ULTRASTRUCTURAL MANIFESTATIONS OF THE THROMBOHEMORRHAGIC SYNDROME IN THE LUNGS IN ENDOTOXIN SHOCK

É. A. Bardakhch'yan and N. G. Kharlanova

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The need to study septic shock and its mechanisms highlights the importance of the study of its experimental model, namely endotoxin shock (ETS). An important stage of endotoxemia is a disturbance of the clotting system of the blood, and the shock itself may be either the cause or the effect of disseminated intravascular clotting [10, 13]. We have observed this phenomenon in animals with ETS on electron-microscopic study of the kidney [1], brain [7], myocardium [8], and adrenal medulla [5] and cortex [3, 4]. The mosaic character of the blood clotting disorders in ETS is accompanied by a paradoxical combination of thrombotic and hemorrhagic disturbances and it assumes particular importance in the lungs which, beside their respiratory function, also perform a number of nonrespiratory functions, connected with regulation of the synthesis, release, and inactivation of certain biologically active substances and vasoactive compounds [9, 11].

It was therefore decided to undertake an ultrastructural study of intravascular changes in the pulmonary capillaries in the course of ETS.

EXPERIMENTAL METHOD

Experiments were carried out on rats, rabbits, and dogs. The rabbits and dogs received an intravenous injection of 5 mg/kg of Escherichia coli lipopolysaccharide; the rats received the same lipopolysaccharide by injection into the caudal vein in a dose of 2 mg/100 g body weight. The animals were killed with a lethal dose of pentobarbital after 30 min and 5 h (10 animals of each species) and 3 days (10 rats). In control experiments (three animals in each group) sterile physiological saline was injected. Pieces of the lungs, after fixation with glutaraldehyde and OsO_4, were dehydrated and embedded in Epon. Sections were stained with uranyl acetate and lead citrate and studied in the JEM-100S electron microscope.

EXPERIMENTAL RESULTS

In the initial period of ETS (after 30 min) many micropinocytotic vesicles appeared in the cytoplasm of the endothelial cells, accompanied by clasmatosis of the microvilli, edema of the cell cytoplasm and, in some cases, desquamation of the endotheliocytes. The mechanism of desquamation of the endothelial cells is rather complex, although universal, and it is evidently realized in the capillary and arteriolar segments of the vascular system, through the indirect action of certain biologically active substances and lysosomal enzymes, induced under the influence of hypoxia, hypoxemia, and acidosis [2].

Defects of the vascular wall lead to increased permeability of the air-blood barrier. However, the outflow of blood cells may also occur even without any visible (even in the electron microscope) damage, due to a mechanism of diapedesis (Fig. 1a, b). Large concentrations of platelets are found in the lung capillaries, and usually they appear to be degranulated (Fig. 1c).

