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ULTRASTRUCTURAL CHANGES IN THE MICROCIRCULATORY BED OF THE LUNGS IN ENDOTOXIN SHOCK

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Endotoxins are known to possess definite affinity for blood vessels and they damage capillaries through their direct toxic action on the endothelium [2, 3, 9]. The development of endotoxin shock (ES) is considered to be accompanied by disturbance of the systemic hemodynamics, by hypoperfusion of the internal organs, secretion of biologically active substances into the blood, and by disseminated intravascular clotting followed by fibrinolysis, and so on [4, 12, 13].

The reaction of the microcirculatory system to endotoxinemia is most marked in the lungs, whose vascular receptors are particularly sensitive to catecholamines and vasoactive substances [13].

Considering that the lungs are a target organ for introduced endotoxin, it was decided to undertake an electron-microscopic study of lesions of the microvessels in the course of ES.

EXPERIMENTAL METHOD

Experiments were carried out on rats, rabbits, and dogs. Rabbits and dogs were given an intravenous injection of 5 mg/kg of *Escherichia coli* lipopolysaccharide and rats were

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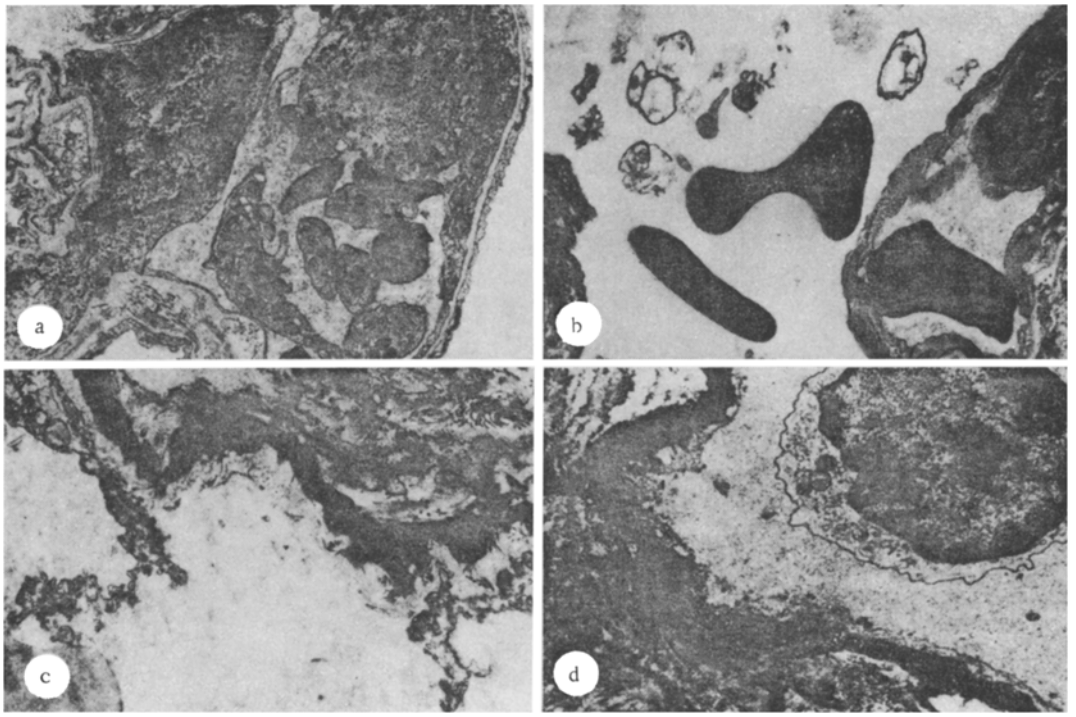

Fig. 1. Initial period of ES: a) thrombosis of alveolar capillary; b) erythrocytes in alveolar space, where destroyed surfactant also can be seen; c) separation of endothelial cells; d) denudation of the subendothelium. a, b) 4200×; c, d) 7000×.

given an injection of the same lipopolysaccharide into the caudal vein in a dose of 2 mg/100 g. The animals were killed after 30 min (18 dogs, 10 rabbits, and 10 rats), 5 h (10 dogs, 10 rabbits, 10 rats), and 3 days (10 rats) with a lethal dose of pentobarbital. In control experiments (3 in each group) sterile physiological saline was injected.

