REACTIVE CHANGES IN THE LIVER STROMA IN RESPONSE TO ZYMOSAN

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KEY WORDS: zymosan; mononuclear infiltration; Kupffer cells; monocytes.

Recent investigations have shown that administration of polysaccharides such as zymosan, glucan, prodigiosan, etc., causes the onset of tachyphylaxis, when the resistance of the organism to infection, irradiation, tumors, blood loss, and trauma, is increased [1, 2] and repair processes are stimulated [3]. There is reason to suppose that the liver reticuloendothelial system (RES) plays an important role in the formation of tachyphylaxis.

It was accordingly interesting to determine what changes take place in the liver stroma under the influence of zymosan, one of the most effective inducers of tachyphylaxis. The investigation described below was devoted to the study of this problem.

EXPERIMENTAL METHOD

Male (DBA×C57BL)F_1 mice weighing 18-22 g were used. Zymosan was injected intravenously in a dose of 2 mg per animal in 0.5 ml of 0.85% NaCl solution. The animals were killed 5-9 at a time 4, 24, and 48 h and 5, 9, 15, and 21 days after injection of zymosan. Sections through the liver, spleen, and thymus, 5-8 μ thick, were stained with hematoxylin and eosin.

TABLE 1. Mean Number of Cells in Foci of Infiltration and Area of Liver Occupied by Foci at Different Times after Injection of Zymosan (M ± m)

Parameter	Time after injection of zymosan, days		
	1	2	5
Mean number of cells in foci of infiltration	7±0,912	18±1,384	33±3,02
Area of section occupied by foci, %	0,34±0,114	4,1±0,83	25,6±3,84

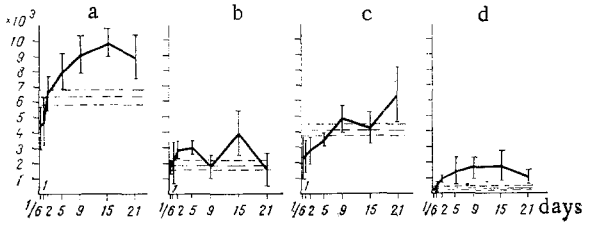

Fig. 1. Leukocyte (a), granulocyte (b), lymphocyte (c), and monocyte (d) counts after injection of zymosan.

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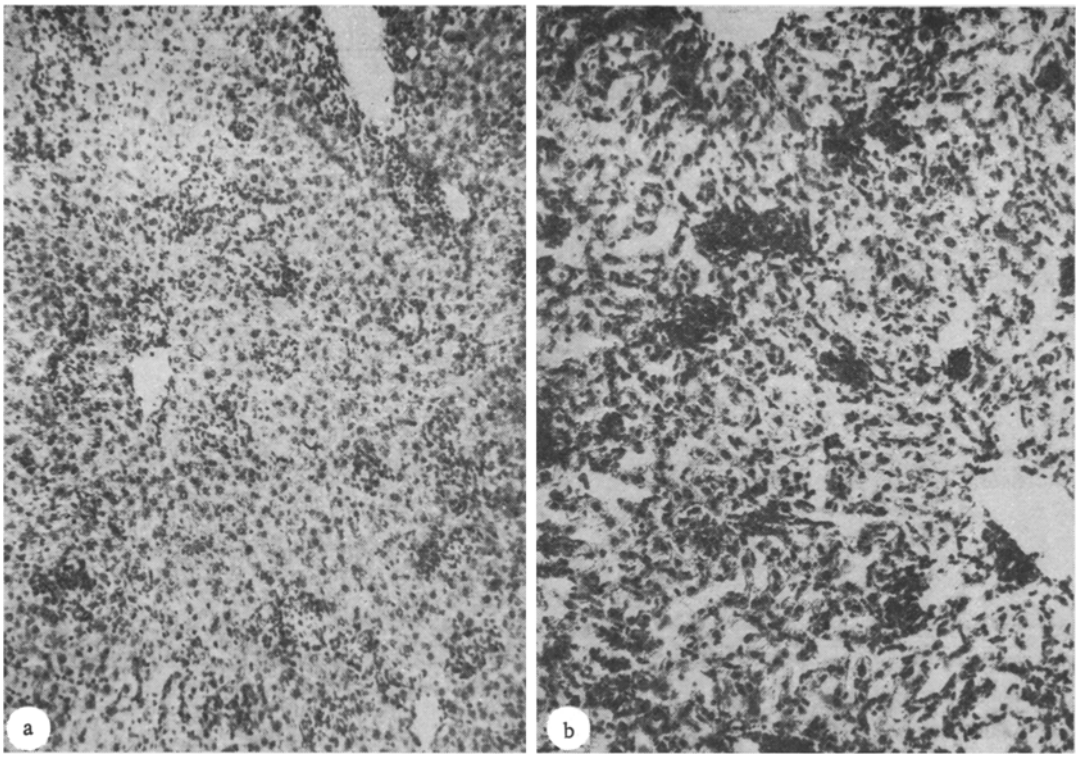

Fig. 2. Structure of liver after injection of zymosan: a) 2 days after injection. Gomori's test for acid phosphatase, 160×; b) 5 days after injection. Hematoxylin-eosin, 160×.

