MORPHOLOGY AND PATHOMORPHOLOGY

Peritoneal Endotoxicosis, Morphology, and Morphogenesis of Biosystem Involvement in Experimental Animals

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Experimental acute peritonitis was induced by single and repeated injections of 1.5-3% fecal autosuspension in the abdominal cavity of white rats. The blood content of medium-weight molecular proteins, which increases 1.5-2-fold during the course of entry of toxic products into the blood over 1-3 days, was measured. Examination of the vascular system and parenchymatous elements of many organs helped reveal three morphogenetic mechanisms of their injury: hyperfunction of cells, followed by their depletion and death; the direct action of toxic products on membranous structures of cells due to severe impairment of the histohematic barrier at the level of the microcirculation; the destructive effect of hypoxia due to the development of the disseminated intravascular coagulation syndrome.

Key Words: *experimental peritonitis; endotoxicosis; visceral involvement*

One of the principal contributors to the pathogenesis of acute peritonitis (AP) is endotoxicosis, including three components: the source of intoxication (bacterial toxins, products of abdominal organ tissue degradation, medium-weight molecular proteins, etc.), the circulation of toxins in the body, and their action on target organs, involving injury to highly specialized visceral structures. The release of lysosomal enzymes (hyperfermentemia) and of bioactive substances associated with it leads to a second wave of intoxication with the formation of a "vicious circle" [2,3] which aggravates the course of the disease. However, our knowledge of the morphogenetic mechanisms of involvement of the smallest biosystems in AP is inaccurate and incomplete. Special attention should be paid to the adequacy of AP reproduction and experimental verification of the endogenous intoxication syndrome.

The purpose of this research was to define the criteria for assessing endogenous intoxication in experimental animals and to identify the mechanisms of the injurious action of some endotoxins on the structural components of organs and tissues.

MATERIALS AND METHODS

Experiments were carried out with 120 outbred male white rats. AP was induced as described previously [9], with our modifications [4]. To induce AP, 1.5-2.0 ml of 3% fecal autosuspension were injected in the abdominal cavity (group 1). The "hypertoxicosis" state was attained either by a single injection of fecal suspension of an increased concentration, or by repeated injections thereof during 24 h (group 2). The animals were sacrificed under chloroform narcosis 3, 6, 12, and 24

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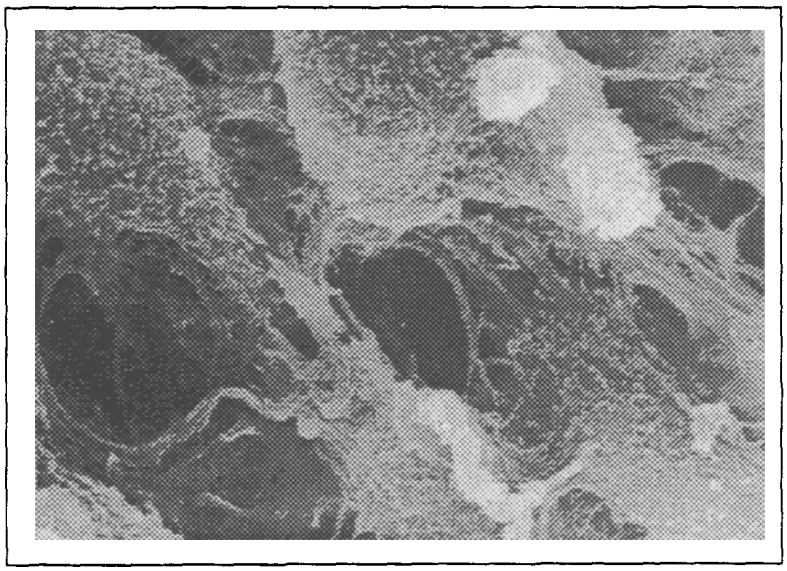

Fig. 1. Dilated stomata in the diaphragmatic part of the peritoneum on AP day 1. Scanning electron microscopy. ×3000.

h and 3, 6, and 10 days after the first injection. Each experimental series consisted of 5 to 10 animals. Mortality was 10% in group 1 and 25% in group 2. Five rats intraperitoneally injected the same amount of normal saline were controls.