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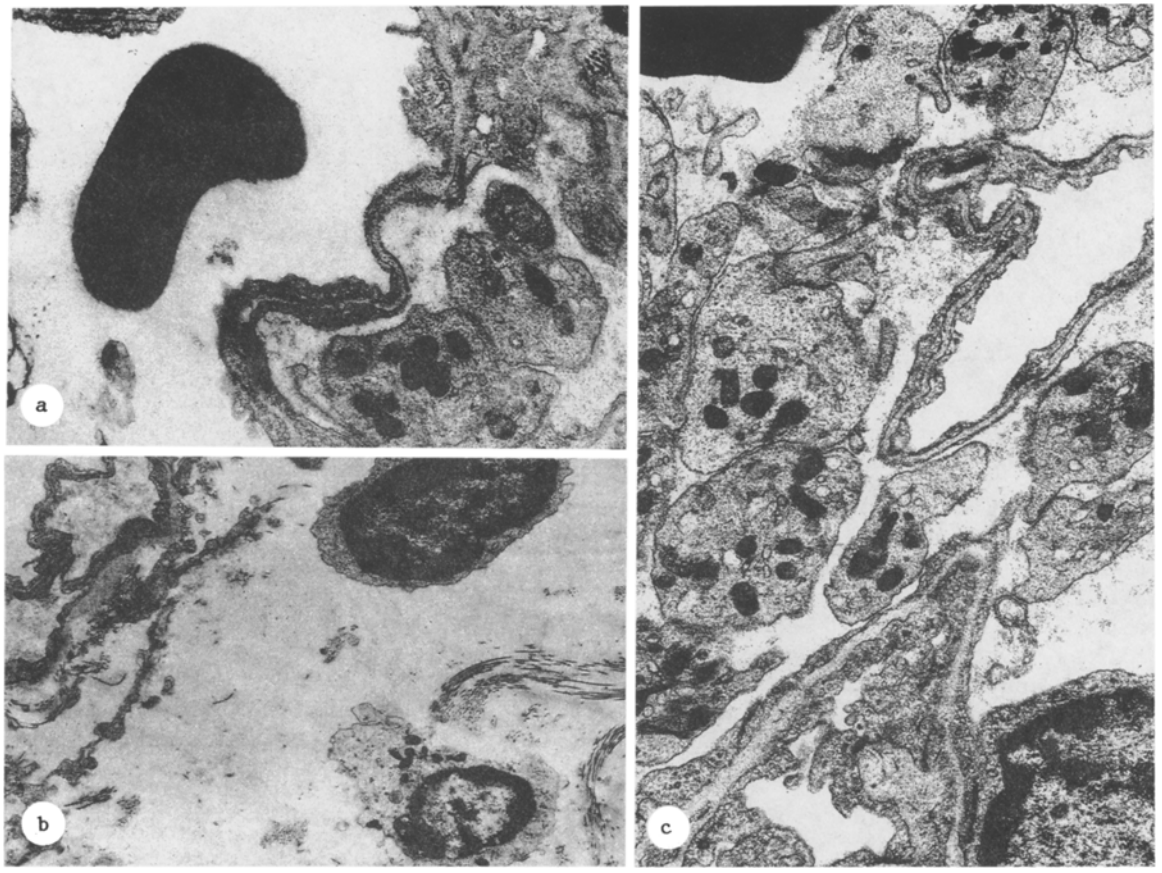

Fig. 1. Ultrastructure of lungs in initial period of ETS. a) Appearance of erythrocyte in alveolar space. 7000 ×. b) Appearance of leukocytes in alveolar space. 3500 ×. c) Aggregation of platelets in capillary lumen. 7000 ×.

Diapedesis of erythrocytes is a passive process as a result of pressure of the blood in a situation of ever-increasing capillary permeability, whereas diapedesis of leukocytes is an active process of their migration through interendothelial spaces [6].

The results of the ultrastructural studies prove conclusively that besides hemorrhages of erythrocytes and sequestration of polymorphonuclear leukocytes, an important role in the development of acute respiratory failure is played by aggregation and adhesion of the blood cells and also thrombosis of the pulmonary capillaries. Close correlation exists between the blood adrenalin level and platelet aggregation; in particular, the hypercatecholaminemia associated with ETS stimulates thrombus formation [2].

Intravascular clotting, in which fibrin in precipitated in the alveolar capillaries (Fig. 2a) and has its characteristic period spacing, on average 20-22 nm (Fig. 2b), deserves special attention in the intermediate period of shock (after 5 h). Ultrastructural changes also were recorded in the platelets (vacuolation of the cytoplasm, reduction of the organelles, swelling of the mitochondria destruction of the plasma membrane), and were accompanied by their standard reaction: release of a specific platelet component — the so-called thromboplastic factor. Adhesion and aggregation of platelets and the formation of mxied thrombi, connected with deposition of fibrin, and together constituting a phenomenon of viscous metamorphosis, were frequently observed (Fig. 2a).

The predominant deposition of fibrin in the pulmonary arterioles also was confirmed by the results of 51 autopsies of persons dying from septic shock [13]. This affinity of fibrin precipitation in shock can evidently be explained by the ability of the lungs, in extremal situations, to assume the function of an organ responsible for additional filtration by-products from the venous network [13].

