growing volumetric share of secondary lysosomes in hepatocytes, endotheliocytes, and Kupffer's cells in iron-deficiency anemia creates a high background of lysosomal enzymes in the blood plasma [6] and is a risk factor during cell exposure to membranotropic and lysosomotropic agents; therefore, the use of lysosomotropic drugs containing iron ions characterized by prooxidant properties in the treatment of patients with iron-deficiency anemia [10] may exacerbate cell membrane damage and stimulate the development of destructive processes.

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Morphological Criteria of Reduced Permeability and Trophism of Cirrhotically Changed Rat Liver and their Increase under the Influence of Pyrogenal and Pyridoxal Phosphate (Morphometry and Transmission and Scanning Electron Microscopy Findings)

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Lack of data on fine microcirculatory changes in the liver in cirrhosis [10,12] and on the pathogenetic role of hypoxic ultrastructural disorders dictates the need to seek the origin of the microcirculatory disorders occurring in the organ in this disease. Development of research in this field using morphometry and transmission and (especially) scanning electron microscopy is impeded by insufficient knowledge of drug effects on the permeability and trophism of the cirrhotically altered liver.

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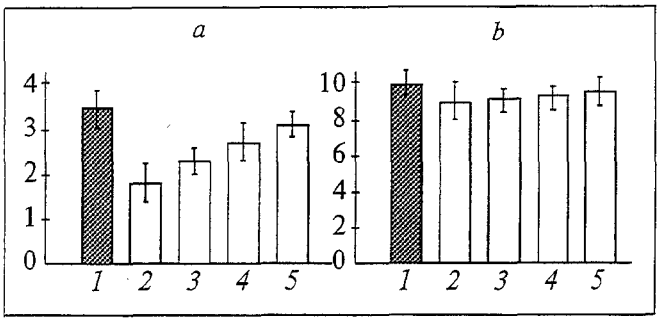


Fig. 1. Relative sinusoid (*a*) and sinusoidal cells (*b*) volumes (in %) on semithin epon slices of rat liver. *1*) intact control rat liver; *2*) rat liver in CCl_4–induced cirrhosis; *3*) cirrhotic rat liver 20 days after CCl_4 discontinuation; *4*) cirrhotic liver of rats injected pyrogenal for 20 days after CCl_4 discontinuation; *5*) cirrhotic liver of rats injected pyridoxal phosphate for 20 days after CCl_4 discontinuation.

This circumstance prompted us to undertake the present investigation using a complex of methods aimed at examining changes in the sinusoids, stromal atypical carcass, and parenchyma of the cirrhotic liver under the influence of pyrogenal, a tissue permeability activator and antifibrotic agent [7], and pyridoxal phosphate, an activator of energy substrates and amino acid metabolism in hepatocytes and a stimulant of liver detoxifying properties [8] in CCl_4-induced cirrhosis of the liver.

MATERIALS AND METHODS

Male Wistar rats weighing 180 to 200 g were used in experiments. Cirrhosis of the liver was induced by chronic inhalation exposure to CCl_4 (twice a week, 4 h a day for 6 week) in hermetic chambers 400 liters in volume with a CCl_4 concentration in the chamber air of 200 mg/liter [11]. For 20 days after CCl_4 discontinuation, when all symptoms of cirrhosis were already evident [2,5,6], pyrogenal (in a dose of 15 MAP (membrane action potential) per 100 g body weight) or pyridoxal phosphate (in a dose of 2 mg/100 g body weight) was intramuscularly injected to rats 8 times. Controls were liver samples from intact rats kept in similar experimental chambers without CCl_4 for the same period and cirrhotic liver samples from rats similarly exposed to CCl_4 but administered no drugs 2 and 20 days after CCl_4 discontinuation. Liver samples for transmission and scanning electron microscopy were treated by standard routine methods. For scanning electron microscopy the samples were dehydrated by freeze-drying [9] and shadowed with carbon and gold. Ultrathin slices prepared with an LKB-8800 ultratome were studied after double contrast staining under a JEM-7A electron microscope and the samples for scanning under a JEM-100S/SEG electron microscope with an ASID-5 scanning attachment. Using a quadratic test system [13], the relative sinusoid and sinusoidal cell volumes and the counts of these cells in a 80×80 μ test area were determined on semithin epon slices stained with toluidine blue.

