EFFECT OF *E. coli* ENDOTOXIN ON ERYTHROCYTE MORPHOLOGY AND BLOOD IRON LEVEL IN RATS

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

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An important place in the pathogenesis of polyvisceral lesions caused by endotoxin is ascribed to disturbances of the microcirculation and changes in the rheologic properties of the blood [1, 3]. In turn, blood flowability is determined by the number and functional state of the erythrocytes [4]. The development of endotoxemia in dogs is accompanied by a significant increase in the content of lipid peroxides in the erythrocytes, an increase in microviscosity of their membranes, and a decrease in their deformability [15]. A similar trend of this last parameter also is observed in septicemia in man and mice [10]. It has also been shown that the electric charge on the erythrocyte surface falls in rabbits with endotoxin shock [13]. These physicochemical changes in the red blood cells must be reflected in their structure, transmembrane ion transport, adhesiveness, performance of their acid-transporting function, and so on. However, no morphologic studies of erythrocytes have been undertaken during exposure to endotoxin. There is only a single communication in the literature on the effect of cholera toxin in vitro on changes in ionic composition and shape of erythrocytes [5].

The aim of the present investigation was accordingly an ultrastructural study of erythrocytes in capillaries of various internal organs, of the dry mass of red blood cells in films, and of the serum iron concentration in the course of experimental endotoxemia.

EXPERIMENTAL METHOD

Experiments were carried out on 36 male rats weighing 200 g. Endotoxin (lipopolysaccharide – LPS) of *E. coli* was injected into the caudal vein in a dose of 2 mg/100 g body weight. Erythrocytes were studied in capillaries of the sensomotor cortex and central gray matter, atrial and ventricular myocardium, lungs, liver, and kidneys. Material was taken after 30 min (initial stage of shock, 10 rats), 5 h (intermediate stage of shock, 10 rats), and 3 days (stage of late endotoxemia, 10 rats). Sterile physiological saline was injected in the control experiments (three rats in each group). The serum iron concentration was calculated by Wahlquist's method in the modification of Hagberg and Efimova. The erythrocyte dry mass of the rats was determined on unstained blood films by means of a "Peraval Interfaco" interference microscope (Carl Zeiss, Jena) [2]. Material for electron microscopic study was fixed in glutaraldehyde and osmic acid, dehydrated, and embedded in Epon. Sections cut on the LKB 8800 ultramicrotome were stained with uranyl acetate and lead citrate and examined in the IEM-100S electron microscope. The results were subjected to statistical analysis by Student's test by computer.

EXPERIMENTAL RESULTS

The development of endotoxin shock is manifested as a syndrome of disseminated intravascular clotting, characterized by microthrombosis and deposition of fibrin in the capillaries of certain target organs, and also as

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TABLE 1. Changes in Dry Mass and Concentration of Solid Residue in Erythrocytes and Serum Iron Concentration of Control Rats and at Different Periods of Endotoxin Shock

Parameter	Background	Time after injection of endotoxin		
		30 min	5 h	3 days
Control (n = 3)				
Dry mass (pg)	31,6±2,2	31,9±2,3	31,7±2,1	32,1±2,3
Concentration (pg/μ^3)	0,34±0,02	0,35±0,03	0,35±0,02	0,36±0,02
Iron concentration (µg%)	112,8±3,7	117,1±3,5	114,7±3,3	110,4±3,9
Experiment (n = 10)				
Dry mass (pg)	32,3±2,4	25,4±1,2*	19,6±1,6*	25,3±3,2*
Concentration (pg/μ^3)	0,36±0,03	0,28±0,01*	0,22±0,02*	0,28±0,04*
Iron concentration (µg%)	106,6±3,2	70,1±2,9*	45,3±2,9*	83,4±5,9*

Legend. *$p < 0.05$ – significant differences compared with background; n) number of experiments.

hemolysis of erythrocytes. The arrangement of the organs in decreasing order of severity of hemolysis in the microcirculatory bed is: lungs → kidneys → myocardium → liver → brain.

