ULTRASTRUCTURAL CHANGES IN THE TISSUE-BLOOD BARRIER IN THE LIVER IN THE EARLY PERIOD AFTER ENDOTOXIN INJECTION

Yu. A. Barshtein, Yu. V. Persidskii, and M. I. Grutman

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Endothelial cells are structures which are among the first to interact with infectious agents and toxic substances during the development of an infectious process. Changes in the microcirculatory bed of the liver, like the response of individual types of liver cells to pathogenic microorganisms and of their metabolic products are interesting because it is the liver which carries the main load relative to inactivation of toxins and metabolites of inflammation. The accessibility of the hepatocytes for exposure to toxic products, together with the degree of their uptake by macrophages (Kupffer cells), is determined by the permeability of the tissue-blood barrier in the liver. Endothelial cells of sinusoids form numerous pores and extensive fenestrae with a single membrane, through which substances are transported into the Disse's spaces to hepatocytes. The number and diameter of the fenestrae and pores vary depending on the species of animal [5, 6], and their number also changes in pathological states [1, 4, 8, 9] and under the influence of various substances [3, 10, 11].

Although changes in permeability of the tissue-blood barrier in the liver play an important role in metabolism, the mechanisms determining the response of the endothelial cells of the sinusoids and the porosity of the barrier have not yet been explained. According to Steffan and co-workers [10], this is due to the difficulty of studying these processes in vivo. We therefore decided to study the reactions of epitheliocytes of the sinusoids and of other types of liver cells at intervals during the early stage after injection of *Salmonella typhimurium* endotoxin, for this endotoxin induces a number of biological effects which accompany Gram-negative infection.

EXPERIMENTAL METHOD

CBA mice weighing 18-20 g were given an intraperitoneal injection of an ultrasonic lysate of *S. typhimurium*, sterilized by filtration through membrane filters. Its principal active component is an endotoxin. The dose (2 LD_{50}) was chosen in order to give a gradually developing effect of a disturbance of the circulation. The mice were anesthetized with hexobarbital in a dose of 60 mg/kg 15, 33, 45, and 60 min later, laparotomy and thoracotomy were performed, and a cannula was introduced through the left ventricle into the aorta, where it was fixed. Meanwhile an incision was made in the inferior vena cava above the point where it receives the hepatic vein. For 20 sec the mouse was perfused with Eagle's medium with the addition of 10 U heparin at a temperature of 37°C and at the rate of 2 ml/min, after which perfusion was continued with a fixative (2.5% glutaraldehyde in Eagle's medium) for 15 min. Mice receiving an intraperitoneal injection of physiological saline (0.2 ml) served as the control. Fragments of tissue from different parts of the liver, with a volume of 2 mm^3, were fixed in the same solution for 24 h at 4°C. Some of them were washed in 0.1 M cacodylate buffer and then dehydrated in ethanol of increasing concentration and dried, secured with current-conducting glue to stages, and sprayed with gold (thickness of layer 20 nm) in a JFC-1100 apparatus ("Jeol," Japan). The material was examined in a JAMP-10S scanning electron microscope ("Jeol"). Other pieces were postfixed in 1% OsO_4 solution in cacodylate buffer, dehydrated in ethanol of increasing concentration, and mounted in Epon 812. Ultrathin sections were stained with a saturated solution of uranyl acetate and lead citrate and examined in ÉMV-100 (USSR) and JEM-100 CX 11 ("Jeol") transmission electron microscopes.