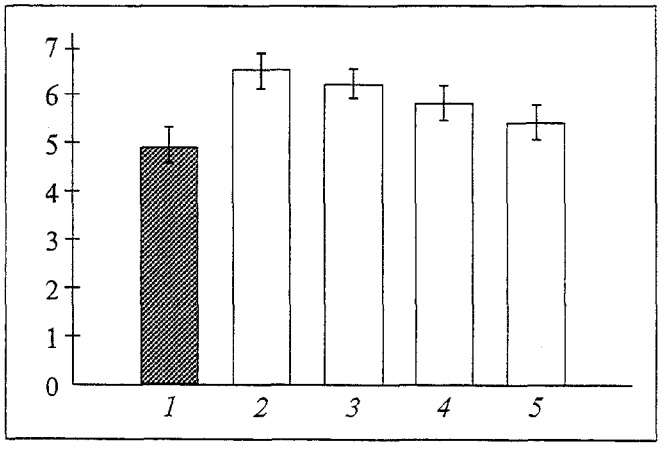


Fig. 2. Number of sinusoidal cells in a test area on semithin epon slices of rat liver. Notation as in Fig. 1.

RESULTS

Morphometric and ultrastructural study of the liver of control rats in inhalation chambers kept without CCl_4 showed no morphological shifts in comparison with intact rat liver. Morphometric examination of semithin epon slices of cirrhotic liver showed a reliable (by more than one third) reduction of the total relative sinusoidal volume (Fig. 1, *a*)., markedly differing from the control even 20 days after the exposure, this undoubtedly reducing the trophics and oxygenation of the organ. Moreover, the relative volume of sinusoidal cells was noticeably reduced (Fig. 1, *b*) and their count increased (Fig. 2) in cirrhotic liver; these two phenomena might be interrelated in view of the reduced blood supply to the endothelial cells on account of the deteriorated microcirculation and, possibly the concomitant compensatory increase in the counts of endotheliocytes or Kupffer's cells. Kupffer's cells are known to migrate under certain conditions as precursor monocytes and to become incorporated in the sinusoidal wall or to proliferate. The manifest reduction of the microcirculation augmented the observed reduction of the number of fenestrae and pinocytic vesicles in endothelial cells. Visually a clear-cut difference was observed between the sizes of sinusoids of the multilobular pseudolobules, where they were sharply narrowed, and of regenerate monolobular nodules with widened sinusoids (Fig. 3, *a*). Moreover, evident differences were seen in the architectonics of the pseudolobule hepatocyte surface microrelief (Fig. 3,

