ERYTHROCYTE MEMBRANE AND CONFIGURATION CHANGES IN BURN SEPTICOTOXEMIA

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An important role in the pathogenesis of severe burn trauma is played by complex changes, so far little studied, in the blood cells, especially erythrocytes. In the clinical picture of burns changes such as anisocytosis, poikilocytosis and, sometimes, hypochromia are typical. The genesis of the anemia which frequently arises is not clear. Recent investigations have shed light on some aspects of changes in erythrocytes which are attributed to the action of various factors characteristic of burns: deformation of erythrocytes in the microcirculatory system as a result of disturbances of the latter [6, 11], release of catecholamines into the blood stream [15], accumulation of prostaglandins [4], and changes in the content of lipids and lysolecithins in the erythrocyte membrane [9].

Some therapeutic measures undertaken in the treatment of burns and, in particular, mass transfusions with dextran and local therapy with preparations of the betadine group or sulfadiazine, disturb metabolism in the erythrocyte membrane on account of the development of acidosis and hyperosmolarity of the blood, and may contribute to the development of acute anemia [5, 15]. The direct action of heat, as experiments *in vitro* [5] have shown, causes changes in erythrocyte configuration.

However, no systematic morphological study of the time course of changes in configuration of the erythrocytes and the state of their membrane during severe burn trauma has yet been undertaken, and this was accordingly the aim of the study described below.

EXPERIMENTAL METHOD

Changes in configuration of erythrocytes from peripheral venous blood and in the state of the membranes were studied by scanning electron microscopy in 16 patients with severe thermal burns of the III-IV degree (from 25 to 45% of the body surface) between 15 and 50 days after trauma. Four patients showed changes to some degree or other in their red blood: anisocytosis and poikilocytosis; in six of them, in a state of septicemia, hypochromia and signs of anemia were found, with a hemoglobin concentration down to 5.8-6.5 g%. Erythrocytes of six healthy donors were used as the control. For a more rapid and qualitative study of erythrocytes a modified method [2] of fixation of the material was used. A drop of blood was applied from a thin pipet to the polished surface of a metal support, namely the holder of the scanning attachment of the IEM-100CX microscope. The drying material was placed for 20 min in a bottle with 4% glutaraldehyde solution, made up in 0.1 M cacodylate buffer at pH 7.4, postfixed in 1% OsO_4 solution, and dehydrated in alcohols and acetone (15 min in each). The support was then removed from acetone and kept for 10-15 sec in liquid nitrogen. The supporting holder was then secured to the revolving stage of an IEE-40 vacuum evaporator and stained with copper. The resulting preparation was examined in the scanning system of the IEM-100CX microscope, with an angle of inclination of 45°, accelerating voltage of 15 kV, and magnification from 5000 to 15,000. To determine the relative proportions of different forms of erythrocytes, the different forms were counted by the method in [1] and the ratio between them expressed in percent.

Some material for investigation by transmission electron microscopy was fixed, dehydrated, and embedded in Araldite by the usual method. The stained ultrathin sections were examined in the IEM-100CS electron microscope with a magnification of between 10,000 and 80,000.

