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RELATIONSHIP BETWEEN STRUCTURE, MICROCIRCULATION, AND TISSUE VASCULAR PERMEABILITY OF THE LIVER IN EXPERIMENTAL TOXIC HEPATITIS

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Biomicroscopic investigation of the liver showed that in experimental toxic hepatitis induced by heliotrine, very early and marked disturbances of the microcirculation arise, before injury to the parenchyma. Progression of the disturbances of the microcirculation and tissue vascular permeability, leading to hypoxia of the liver tissue and disturbance of liver metabolism, play a dominant role in the intensification of the pathomorphological changes and conversion of the acute pathological process in the liver into chronic.

KEY WORDS: *Microcirculation; tissue vascular permeability; toxic hepatitis; heliotrine.*

The object of this investigation was to study some mechanisms of the formation and development of the liver lesions during poisoning by heliotrine, which produces a picture very similar to that of epidemic hepatitis in man [1-6].

EXPERIMENTAL METHOD

Experiments were carried out on 115 albino rats of both sexes weighing initially 130-200 g. Heliotrine was given as a single subcutaneous injection in a dose of 25 mg/100 g body weight. Tests were carried out during the first 3 h, after 24, 48, and 72 h, and also after 7, 10, 20, 30, and 90 days. The microcirculation of the liver was studied by intravital luminescence microscopy [8], tissue vascular permability by injection of luminescent serum (0.7 ml intravenously), and the diameter of the microvessels by scanning photometry [3]. Liver sections were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS AND DISCUSSION

A uniform dilatation of both terminal afferent vessels and sinusoids and also of the hepatic and collecting venules was observed 10-15 min after injection of heliotrine. Later the vasodilatation increased and the blood flow was slowed. The state of aggregation of the blood cells (a juxtamural arrangement of the leukocytes was observed in the microvessels after 40-50 min) and the tissue vascular permeability showed parallel changes. Juxtamural ad-

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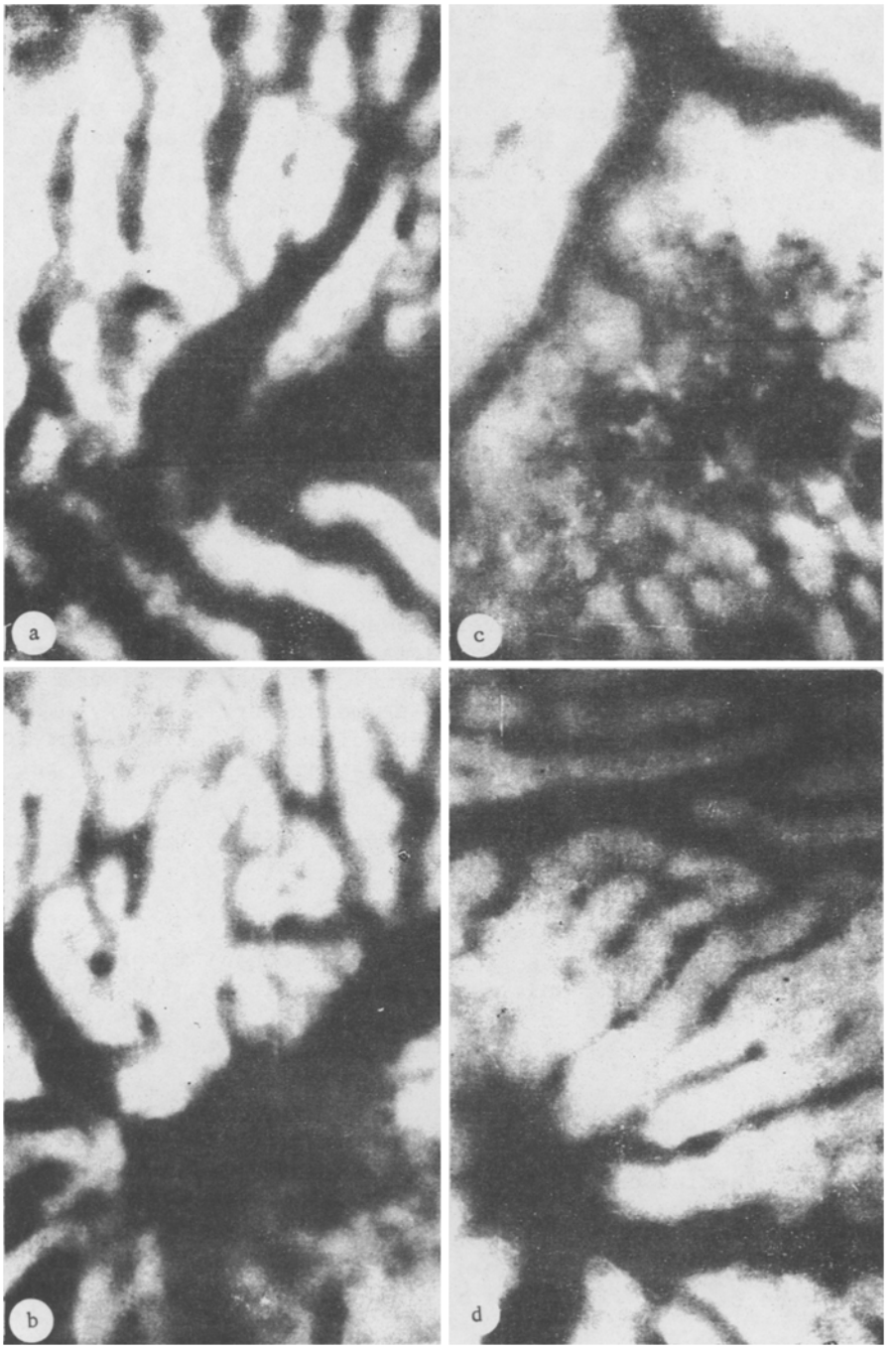