Disturbances in the hemostasis system in ETS are reflected in the rheologic properties of the blood. A progressive increase in the aggregating power of the platelets and erthyro-

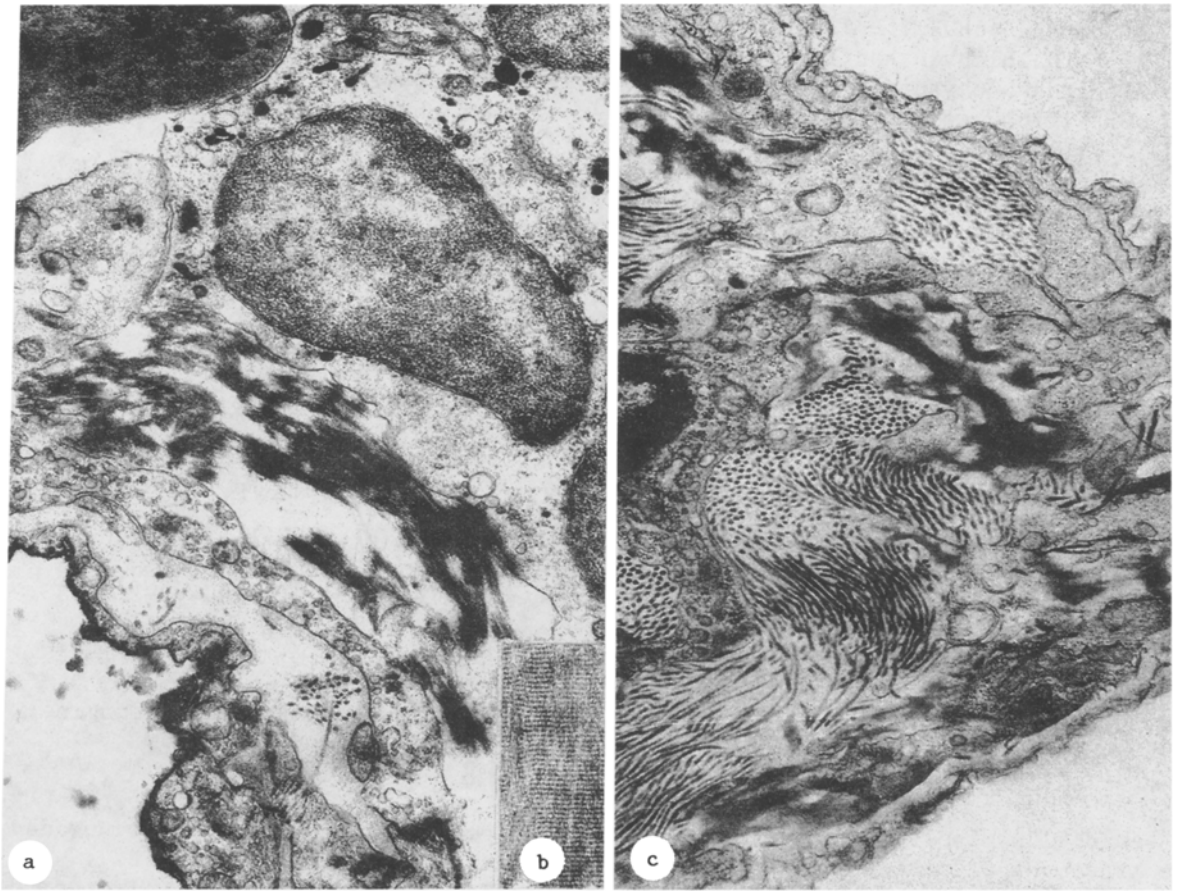

Fig. 2. Ultrastructure of lungs in intermediate period of ETS (a, b) and in stage of late endotoxemia (c). a) Deposition of fibrin, connected with platelet and polymorphonuclear leukocytes. 7000 ×. b) Structure of fibrin, with period spacing. 70,000 ×. c) Interstitial fibrosis and appearance of fibrinoid material. 10,000 ×.

cytes increases the viscosity of the blood, and a change in its flowability and destruction of cell membranes with release of aggregation stimulators lead to maintenance of a chronic syndrome of disseminated intravascular clotting and a consumption coagulopathy [10]. On the other hand, there is evidence that the formation of a "platelet carpet" in the zone of vascular damage potentiates their alteration, which is brought about by secretion of serotonin, histamine, beta-thromboglobulin, thromboxane A_2, and other factors, directly or indirectly damaging the vascular wall, from the platelets [10].