In the first 30 min after injection of endotoxin no ultrastructural disturbances of the erythrocytes were found. However, on cytointerferometry the dry mass and concentration of solid residue of red blood cells, i.e., the ratio of dry mass to volume of erythrocytes, decreased from 32.3 ± 2.4 to 25.4 ± 1.2 pg ($p < 0.001$) and from 0.36 ± 0.03 to 0.28 ± 0.01 pg/μ^3 ($p < 0.05$) respectively (Table 1). As Table 1 shows, the shift took place mainly on account of reduction of the dry mass and, to a lesser degree, a change in volume. The interferometric data agree with the results of biochemical observations, which also revealed a decrease in the serum iron concentration ($p < 0.001$) (Table 1).

In the intermediate stage of endotoxemia (after 5 h), by contrast with the initial period, hemolyzed erythrocytes were constantly found in capillaries of the myocardium, lungs, and kidneys (Fig. 1a, b). Permeability of the tissue-blood barriers in the lungs and myocardium was so high that they were found also in the interstitial space or in the alveolar lumen (Fig. 1c, d), where fibrin also was frequently seen (Fig. 1d). In fact, red blood cells as such were absent – instead of them only collapsed membranes remained, shaped like loops. It is difficult at present to answer the question whether extravasation of already hemolyzed erythrocytes took place or whether the cells underwent hemolysis in the stroma. Whatever the case, these cells did leave the vessels, although their injury within the stroma or even within the lumen of the alveoli cannot be ruled out. At this stage of endotoxemia the greatest decrease in values of the dry mass and concentration of the solid residue in the erythrocytes took place, to 19.6 ± 1.6 pg and 0.22 ± 0.02 pg/μ^3 respectively. The lowest serum iron concentration also was found, namely 45.3 ± 2.9 μg% ($p < 0.001$) (Table 1).

The stage of late endotoxemia (after 3 days) was marked by the appearance of erythrocytes of ameboid shape – so-called echinocytes (Fig. 2a), which are usually less osmiophilic than the other red blood cells. A particular feature of the reaction of these erythrocytes to LPS is that the branching evaginations of their membrane usually undergo microclasmatosis. It is interesting to note that the same fate befalls echinocytes located in the alveolar space (Fig. 2b). Such changes of shape are evidently reflected in the character of the junctions between the cells, in the form of intravascular aggregation of the cells with "rouleaux" formation and sludging.

The cytointerferometric study of the erythrocytes showed that the dry mass and concentration of solid contents in them, and also the serum iron concentration were higher than at the previous stage, but much lower than the background values ($p < 0.05$) (Table 1).

So far as the reaction of theerythrocytes is concerned, the probable mechanism of what may be called their reactive lysis may be indirect, through activation of complement. An important role in this situation is ascribed to the C5b-9 lytic active complex, which has the form of a cylindrical macromolecule 15 nm high, 10 nm in internal diameter, and with walls about 1 nm thick [8]. When it is introduced into the lipid bilayer of the erythrocyte membrane, the main change in it under the influence of complement is the formation of a perforating core, the wall of which is formed by the C5b-9 complex. After lysis of part of the plasmalemma pores or channels are formed, through which rush streams of salts and water, causing osmotic changes [8].

