action of acetylcholine affects not only potassium permeability. It has recently been found that acetylcholine can also affect the slow sodium–potassium current in pacemaker cells [4], but no systematic investigations of this aspect of the action of acetylcholine have yet been undertaken. Further studies are needed to explain the internal mechanism of the opposite cholinergic influences on the heart.

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DISTURBANCE OF HEPATOCYTE MORPHOLOGY AND FUNCTION AND OF THE HEPATIC MICROCIRCULATION DURING LONG-TERM EXTRACORPOREAL CIRCULATION IN DOGS

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A tendency has recently been noted for the function and structure of individual organs and systems to be studied more closely in connection with extracorporeal circulation. However, the state of the liver has received unjustifiably little attention in these investigations.

Meanwhile, a long-term extracorporeal circulation is by no means without its effect on the organism and often leads to dysfunction of the liver [2-4, 6, 9]. Even an adequate artificial circulation may be accompanied by disturbances of the hepatic and, in particular, the portal blood flow [3].

Activity of the liver is determined mainly by its blood supply [5]. Most investigators note the development of a "homologous blood" syndrome in dogs, manifested as a rise of portal pressure and the almost total cessation of the outflow of blood from the liver [4, 6, 7]. This phenomenon also takes place in man, although it is much less marked [6, 9].

The data mentioned above, and also the inadequate information in the literature on metabolism of the liver during long-term extracorporeal circulation, are responsible for the increased interest in this problem.

The object of this investigation was to study morphological, functional, and enzymic changes in the dog liver during long-term extracorporeal circulation.

EXPERIMENTAL METHOD

Seven adult male dogs were used. Extracorporeal circulation was formed in four animals in the usual way by an apparatus with a counterflow foam-film oxygenator and roller pump for a period of 3 h. The apparatus was filled with fresh donor's blood and gelatinol

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with a hemodilution of 25%. The volume velocity of perfusion was maintained at the rate of 2.4 liters/m² body surface. Three intact dogs under general anesthesia (without extracorporeal circulation), served as the control. Morphine-hexobarbital and intubation anesthesia (halothane with oxygen — 2 vol.%) were used for anesthesia.

The liver function was assessed by Sergeeva's function test (Author's Certificate 547209), which is based on injection of a vital dye (neutral red) into the system of the portal vein. By this test the degree of damage to the liver parenchyma and microcirculatory disturbances in the organ could be characterized qualitatively and quantitively, and changes in pH could also be recorded. For morphological study pieces of liver were fixed in 10% formalin and sections were stained with hematoxylin and eosin. Activity of succinate dehydrogenase (SDH) (by the method of Quaglino and Hayhoe) and of acid (AcP) and alkaline (AlP) phosphatases, by the method with azotol* phosphate, also were determined in cryostat sections 7 μ thick. Activity of the enzymes was estimated on the 4-Microvideomat television apparatus, controlled by a 720 C computer. Parallel with the above, during the extracorporeal circulation the morphological and biochemical changes in the peripheral blood were studied.

EXPERIMENTAL RESULTS

After the extracorporeal circulation had been functioning for 3 h, marked disturbances of the microcirculation were found in the liver. In extensive areas of the liver venous congestion was observed. The diameter of the sinusoidal capillaries was increased, frequently they were club-shaped, indicating irregular filling of the vessels with blood. A sludging syndrome was observed in many sinusoids. The data on accumulation of neutrophilic leukocytes in the intralobular hepatic capillaries deserve attention. They were diffusely arranged in the liver in chains, filling the lumen of the sinusoids, or they formed leukocytic thrombi (Fig. 1). Vessels surrounded by a halo of neutrophilic leukocytes, as a result of their migration through the vessel walls, could also be observed. These findings are in harmony with marked neutrophilic leukopenia and they can be explained by disturbance of the rheologic properties of the blood and accumulation of a large proportion of the leukocytes in hepatic microvessels.

Some vessels were seen which were filled entirely with plasma, with hardly any blood cells, evidence of slowing of the blood flow. In two dogs the plasma was brownish in color.