Fig. 1. Biomicroscopic picture of liver in experimental toxic hepatitis (75×): a) 3 h after injection of heliotrine; b) 24 h, c) 72 h, d) 30 days after injection. Remainder of legend in text.

hesion of the cells together with intravascular aggregation of the erythrocytes, sharply reduced the lumen of the vessels; in some parts of the microcirculation belonging to afferent terminals and sinusoids movement of the blood was to-and-fro in character, and after 180 min the blood flow in these areas ceased completely (Fig. 1a). After injection of luminescent serum (60 min after injection of heliotrine) the outlines of the blood vessels became clear, the blood cells gave intense emerald green fluorescence, and after a further 15-20 min fluorescent granules were retained in the terminal afferent vessels and sinusoids. Granularity of this type was detected over a considerable extent of the capillaries, and after 60-90 min outside the capillaries also. Pathomorphologically marked circulatory and degenerative-inflammatory changes were observed after 3 h (Fig. 2a).

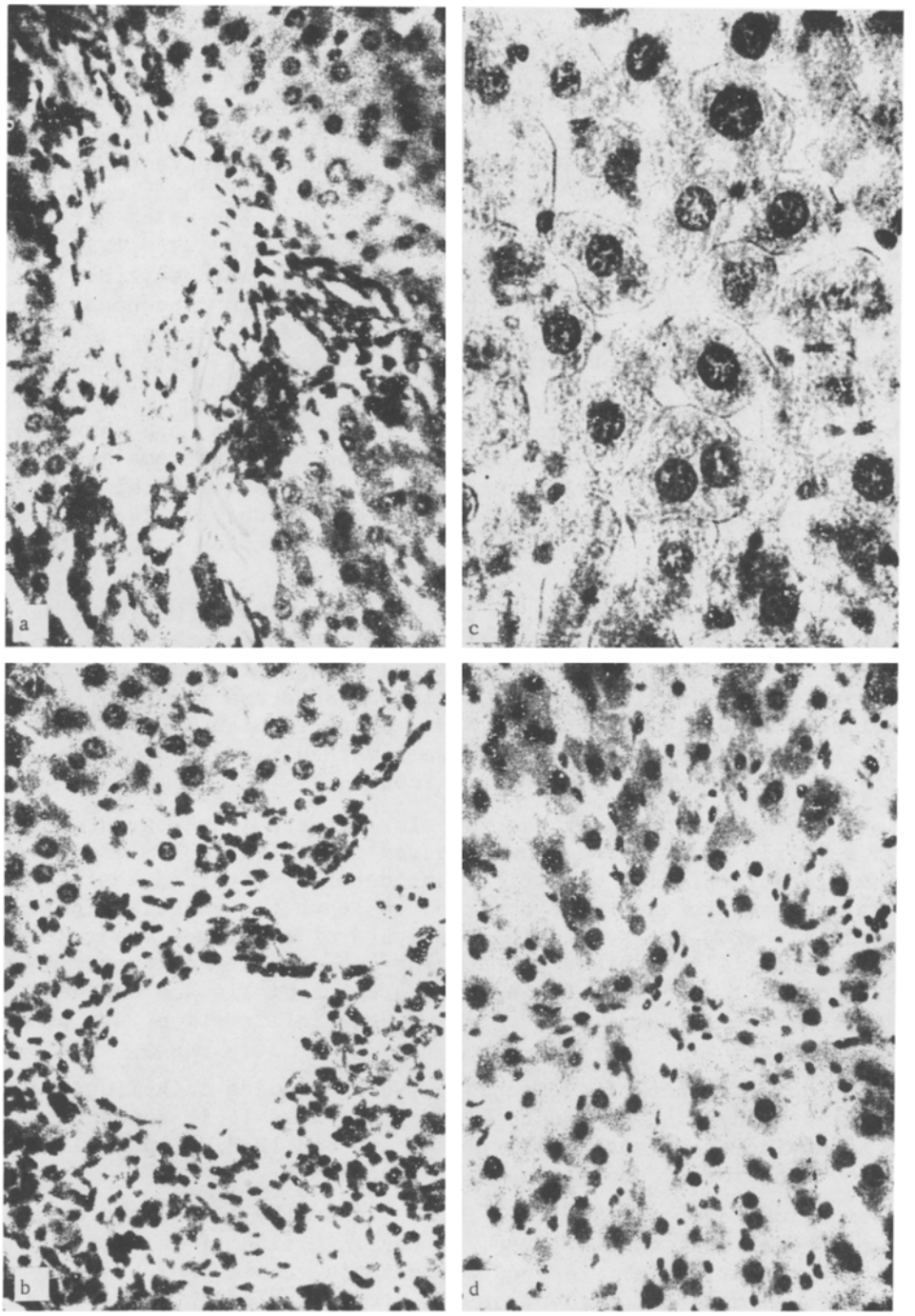

Fig. 2. General morphological picture of liver in experimental toxic hepatitis: a) 3 h after injection of heliotrine (200×): b) 24 h (200×), c) 72 h (400×), and d) 90 days (200×) after injection. Hematoxylin—eosin. Remainder of explanation in text.

After 24 h most vessels of the liver were dilated and congested with blood, the blood flow was considerably slowed, and stasis was observed in some places, where it was most probably due to intravascular aggregation of the blood cells and microhemorrhages. In some areas near the hepatic