ULTRASTRUCTURAL BASIS OF THE ACTION OF ENDOTOXIN ON ENDOTHELIAL CELLS

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KEY WORDS: endotoxin; endothelial cells; shock; permeability.

In the initial period of endotoxin shock, permeability of the various tissue–blood barriers is increased [1-3, 6]. By analysis of the data, three mechanisms of this process have been postulated: the first – on account of the formation of multiple micropinocytotic vesicles, the second – connected with diapedesis, when blood cells leave the capillaries without any visible disturbances of their wall [8], the third – associated with more severe disturbances of ultrastructure: from slight separation of the endothelial cells [9] to the appearance of large areas of perforations measuring almost 20 μ [6].

At the same time, the vascular phenomena induced by endotoxin have been shown to produce a more varied picture of changes in the endotheliocytes which, in turn, induce a cascade of vasculo-hematologic disturbances, responsible for damage to the functional elements of the corresponding target organs.

Our single observations, made on desquamated endothelial cells in arterioles of the kidneys and adrenals in endotoxemia, suggested the need for a more detailed analysis of the character of their desquamation in another parenchymatous organ – the liver – in the intermediate period of endotoxin shock, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Endotoxin shock was obtained in 10 adult anesthetized dogs weighing 7-10 kg, into which typhoid or *E. coli* endotoxin was injected intravenously in a dose of 5 mg/kg. Two dogs receiving an equivalent volume of sterile physiological saline served as the control. The arterial pressure was recorded by means of an ultrasonic pressure transducer by the direct method [7].

Circulating endothelial cells were counted in the blood 5 h after injection of endotoxin or physiological saline by the method in [11] in the writers' modification. For this purpose, 5 ml blood was added to 10 ml of buffered sodium citrate solution. The erythrocytes were hemolyzed with 1% saponin solution in 50% ethanol, and the remaining "leukocyte concentrate" was centrifuged at 5000 rpm for 15 min. The residue was transferred to slides, films were stained by the Romanovsky–Giemsa method, and they were studied for the presence of desquamated endotheliocytes, which were counted per 100 blood cells. The result was considered to be positive if more than five endothelial cells were found on the slides, and negative if their number did not exceed two [10]. The numerical results were subjected to statistical analysis.

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TABLE 1. Number of Desquamated Endothelial Cells in Blood Films from Control Dogs and during Intermediate Period of Endotoxin Shock

Parameter	Control (n=3)		Experiment (n=10)	
	background	5 h after injection of physiological saline	background	5 h after injection of endotoxin
Desquamated endothelial cells (per 100 blood cells)	2,8±0,42	3,1±0,45	3,0±0,63	18,2±1,04*

Legend. Asterisk indicates significance of differences relative to background ($P < 0.001$). n) Number of experiments.

