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## ULTRASTRUCTURAL CHANGES IN THE ERYTHROCYTES AND PLATELETS

OF PATIENTS WITH SEVERE THERMAL BURNS DURING THE BURN SHOCK PERIOD

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An important role in the pathogenesis of burns is played by complex and little-studied changes in the blood cells, especially erythrocytes. Meanwhile information on these changes is essential for the elucidation of the pathogenesis of the first stage of burn shock, which is characterized by poikilocytosis, anisocytosis, and microcytosis, all of which can be observed under the light-optical microscope. It is in the period of burn shock that the action of erythrocyte-damaging factors such as acidosis and hyperosmolarity of the blood [7], release of catecholamines into the blood [12], and changes in the serum concentrations of lipids and lysolecithins [10] is observed. Massive infusion therapy, especially injection of preparations of the dextran type, may also undoubtedly play a definite role in the changes affecting erythrocytes [8]. The possibility cannot be ruled out that it is these same processes which lead to the development of acute anemias and various blood coagulation disturbances often observed in severe burns. In this context considerable interest is attached to changes in configuration and structure of the erythrocytes, the state of their cell membrane, with the functioning of which are linked such important factors as adhesion of the cells, transmembrane ion transfer, and so on, as well as changes in the platelets.

The aim of this investigation was to study the dynamics of changes in configuration of erythrocytes in the peripheral venous blood of patients with severe thermal burns of the skin during the period of burn shock, and also to study changes in platelet structure.

## EXPERIMENTAL METHOD

Erythrocytes were taken for study from the peripheral venous blood obtained from 12 patients with severe thermal burns of the III-IV degree, affecting 35-60% of the body surface, during the period of burn shock. Two of these patients died at the end of the period

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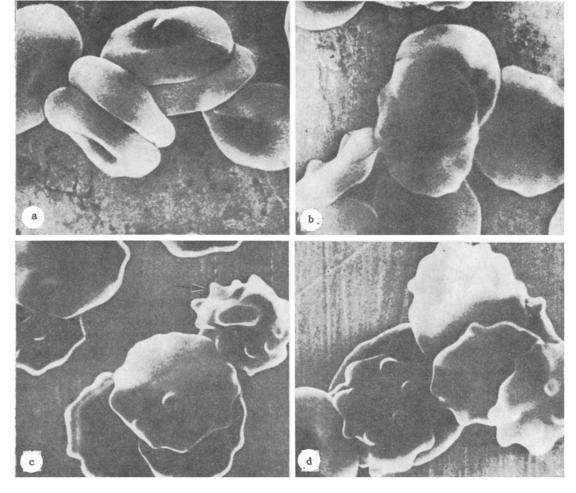


Fig. 1. Changes in configuration of erythrocytes in patients during burn shock (8000 ×). a) Typical biconcave discocytes (normocytes) from normal donated blood, b) numerous ovalocytes and cristate erythrocytes in peripheral blood of patients during burn shock, c) increase in number of spherocytes and microechinocytes in peripheral blood of patients during burn shock, during life, d) numerous spherocytes and echinocytes in blood of patients recovering from shock but subsequently dying from septicemia.

of burn shock, and three patients died during burn septicotoxemia on the 15th-25th day after burning. The blood was subjected to combined scanning and transmission electron microscopic investigation. The scanning electron-microscopic investigation was carried out with the aid of an original rapid fixation method [1]. Transmission electron-microscopic investigation followed the usual procedure. During scanning electro-microscopy the various types of erythrocytes were counted by the method in [2]. Blood from five healthy blood donors was used as the control.

## EXPERIMENTAL RESULTS

The study of the donors' erythrocytes (control) revealed that in all cases most erythrocytes were normocytes — biconcave discoids with symmetrical depressions, a thin, smooth, homogeneous membrane, and uniform density of their matrix. Erythrocytes of oval shape (ovalocytes) were seen comparatively rarely (up to 5-10% of all the cells). A few hemispheric protuberances which, according to some workers [11], reflect the aging process of the erythrocytes, could be seen on the membrane of only a few cells (Fig. 1a).