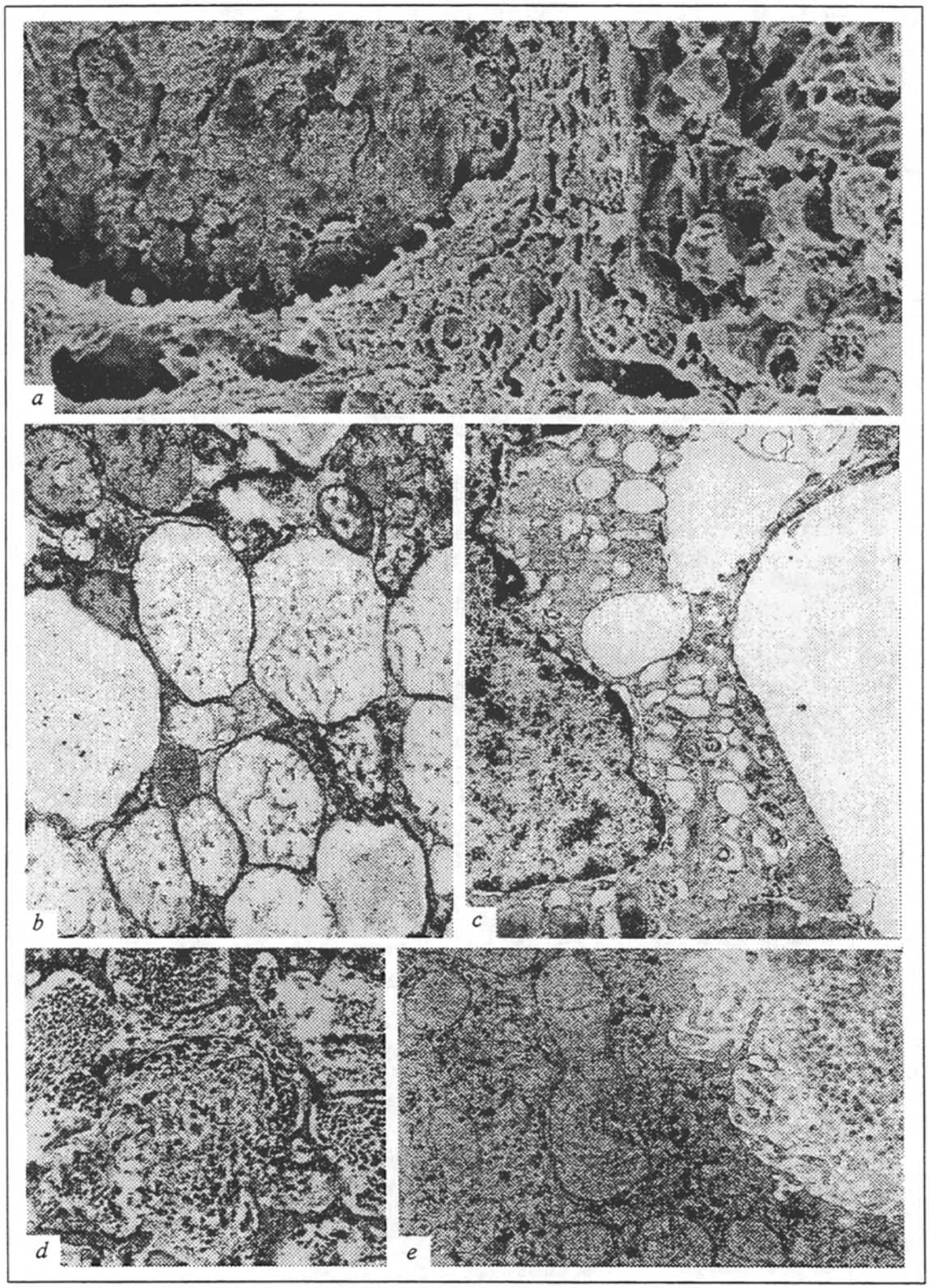


Fig. 3. Ultrastructure of cirrhotic rat liver 2 (*a–d*) and 20 days after discontinuation of CCl_4 inhalations. *a*) scanning electron microscopy, *b–e*) transmission electron microscopy; *a*) pseudolobular (left) and regenerate nodule (right) fragments separated by a septum with abnormal pseudolobular shunting vessels (bottom), ×1120; *b*) fragment of pseudolobular prenecrotically altered hepatocyte, ×20,000; *c*) destructively altered pseudolobular endotheliocyte, ×14,000; *d*) fragment of pseudolobular septum with densely packed connective tissue elements and abundant collagen, 36000; *e*) fragment of pseudolobular hepatocyte with signs of spontaneous repair of ultrastructures; collagen lysis in Disse's space, ×20,000

