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Specific Features of Ultrastructural Changes in Cells of the Rat Mononuclear Phagocyte System in Experimental Endotoxemia

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The development of endotoxin-induced shock (ES) is accompanied by the failure of many organs, resulting from systemic hypotension, hypoperfusion of various target organs, disseminated intravascular coagulation, multiple intracellular alterations, and even cell death (necrosis) [2,3,5]. Doubtless, both the course and the outcome of ES significantly depend on the capacity of the detoxification systems to neutralize the endotoxin (lipopolysaccharide) and to eliminate the consequences of its action.

Accordingly, the ultrastructural study of alveolar macrophages (AM) and stellate reticuloendotheliocytes (SR) during endotoxemia is of special interest as these are essential components of the mononuclear phagocyte system (MPS) naturally adapted for the performance of a clearing function in the organism.

MATERIAL AND METHODS

Experiments were carried out on 39 rats of both sexes weighing 200-250 g. *Escherichia coli* endotoxin in a dose of 2 mg/100g was injected into the caudal vein. The animals were sacrificed 30 min (initial phase of shock), 5 hours (middle phase), and 3 days (phase of late endotoxemia) post-injection, using a lethal dose of nembutal. The control animals received sterile saline. Ten experimental and three control rats were sacrificed at each time. From different parts of each lung and from the liver samples were taken, five per organ. The samples were treated with glutaroosmic fixative and, after dehydration, embedded in Epon 812. The sections obtained using an LKB 8800 ultramicrotome were contrasted with uranyl-acetate and lead citrate and analyzed in a JEM-100S electron microscope. For the light microscope analysis, semithin sections were produced from the same tissue blocks and stained with toluidine blue and azure 2. In addition, the tissue samples were fixed in a 10% solution of neutral formalin and Carnoy fluid. The celloidin-paraffin sections were stained with hematoxylin-eosin; acid phosphatase activity was assessed after Gomori.

RESULTS

No ultrastructural changes were revealed in the AM and SR of the control animals receiving sterile saline throughout the period of observation. Half an hour after endotoxin challenge (initial phase of ES), in the experimental animals the changes were limited to microcirculatory disturbances and dystrophic impairment of the alveolar and parenchymatous cells. In the liver sections, as a rule, moderate and high activity of acid phosphatase was indicated after the Gomori staining in the form of enlarged and fused granules in the cytoplasm of the hepatocytes and SR.

At the ultrastructural level, the initial alterations in the lungs were characterized by the release of lamellar bodies from the cytoplasm of type-II pneumocytes and degradation of the surfactant. The latter could first be detected in the alveolar spaces; then it underwent phagocytosis by AM and was further destroyed with the aid of the cellular lysosomes (Fig. 1,a).

AM are known to be extremely sensitive to endotoxin and to phagocytize it even when endotoxin is injected in small quantities [8]. According to the data of electron microscopy, as early as 30 min after the injection of labeled endotoxin, tubular structures consisting of parallel membranes placed 5 nm apart are seen in the SR cytoplasm [14]. The capture of endotoxin was almost exclusively mediated by SR and, to much lesser degree, by the endothelial and parenchymatous cells.