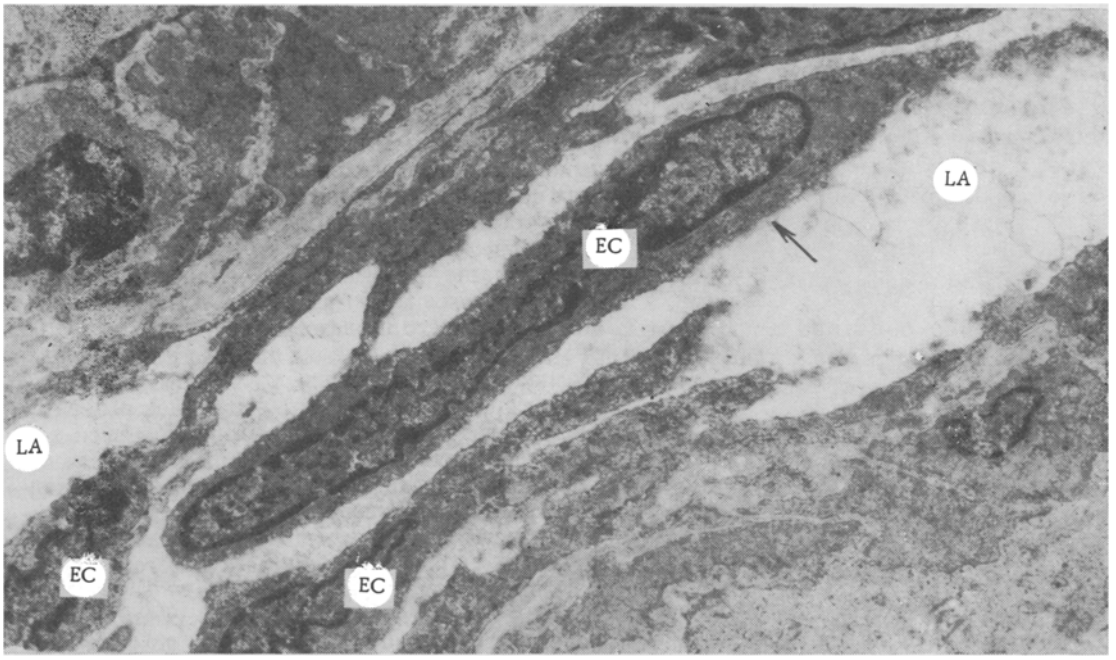

Fig. 1. Fragment of arteriole in longitudinal section during intermediate period of endotoxin shock. LA) Lumen of arteriole, EC) endothelial cells (arrow indicates a desquamated endothelial cell). 2800 ×.

Pieces of liver were fixed in a 3% solution of glutaraldehyde in 0.1 M phosphate buffer, postfixed in a 1% solution of osmic acid in Millonig's buffer at 4°C, dehydrated in alcohols, and embedded in Epon-812. Sections cut on the LKB-8800 Ultramicrotome were stained on grids with uranyl acetate and lead citrate and examined in the IEM-100S electron microscope.

EXPERIMENTAL RESULTS

Intravenous injection of the endotoxins into dogs of the experimental group caused the arterial pressure to fall after 30 min (the initial period of endotoxin shock) to 60 ± 10 mm Hg ($P < 0.001$), when, after a small rise, it soon began to fall gradually again, to reach 20 ± 5 mm Hg ($P < 0.001$) by the end of the 5th hour (the intermediate period of endotoxin shock). All dogs also developed characteristic symptoms described previously in animals of other species [12].

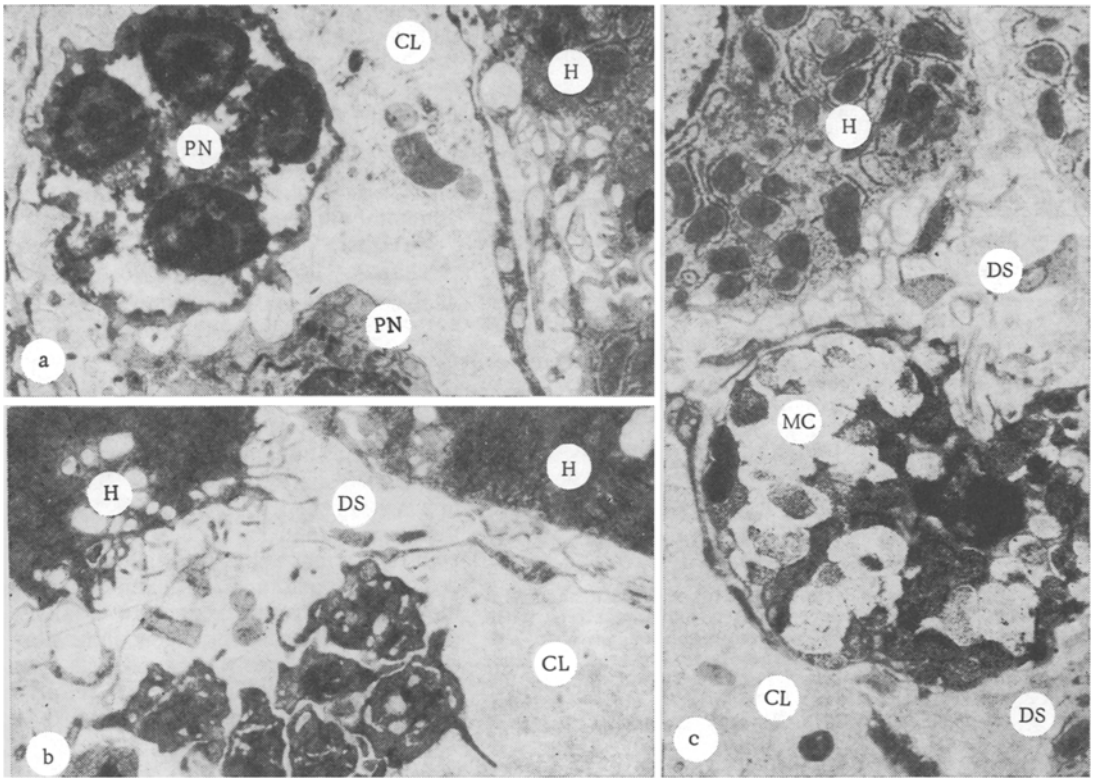

Fig. 2. Ultrastructure of degranulated polymorphonuclear neutrophils (a), platelets (b), and mast cell (c) in intermediate period of endotoxin shock. PN) Polymorphonuclear neutrophils; P) platelets; MC) mast cell; CL) capillary lumen; H) hepatocytes; DS) Disse's space. Magnification: a) 4200, b) 7000, c) 3500.

