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MORPHOLOGICAL AND BIOCHEMICAL INVESTIGATION OF THE LIVER IN SYSTEMIC ENDOTOXEMIA

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KEY WORDS: liver; metabolism; endotoxin.

Endotoxemia, which accompanies various diseases (septicemia, diffuse peritonitis, gangrene, etc.), leads to the development of multiple organ failure in 40-85% of surgical patients [1, 6, 14]. An important stage in the pathogenesis of injury to organs and tissues, including the liver, is systemic endotoxemia as a result of destruction of the saprophytic and pathogenic intestinal microflora [4, 12].

The aim of this investigation was to study the structure and metabolism of the liver tissue of dogs in the course of systemic endotoxemia.

EXPERIMENTAL METHOD

Experiments were carried out on 11 mongrel dogs weighing 13-18 kg, which received an intravenous injection of *E. coli* lipopolysaccharide (LPS) in a dose of 2 mg/kg, and each dog underwent liver biopsy 0, 1, 3, 5, and 7 h thereafter. Each biopsy specimen was divided into two parts, one of which was fixed in 10% neutral formalin and embedded in paraffin wax. Microtome sections were stained with hematoxylin and eosin. From the other part of the biopsy material a homogenate was prepared, in which the content of albumin, total protein, bilirubin, and glucose and also activity of lactate dehydrogenase (LDH), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (GTP), and alkaline phosphatase (AlP) activity were determined using an "Express-550" automatic biochemical analyzer (Ciba Corning, England). The numerical results were subjected to statistical analysis by computer.

EXPERIMENTAL RESULTS

Microscopic study of the liver in endotoxemia showed progressive changes in the microcirculatory system and parenchyma of the organ. Only 1 h after injection of LPS, irregular congestion of the portal vessels, especially branches of the portal vein, was noted. In individual portal tracts there were small areas of hemorrhages, which increased in size after 3 h, and by 5-7 h they had even spread to the periportal regions. After 3 h uneven dilatation and congestion of the sinusoidal vessels and hepatic venules, aggregation of erythrocytes, and foci of leukocytic stasis were observed. After 5 and

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TABLE 1. Biochemical Characteristics of Liver Tissue in Endotoxemia (M ± m, per g tissue)

Parameter (μmoles/liter)	Control	Duration of toxemia, h			
		1	3	5	7
Albumin	2.58±0.18	2.83±0.17*	3.41±0.21.	2.30±0.14*	2.32±0.16*
Total protein	12.1±1.4	11.5±0.8*	18.6±1.9	12.2±1.5*	12.6±1.5*
Billirubin	5.95±0.41	4.79±0.32	10.55±0.92	4.82±0.37*	7.31±0.51
Glucose	435.2±34.1	680.1±57.0	481.4±42.2*	685.2±56.4	90.5±12.6
LDH	35823.3±3214.6	34617.8±3127.4*	39513.1±3221.7*	45378.4±3462.1	39535.3±3328.9*
ALT	13571.7±928.4	14919.0±1015.2*	26217.4±1836.1	640.4±78.5	6669.2±521.3
GTP	312.7±26.2	269.1±24.3*	241.3±21.1	254.9±20.3*	241.1±19.8
AIP	647.4±58.8	1192.5±92.0	1963.5±156.4	3127.6±315.6	3459.2±327.9

Legend. All values except those marked by an asterisk differ significantly from control.

7 h focal concentrations of polymorphonuclear leukocytes were observed in individual portal tracts and in areas of hemorrhages. Injuries to hepatocytes consisted of albuminoid (cloudy-swelling and vacuolar) degeneration and monocellular necroses, mainly involving periportal cells, after 1 h, focal necroses after 3 h, and solitary periacinar necroses 5-7 h after the beginning of toxemia. Stellate endotheliocytes and endothelial cells increased in size 3 h after injection of LPS and their nuclei appeared pycnotic.