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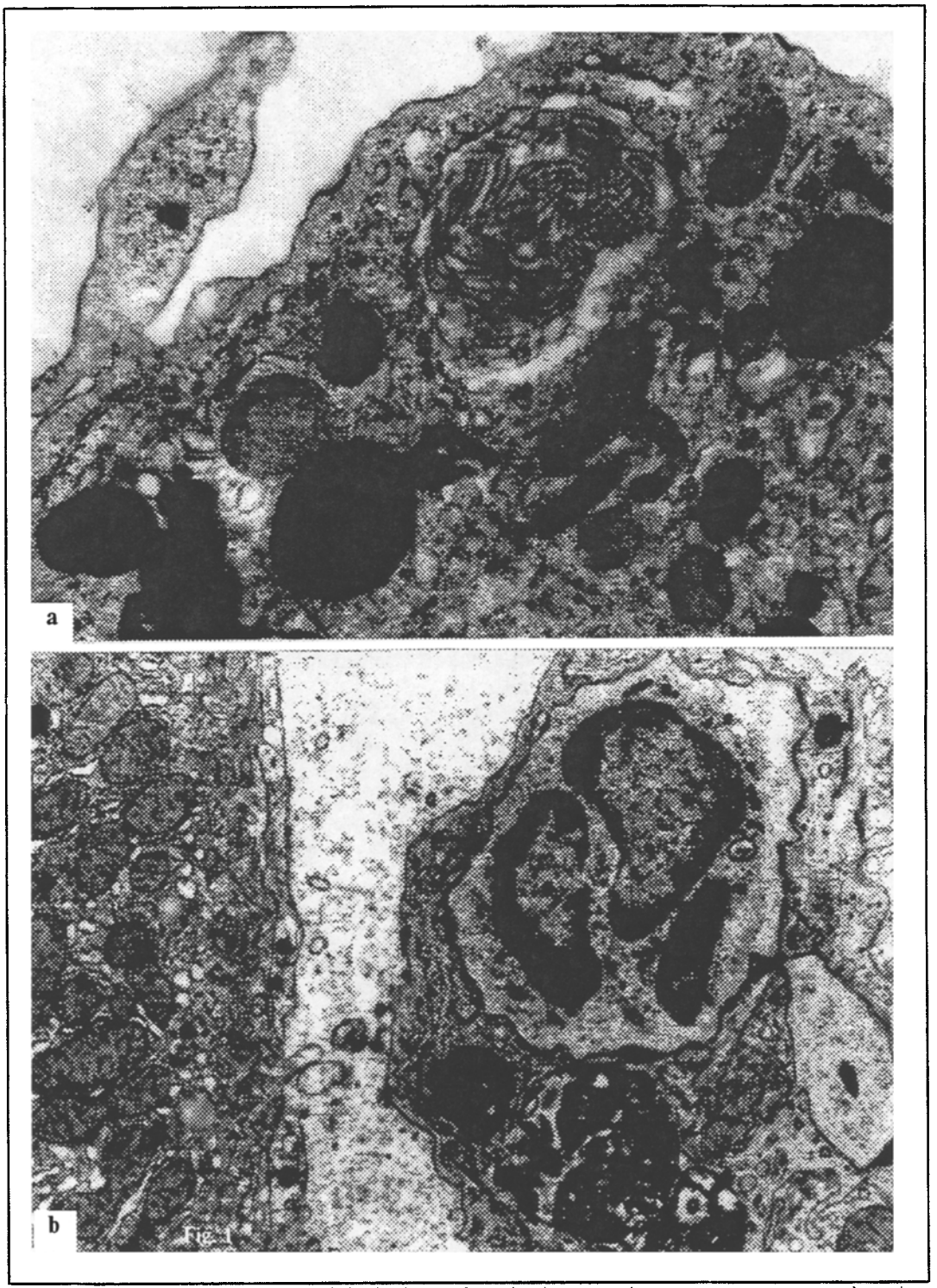

Fig. 1. Initial phase of endotoxin-induced shock. a) part of an alveolar macrophage, containing in its cytoplasm the surfactant membranes. x 28,000; b) phagocytosis of polymorphonuclear leukocyte by stellate reticuloendotheliocyte. x 11,000.

During this early period of ES, in the sinusoidal capillaries the first morphological signs of disseminated intravascular coagulation (DIC) syndrome, expressed as thrombogenesis and aggregation of fibrin filaments, were revealed. Some blood cells and fibrin underwent phagocytosis by SR (Fig. 1,b). Interestingly enough, some quantity of fibrin was also ingested by the hepatocytes, where fibrin could be detected in the dilated tubules of the granular cytoplasmic reticulum.

Thus, in the initial phase of ES, the interactions between lung and liver macrophages and the corresponding epithelial cells within the framework of the stromal-parenchymatous contacts were clearly traced. The formation of "tandems" between the