In dogs receiving physiological saline the blood pressure remained practically unchanged; the number of desquamated endothelial cells remained the same as in the initial state, and the ultrastructure of the liver corresponded to the pattern described previously [4].

In the intermediate period of shock, examination of the films, irrespective of the endotoxins used, showed a sixfold increase in the number of desquamated endotheliocytes (Table 1). Electron-microscopic investigation of the liver revealed different stages of successive separation of endothelial cells from the basement membrane in the arterioles and sinusoidal capillaries. This process was particularly demonstrative in the arterioles, where the cytoplasmic "bridge" could be seen to become gradually thinner with progressive detachment of the endotheliocytes, and the cells protruded far into the lumen of the vessel and finally were detached (Fig. 1).

The nuclei of the desquamated endotheliocytes became drop-like in shape, and their surface facing the basement membrane was usually considerably narrowed whereas the opposite surface was widened. On the basis of this feature it is easy to differentiate the corresponding surface of the desquamated endotheliocytes.

Degranulated polymorphonuclear neutrophils (Fig. 2a) and concentrations of platelets (Fig. 2b) could be seen in the sinusoidal capillaries. Mast cells, whose granules were uniform in density or were completely empty, reflecting different degrees of liberation of mediators (labrokines), could be seen in the Disse's space (Fig. 2c).

The possible mechanisms of alteration of the endothelial cells thus become clear. First, they may be connected with the direct action of endotoxin, which contributes to separation of the endotheliocytes from the basement membrane in tissue culture [13]. Second, injuries to the endothelial cells may be due to vasoactive substances of platelets and mast cells or to lysosomal enzymes of leukocytes [5].

Consequently, the results indicate that destructive disturbances of the blood vessels are most probably the result of interaction between the endothelium and blood cells. However, it is also evident that the possibility of any such changes must be potentiated by hemodynamic stress and by other preceding lesions caused by such stress.

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ULTRASTRUCTURE OF THE PERI-INFARCTED ZONE OF THE MYOCARDIUM

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KEY WORDS: ischemia and infarction of the myocardium; peri-infarct zone; ultrastructure of cardiomyocytes; intracellular regeneration.

Particular attention is currently being paid to the peri-infarct zone (PIZ) of the myocardium, bordering on the infarct [3, 4, 6], as the point of application of various therapeutic procedures aimed at limiting the spread of necrosis of cardiomyocytes. The undisputed beneficial clinical effect of these procedures [10] calls for further structural and functional analysis of processes taking place in PIZ in the course of spontaneous experimental occlusive myocardial infarction and under the influence of drugs. However, the structural characteristics of PIZ have not yet been adequately studied and changes in the cardiomyocytes at the periphery of zones of necrosis of heart muscle and on the boundary with normal tissue are variously interpreted [6].

The aim of this investigation was to study the dynamics of ultrastructural changes taking place in PIZ of the myocardium in dogs after occlusion of the coronary artery.

EXPERIMENTAL METHOD

Occlusive myocardial infarction was induced in 26 mongrel dogs weighing 16-20 kg by ligation of the anterior descending branch of the left coronary artery in its middle third [2]. Material for morphologic investigation was taken 2, 4, 6, 8, and 12 h, 1, 2, 3, and 15 days, and 1 month after occlusion of the coronary artery. Samples of myocardium were excised from the center and periphery of the area of ischemic necrosis, from PIZ, and from areas of myocardium remote from the zone of infarction and were fixed in a 12% solution of neutral formalin. Sections cut from blocks of tissue embedded in paraffin wax were stained with hematoxylin–eosin and by the PAS reaction, with counterstaining of the muscular nuclei with hematoxylin, and embedded in polystyrene. The investigation was carried out in direct and polarized light by means of a Docuval universal light microscope (Carl Zeiss, East Germany). Specimens for electron-microscopic study were fixed in cold 4% paraformaldehyde in 0.1 M phosphate

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