Many (up to 40-60%) modified forms of erythrocytes were observed to appear in the peripheral blood of patients with severe burns admitted in a state of burn shock to the A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR. These were mainly deformed erythrocytes — ovalocytes, "cristate" forms, and dome-shaped normocytes. Some of these adhered together to form unique "rouleaux" of two to four erythrocytes. In

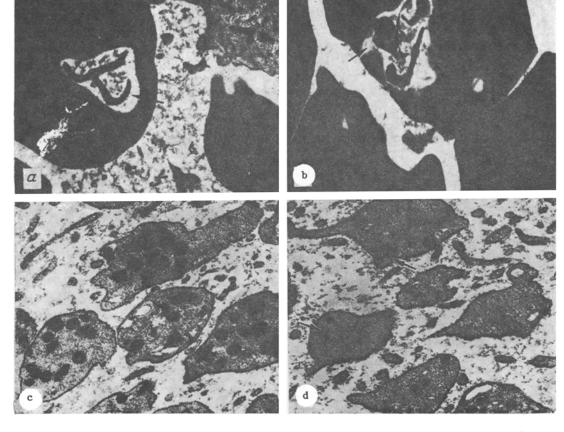


Fig. 2. Changes in matrix of erythrocytes and platelets in patients during burn shock  $(12,000 \times)$ . a, b) Appearance of inclusions (arrows) in matrix and of erythrocyte sludge; c) typical platelets from donated blood; d) sharp decrease in number of cytogranules (arrow) in matrix of platelets from burned patients during period of shock.

some of them the symmetry of the concavity was disturbed and ridges appeared around the edges of the concavity. Festooning of the cell membrane and adhesion of the cells into separate conglomerates were observed in many cells (Fig. 1b). In two patients, who died in the period of burn shock, besides deformed erythrocytes of the types mentioned above, the appearance of "elongated" cells and destructive forms (spherocytes) was observed. Meanwhile, microcytes with a modified membrane, an uneven matrix, and cells of the echinocyte type, with multiple protrusions of the cell membrane, appeared in the blood stream.

A quite distinct parallel was noted between the severity of burn shock and the increase in number of deformed and structurally degenerate forms of erythrocytes (spheroids). In patients dying in the period of shock the number of modified forms of erythrocytes reached 55-60% in a preparation, whereas in seven patients, who recovered from shock and subsequently were cured, this parameter did not exceed 35-40% during shock (Fig. 1c). Three patients recovered from shock only to due subsequently. All patients had extensive and deep burns affecting more than 40% of the body surface, and one patient, who died from pneumonia on the 15th day after burning, had burns of the respiratory passages as well as burns of the skin. Two patients died from septicemia on the 15th-25th day after trauma. Besides numerous deformed erythrocytes, up to 15-20% of structurally degenerate forms appeared in the peripheral blood of these patients during the period of shock: ranging from spheroids to spheroechinocytes and spherostomacytes, the number of which subsequently reached 60% (Fig. 1d).

Investigation of erythrocytes in the transmission electron microscopy revealed structural changes in the cells closely connected with changes in their configuration and the microcirculatory changes. During sludging of the erythrocytes, cells with altered configuration and destruction of the matrix were found, and includions appeared in the matrix (Fig. 2a). Most frequently these inclusions were observed in degenerative forms of erythrocytes, namely

spherocytes (Fig. 2b) and spheroechinocytes. Definite changes also were observed in the ultrastructure of the platelets. These consisted of a decrease in the number of cytogranules, swelling of the mitochondria, and an increase in the number of cytoplasmic outgrowths. In some places aggregation of platelets into groups of four to six cells was observed. These changes were interpreted as reversible and they are not specific for burns [13]. However, the presence of platelet aggregates combined with the changes in the erythrocytes observed above, and the hemoconcentration typical for the clinical picture of burns, predispose to the formation of capillary stasis and thrombosis during the period of shock.

Thus considerable changes in the configuration and structure of the erythrocytes. closely linked with the severity of burn shock, are observed in patients with severe thermal burns. Many of these cells are deformed erythrocytes: ovalocytes, cristate ovalocytes, dome-shaped normocytes. There is no doubt that the appearance of these forms in the period of burn shock is due to many different causes, possibly connected with the chemical features of the blood in these patients during burn shock. However, there is no doubt that a definite role in the genesis of these forms of erythrocytes may be played by microcirculatory disturbances, deformation of the capillary lumen, stasis, and sludging of the erythrocytes, which are typical of burn shock. Burns are characterized by reduced deformability of the erythrocytes [9], i.e., their ability to be deformed and to regain their normal shape is reduced (without the onset of pathological changes in them). Under normal conditions erythrocytes possess this ability to a very high degree. Under conditions of a disturbed microcirculation, the vulnerability of the erythrocytes as they pass through deformed capillaries is unavoidable, and they undergo considerable deformation. Deformation of the erythrocytes, and also other damaging factors, lead to the development of structurally degenerate types of erythrocytes. This may evidently account for the dependence of the number of deformed erythrocytes on the severity of the course of burn shock which, as we know, is determined primarily by the degree of the microcirculatory disturbances [3, 4, 6]. The mechanisms of burn shock largely determine the severity of the subsequent course of the disease (the so-called "after-effect" of shock). Elucidation of the structural changes in the erythrocytes and platelets during shock, which are examined together under the light microscope, is thus of undoubted importance for the treatment of burns.

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