The level of medium-weight molecular (MWM) proteins was measured in the serum with an SP-46 spectrophotometer at wavelength 254 nm [10]. At autopsy smears from the peritoneum and blood were stained as described previously [7] in order to detect the level of cationic proteins in granulocytes. Specimens of different portions of the peritoneum, liver, kidneys, heart, spleen, hypothalamic area, pituitary, and adrenals were taken for morphological examination. The fragments were embedded in paraffin and stained with hematoxylin-eosin, picrofuchsin, Schiff-iodine acid, paraldehyde fuchsin with azan staining after Heidenhain, and with sudan IV after Leigh. Enzymes were detected in frozen cryostat slices: succinate dehydrogenase (SDH), NAD^+ and NADP-diaphorases, glucose-6-phosphate dehydrogenase (G-6-PD), and acid and alkaline phosphatases. Histochemical reactions were quantitatively assessed by scanning (30 or more cells of different organs) with an MUF-5 radiation microspectrophotometric device. Digital data were statistically processed using designated computer software. A polarization microscope was used to study the myocardium.

Part of the material was fixed in 2.5% glutaraldehyde on Hanks buffer (pH 7.3) and then in 1% osmic acid, dehydrated in ascending alcohols, and embedded in epon. Ultrathin slices were made with an LKB-III ultratome, contrasted with uranyl acetate and lead citrate, and studied under JEM-100C and EMB-100AK electron microscopes. For scanning electron microscopy, fragments of organs were fixed in 4% paraformaldehyde, dehydrated by the critical point passage technique, sprayed with platinum by the ionic the bombarding method, and examined under a 840A electron microscope.

Peripheral blood corticosterone and adrenocorticotropic hormone (ACTH) were radioimmunoassayed using RIN-B-^{3}H (Institute of Bioorganic Chemistry, Belarus) and ACTHK-PR "CIS" (France) kits, respectively. Results were statistically processed after Student.

RESULTS

The count of bacterial corpuscles in the abdominal cavity rapidly increased during the first day of

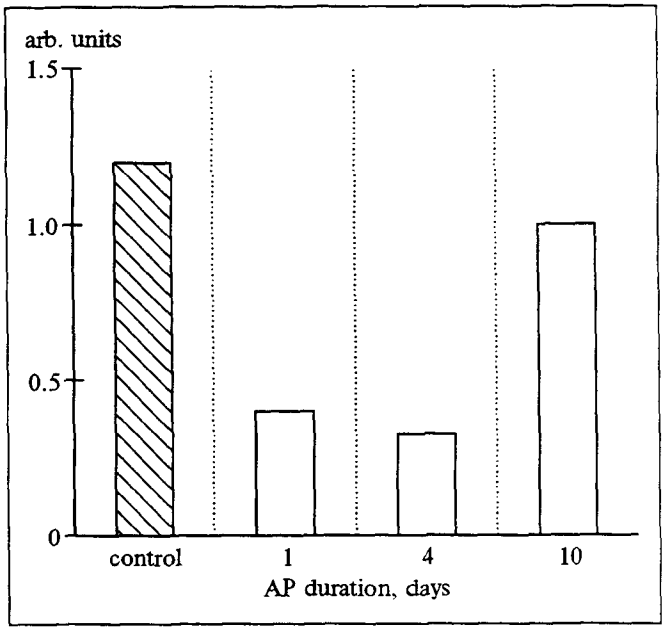

Fig. 2. Level of cationic proteins in the peripheral blood neutrophils in the course of AP.

the experiment, reaching the maximum by the end of this day. Later the number of bacteria gradually decreased. Examination of the peritoneum under the electron microscope showed that as early as 3 h after injection mesotheliocytes were sharply reduced, the number of microvilli on their surface had decreased, and large spaces had appeared between cells, allowing the abdominal contents to spread outside the abdominal cavity. The stomata of the diaphragmatic part of the peritoneum were markedly dilated (Fig. 1) and microorganisms were often seen in their lumina. During the subsequent 6-12 h mesotheliocytes at some sites were necrotized and desquamated, denuding the connective tissue base.