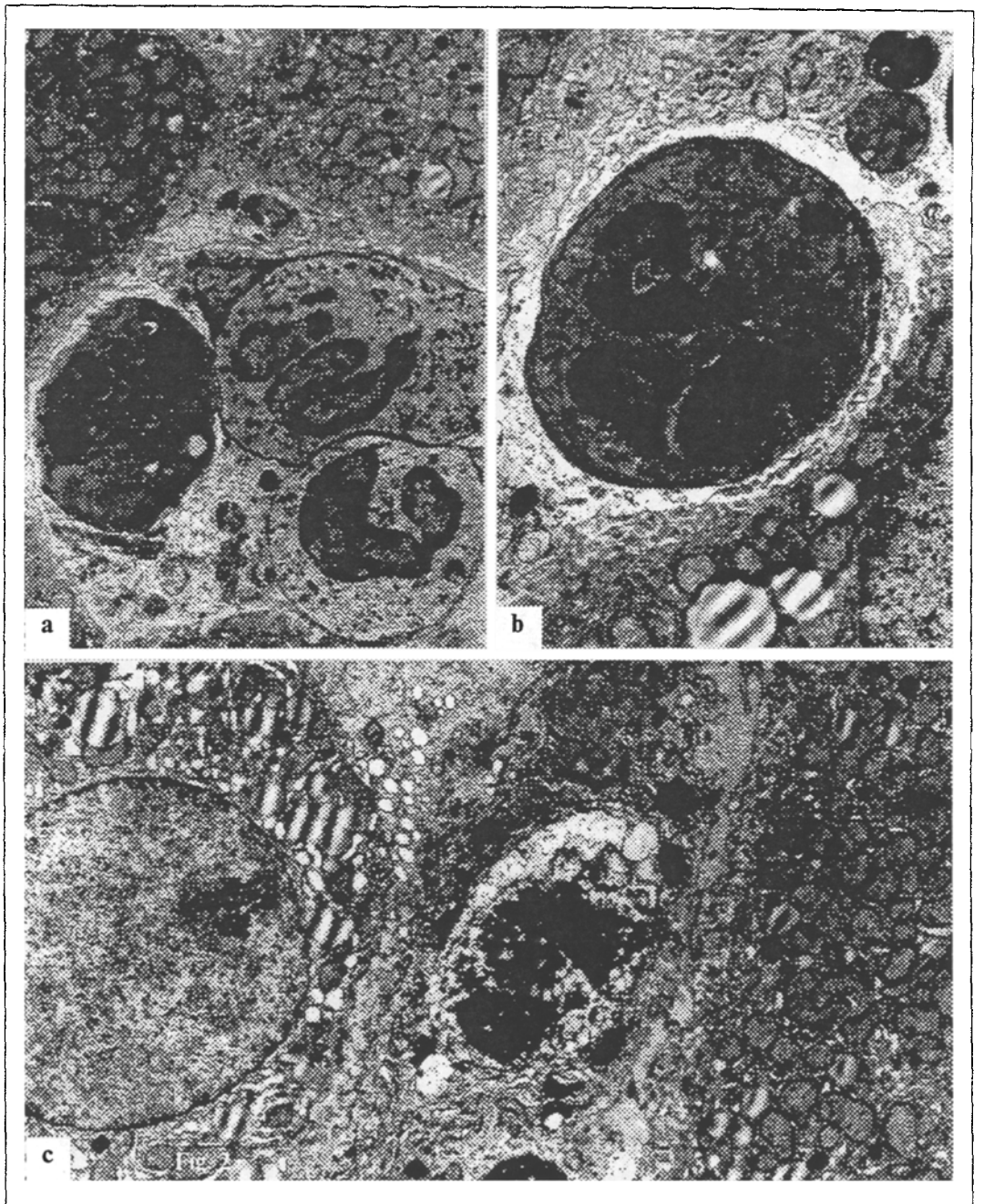

Fig.2. Middle phase of endotoxin-induced shock. a) the cytoplasm of the stellate reticuloendotheliocyte contains three unchanged polymorphonuclear leukocytes. x 3600; b) internalized leukocyte in giant lysosome. x 4800; c) destroyed leukocyte in cytoplasm of stellate reticuloendotheliocyte; note also the fibrin filaments. x 5300.

organ-specific macrophages and specialized parenchymatous cells (AM-pneumocyte and SR-hepatocyte) increases the clearing functions of the lung and liver compartments of the MPS. Similar microsystems exist in other organs as well, e.g., in the brain (microgliocyte-neuron), ensuring the structural homeostasis of the nervous tissue [4,7].