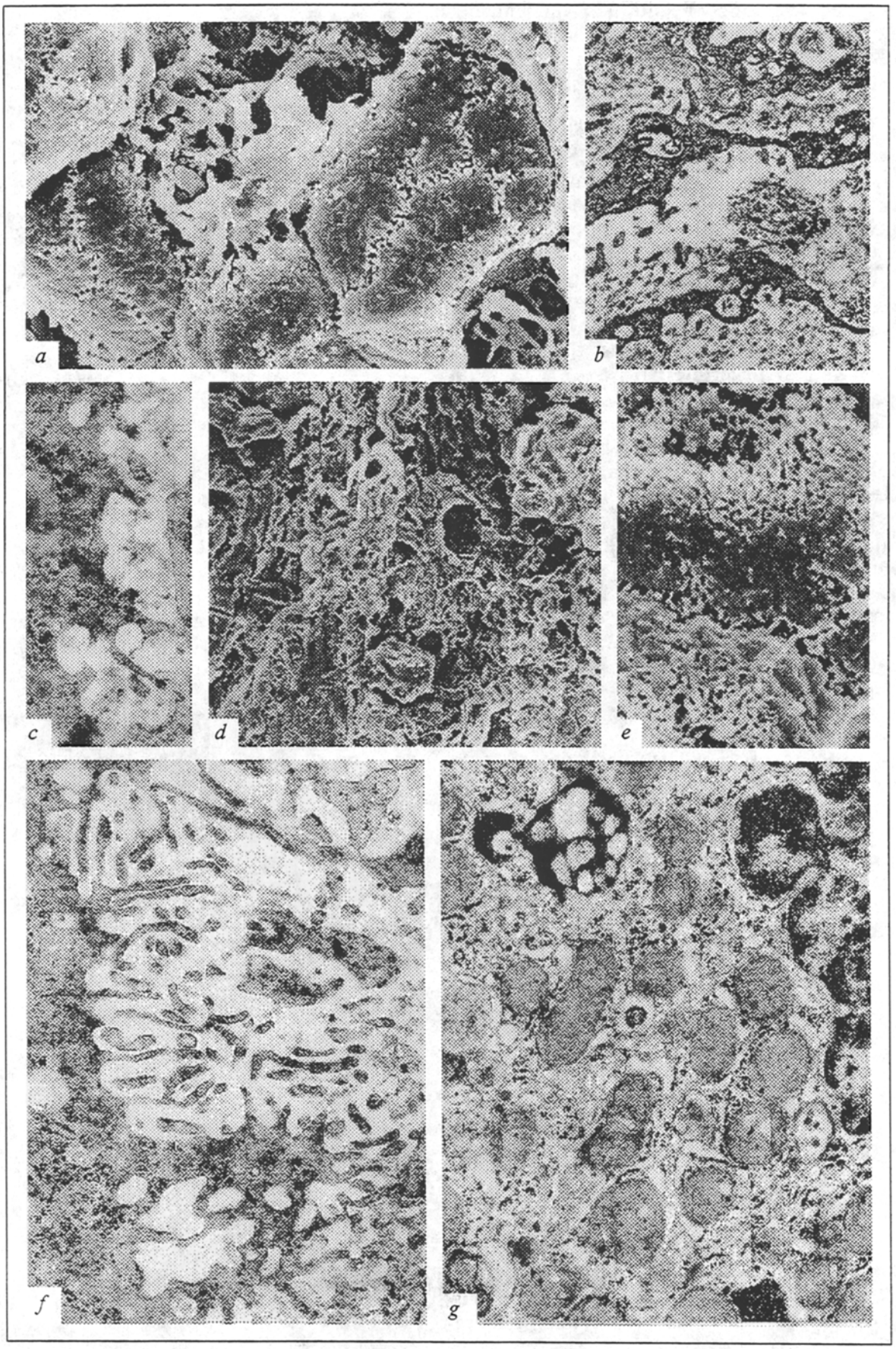
a) which characterized the leveling of the hepatocyte surface in pseudolobules and the wavelike relief of the adjacent plasmalemmas, as well as the manifest microvilli of hepatocyte exchange in regeneration nodules. Marked deterioration of the microcirculation led to the frequent detection in multilobular pseudolobules of hepatocytes with swollen and partially destroyed mitochondria (Fig. 3, *b*), with solitary cristae in their martix and calcium phosphate accumulations indicative of prenecrotic changes in the cells [4] shown by electron contrast examination. Evidently destructive hypoxic changes in the ultrastructure of some endotheliocytes were seen with marked impairment of the endoplasmic reticulum and mitochondria (Fig. 3, *c*). Pinocytic vesicles, transendothelial metabolism indicators, were rarely detected in such endotheliocytes. Evidently, destruction of some of the endothelial cells resulted in breaches in the sinusoidal lining, and the presence of these breaches may be connected with the reduced angiogenic potential of the cirrhotic liver commonly capable of rapidly liquidating such breaches. No doubt, impairment of the hematoparenchymatous barrier may result in exosure to numerous blood factors disturbing liver homeostasis and the immune status. It is important to note that the well-known sign of liver cirrhosis observed in such cases, sinusoidal capillarization, that is, formation of basal membranes in sinusoids which are commonly absent in rats, becomes a compensatory adaptive phenomenon "closing" the breaches forming in the sinusoidal lining. Local swelling are as a rule observed in bypassing vessels of the pseudolobular septa (Fig. 3, *a*), these swelling indicating alternations in vascular diameter that evidently impair laminar blood flow in these vessels, possibly leading to cavitation injuries of the endotheliocyte plasmalemmas. The septa were characterized by densely grouped connective tissue elements (Fig. 3, *d*) indicative of the degree of liver fibrosis.