Pieces of the lungs were fixed with glutaraldehyde and osmium, dehydrated, and embedded in Epon. Sections cut on the LKB 8800 ultramicrotome were stained on grids with uranyl acetate and lead citrate and examined in the JEM-100S electron microscope.

EXPERIMENTAL RESULTS

On electron-microscopic investigation microcirculatory disturbances were clearly distinguished: cessation of the blood flow, sludging, hyperemia, and thrombosis. It is an interesting fact that a well defined thrombohemorrhagic syndrome was present in the initial (30 min) and especially in the intermediate (5 h) periods of ES, when thrombosis with fibrin precipitation in the alveolar capillaries (Fig. 1a) and hemorrhages into the stroma and alveolar spaces (Fig. 1b) were present simultaneously.

It must be emphasized that vascular thrombosis of the microcirculatory bed of the lungs and fibrin precipitation were more marked in rabbits and rats than in dogs. In dogs with ES, hyperfibrinolysis predominated during the first 30 min, as shown by the considerable shortening of the euglobulin lysis time, the presence of fibrin degradation products, and the marked hypofibrinogenemia [8]. Meanwhile, toward the end of the first hour, the early primary activation of the fibrinolytic system continued, and was joined by thrombocytopenia and lengthening of the partial thromboplastin time [8]. The less frequent discovery of microthrombi in dogs also is associated with high fibrinolytic activity [6]. Finally, another important factor is fibrinogenolysis, connected with an extremal increase in activity of the anticlotting system in shock [5].

So far as hemorrhages are concerned, one possible cause of their appearance may be a consumption coagulopathy, for which microthrombi are the material substrate, although they are not always identified at autopsy because of activation of fibrinolysis [14]. Another cause of hemorrhages is not so much changes in the clotting system of the blood as structural disturbances of the air-blood barrier itself. In ES, for instance, within a few minutes large areas of extravasation are observed in the stroma of many organs, where they arise long

