The result of the action of eleuterococcus on biological rhythms of erythrocyte, hemoglobin, and leukocyte concentrations thus depends on the time during the 24-hour period of its administration. Changes in the biological rhythms of these peripheral blood parameters took place only if the preparation was given to the animals in the afternoon.

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EFFECT OF ENDOTOXIN ON OXIDATIVE METABOLISM OF POLYMORPHS

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The endotoxemia which often accompanies diseases of bacterial origin is connected with the appearance of an endotoxin (a fragment of the wall of Gram-negative bacteria) in the blood stream. The lungs are affected before other organs and most severely by endotoxemia. The primary target cells for endotoxin are considered to be the endotheliocytes of the alveolar capillaries. Results obtained in studies on cultures of endothelial cells indicate the absence of any direct cytotoxic action of the endotoxin over a wide range of concentrations [12]. However, another view also is widely held [2]. A characteristic feature of endotoxemia is the leukopenia which has already appeared by the end of the first hour [4]. Meanwhile, as has repeatedly been

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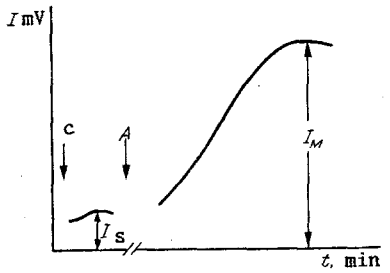

Fig 1. Chemiluminescence curve (diagram). C) Cells, A) activator I_s) Spontaneous chemiluminescence, I_m) activated chemiluminescence.

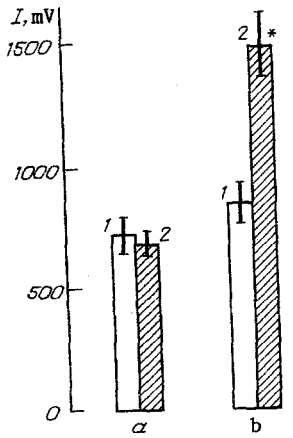

Fig. 2. Chemiluminescence response of neutrophils obtained from animals with endotoxemia lasting 1.5 h (shaded columns) compared with neutrophils from intact animals (unshaded columns). a) I_s, b) I_m. Mean error of mean $m = \sigma\sqrt{n}$ used as confidence interval. *$p < 0.05$.

pointed out, polymorphonuclear leukocytes (polymorphs) accumulate in large numbers in the lumen of the pulmonary capillaries. The sharp increase in the number of neutrophils in the lungs in the early stages of endotoxemia suggests that these cells are involved in the damage caused to the endothelial lining of the pulmonary microvessels. Neutrophils are known to be able to respond to particular agencies by a sharp increase in oxygen and glucose uptake, i.e., by a respiratory burst — and by the massive production of active forms of oxygen (AFO): the superoxide anion-radical (O_2), the hydroxyl radical (HO), and hydrogen peroxide (H_2O_2). AFO play an important role in tissue damage in various states (inflammation, postischemic syndrome, sepsis). Results obtained previously [1] show that injection of endotoxin stimulates oxidative metabolism of the neutrophils.

The aim of this investigation was to discover whether endotoxin can itself induce an increase in APO production neutrophils, or whether it exerts its action indirectly through other biological factors.

EXPERIMENTAL METHOD

Experiments were carried out on peripheral blood neutrophils of 15 male albino rats weighing 260-390 g. The leukocytes were separated from erythrocytes by allowing heparinized whole blood to stand with gelatin. The resulting leukocyte suspension was applied to a Ficoll-Verografin gradient ($d = 1.078$ g/cm^3) and centrifuged for 55 min (400g, 20°C). The few erythrocytes present in the residue were hemolyzed with distilled water (30 sec, 4°C). The osmolarity of the solution was restored with 0.6 M NaCl, after which the cells were twice washed with Hanks' solution (pH 7.4, 400g, 10 min). The number of viable cells was determined by studying ingestion of trypan blue. It amounted to 95-98% before and after incubation of the cells in the medium

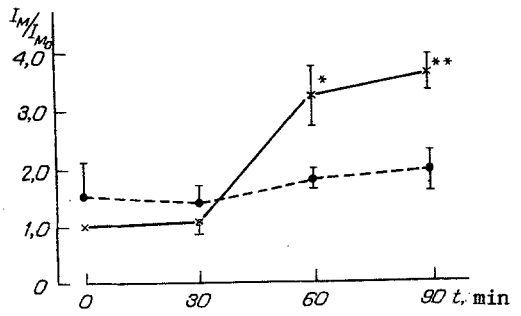

Fig. 3. Time course of chemiluminescence response during incubation of neutrophils obtained from intact animals with (broken line) and without endotoxin (continuous line). *$p < 0.05$, **$p < 0.01$.

for 2 h at 37°C. Incubation of leukocytes with endotoxin very slightly reduced (to 87%) the number of viable cells. AFO production by leukocytes was estimated by measuring chemiluminescence on a 1251 luminometer (LKB, Sweden). The luminol concentration in the medium of measurement (Hanks' solution, pH 7.4) was $2 \cdot 10^{-5}$ and $5 \cdot 10^{-4}$ M. With constant mixing at 37°C and with a final cell concentration of $0.2\text{-}0.8 \cdot 10^6$/ml, the light sum of luminescence in 10 sec was measured. Spontaneous chemiluminescence (I_s) — before addition of the activator to the medium, and so-called maximal chemiluminescence (I_m) — after addition of the activator, were estimated (Fig. 1). In the experiments of series I activity of neutrophils isolated from the blood of rats receiving *E. coli* endotoxin intravenously in a dose of 1 mg/kg 1.5 h previously, was compared with that of neutrophils of intact animals. The cells were stimulated with latex. In series II cells isolated from intact animals were incubated with endotoxin in vitro (final concentration 0.01 mg/ml) and the latex was added immediately after the endotoxin (0 min), and 30 min, 1 h, and 1.5 h after the beginning of incubation with the endotoxin. Cells from the same animals, incubated without endotoxin, served as the control.