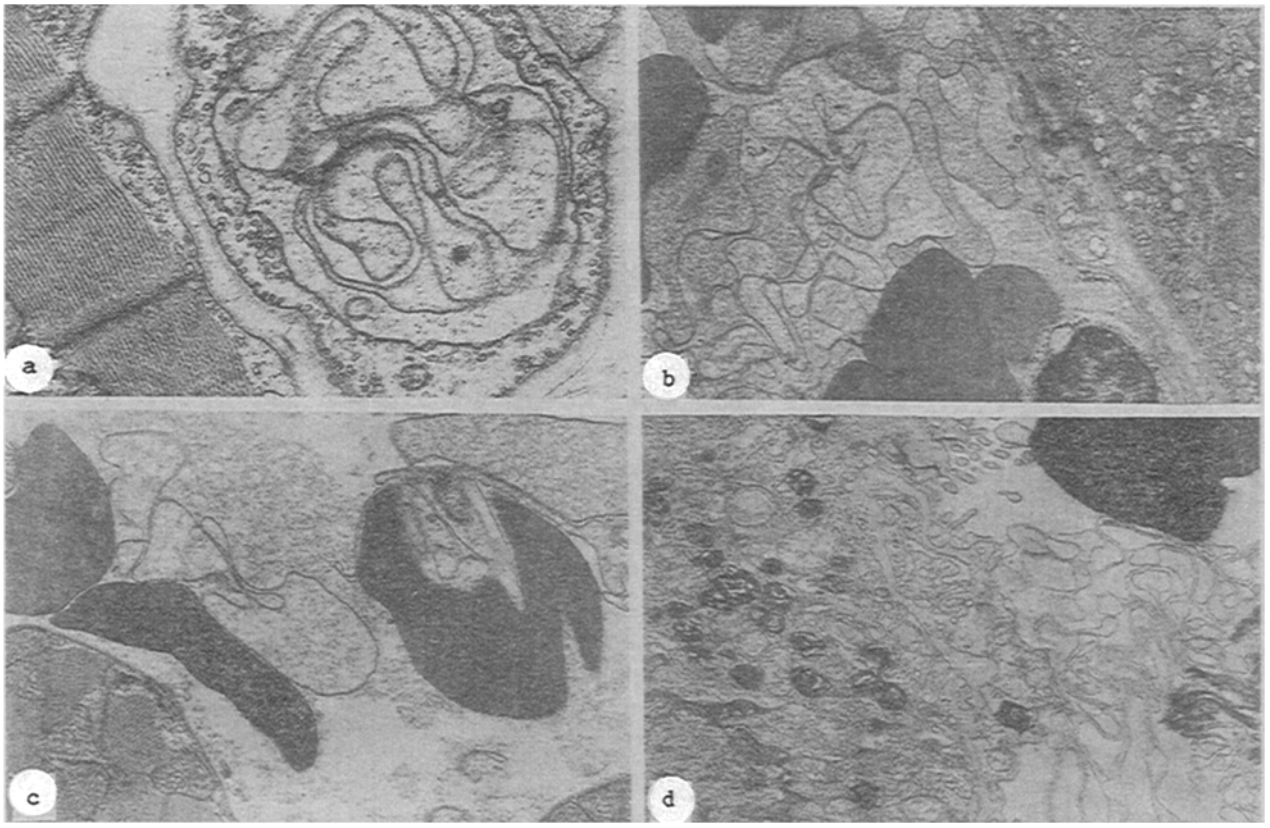

Fig. 1. Ultrastructural changes in erythrocytes in intermediate stage of endotoxin shock: a) hemolyzed erythrocytes in myocardial capillary. 10,000×; b) hemolysis of erythrocytes in renal intertubular capillary. 2800×; c) appearance of undamaged and hemolyzed erythrocytes in interstitial space of myocardium. 5600×; d) hemolyzed erythrocytes and fibrin in alveolar lumen of lungs. 5600×.

However, injury to the erythrocyte membrane can take place even without the involvement of complement, through the direct action of LPS subunits, and especially those that are richest in lipid A. This situation is perfectly acceptable, for receptors for LPS are present on the erythrocyte surface [14]. Moreover, the stability of this process has been established [9]. A study of interaction between rabbit erythrocytes and LPS from a mutant strain of *Salmonella minnesota* Re 595, deprived of its O-antigenic polysaccharide part but containing lipid A and the trisaccharide 2-keto-3-deoxyoctoic acid, showed that initially LPS binds with the membranes, after which it undergoes molecular reorientation within the lipid bilayer of the erythrocyte membrane. Insertion of LPS into the membrane leads to its destabilization and to modification of the cellular response [9].

Finally, in the case of development of septic shock in patients or in model experiments with living microorganisms, the cause of damage to the erythrocytes may be, besides the factors mentioned above, membrane-active cytolysins (hemolysins). It must be emphasized that the hemolysin of *E. coli* is the best studied of all the cytolysins of Gram-negative bacteria [11]. It has the ability to form pores 1.5-3.0 nm in diameter both in the erythrocyte membrane [7] and in lipid bilayer membranes consisting of purified phosphatidylcholine [12]. It has recently been shown that the average life span of the pores (channels) formed by one molecule of a hemolytically active form of hemolysin in an artificial membrane is 2 sec [6].