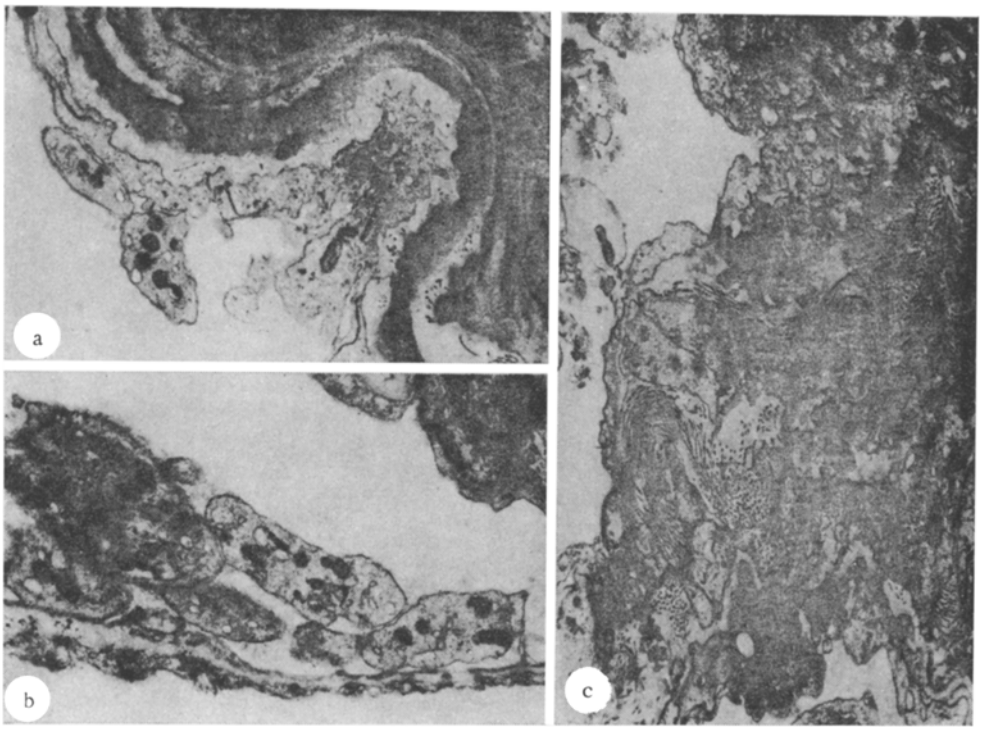

Fig. 2. Ultrastructure of lungs in initial period of ES (a, b) and in stage of late endotoxemia (c). a) Fixation of platelets to intact endothelium. 7000×; b) formation of a "platelet carpet." 10,000×; c) interstitial fibrosis. 5600×.

before the first signs of consumption coagulopathy are recorded. Literally with a few seconds after injection of endotoxin, fragmentation of the walls of the small venules takes place. The most remarkable thing is that the blood flow is maintained during bleeding, and the site of rupture later ceases to be detectable morphologically and thrombus formation does not occur [15].

Electron microscopy revealed not only the "explosive" character of the increase in permeability of the microcirculatory bed, but also, in other cases, the possible sequence of pathological reactions at the vessel wall level. For instance, considerable separation of the endothelial cells (Fig. 1c) and their detachment with exposure of the subendothelium (Fig. 1d) could sometimes be observed. Serotonin and histamine, released on degranulation of platelets and tissue basophils (mast cells), as well as other biologically active substances [1, 13], take part in the labilization of the air-blood barrier.

Desquamation of endotheliocytes also takes place in the lung arterioles. We observed a similar phenomenon previously in certain other organs, and a sixfold increase in the number of desquamated endothelial cells in blood films [2]. Desquamation of endothelium leads to contact between the blood cells and collagen, which is a powerful inducer of the reaction of release of biologically active substances and of platelet aggregation and adhesion [16].

However, platelets can also adhere to intact endothelium. This may happen to single platelets (Fig. 2a) or to cells forming a cover in one or two layers (Fig. 2b). In the latter case a cell monolayer or bilayer, which has been called a "platelet carpet," is formed [11].

The results can be most adequately explained by changes in the plasmalemma of the damaged endothelial cells, even despite the absence of changes in them that are visible in the electron microscope. In this case certain receptors and certain cell membrane lipids bind with effector molecules into what are called receptor areas or "operational units" [7]. The plasma membrane behaves as a control center, biochemically transforming the signal from the cellular microenvironment and triggering the cell response like a relay [10].

Further progression of the lesions of the microcirculatory bed, with reduction of the blood flow and rheologic disturbances takes place in the intermediate period of ES. The addition of syndromes of disseminated intravascular clotting and of consumption coagulopathy leads to the long-term existence of microthrombi and hemorrhages, which arise, if not strictly simultaneously, at least (so far as the manifestations are concerned), parallel to one another.

In the late period endotoxemia (3rd day) a qualitatively new feature is the presence of numerous fibrous elements, consisting mainly of collagen fibers and stromal cells of the fibroblast series and their processes, against the background of interstitial edema (Fig. 2c). Type II alveolocytes proliferate and occupy a larger surface area than in the control. The epithelium thickens at the same time. Sometimes interstitial fibrosis is combined with the extracellular localization of lysosomes.

Consequently, at the stage of the process we have studied the most characteristic feature is the formation of a powerful perivascular barrier at the level of the blood vessels of the microcirculatory bed. The formation of this additional obstacle on the blood-tissue boundary disturbs the gas exchange in the lungs and is one cause of the acute pulmonary failure associated with the action of endotoxins.

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