EXPERIMENTAL RESULTS

The results of the experiments of series I, in which the ability of neutrophils to produce AFO was estimated after circulation of the endotoxin in the blood stream for 1.5 h, are shown in Fig. 2. It was found (Fig. 2a) that the values of I in this case were virtually identical, and, moreover, in neutrophils obtained from animals with endotoxemia, they were actually a little below the control levels. Meanwhile, I of neutrophils obtained from animals with endotoxemia lasting 1.5 h was significantly higher than the control (Fig. 2b).

The results of the experiments of series II, in which neutrophils obtained from intact animals were incubated with and without the endotoxin for 1.5 h are given in Fig. 3. The data reflecting the time course of chemiluminescence are represented on this graph as values of I_m, normalized relative to the maximal value of chemiluminescence during stimulation of intact neutrophils, at the zero moment of time (I_{m0}). In this way it is possible to allow for the initial ability of the neutrophils to respond by AFO production to stimulation by latex. The results show that the chemiluminescence response did not begin to differ significantly until 1 h after the beginning of incubation of the cells in the medium. It is interesting to note that chemiluminescence values recorded on incubation of the cells with endotoxin virtually did not increase with time (broken curve), and the corresponding control values increase more than threefold. Depending on the duration of incubation in medium with and without endotoxin, I_s varied, but not significantly.

Activated polymorphs are regarded as the main factor damaging the endothelium of the lung microvessels in situations associated with the appearance of bacterial endotoxin in the blood [6, 8, 11]. Granulocytic exhaustion significantly inhibits the increase in permeability of the lung capillaries for protein and reduces the degree of edema developing in endotoxemia [4]. Endotoxin itself, as investigations in endothelial cell cultures [1] have shown, evidently has no marked cytotoxic effect, but addition of polymorphs to the medium leads to distinct damage to the endotheliocytes, especially in the region of their junctions with neutrophils. It can be taken as proven that endotoxin can activate neutrophils, in particular, by increasing the production of active forms of oxygen [5, 9]. The results obtained in our experiments do not allow an unequivocal conclusion. Incubation of neutrophils with endotoxin in serum-free medium did not stimulate AFO production and did not increase their production in

response to another activator (latex). Moreover, the ability of the cells to produce AFO during stimulation by latex was actually reduced in the presence of endotoxin. Possibly the concentration of endotoxin which we used in experiments in vitro (0.01 mg/kg) was too large. It has been shown [2], high concentrations of endotoxin, unlike low, can inhibit phagocytosis. We also know that intensive production of H_2O_2 in vitro by phagocytic cells requires their fixation to substrate [7]. The data obtained by the study of the effect of endotoxin on alveolar macrophages is evidence that in the presence of endotoxin the ability of the cells to adhere to glass is reduced [5]. The study of the AFO-producing function of polymorphs from animals receiving the endotoxin beforehand in a dose usually causing damage to lung tissue, gave different results. Stimulation of such neutrophils by latex was accompanied by a much greater (by 1.5 times) degree of AFO production than in the control.

The neutrophil population present in the lumen of blood vessels is known to be heterogeneous. Two pools can be distinguished in it: circulating in the blood stream and marginal, or juxtamural. The marginal pool consists of cells with more or less firm connections with the endothelium. The marginal pool is particularly large in pulmonary vessels, where it may reach 70% or more [3]; more than 20% of the cells, moreover, have sufficiently firm connections with the endothelial lining. Injection of endotoxin induces rapid and intensive accumulation of polymorphs in the lungs [10]. Adhesion of neutrophils to the endothelium, like their subsequent migration into the tissue, is the initial phase of the cell response to all kinds of stimulators, i.e., their activation. This means that the most active fraction of neutrophils, primarily from the marginal pool, accumulates in the lungs in response to injection of endotoxin. During prolonged circulation of endotoxin in the blood, neutrophils circulating in the blood must join the marginal pool. The marked neutropenia, which is highly characteristic after circulation of endotoxin for 1 h [4], is evidently connected with the process of successive transition of activated cells into the marginal pool and their accumulation in the microvessels. It can be accepted that after endotoxemia for 1.5 h, which existed in our experiments the least active fraction of neutrophils or cells least capable of activation remained in the circulating blood. Nevertheless, even in this fraction an appreciable increase was observed in AFO production in response to stimulation by latex. Thus in vivo endotoxin can potentiate AFO production by neutrophils in response to stimulation of phagocytosis Since no such effect was found in serum-free medium it can be tentatively suggested that the activating action of endotoxin on leukocytes was mediated by plasma components, such as the C_{3a}- and C_{5a}-components of the complement system, which may be activated by endotoxin and which, in turn, are sufficiently powerful activators of neutrophils [9]. It can also be postulated that activation of leukocytes in the pulmonary microvessels involved the participation of the pulmonary endothelium, and the factor activating the cells may be metabolites of arachidonic acid: thromboxanes and leukotrienes. As was shown in [12], their release by endotheliocytes is stimulated by endotoxin.

Thus the stimulating effect of endotoxin on polymorphs in vivo is indirect.

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