Five hours after endotoxin administration (middle phase of ES) the mentioned structural alterations persisted and had progressed [2,3,6]. Histochemical analysis of the liver revealed a preserved high activity of acid phosphatase, especially in the SR. Judging by the ultrastructural changes in AM and SR (increase in the number and size of cells, development of multiple protrusions on the plasmalemma, enhanced phagocytic and lysosomal activity), these cells should be considered as activated macrophages, differing by morphological criteria from the population of resident macrophages. However, while AM engulfed the massively degrading surfactant and cell debris (mostly type-II pneumocytes), SR phagocytized blood cells (mainly polymorphonuclear leukocytes). The activation of SR was so pronounced

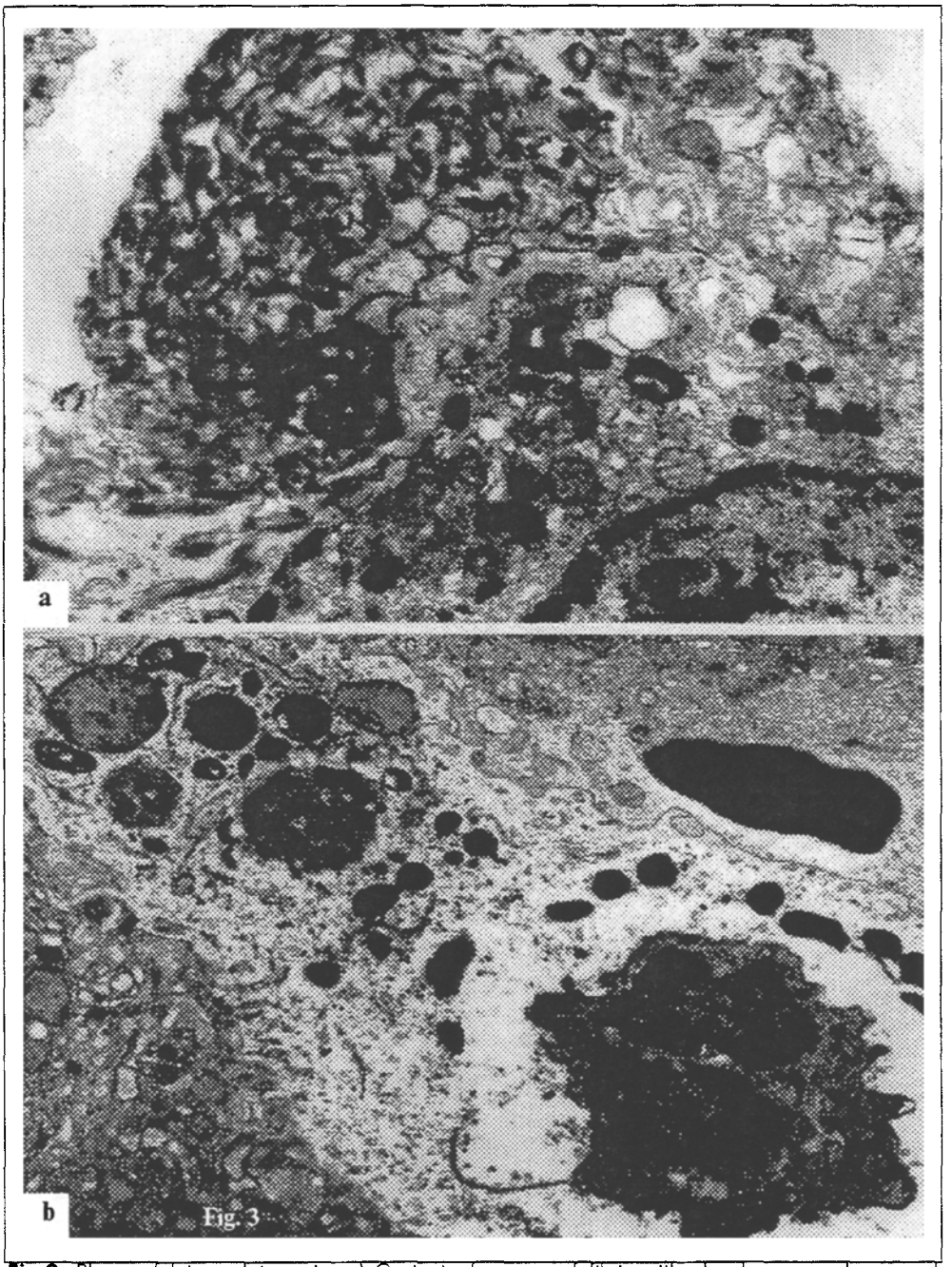

Fig.3. Phase of late endotoxemia. a) Contact of masses of fibrin with alveolar macrophage and internalization into the cytoplasm. x 19,000; b) acute activation of lysosomal apparatus, leading to destruction of stellate reticuloendotheliocyte. x 8000.