A study of MWM in the peripheral blood of adult animals showed an increase in their level from 0.18±0.01 (control) to 0.24±0.02 (in 24 h) and to 0.28±0.03 arb. units by the end of day 3. The level of MWM in the blood then fell, though not as far as to reach the initial level even by day 10 of the experiment (the difference is statistically reliable). On the other hand, the level of cationic proteins in the peripheral blood neutrophils dropped markedly (Fig. 2).

Under conditions of "hypertoxicosis" the MWM level rapidly increased during 3 days (Fig. 3, *a*).

The vascular bed of all the examined organs underwent marked rearrangement. This involved, first of all, intensification of micropinocytosis, local injury to the endotheliocyte vascular surface, and development of the disseminated intravascular coagulation syndrome. These changes were most typical of day 1 of the experiment, whereas later on, destructive changes developed in the endothelial cells (plasmatic impregnation of their hyaloplasm, dilatation of the "pores" in the endothelial lining 60-80 nm in diameter, disrupted cell-to-cell contacts). This resulted in the penetration of plasma (Fig. 4) containing toxic products and enzymes in the perivascular space and their direct action on the parenchymatous elements of the viscera. More pronounced signs of edema and erythro- and leukodiapedesis were characteristic of "hypertoxicosis," in which the vascular wall frequently underwent fibrinoid necrosis.

The effects of some endotoxins, specifically, of MWM, have been fairly well studied. The low molecular weight of MWM permits them to penetrate easily through the plasma cell membrane, causing submicroscopic disorganization of the mitochondria with disorders of oxidative phosphorylation [1,5,6]. This is particularly evident upon electron microscopic examination of the viscera. For example, changes in specialized cellular elements of the studied organs are recorded as early as during the first hours of AP. Neurons and terminals of the median eminence and caudal neurohypophysis intensively lose neurosecretion granules in the supraoptic and paraventricular (in large- and small-cell populations) nuclei of the anterior hypothalamus. In adenohypophyseal corticotropocytes hormonal granules are accumulated in the