Hence, the totaly of morphometric and subcellular findings in cirrhotic liver indicates the principal pathogenetic role in cirrhosis to be a drop of the microcirculation level, leading to hypoxic and atrophic injuries in both stromal and parenchymal cells and augmenting the intrahepatic shunting characteristic of the cirrhotic liver [5,6]. Discoordination of intracellular repair in some endotheliocytes of cirrhotic liver blood vessels and, possibly, a reduced level of their proliferation, resulting in impairment of the hematoparenchymatous barrier, appear to be the initiating and ground-laying elements in the development of a chronic inflammation followed by cirrhotic degeneration of the organ. Portocaval blood flow shunting in the liver forming as a result of parenchymatous necrosis and septal fibrosis appears only to aggravate the development of cirrhosis of the organ.

Twenty days after CCl_4 was discontinued a reliable increase of sinusoidal relative volume was observed in the cirrhotic liver (Fig. 1, *a*) which seemed to reflect an improvement of the blood supply to the liver in view of the absence of hepatotropic toxin, the sinusoidal cell volume being unchanged (Fig. 1, *b*) and the count somewhat reduced (Fig. 2). The improved microcirculation seemed to be conducive to reducing the liver parenchyma necrosis seen on semithin slices, the degree of liver fibrosis, and the number of pseudolobules. Increased oxygenation and trophism of the liver promoted positive subcellular shifts in the structure of pseudolobular hepatocytes (Fig. 3, *e*). Besides an increase in the number of mitochondrial cristae, individual glycogen granules appeared in these cells which were absent in cirrhotic liver pseudolobule cells immediately after CCl_4 exposure was discontinued; elongated microvilli appeared on cellular exchange poles and surfaces facing Disse's spaces (Fig. 3, *e*). Detection in these cells of collagen fibrils with blurred profile and of floccular material (Fig. 3, *e*) seems to be a result of cell lysis. These features detected in the liver 20 days after CCl_4 discontinuation appear to reflect the initial manifestations of reversible changes in the atypical ogran and of its spontaneous repair directly associated with blood supply improvement.

A course of pyrogenal treatment administered to cirrhotic rats during 20 days after the posttoxic period of the experiment led to an increase in the relative volume of liver sinusoids (Fig. 1, *a*), reduced the count of sinusoidal cells (Fig. 2), and extended the hepatocyte intercellular spaces (Fig. 4,

Fig. 4. Changes in cirrhotic rat liver ultrastructure under the influence of courses of pyrogenal (*a–c*) and pyridoxal phosphate (*d–g*) therapy administered for 20 days after discontinuation of CCl_4 inhalations. *a, d, e*) scanning electron microscopy; *b, c, f, g*) transmission electron microscopy. *a*) improved architectonics of pseudolobular hepatocyte surface ultrastructure with expansion of intercellular spaces; sinusoid dilatation and enhanced fenestration of walls, ×5000; *b*) fragment of pseudolobular loosened septum with signs of collagen lysis in septal intercellular spaces, ×6000; *c*) hepatocyte fragment in exchange pole zone with cytophagic vacuoled, ×20,000; *d*) ingrowth of individual and trabeculae–arranged hepatocytes and of sinusoidal swellings into loosened pseudolobular septa, ×1000; *e*) extensive growth of microvilli forming craterlike structures on pseudolobular hepatocyte exchange poles, ×8000; *f*) fragment of pseudolobular hepatocyte exchange pole with numerous elongated microvilli, ×20,000; *g*) fragment of pseudolobular hepatocyte with subcellular signs of ultrastructure recovery, ×20,000.