venules the number of functioning capillaries was considerably reduced. In the hepatic and collecting venules the blood flow was considerably slowed, their diameter was increased, and the juxtamural layer appeared edematous (Fig. 1b). Labeled proteins were observed leaving the region of the terminal afferent vessels and appearing in the hepatocytes. Morphologically the picture was one of acute hepatitis. Circulatory disorders (stasis of blood, rupture of blood vessels, and hemorrhages into the stroma) increased in

severity. Massive foci of lymphohistiocytic infiltration appeared around the vessels and bile ducts, the trabecular structure was disturbed, and marked signs of granular and vacuolar degeneration developed (Fig. 2b).

After 48-72 h the vascular pattern in some areas became indistinguishable because of foci of hemorrhage; in other areas only slowing of the blood flow was observed (Fig. 1c). Around the hepatitic venules areas which had lost the autofluorescence and shape characteristic of intact hepatocytes were found and the number of functioning capillaries was greatly reduced. Between 30 and 50 min after injection of the luminescent serum, the areas which had lost the characteristic pattern of the liver were permeated with labeled protein. Infiltration with lymphocytes, histiocytes, and plasma cells increased in intensity, and the trabeculae lost their complex structure and became fragmented. The hepatocytes were greatly swollen and showed clear signs of granular and vacuolar degeneration. Cells located near the central veins were in a state of necrobiosis. The Kupffer cells were swollen and desquamated (Fig. 2c).

