hepatocytes and connective tissue elements into the intercellular space and participates in extracellular resorption of fibrous tissues [13-15].

Our previous studies of the liver from old rats showed that hepatocytes are involved in collagen resorption (data not published). DS often contained collagen fibrils, and the cytoplasm of sinusoidal cells included superfine tonofilaments similar to those in collagen-producing fibroblasts. These data and previous reports [29,31,37] indicate that sinusoidal cells are involved in the formation of collagen fibers. Vacuoles containing fragments of swollen collagen fibrils were also found in the cytoplasm of hepatocytes adjacent to DS.

Ultrastructural studies of liver cells taken at various stages of CCl_4-induced liver cirrhosis demonstrated the formation of connective tissue fibrils not only around dead hepatocytes (replacement fibrosis) but also nearby undamaged hepatocytes. The cytoplasm of KC contained tonofilaments similar to those found in old rats [4]. KC and Ito cells under certain pathological conditions can be transformed into collagen-producing myofibroblasts [29].

After CCl_4 administration, collagen fibrils were transported into hepatocyte cytoplasm. Examination of regeneration hepatomas showed that striated collagen fibrils were arranged along the smooth (without microvilli) surface of hepatocytes, and the Golgi apparatus with enlarged cisterns was seen near collagen fibrils indicating that hepatocytes can secrete proteolytic enzymes. Zigzag-shaped invaginations of hepatocyte membranes had ampoule-like dilatations containing partially swollen collagen fibrils (Fig. 2). Golgi complexes or enlarged SER cisterns and mitochondria were seen in the hepatocyte cytoplasm. Collagen fibrils were also found in DS between 2 hepatocytes isolated from the sinusoid by endothelial cell. The sinusoid included