The developing systemic endotoxemia led to marked disturbances of metabolism of the liver tissue (Table 1). There was a sharp decrease in the glucose concentration until 7 h, the main cause of development of hyperglycemia observed in endotoxic shock [5]. In the preceding period, its content varied. An increase in the glucose content in the liver tissue is in agreement with the well-known facts that glycogenolysis is enhanced in the liver in endotoxemia [15], probably for several reasons: stimulation of this process by prostaglandin D_2, which is produced by Kupffer cells, activated by endotoxin [9], increased glycogen phosphorylase activity due to its more rapid reactivation [8], and also intensification by endotoxin of gluconeogenesis [10], which takes place against the background of depressed phosphoenolpyruvate kinase activity and increased secretion of glucagon and corticosterone [16].

The increase in the total protein concentration during the first 3 h by 53.7% compared with the control ($p < 0.05$) was due not only to a compensatory intensification of albumin synthesis by 32.1% of its initial level ($p < 0.05$), but also, evidently, by the more rapid formation of other proteins, including globulins, fibronectin, acute phase proteins, and other mediators of inflammation [13]. The subsequent decrease in the concentrations of albumin and total protein was probably connected both with potentiation of proteolysis [3] and with depression of the protein-synthesizing function, which is in agreement with morphological evidence of increasing alteration of hepatocytes and sinusoidal cells.

A fall in the bilirubin concentration by 19.5% was observed 1 h after injection of the endotoxin, but after 3 h this was replaced by an increase of 1.8 times compared with the physiological normal value ($p < 0.01$). This may be connected with the marked hemolysis of erythrocytes in regions of hemorrhages observed in the liver, and also with a disturbance of bile secretion as a result of inhibition by endotoxin of cell membrane Na,K-ATPase activity [7]. The subsequent fall in the bilirubin concentration in the liver tissue was accompanied by marked hyperbilirubinemia. The increase in LDH activity observed, especially 5 h after injection of LPS, suggests intensification of anaerobic glycolysis due to the circulatory hypoxia developing in endotoxemia [11]. The progressive rise of AIP activity (by 5.3 times after 7 h) is evidence of increased permeability of the cell plasma membranes, developing under hypoxic conditions and linked mainly with elevation of the level of lipid peroxidation, intensification of which is observed during the first few hours after injection of endotoxin [2]. The increase in membrane permeability of the cells and their organelles leads to the more rapid entry of intracellular enzymes into the blood stream, for the high blood enzyme levels thus produced may be regarded as an indication of disturbance of liver structure. In our investigations a decrease in GTP and ALT activity was observed starting with 3-5 h after injection of the endotoxin, in agreement with the results of the morphologic investigation concerning the development of dystrophic and necrotic changes in the hepatocytes.

Injection of *E. coli* LPS into dogs thus causes marked morphologic injuries to the liver tissue, which correlate with disturbance of its metabolism, and which lie at the basis of disturbance of liver function in systemic endotoxemia. The parallel development and progression of structural and metabolic disturbances suggests that both direct injury to the liver cells, leading to their ultimate death, and disturbance of coordination between metabolic processes in the hepatocyte, leading to its functional insufficiency, play a role in the genesis of hepatic failure. It is important to note that the distur-

bances described above develop as early as in the first few hours after injection of endotoxin; consequently, hepatic failure formed long before the appearance of its clinical manifestations.

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MORPHOLOGY OF TISSUE COMPONENTS OF LAYERS OF THE RAT MYOCARDIUM IN THE EARLY STAGES OF MYOCARDIAL HYPERTROPHY

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KEY WORDS: myocardial layers; hypertrophy of the heart; morphometric analysis.

An elegant hypothesis has now been put forward to explain the dynamics of functional and structural changes in the myocardium during the development of adaptive reactions [4, 5]. However, most investigations have not taken account of local differences in the structure of the myocardium, despite data in the literature on structural and metabolic differences between its layers, and also differences in their resistance to experimental procedures [1, 7, 9-11].

This paper describes a morphometric study of different layers of the left ventricular myocardium of Wistar rats at the beginning of the stable stage of myocardium hypertrophy [4], caused by narrowing by 50% the lumen of the abdominal aorta, in order to identify differences in the response of tissue components of the subendocardial, subepicardial, and intermediate layers to changes in the intensity of cardiac function. Isolation of the layers and zones for the investigation was described previously [6].

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