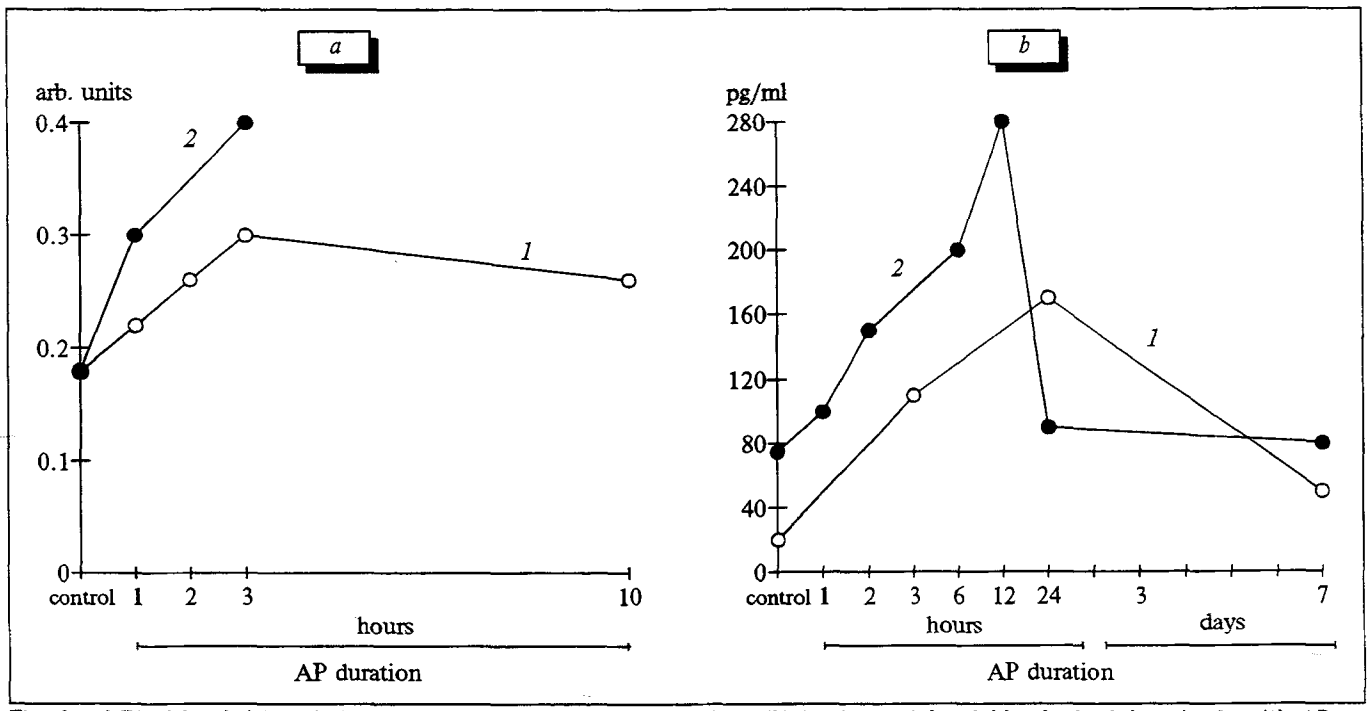

Fig. 3. MWM level (*a*) and time course of hormonal concentrations (*b*) in the peripheral blood of adult animals with AP. *a*: *1*) usual course of AP, *2*) AP with "hypertoxicosis"; *b*: *1*) ACTH, *2*) corticosterone.

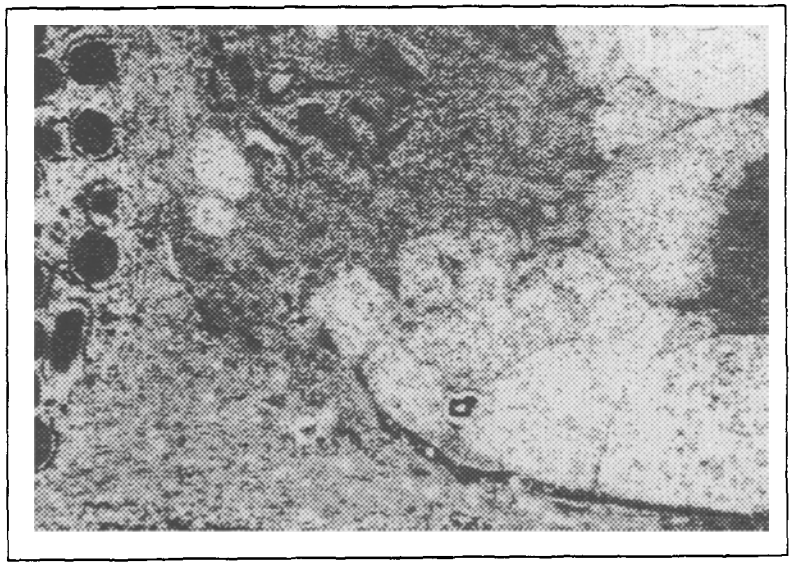

Fig. 4. Impregnation of endotheliocyte cytoplasm and perisinusoidal space of the liver with plasma proteins. AP day 2. Electronogram. ×3600.

plasmolemmas, and their exocytosis is observed. The content of lipids is drastically reduced in the liposomes utilized for steroidogenesis intensification in the adrenocortical bundle zone cells. Morphological criteria of increased functional activity of the neuroendocrine and endocrine systems are confirmed by the findings of radioimmunoassay which indicate an increase of ACTH and corticosteroid concentrations in the peripheral blood (Fig. 3, *b*). However, after 12 and, especially, 24 h the first signs of destruction are observed in these cells, manifested by hyaloplasm edema, nuclear pyknosis, swelling and destruction of mitochondria, and a corresponding drop in the level of adaptive hormones.