In the stage of late endotoxemia (after 3 days) a qualitatively new feature was interstitital fibrosis. Fibrinoid changes characterized by the appearance of a considerable volume of fibrinoid material were recorded simultaneously and in large quantities (Fig. 2c). This fibrinoid consists of swollen, homogeneous, chaotically arranged fragments of collagen fibers and fibrin, later undergoing polymerization.

The development of ETS was thus characterized by increased permeability of the air-blood barrier and by hemorrhages into the lungs. The inclusion of a clotting component adds to the disturbance of the intrapulmonary hemodynamics and aggravates it, and promotes the formation of a powerful perivascular barrier at the level of the microcirculatory bed. The formation of this additional obstacle on the air-blood boundary disturbs the gas exchange in the lungs and, at the same time, has a marked effect on their selective ability to ingest, store, and inactivate various vasoactive compounds. The consequent overloading and exhaustion of the nonrespiratory functions leads to the formation of actue pulmonary failure under the influence of endotoxin.

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EFFECT OF D-PENICILLINAMINE ON HEPATOCYTE ULTRASTRUCTURE AND ON STATE OF THE GROUND SUBSTANCE IN EXPERIMENTAL CIRRHOSIS OF THE LIVER IN RATS

A. S. Loginov, E. D. Makar'eva, T. V. Skobeleva, and M. A. Tumanyan

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D-penicillinamine (DPA), a metabolite of benzylpenicillin hydrolysis, is nowadays used for the treatment of patients with primary biliary cirrhosis of the liver (PBC). The use of DPA in PBC rests on the broad spectrum of its action: It lowers the level of circulating immune complexes and has a copper-eliminating action [2, 6, 10], it depolymerizes pathological macroglobulins, and inhibits collagen synthesis [2, 3, 5]. However, data on the efficacy of DPA in patients with PBC are often contradictory and there are no precise opinions about side effects arising during the use of this compound. Only solitary reports of the action of DPA on hepatocyte ultrastructure could be found in the accessible literature [11].

The aim of this investigation was to study the action of DPA on hepatocyte ultrastructure and on the state of the ground substances in the liver of rats with experimental cirrhosis.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing initially 140-160 g. Cirrhosis was produced by subcutaneous injection of CCl_4 in olive oil (1:1) in a does of 01. ml/100 g body weight, twice a week for 6 months. DPA was injected perorally in a does of 200 mg/kg 5 times a week from 2 to 4 and from 2 to 6 months of the experiment. Rats with liver damage induced by CCl_4 and intact animals, kept under the same conditions as the experimental animals served as the control. For electron-microscope investigation the rat liver was prefixed by perfusion with 0.5% gluataraldehyde in 0.1 M cacodylate buffer (pH 7.2) through the portal vein for 15-20 min [1]. The liver was washed with the same buffer for 10 min by intravascular perfusion, after which tissue fragments were fixed with 1% OsO_4 for 2 h. The tissue fragments were dehydrated in a series of alcohols of increasing concentration and embedded in Araldite. Sections cut on an Ultracut F ultramicrotome ("Reichert") were stained with uranyl acetate and lead citrate and examined in the JEM-1200 EX electron microscope with accelerating voltage of 80 kV and 30-μ aperture diaphragm. Uronic acids (UA) in the liver tissue were determined by the carbazole method [7], and activity of N-acetyl-β-D-glucosaminidase (β-NAG) was studied by Weissman's method in our own modification for the liver [4].

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