Thus the development of endotoxin shock is accompanied by reduction of the dry mass and concentration of solid residue in erythrocytes. Endotoxin also reduces the content of iron in the blood serum, which can be used for hemoglobin synthesis. Injury to the erythrocyte membrane leads to loss of hemoglobin and erythrocytic thromboplastin by the red blood cells, as a result of which collapsed membranes of an irregular shape remain. The gross

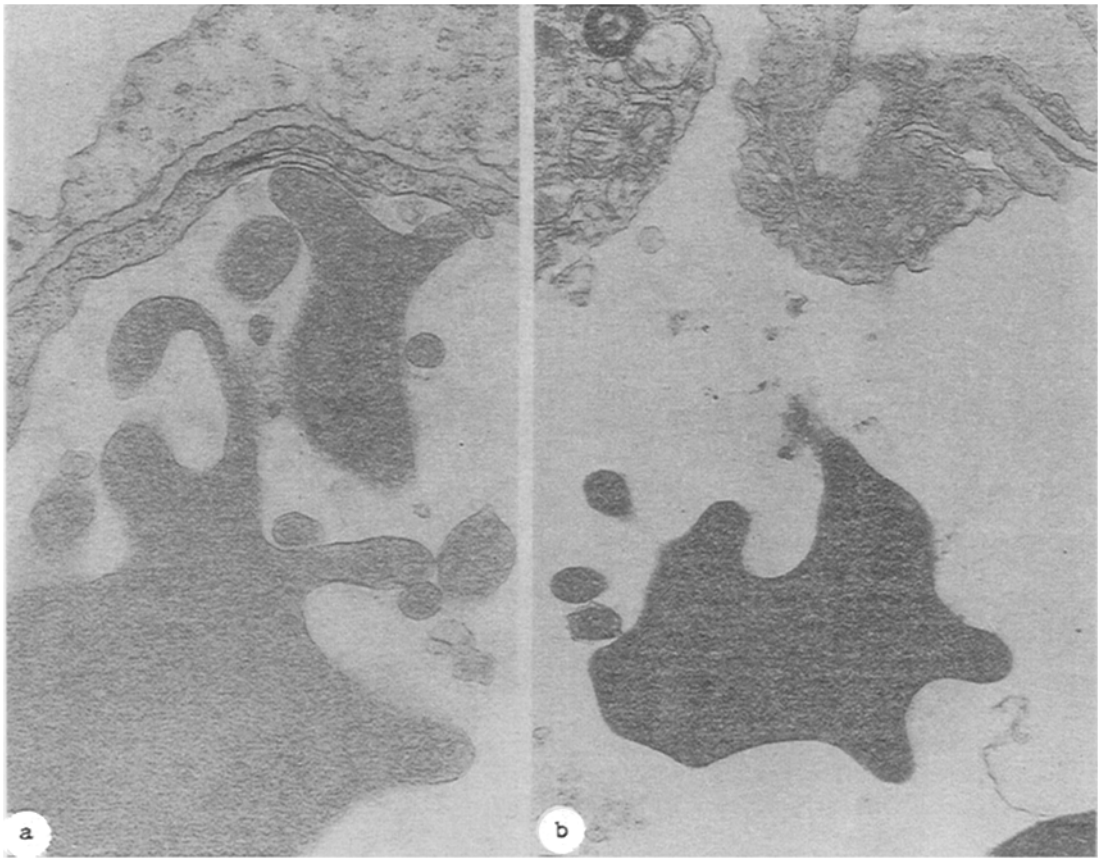

Fig. 2. Ultrastructural changes in erythrocytes at stage of late endotoxemia: a) appearance of erythrocytes of ameboid shape (echinocytes) in lumen of lung capillary. 10,000×; b) release of echinocyte into alveolar lumen. 5600×.

morphologic changes in the erythrocytes are quite stable in character. The appearance of echinocytes enlarges their surface of contact and thereby increases the mechanical strength of the aggregates and strengthens the aggregating powers of the blood cells.

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