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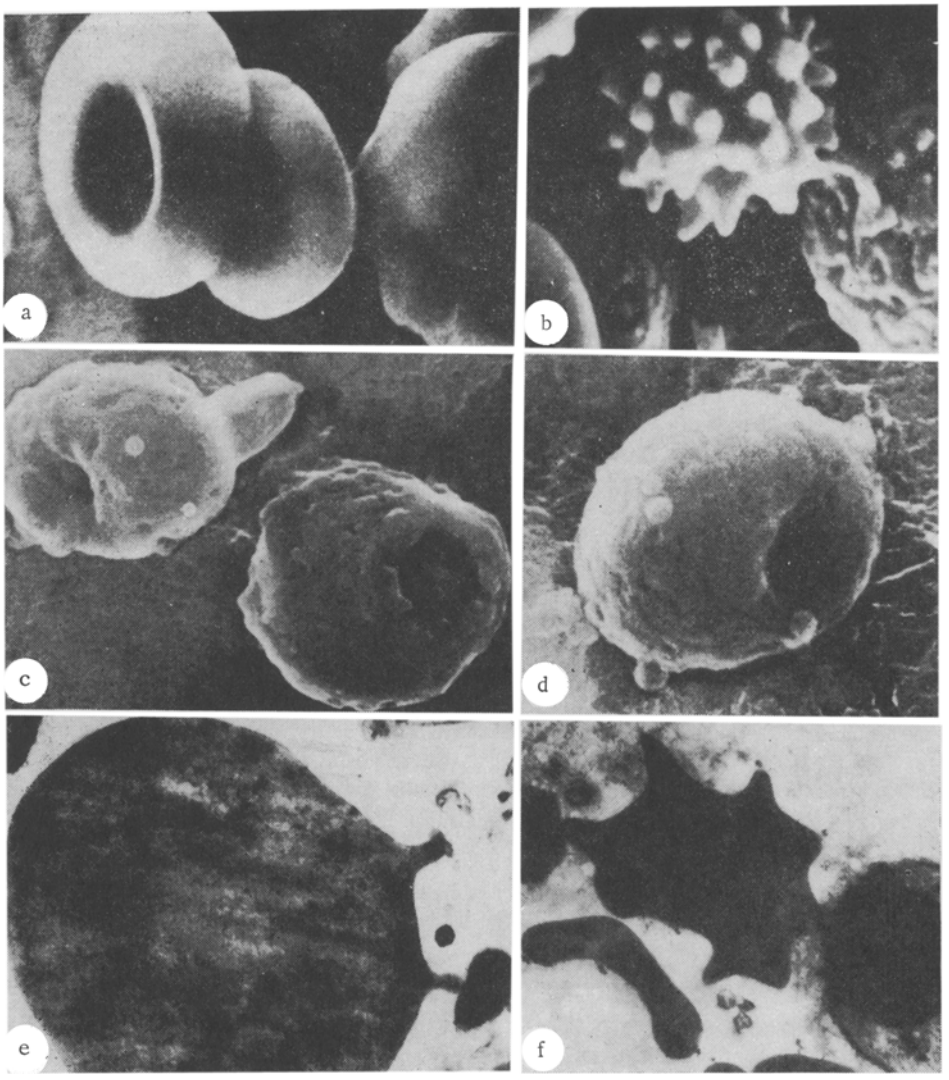

Fig. 1. Transformation of erythrocytes in blood of burned patients: a) normal erythrocytes (discocytes), b) echinate erythrocyte (echinocyte), c) altered erythrocyte consisting of a stomatocyte with "pseudopodium"-like projection of membrane, d) spherostomatocyte with indentations and vesicles on the membrane, e, f) sections of echinocyte-like erythrocytes in transmission microscope. Magnification: a-d) 8000; e, f) 10,000.

EXPERIMENTAL RESULTS

The study of the donors' erythrocytes (control) showed that they were predominantly normocytes (biconcave discocytes with a smooth edge and homogeneous membrane (Fig. 1a). Ovalocytes were comparatively rare (not more than 5%). Only in solitary cases were ovalocytes with a few hemispherical projections of the membrane and solitary short outgrowths, which evidently reflect the process of natural "aging" of healthy human red blood cells found.

Besides unchanged normocytes, the blood of the burned patients also contained many altered cells. These included many ovalocytes, dome-shaped normocytes (cells with a symmetrical obliteration of their concavity and with a unilateral dome-shaped projection of the disk wall, and also cells with an uneven edge and with an asymmetrically curved pole).

Many of the altered cells were erythrocytes in different phases of spheroid transformation. Transformation of the cell began with the appearance of uneveness of the outline of the discocyte and unilateral or bilateral obliteration of the biconcavity. Meanwhile the homogeneity of the membrane was disturbed: Initially single, later more numerous small hemispherical projections of the cell membrane appeared on the surface of the cell. Later they could increase in length and be converted into short rods with a semicircular tip — echinate forms. As a result of deformation the normocyte initially had an asymmetrical discoid appearance, and later became an echinocyte.

Later the cells gradually swelled and assumed the form of an echinate spherocyte (spheroechinocyte). According to data in the literature [2], the different final forms of spherocyte transformation of the normocyte are stages of irreversible destruction of the cells (Fig. 1b). A particular feature of the destructive spheroid forms of erythrocytes in severe burn trauma in the stage of burn septicotoxemia is the absence of typical "needle-like" outgrowths on the surface of the spherocyte, such as are observed during aging of donors' blood [2].

A unique form of irreversible erythrocyte transformation was observed in the blood of patients with severe skin burns, namely spherostomatocytes. These cells are characterized by a spherical shape, with the formation of an extensive depression or "stoma" on one side and by a marked asymmetrical projection of the membrane on the opposite side. Sometimes this projection looks like a "pseudopodium." The structural heterogeneity of the spherostomatocyte membrane is very clearly distinguishable: Dilated pores and indentations, small hemispherical and pectinate projections and defects could be seen in the thickness of the cell membrane (Fig. 1c, d). Characteristically the morphological structural heterogeneity of the erythrocyte cell membrane increases appreciably during its transformation and during the development of destructive changes and forms.