Kupffer cells were counted in 50 fields of vision by means of a morphometric grid under a magnification of 900. The total number and area of foci of infiltration were determined at the same time. To determine the mitotic index 5000 hepatocytes were counted under a magnification of 900. Gomori's test for acid phosphatase was carried out on frozen sections of the liver. For electron microscopy the liver was perfused with 1.5% glutaraldehyde solution in phosphate buffer (pH 7.4) by Wisse's method [11]. The material was embedded in Araldite. Ultrathin sections stained with lead citrate were examined under the JEM-100B electron microscope. To identify sinusoidal cells, the morphological criteria of Wisse [7] were adopted. Blood was taken from the retro-orbital sinus to determine the leukocyte count and formula. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The weight of the liver began to increase 2 days after injection of zymosan and reached a maximum by the 9th day. The weight of the spleen also increased, i.e., hepatosplenomegaly developed. The weight of the thymus, on the other hand, decreased by almost one-third by the 2nd day, after which it recovered and even showed a tendency to increase.

Leukopenia developed 4 h after injection of zymosan, but later it was replaced by leukocytosis (Fig. 1). The fact will be noted that in all animals, starting from the 2nd day and throughout the experiment, the absolute number of monocytes in the blood was increased, up to a maximum 1-2 weeks after zymosan stimulation.

Clusters of a few mononuclear cells, with perivascular and intralobular distributions, appeared in some hepatic sinusoids after 24 h. High acid phosphatase activity was detected (Fig. 2a) in the mononuclear cells forming the foci of infiltration. The area occupied by these foci gradually increased and by the 5th day it amounted to 25% of the total area of the section (Table 1).

Until the 5th day the foci of infiltration were local in character (Fig. 2B), but after 9 days they became blurred in outline and appeared to creep over the liver tissue. The number of foci increased almost fivefold from the 2nd to the 5th days of the experiment. The number of Kupffer cells also increased between the 2nd and the 5th days. At the peak of infiltration, i.e., 9 days after injection of zymosan, mitoses appeared in the hepatocytes ($4.1 \pm 0.46^{\circ}/_{oo}$). Regression of the process was observed after 15 days.