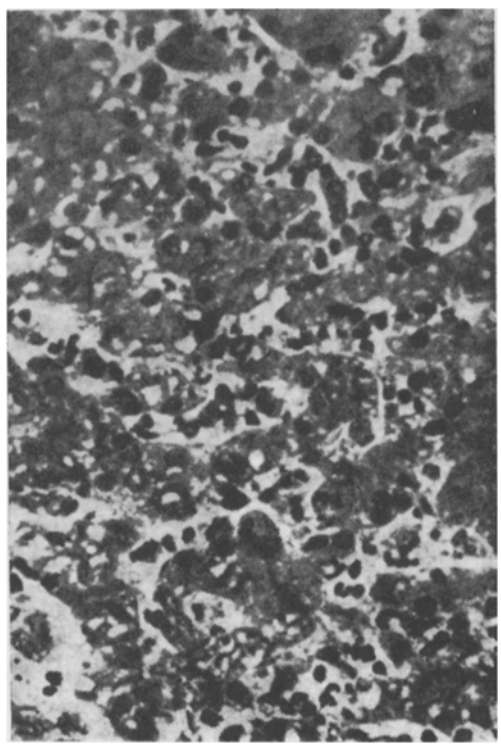

Fig. 1. Accumulation of neutrophilic leukocytes in sinusoidal capillaries of liver. Here and in Fig. 2: hematoxylin—eosin, 100 ×.

*β-hydroxynaphthoic anilide.

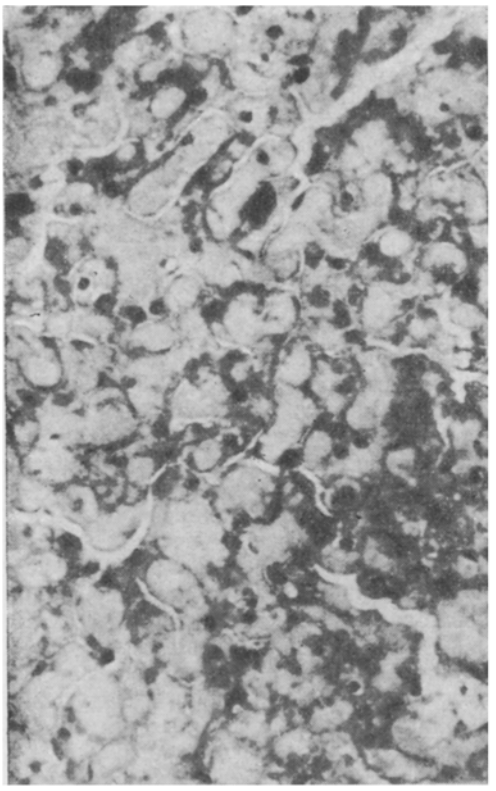

Fig. 2

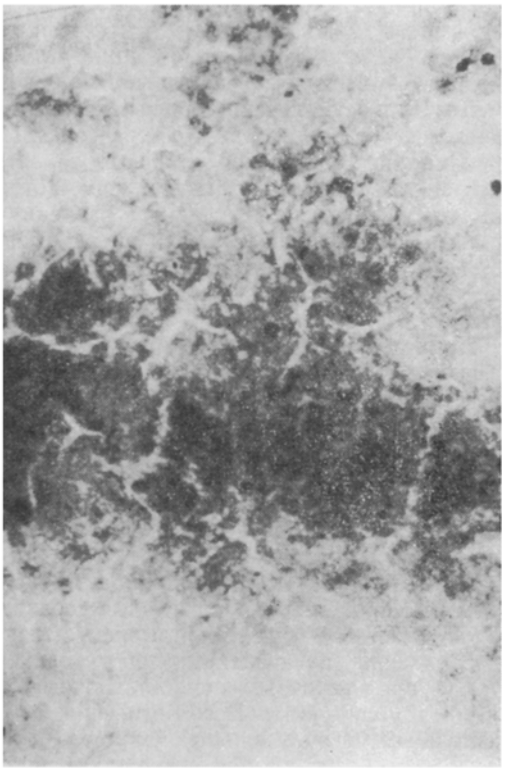

Fig. 3

Fig. 2. Necrobiotic changes in hematocytes.