In other organs partial colliquative necrosis of hepatocytes, hydropic dystrophy of nephrocytes, defects in the outer membrane of lymphocytes, swelling and degradation of mitochondria, and subsegmentary contracture changes in cardiomyocytes were noted on day 1 of the experiment. On days 2-3 of AP destructive processes were the most expressed, characterized by lobular necrosis of the liver, necrotic nephrosis, zonal contracture injuries to cardiomyocytes, and lysis of lymphocytes. By this time the levels of MWM in the blood and vascular disorders were maximal.

The energy provision of the cells appreciably changed, reflecting a unidirectional time course for all the tested organs (suppression of tissue respiration). For example, in the adrenocortical bundle zone the NAD^+ level dropped by the end of day 1 from 0.382±0.04 (control) to 0.263±0.01 arb. units ($p<0.05$), G-6-PD from 0.502±0.02 to 0.499±0.05 arb. units ($p>0.05$), and SDH from 0.324±0.013 to 0.285±0.007 arb.units ($p<0.05$) with an increase on day 3. This appeared to be due to the release of some enzymes into the regional bloodflow, which fact is confirmed in V. A. Popov's report [8], demonstrating hyperfermentemia as early as 24 h after AP onset.

Hence, an important component of peritoneal endotoxicosis in AP is impairment of peritoneal mesotheliocytes with a marked dilatation of stomata, which leaves the way open for intensive entry of bacterial and tissue degradation products into the common bloodstream. This leads to a drop of the level of cationic proteins in the peripheral blood neutrophils. The developing hyperfermentemia and depression of the body's adaptive systems still further aggravate the status of experimental animals with AP. The severity of endotoxicosis in such animals is to a considerable degree characterized by the level of MWM in the peripheral blood. Specifically, the "hypertoxicosis" status is associated with an appreciable increase of the MWM level in the blood *vs.* that in AP induced by a single injection of fecal suspension.

Injury to the smallest biosystems of the organism is related to at least three morphogenetic mechanisms: 1) cell hyperfunction followed by depletion and death; 2) a direct effect of toxic substances on cell membrane structures due to profound involvement of the histohematic barrier of microvessels; and 3) a destructive effect of dyscirculatory disorders and hypoxia on the organs, tissues, cells, and subcellular structures.

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Oxidative Metabolism of Neutrophilic Polymorphonuclear Leukocytes in Experimental Massive Pulmonary Embolism

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Oxidative metabolism of neutrophilic polymorphonuclear leukocytes circulating in the blood was studied by chemiluminescent analysis and the nitroblue tetrazolium reduction test. Pronounced activation of oxidative metabolism of polymorphonuclear leukocytes was observed by the third and sixth hours of massive embolism of the pulmonary arteries. Comparison of the examined parameters of oxidative metabolism of leukocytes isolated simultaneously from venous and arterial blood indicates a delay of the most active cell fraction in pulmonary vessels during massive embolism. The transfer of functionally active leukocytes from the circulating to the marginal pool of the lungs suggests their participation in damaging the pulmonary microvascular endothelium and in increasing its permeability.

Key Words: *massive pulmonary embolism; neutrophilic polymorphonuclear leukocytes; lung vessels; endothelium*

Damage to the endothelial lining of microvessels is thought to be the principal factor in the development of the respiratory distress syndrome in adults with various diseases [17]. Numerous reports attest that endothelial damage is associated with the activation of oxidative metabolism in neutrophilic polymorphonuclear leukocytes (NPMNL) and the production of reactive oxygen species (ROS) by them [11,13,14,21]. We have shown that acute massive embolism of pulmonary arteries (MEPA) is paralleled by neutrophil accumulation in the lumen of alveolar capillaries, by numerous local impairments of the endothelium, and by manifest interstitial edema [2]. The aim of this work was to investigate NPMNL oxidative metabolism and the possible role of ROS generated by these cells in damage to the endothelium of pulmonary microvessels in experimental MEPA.

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MATERIALS AND METHODS

Mongrel dogs weighing 15 to 20 kg were used in experiments which were carried out under conditions of closed chest and spontaneous respiration. For

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