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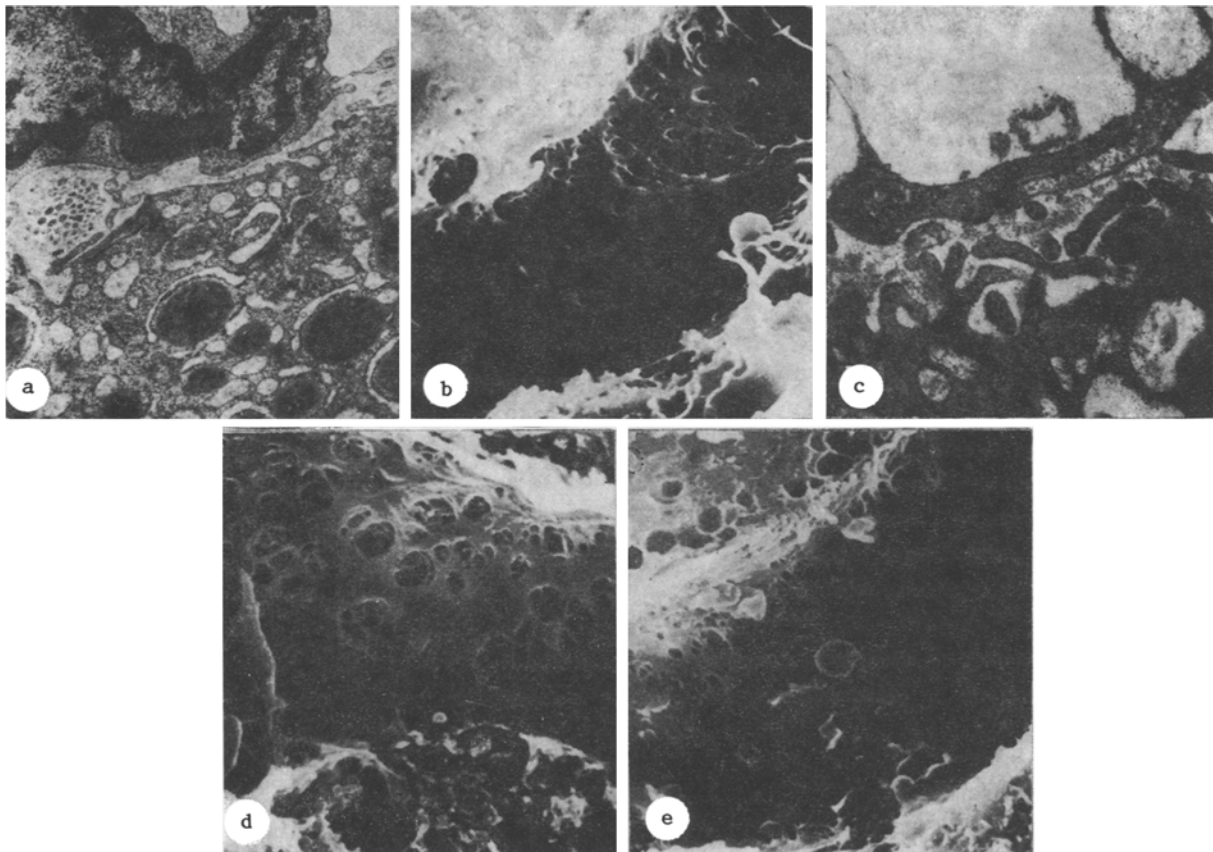

Fig. 1. Changes in endothelial cells of liver sinusoids during 60 min after injection of endotoxin: a) uneven contour of cytoplasmic membrane and karyolemna of endothelial cell of a sinusoid, increased content of cisterns of smooth endoplasmic reticulum — 15 min after injection of endotoxin, 21,000×; b) increased number of pores on surface of endotheliocyte of sinusoid 15 min after injection of endotoxin, 6600×; c) vacuolation of cytoplasm of endothelial cell of sinusoid — 60 min after injection of endotoxin, 24,000×; d) increased number of pores in endothelial cell, platelets in lumen of sinusoid 60 min after injection of endotoxin, 8600×; e) numerous vesicles present on surface of endothelial cell — small fatty inclusions are visible in hepatocyte on fracture surface; 60 min after injection of endotoxin, 7800×.

EXPERIMENTAL RESULTS

In the early stages after injection of the endotoxin (15 min) changes were observed in the shape of the endothelial cells of the sinusoids, accompanied by some increase in volume of the nucleus-containing zone, and an increase in the content of elements of the smooth endoplasmic reticulum in the cytoplasm; bundles of microfilaments were found beneath the cytolemma. The karyolemm and cytolemma were characterized by an uneven outline (Fig. 1a). The cytoplasm of the endotheliocytes appeared to be drawn toward the nucleus. Under the scanning electron microscope, fenestrae and pores with the characteristic cluster arrangement of the latter were observed in greater numbers than normally on the surface of the endothelial cells of the sinusoids (Fig. 1b). The pores also were enlarged compared with the normal size.

During transmission electron microscopy 45-60 min after injection of the endotoxin the nuclei of the endothelial cells of the sinusoids preserved their uneven outline with condensation of chromatin granules beneath the nuclear membrane. The cytoplasm was characterized by the presence of vacuoles and of microfilaments and by pinching off of small areas of cytoplasm into the lumen of the sinusoids (Fig. 1c). The abdominal surface of the cytolemna also became uneven and formed wide outgrowths, which was accompanied by widening of Disse's space. Under the scanning electron microscope the dimensions and number of the pores increased even more, and at the same time zones on the surface of the endothelial cells without these formations were found (Fig. 1d, e).