Fig. 3. Intensification of neutral red absorption by hepatic parenchyma. Neutral red, 100 ×.

Often conglomerates of protein and blood cells could be seen in the hepatic venules. Thrombi of this sort obstructed vessels of different caliber and intensified the disturbance of the hemodynamics. Zones of blocking of the microcirculation accounted for 75.2 ± 8.1% (compared with 36.0 ± 5.5% in intact animals). A large part of the hepatic parenchyma thus ceased to be supplied with blood and it was in a state of ischemia.

The structure of the organ was disturbed and the hepatic trabeculae were disorganized. Degenerative changes were observed in the hepatocytes. They lost their distinct outlines and their polygonal shape, and signs of intracellular edema were observed. Necrobiotic changes were particularly marked in hepatocytes located near the central veins. Pycnosis of the nuclei was found in some cells but in the overwhelming majority of the degeneratively changed hepatocytes, lysis of the cells was observed (Fig. 2). Accumulations of cell debris composed of isolated nuclei and fragments of cytoplasm were observed in the sinusoidal capillaries. In areas of the hepatic parenchyma with marked disturbances of the circulation, grains of pigment accumulated in the Kupffer cells and gave a positive reaction for Prussian blue. The deposits of iron-containing pigments in the Kupffer cells indicated destruction of erythrocytes.

The plasma hemoglobin level at the end of the period of extracorporeal circulation reached 120 mg/100 ml plasma ($P < 0.1$), in agreement with the morphological data given above. These observations indicate a role of the liver in neutralization of hemoglobin and its breakdown products in the presence of excessive hemolysis.

In preparations stained with neutral red extensive regions of affected hepatic parenchyma were discovered. Absorption of the vital dye was sharply intensified both by the nuclei and by the cytoplasm of the parenchymatous cells (Fig. 3). Since neutral red is an indicator dye, the crimson color of the stained tissue indicated a shift of pH toward the acid side and the development of acidosis in the affected areas of the liver.

The shift of the acid–base balance of the liver tissue in in agreement with the results of tests on blood flowing from the liver, when the development of metabolic acidosis also was observed. Meanwhile blood flowing from the brain and heart preserved its acid–base balance for a long time at the original level. These data, together with observations in which the presence of acidosis was recorded in muscle and lung tissues [1], are evidence of a redistribution of the blood flow in favor of vitally important organs during the extracorporeal circulation, confirming data in the literature [4, 6]. The total absorption capacity of the liver cells was increased by 67.03 ± 8.4% ($P < 0.05$). Damage to the endothelial layer was observed in many of the vessels: It was viable, and in some places endotheliocytes had desquamated into the lumen of the vessel. Absorption of vital dye by endotheliocytes also was increased, and the stained nuclei was sharply outlined. Throughout the parenchyma multiple hemorrhages were observed — from small, due to diapedesis, to large following injury to the vessel walls.

Testing for SDH activity revealed an increase in size of the formazan granules and diffusion of the enzyme in the cytoplasm of the parenchymatous cells. Cells in which virtually no formazan granules were present could be seen in some places. Toward the end of the period of observation SDH activity was reduced by 41.2 ± 3.7%. Testing for AlP activity revealed diffuse and intensive staining of the cytoplasm of the hepatocytes. AlP activity in the blood plasma also was increased. Activity of this enzyme was increased by 28.5 ± 3.8%. Activity of AcP was increased by 17.4 ± 2.7%; this enzyme was detected in the form of large granules in the cell cytoplasm. The changes in enzyme activity observed are evidence of injury to intracellular structures.

This investigation showed that under the influence of long-term extracorporeal circulation various interconnected morphological and functional changes arise in the liver of dogs. Hemodynamic and structural changes revealed in the hepatic parenchyma are linked primarily with disturbance of the regional circulation. Consequently, one of the important conditions for normal function of the liver and also, evidently, of other organs during extracorporeal circulation, is correction of the circulation in terminal regions of the vascular system.

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