a
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a). Marked widening of sinusoids and enhanced fenestration of their walls in reticular plate zones were observed. Pseudolobular septa disintegrated, and collagen lysis was observed in septal intercellular spaces (Fig. 4, *b*) and Disse's spaces with collagen flocculation (Fig. 4, *c*). The number of pseudolobules was noticeably reduced and that of regeneration nodules increased in semithin slices of the liver. The pseudolobular hepatocyte surface became less folded and the cell surface ultrastructural architectonics improved; specifically microvilli at cellular exchange poles grew longer (Fig. 4, *a*) and numerous cytophagic vesicles appeared (Fig. 4, *c*). Signs of pseudolobular hepatocyte subcellular repair became even more manifest than in control rat liver. It is noteworthy that pyrogenal administration after chronic CCl_4 exposure raised the serum albumin level and decreased the β-globulin content, thus stimulating regeneration processes [1]. The observed increase in the number of pinocytic vesicles in endotheliocytes was indicative of improved transendothelial metabolism. Activation of the above-mentioned subcellular phenomena is known to indicate an enhanced supply of plastic materials to the cells, that is, it reflects improved trophism of both the parenchymal and stromal cells. Improvement of the microcirculation conditions in the liver appeard to increase the oxygen supply to the hepatocytes, this boosting the metabolic processes and resulting in the activated resorption of excess collagen. It should be noted that thinning of the connective tissue cords around the pseudolobules was observed in rats treated with pyrogenal after a three-month exposure to CCl_4 [1].

A course of pyridoxal phosphate had a still more maarked effect on the recovery of cirrhotic liver terminal vessels and parenchymatous cell ultrastructure. Administration of this drug resulted in an even more pronounced increase of sinusoidal relative volume (Fig. 1, *a*) and almost normalized the count of sinusoidal cells (Fig. 2). Sinusoids and hepatocytes grew into loosened pseudolobular septa, possibly accounting for both the increase in the volume of sinusoids by activation of terminal angiogenesis and the participation of the organ parenchyma in stromal excess resorption. The detected changes in the structure of collagen, whose fibrils had a variable diameter and a feltlike structure not only in Disse's spaces but in the pseudolobular septa as well, confirmed this hypothesis (Fig. 4, *d*). The frequent detection in endotheliocytes of centrioles, mitosis organelles, also appears to reflect endothelial hyperplasia. Numerous folds and processes on the endotheliocyte surface and the presence in them of multiple pinocytic vesicles and vesicles forming transendothelial channels demonstrated a still higher intensity of transendothelial metabolism than under the influence of pyrogenal. The detected volumetric and spatial characteristics of collagen fibers in the gaps between cells were indicative of their primary extracellular lysis in the process of resorption, this confirming previous data [3]. Intensive development of microvilli forming "craters" (Fig. 4, *e*) with elongate microvilli and their conglomerates (Fig. 4, *f*) at the exchange poles of some pseudolobular hepatocytes was a manifestation of stepped-up metabolims. Such hepatocytes were characterized by folded nuclear membranes (Fig. 4, *g*), this being typical of higher nuclear-cytoplasmic ratios. On the subcellular level these cells showed signs of ultrastructure repair, expressed in abundant autophagosomes and secondary lysosomes with fragments of impaired organelles, the presence of reduced lipid droplets, the appearance of elongated cristae in the mitochondria, hyperplasia of Golgi apparatus components, and the appearance of polysome accumulations around cell nuclei (Fig. 4 , *g*).

Hence, cources of pyrogenal or pyridoxal phosphate treatment resulted in the repair of blood terminals, and an increase of permeability and trophism of the cirrhotically altered liver. These drugs to varying extents reduced the degree of structural atypism of the organ and helped eliminate atrophic and hypoxic subcellular disturbances, making the cirrhotic changes in the liver reversible. These data confirm the key pathogenetic role of microcirculatory disorders in the development of cirrhosis of the liver.

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