CHANGES IN SOME MEMBRANE PROPERTIES AND STRUCTURE OF ERYTHROCYTES IN SEVERE EXPERIMENTAL BURNS

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Generalized damage to cellular and intracellular membranes in thermal burns is the universal mechanism of development of the cascade of pathological processes in the severely burned individual [3]. During the first minutes after an extensive deep burn, at least two mechanisms of injury to the lipid layer of the biological membranes interact in the blood: highly active phospholipases are liberated from cells destroyed by the action of heat and processes of lipid peroxidation are intensified by products of free-radical reactions [1, 2, 12]. As a result of a primary change in membrane structure the resistance of the membrane to mechanical action, to osmotic pressure, and to peroxide compounds may be reduced. In burns the resistance of the membrane to mechanical action, to osmotic pressure, and to peroxide compounds may be reduced. In burns the resistance of the erythrocyte membrane, which is linked with properties so important for function of the erythrocyte as its integrity and shape, has virtually not been studied. All that is known is that the number of erythrocytes of the normal shape (diskocytes) in the blood of burned patients during the period of shock falls, and the number of transitional and degenerative forms, with a tendency toward hemolysis and aggregation and having lost most of their ability to transport oxygen, rises [4]. The presence of pathologically changed erythrocytes in the blood soon after burning may be due to destabilization of their membranes by membrane-damaging factors, a change in electrical potential of the membranes [7, 10] or (later), disturbance of hematopoiesis. Under clinical conditions, in the earliest times after burning, when relations to carry out investigations, and that is why the present study was of an experimental nature. We studied the resistance of the erythrocyte membrane to peroxide hemolysis, the morphology of erythrocytes, and some of their electrical properties linked with integrity of the cells [6].

EXPERIMENTAL METHOD

Experiments were carried out on 60 male Wister rats weighing 150-180 g. A burn of the IIIb degree (20% of body surface) was inflicted on 60 male rats by the flame of a spirit lamp with an exposure of 50 sec. The investigation was carried out 15, 30, and 60 min and 24 h after burning. Blood samples were fixed in 2.5% glutaraldehyde, treated with 1% OsO_4 solution, dehydrated, sprayed with gold, and examined in the scanning electron microscope.

TABLE 1. Resistance of Erythrocyte Membrane to Peroxide Hemolysis in Control (Intact) and Burned Rats (M ± m)

Group of animals	Peroxide hemolysis %	p	Change, in %
Control rats	16,7±1,66		
Burned rats time after burning:			
15 min	34,4±2,1	<0,001	+104
30 min	39,4±3,6	<0,001	+136
60 min	24,2±2,7	<0,05	+44
24 h	28,6±4,8	>0,05	

Legend. Significance of differences and percentage of changes calculated relative to control.

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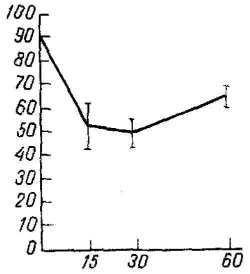

Fig. 1

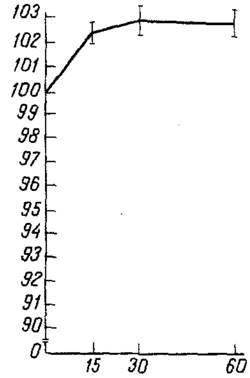

Fig. 2

Fig. 1. Changes in polarization time of rat erythrocytes during 1st hour after burning. Abscissa, here and to Fig. 2: time after burning (in min); ordinate, polarization time of erythrocytes (in sec).

Fig. 2. Changes in specific volume resistance of rat erythrocytes during 1st hour after burning. Ordinate, specific resistance (in Ω·cm).

The number of different forms and types of erythrocytes was counted and expressed as percentages of the total [8]. The resistance of the erythrocyte membrane to peroxide hemolysis was studied by the method in [5, 9]. The polarization time of the erythrocytes during measurement of their electrical density and specific volume resistance was investiated on the BRIZ-1 instrument at 37°C. A test specimen of erythrocytes was prepared by selecting sedimented erythrocytes from heparinized blood and diluting 1 volume of them in 3 volumes of phosphate buffer (pH 7.4).

EXPERIMENTAL RESULTS

Data on resistance of the erythrocyte membrane to peroxide hemolysis are given in Table 1.

It will be clear from Table 1 that 15 min after burning the peroxide hemolysis exceeded by 104% its value in control (intact) rats, evidence of a corresponding decrease in resistance of the erythrocyte membrane. The resistance of the erythrocyte membrane showed an even greater decrease 30 min after burning: there was a corresponding increase in peroxide hemolysis – by 136%. Changes in resistance of the erythrocyte membrane 1 h after burning were much less marked – the increase in peroxide hemolysis was only 44%. Peroxide hemolysis 24 h after burning did not differ significantly from normal.

The results are evidence of a very early but brief decrease in resistance of the erythrocyte membranes to peroxides after thermal burning. The marked hemolysis which develops at these times is evidently the direct result of a decrease in resistance of the erythrocyte membranes.