On the 7th-20th day of the experiment these changes became more marked still and there were extensive areas of hemorrhages with disintegration of the stroma and death of the hepatic parenchyma (hemorrhagic necrosis) in the portal tracts and within the lobules; in other areas most of the hepatocytes were in a state of necrobiosis and necrosis or of granular and vacuolar degeneration. Meanwhile binuclear hepatocytes and mitotic figures also appeared.

By the 30th day the signs of stasis in the microcirculation had subsided a little although the walls of the microvessels were still infiltrated and tortuous, and areas with microhemorrhages still persisted (Fig. 1d). In the hepatitic and collecting venules the blood flow was improved but infiltration of their walls became still more marked. The diameter of the collecting venule was 182.4 ± 3.9 μ compared with 150.7 ± 10.1 μ in the control ($P < 0.02$). Morphologically there was a well-marked picture of chronic hepatitis with disturbance of the trabecular structure of the lobules.

On the 90th day of the experiment the vascular pattern in the greater part of the liver was restored and a fairly rapid blood flow appeared in most of the microvessels, although in some areas the microcirculation was still considerably disturbed. After injection of luminescent serum labeled proteins were observed to leave the vessels into the areas of destruction surrounding them. The trabecular structure of the liver was restored, although many hepatocytes were still in a state of vacuolar and granular degeneration, while those located around the central veins were in a state of necrobiosis and necrosis (Fig. 2d). Here and there in the portal tracts foci of round-cell infiltration were present; the Kupffer cells were proliferating.

These results indicate that microcirculatory disturbances in heliotrine hepatitis precede damage to the parenchyma. The endothelium of the vessels is evidently disturbed first of all, thereby affecting the transcapillary exchange and leading to a disturbance of intracellular metabolic, functional, and structural processes.

These investigations confirm the view [10, 12] that the elementary acinus is an active functional and structural unit of the liver. After injection of heliotrine the greatest changes in fact took place initially around the hepatic venule, which corresponds to zone III of the elementary acinus. Impairment of the microcirculation led to changes in zones II and I of the acinus. When, however, the pathological process in the liver was subsiding a little (on the 90th day of the experiment), hepatocytes in a state of necrobiosis and necrosis were still present in zone III. These results suggest that the phenomena of the hemorrhagic diathesis and of ascites (in the last stages of the disease) observed clinically in patients with heliotrine hepatitis are connected with a disturbance of the microcirculation in the organs of the portal system and, above all, in the liver.

The initial role of microcirculatory disturbances in the development of some forms of toxic liver lesions has also been emphasized by other workers [9, 11, 13].

The worsening of the pathomorphological picture of the liver and the conversion to a chronic pathological process are evidently connected with progressiveness of the disturbances of the microcirculation and of tissue vascular permeability, leading to hypoxia of the hepatocytes [4, 7], disturbance of the energy cycles [2] and of metabolism, with the formation of incompletely oxidized products and of biologically active substances [5].

It will be clear from analysis of these data that the changes arising in heliotrine toxic hepatitis are phasic in character. The first phase is characterized by transient spasms followed by dilatation of the microvessels and disturbance of the cell membranes, i.e., by a disturbance of the intrahepatic circulation at the level of the elementary acini, by increased permeability of the cell membranes, and by the development of circulatory-histotoxic hypoxia, leading to discoordination of intracellular metabolism. The reactions of the microvascular system of the liver were local (since the animals were anesthetized) nervous-reflex in character. The increase in severity of the microcirculatory disturbances was evidently due to the action of products of disturbed metabolism. Considering the extreme shortness of the time interval between development of the disturbances of permeability of the membranous structures and of the microcirculation, this phase can be called "membrane-microcirculatory." The second phase (true alteration) was one of injury to the structure of the intracellular organelles and of the cell as a whole (degeneration—necrobiosis—necrosis), with disturbance of the synchronized activity of the hepatocytes and discoordination of intercellular interrelationships, and the third phase ("terminal") was one of total (over 70% of the volume) functional—structural injury, complete death of the parenchyma of the organ, its exclusion from the life cycle, and its replacement by connective tissue.

Structural changes in the organ naturally may also take place simultaneously, but the distinction of three phases is based on the order and degree of severity of the various structural and functional changes.

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