The investigation showed that the number of modified forms of erythrocytes was 37%, made up of about 10% of various transitional forms, 15% of various echinate forms (echinocytes), and 12% of destructive forms (spherocytes and spherostomatocytes). Definite correlation was found between the severity of the patient's clinical state and the increase in frequency of destructive forms of erythrocytes. Examination of erythrocytes in the transmission electron microscope showed that both unchanged and altered erythrocytes were present; the latter included, in particular, distinctive "echinocyte-like" forms of erythrocytes, resembling a many-pointed star with conical projections of various sizes (Fig. 1f).

In some sections there were individual erythrocytes on whose membrane single thin processes could be seen to make contact with bacillus-like particles in the blood plasma (Fig. 1e). These observations are most interesting. Evidently this could be a case of the focal cytotoxic action of microbial toxins on a heterogeneous cell membrane and on its polysaccharide and protein complexes, followed by a change in the intracellular pressure and by swelling and projection of the cell cytoplasm in the zone of destruction of the membrane.

The results of this electron-microscopic study demonstrate that besides normocytes, many transformed and destructive forms of erythrocytes appear in the blood stream of patients with severe thermal burns of the skin in the period of burn septicotoxemia. The spherostomatocytes which were found are a unique form of destruction of erythrocytes. Investigation of erythrocytes in the transmission electron microscope also revealed some of their forms that had been found during scanning electron microscopy, in particular, echinocytes. This confirms that these forms were really present and were not artifacts. The causes of changes in cell membranes and of deformation and destruction of erythrocytes in severe burn trauma are many and varied. In particular, the "outgrowths" or "spines" on the erythrocyte membrane could arise as a result of the cytotoxic action of proteases and of cytogenic enzymes [10], autoimmune lesions [13], the development of anemia by the patients [12], the action of toxic and infectious factors [7], and disturbances of the acid-base balance during treatment [3]. It is under pathological conditions that the structural heterogeneity of erythrocyte cell membranes increases, evidently on account of disturbance of protein–polysaccharide–lipid complexes of the membranes [8]. All these factors are present to some degree or other in the period of burn septicotoxemia following severe burn trauma, and they may be responsible for the regular changes found in erythrocytes in burn trauma.

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EFFECT OF THYMALIN ON THYMUS MORPHOLOGY AND FUNCTION IN MICE

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In the modern view the thymus produces hormone-like factors which take part in the regulation of the immunocompetent system [5, 8, 9, 12]. Most workers state that these factors are polypeptide in nature [7, 10, 14] and they consider that, as products of the epithelial cells of the thymus, they promote differentiation of stem cells into T lymphocytes both inside and outside the thymus [6, 11, 13].

Since there is little information in the literature on the morphology of the thymus under the influence of thymus factor *in vivo* [12], it was decided to study the effect of thymus polypeptide factor (thymalin) on the morphology and function of the gland in mice.

EXPERIMENTAL METHOD

Thymalin was obtained from the calf thymus by ion-exchange chromatography [2]. It has been shown that thymalin is a substance of basic character with molecular weight of about 5000 daltons and that it consists of 38 amino acid residues [3].

The experimental study was carried out on 36 male CBA mice weighing 18-20 g, divided into two equal groups. Each group of mice was subdivided into experimental and control animals. Thymalin was injected subcutaneously into the experimental mice of group 1 in a dose of 0.05 mg/g body weight in 0.2 ml physiological saline daily for 3 days, and into the experimental mice of group 2 daily for 10 days. This dose was chosen because its administration to mice caused marked stimulation of the immune response to a thymus-dependent antigen, namely sheep's red blood cells (SRBC) [1]. Control animals of both groups received physiological saline by the same scheme. On the 4th and 11th days of the experiment the mice were decapitated. The thymus was weighed, fixed in 10% neutral formalin solution, and embedded in paraffin wax. Sections were stained with hematoxylin and eosin, azure II-eosin, methyl green–pyronine (by Unna's method), and alcian blue, the PAS reaction was carried out, and alkaline phosphatase activity was determined (by Gomori's method) in fresh frozen sections. In the microscopic analysis of the material, areas of cross-section through nuclei of the epithelial cells of cortex and medulla were measured by a graphimetric method using the RA-7 drawing apparatus; the thickness of the cortex and medulla was measured with an ocular micrometer and the ratio between them calculated. The rate index of the thymus (weight of the gland as a proportion of body weight) was determined. The numerical data were subjected to statistical analysis by Student's criterion.

EXPERIMENTAL RESULTS

After injection of physiological saline for 3 days the weight index of the thymus was 2.72 ± 0.21. Lobes of the thymus were large, with clear division into cortex and medulla

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