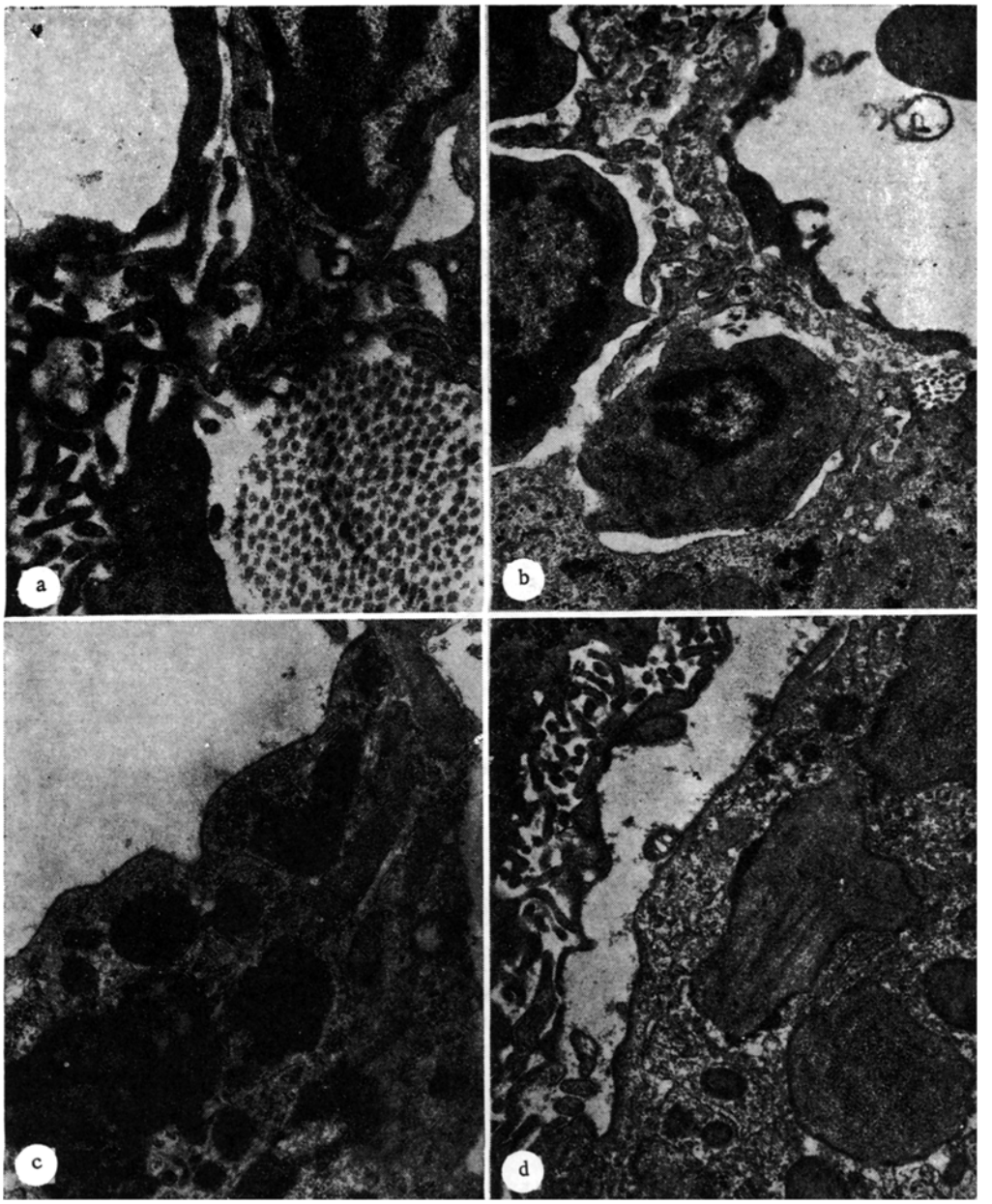

Fig. 3. Electron micrograph of cells of sinusoid 48 h after injection of zymosan: a) region of Disse's space containing collagen fibrils. "Fat-accumulating" cell, 15,500×; b) cluster of monocytes in Disse's space, 14,000×; c) fragment of cytoplasm of endothelial cell, 18,300×; d) fragment of cytoplasm of Kupffer cell. Zymosan inclusions, 14,000×.

Fewer glycogen granules were present in the hepatocytes 48 h after injection of zymosan. The number of lipid drops in the cytosplasm was increased but they were similar in size to those in intact animals. The Disse's spaces were widened. The most substantial changes were found in regions of "fat-accumulating" cells. Bundles of collagen fibrils often could be seen there (Fig. 3a). Usually monocytes could be seen intruding into Disse's spaces in the same region (Fig. 3b). The rough endoplasmic reticulum of fibroblast type was extremely well developed in the fat-accumulating cells. Mitochondria were few in number. There were significantly more lysosome-like granules than in the analogous cells in intact mice. The endothelial and Kupffer cells showed signs of activation of the lysosomal apparatus (Fig. 3c, d).

Zymosan is known to give rise to ultrastructural changes in the macrophages, leading to intensification of their secretory function. As a result the levels of lysosomal enzymes

in the blood serum are raised [4]. Activated macrophages also secrete colony-stimulating factor and monocytopoiesis-inducing factor [5, 6]. Accordingly the process of formation of foci of infiltration in the liver can be represented as follows. The injected zymosan first binds with the Kupffer cells and, in response to its assimilation they secrete factors disinhibiting monocytopoiesis. As a result the number of monocytes in the blood rises. Meanwhile secretion of lysosomal enzymes is stimulated and a high gradient of chemotaxic factors is formed around the stimulated macrophage. On the whole all these changes create the conditions for migration of monocytes from the blood into the liver and their "fixation" at the site of stimulated hepatic macrophages. The possibility cannot be ruled out that the reactive changes now observed in the liver soma play an important role in the formation of a qualitatively new state, that of tachyphylaxis.

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STEREOLOGIC ANALYSIS OF MYOCARDIAL ULTRASTRUCTURE AFTER RESUSCITATION

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KEY WORDS: myocardium; stereology; postresuscitation period; electron microscopy.

The recovery period after clinical death is characterized by disturbance of the functions of many organs and systems of the body. One of the leading pathogenetic factors at the basis of postresuscitation sickness has been shown to be circulatory failure, largely due to a disturbance of myocardial contractility [1, 4, 7].

To study the causes of this phenomenon, an electron-microscopic investigation was made of heart muscle, using morphometric methods to assess ultrastructural changes.

EXPERIMENTAL METHOD

Experiments were carried out on 59 dogs, 14 of which served as the controls. Clinical death for 5 min was produced by massive acute blood loss, and the animals were resuscitated by the method of Negovskii et al. [3]. All experiments were carried out under morphine-hexobarbital anesthesia. The myocardium of the left ventricle was taken for electron-microscopic investigation at the 5th minute of clinical death, during the first 5-10 min after the beginning of resuscitation, and 1.5, 6, and 12 h and 1, 3, 7, and 14 days later. Material was fixed in 25% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in Araldite. Ultrathin sections were examined in the UÉMV-100K electron microscope. Besides the qualitative description of the myocardial ultrastructure, a detailed quantitative analysis of the electron micrographs was made by stereological methods [6]. The relative volumes (Vv), surface densities (Sv), and surface to volume ratios (S/V) were calculated

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