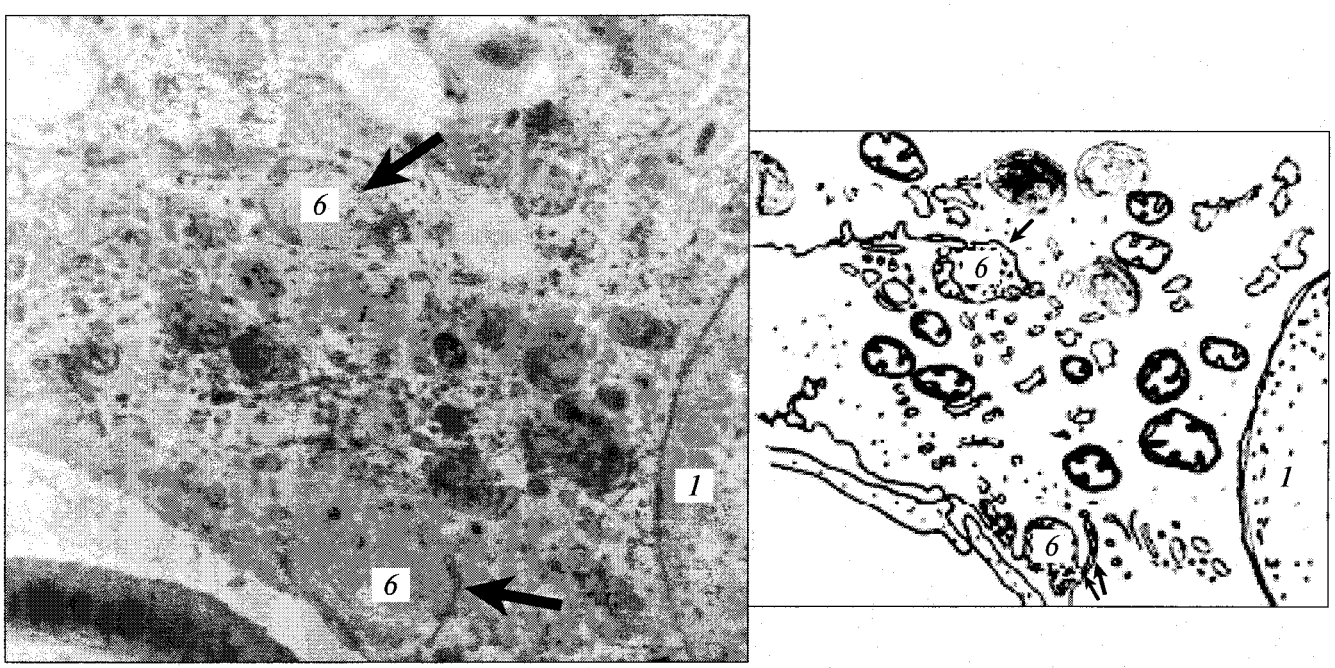


Fig. 2. Retraction of partially swollen collagen fibrils into hepatocyte cytoplasm 12 months after beginning of CCl_4 administration (arrows, ×15,000).

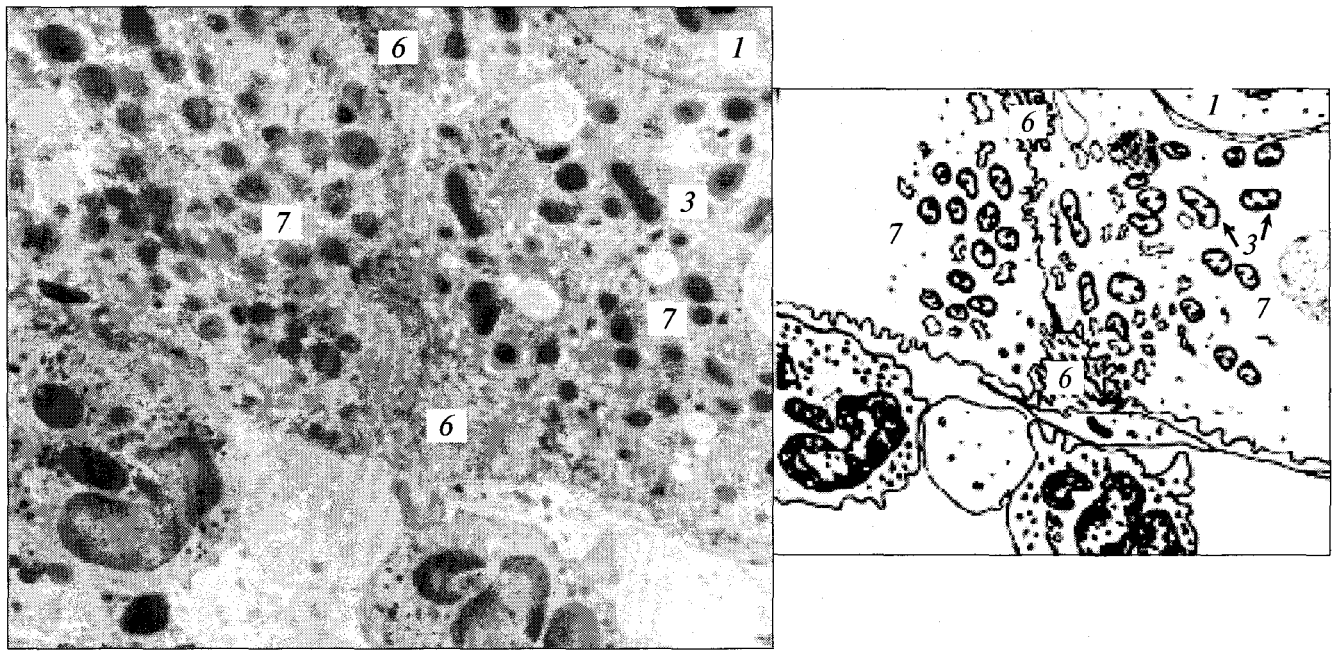


Fig. 3. Two neutrophils nearby collagen fibrils between hepatocytes in regeneration hepatoma after 10 months of CCl_4 poisonong, ×3000).

neutrophils with cytoplasmic processes (pseudopodia) extended toward collagen fibrils (Fig. 3). Granulocytes secrete collagenase and perform extracellular lysis of collagen. Collagenase from polymorphonuclear leukocytes more actively (15-fold) hydrolyse type I than type III collagen, while collagenase from fibroblasts and macrophages displays no such selectivity [35]. Collagen is lysed more rapidly in the presence of a considerable number of inflammatory cells [2]. During fibrosis, neutrophils surround collagen fibrils which attests to their involvement in collagen lysis.