that they each phagocytized two to three cells (Fig. 2,a). The engulfed cells were then wholly internalized in the giant lysosomes (Fig. 2,b), where they underwent digestion, turning into a structureless material, partly or entirely surrounded by an electron-transparent zone (Fig. 2c).

Thus, besides endotoxin clearance, in the middle phase of ES the cells of MPS accomplish the clearing of tissue decay products in the liver and lungs. The high metabolic activity is explained by the phagocytosis and actively proceeding processes of auto- and heterophagy.

Three days following endotoxin injection (phase of late endotoxemia) the activated macrophages in the liver and lungs significantly increased in number. According to the light and electron microscopy data, precisely in this period the concentration of endotoxin (lipopolysaccharide) in these cells reached its maximal share (up to 39%) of the administered dose [9,10].

We established that this period is accompanied by especially pronounced edema and desquamation of the respiratory epithelium (type-I pneumocytes), destruction of surfactant-producing cells (type-II pneumocytes),

fibrinoid impregnation and fibrosis of the stroma, as well as penetration of fibrin usually phagocytized by the AM into the alveolar space (Fig. 3,a). As a rule, the cytoplasm of such cells is packed with multiple secondary lysosomes.

The SR hyperactivity was confirmed by the corresponding ultrastructural reorganization of the organelles and high phagocytic activity directed at the engulfment of destroyed hepatocytes and blood cells. However, sometimes labilization of the lysosomal membranes occurred, resulting in the release of hydrolytic enzymes into the cytoplasm, digestion of its structures, and finally cell death (Fig. 3,b).

It is important to stress, when estimating the ultrastructural alterations in the cells of the MPS liver and lung compartments as a whole, that along with the clearly adaptive reaction of the macrophages [1] fulfilling a protective function in endotoxemia, the accumulation of them in the liver and lungs is considered by some workers as one of the leading injurous factors. Indeed, activated macrophages participate in tissue destruction by means of increased production of catabolic enzymes elastase and collagenase [15]. Activation and exocytosis involve lysosomal enzymes and substances with a broad spectrum of biological action: acid phosphatase, products of arachidonic acid metabolism, cachectin (tumor necrosis factor), monokines, pyrogens, etc. [11-13].

Thus, the state of AM and SR to a great extent determines the characteristic features and the progression of various target organ alterations during the action of endotoxin. The blockade of MPS cells with lipopolysaccharide, fibrin, destroyed cells, and cell debris promotes the manifestation of DIC syndrome and intensifies the morphological signs of tissue damage. Ultrastructural alterations in the microcirculatory bed, pneumocytes, and hepatocytes depend on the potencies of MPS cells for taking part in the catabolic processes. The presence of microsystems consisting of organ-specific macrophages and specialized parenchymatous cells increases the clearing function of the lungs and liver, especially in the initial phase of endotoxemia.

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Effect of Microwaves of Thermogenic Intensity on the Structure of the Blood-Retina and Blood-Brain Barriers

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The neurobiological effects of microwaves and the selectivity of their effect with respect to nervous tissues are due substantially to the primary influences of radiation on the membrane elements of the neuron [8]. The contribution of vascular reactions and changes on the part of the blood-brain barrier to the nervous tissue mechanisms of the effect of SHF energy on the CNS has been variously appraised [1, 9].

The goal of the present investigation was to study the nature and dynamics of the structural changes in the blood-retina and blood-brain barriers (BRB, BBB) after the effect of microwaves of thermogenic intensity in various parts of the visual analyzer, taking into account the high sensitivity of the latter to the given range of electromagnetic radiations [4].

MATERIAL AND METHODS

The experiments were run with sexually mature mongrel guinea pigs and white rats of both sexes, 79 of the former and 30 of the latter, of which 50 and 20

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