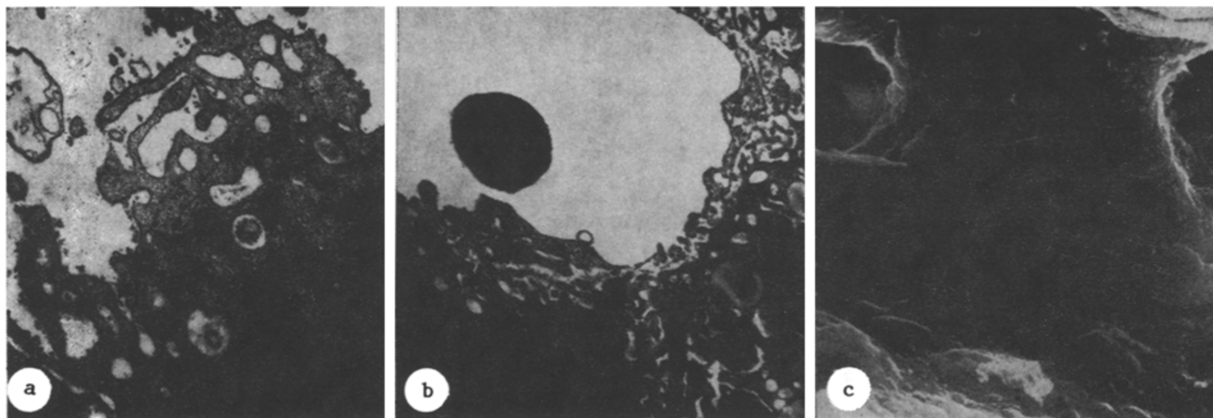

Fig. 2. Changes in Kupffer cells and hepatocytes during 60 min after injection of endotoxin. a) Kupffer cell, enlarged in volume, with increased content of lysosomes and vacuoles, forming rocketlike structures, 20,000×; b) dilatation of tubules of endoplasmic reticulum, small fatty inclusions in cytoplasm of hepatocyte — uneven contour of cytoplasmic membrane of endothelial cell of sinusoid, 7200×; c) appearance of single microvilli on surface of endothelial cells of central vein — places of emptying of sinusoids can be seen, 3000×.

The lumen of the sinusoids in the early period after injection of the endotoxin (15 min) was somewhat dilated and contained single freely lying erythrocytes, single platelets, and lymphocytes. Toward the 60th minute their number increased and the lumen of the sinusoids was dilated. No junctions were present between the blood cells and the luminal surface of the endothelial cells of the sinusoids. At the times chosen for study, no evidence of the formation of fibrin fibrils was observed.

Toward the 15th minute after injection of the endotoxin, a change in shape and an increase in size of the Kupffer cells ware observed and they had an increased content of vacuoles. Their cytolemma formed multiple microvilli. Toward the 60th minute, characteristic rocketlike structures, numerous lyso6omes, and vacuoles were discovered in the stellate reticuloendotheliocytes (Fig. 2a). Under the scanning electron microscope, the enlarged Kupffer cells partly blocked the lumen of the sinusoids and their surface was speckled with microvilli.

In the hepdtocytes between 50 and 30 min after injection, dilatation of the channels of the endoplasmic reticulum was observed, accompanied by a reduction in the glycogen content and an increase in the number of lysosomes and in the number of microvilli facing Disse's space. In the later stages (toward the 60th minute) tiny fatty inclusions appeared in their cytoplasm (Fig. 2b). Some cells were distinguished by the uneven outline of their nuclear membrane and by condensation of chromatin granules beneath the cytolemma, and by an increase in size of the nucleolus.

The study of the state of the endothelial cells of the central venules, lined by continuous endotkelium, revealed very slight changes, visible under the transmission electron microscope, for the first time 60 min after injection of the endotoxin. Among them must be noted a change in shape of the nuclei of some cells and the appearance of individual short microvilli on the luminal surface. Under the scanning electron microscope, a few microvilli were found on the surface of the endothelial cells (Fig. 2c).

The study of changes in the mouse liver cells following injection of *S. typhimurium* endotoxin in a dose of 2 LD_{50} thus revealed an early response of the endothelial cells of the sinusoids and of the Kupffer cells. Changes in the endothelial cells during the first 60 min after injection of the endotoxin took the form of some degree of thickening of the nucleus-containing zone, changes in the shape of the nucleus, and the appearance of microfilaments in the cytoplasm. Parallel with this, a progressive increase was observed in size and number of pores and in the number of fenestrae of the endothelial cells of the sinusoids, while preserving their arrangement in clusters. Before the end of the first hour after injection of the endotoxin the cytoplasm contained separate vacuoles, and areas of cytoplasm were being pinched off into the lumen of the sinusoids. This restructuring of the endothelial cells of the sinusoids in the early periods consisted on the whole of a change in the cytoskeleton and increased flowability of the membranes. Similar changes in endothelial cells, discovered very early, can be characterized on the whole as activation of the endothelial cells of the sinusoids, evidently due to the direct action of the endotoxin on them. It is the early response of the endothelial cells that provides a basis for this assumption, for at these times there were no signs of contact interaction between the endothelial cells and blood cells, or even less, signs of disseminated intravascular clotting. The early response of the endothelial cells thus revealed, on the one hand indicates the

possible existence of endotoxin receptors on their membranes [2] and, on the other hand, confirms the active role of the specialized endothelium of the microvessels of the liver in the realization of the multiple biological actions of endotoxin.

One manifestation of activation of the endothelial cells of the sinusoids is increased porosity of the blood-liver barrier in the early stages after injection of the endotoxin. Signs of a response of the endothelial cells are observed simultaneously with activation of the Kupffer cells, which play a key role in capture and inactivation of endotoxin [7]. At the same time, this restructuring of the endotheliocytes of the sinusoids precedes changes in the endothelial cells of the central venules, individual signs of which do not become apparent until the 60th minute, a fact which confirms the specificity of action of the endotoxin on the endothelial cells of the liver sinusoids.

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