In regeneration hepatomas, DS contained swollen collagen fibrils adjacent to hypertrophied KC with giant Golgi apparatus. It should be emphasized that at all periods of examination of regeneration hepatomas (up to 13 months), Golgi apparatus of KC was sharply enlarged. KC are the main source of hepatic collagenase [16,32]. This enzyme is probably accumulated in hypertrophied Golgi apparatus of KC in regeneration hepatomas, then released into sinusoids, and causes partial lysis of collagen fibrils during fibrosis (especially at the late stages). Our findings agree with the data showing that collagenase activity in the liver during CCl_4-induced cirrhosis increased compared to intact animals [38].

There are intra- and extracellular pathways of collagen lysis [21,36]. The intracellular pathway comprises phagocytosis of collagen fibrils and their degradation in lysosomes. Activated fibroblasts and macrophages synthesizing collagenase and phagocytizing native collagen fibrils possess phagocytic properties [21,35]. To maintain homeostasis in the connective tissue, biosynthesis and catabolism of collagen performed by fibroblasts are in equilibrium [21,24,39] due to antagonistic regulation of their functions [5,17], when the cell produces biologically different and functionally antagonistic substances. In particular, fibroblasts can behave as fibroclasts.

Extracellular lysis and resorption of collagen by hepatocytes and KC became most intensive after termination of CCl_4 poisoning and partial hepatectomy. During CCl_4 poisoning, KC and granulocytes were the main sources of collagenase. After termination of CCl_4 poisoning and partial hepatectomy, hepatocytes appeared to be involved in collagenase secretion, which was confirmed by electron microscopy and histochemical examination [13-15].

Our findings and results of other authors indicate that hepatocytes and KC synthesize [4,28,37] and lyse [1,3,7,8-15] fibrillar proteins, which reflects antagonistic regulation of functions, the general biological feature and a part of the multistage (nervous, hormonal, and humoral) regulation of homeostasis [5, 17,20]. Antagonistic cells were found in various organs [17,20]. Interrelations between the stroma and parenchyma are normally strictly regulated. Under these conditions the synthesis of fibrillar proteins by KC, parenchymal, and Ito cells is inhibited. Disturbances in this system (CCl_4 poisoning) impair this regulatory mechanisms and lead to "the appearance of previously repressed degrees of freedom and certain autonomy" [6]. "Proliferation of connective tissue in chronic diseases is not simple substitution of dead parenchymal cells, but the result of formation of connec-

tive tissue elements due to the absence of inhibitory effects of parenchymal structures" [6]. There are many factors involved in and stimulating fibrogenesis (transforming growth factor β_1 and platelet-derived transforming growth factor) [25,28]. After termination of CCl_4 administration and partial hepatectomy, collagen fibrils are intensely resorbed in cirrhotic liver probably due to activation of reparative processes (intracellular regeneration of hepatocytes and production of collagenolytic enzymes and metalloproteinase activators by hepatocytes, KC, Ito cells, and neutrophils). Thus, cirrhotic and fibrotic lesions can regress after cessation of damaging influences. The synthesis and lysis of collagen occur simultaneously if adverse factors act continuously (as in regeneration hepatomas), but fibrosis dominates over resorption and aggravates the disease. In this case, the course of the disease depends on the strength of damaging agents [19].

Collagen resorption by vaginal epitheliocytes [26], vascular endotheliocytes [40], and decidual cells [42] was described by other authorities. Therefore under certain conditions (that remain to be evaluated), epithelial cells of various origins can lyse collagen and maintain normal interrelations between the stroma and parenchyma.

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