The results of a study of the polarization time of the erythrocytes are given in Fig. 1. A sharp decrese in the polarization time of the erythrocytes took place 15 min after burning, and the decrease was even more marked after 30 min. The polarization time then increased somewhat, but it had not reached the normal value by the end of the 1st hour. These changes are evidence of loss of their normal dielectric properties, characteristic of normal cells with an intact membrane, by a large proportion of the erythrocytes. The specific volume electrical resistance showed a tendency to increase (Fig. 2), and this can be regarded as the result of the rapid formation of complexes, possessing lower electrical conductivity than normal erythrocytes, from healthy and injured cells.

The change in the electrical properties of the erythrocyte membrane and its resistance to damaging factors must be reflected both in the functional characteristics of the erythrocyte and in the configuration of the cell.

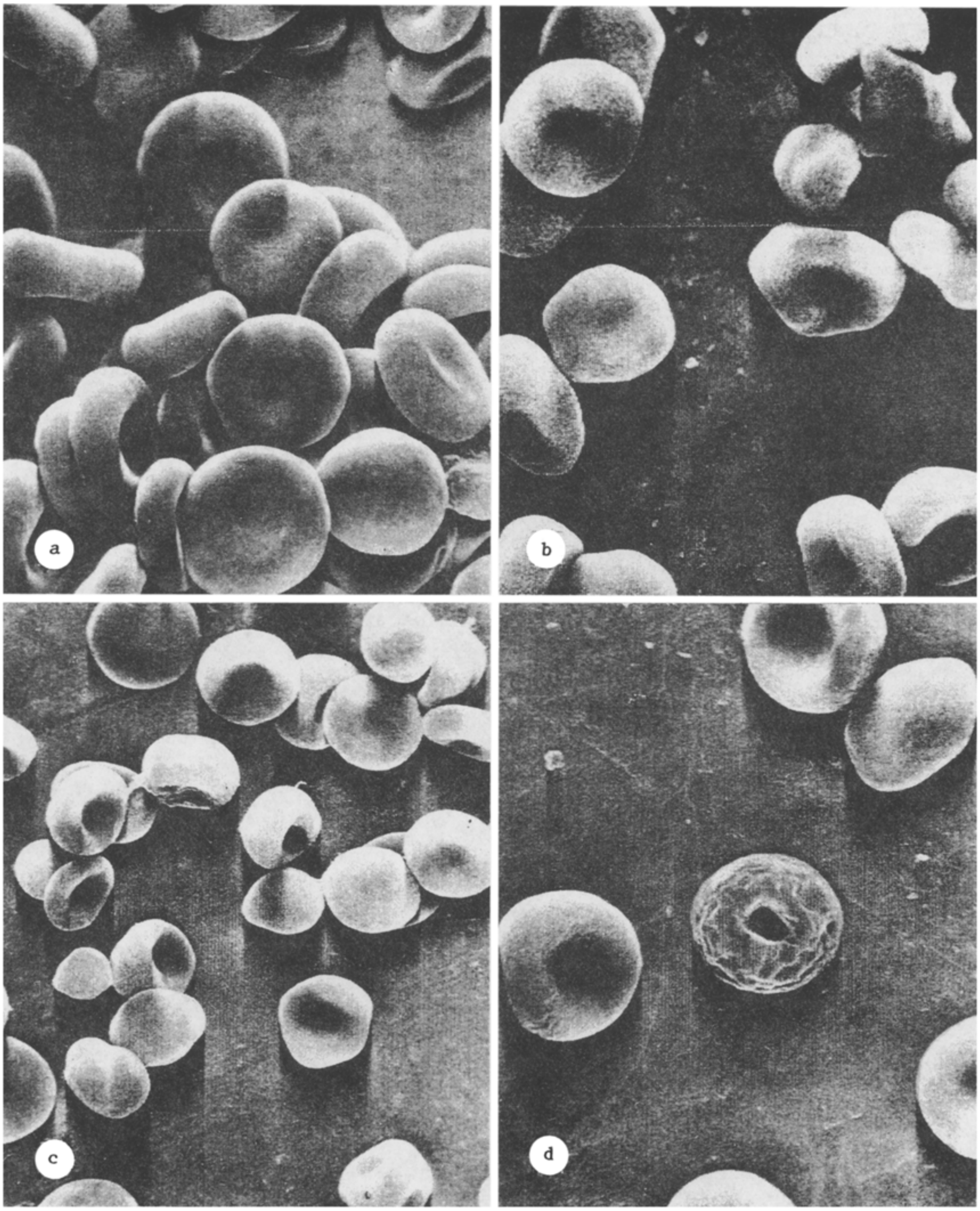

Fig. 3. Rat erythrocytes during 1st hour after thermal burn. a) Erythrocytes of control (intact) rats (3000 ×); b) 15 min after burning (3000 ×); c) 60 min after burning (2000 ×); d) 60 min after burning (3500 ×).

An essential change in the shape of the erythrocytes was revealed in the very early period after burning (Table 2, Fig. 3).

It will be clear from Table 2 and Fig. 3 that the majority (86%) of erythrocytes in the blood of intact rats have the normal biconcave shape with a uniform edge and homogeneous membrane (diskocytes, Fig. 3a). A certain number of changed forms can be explained by the natural evolution of the cells. However, 15 min after burning the number of diskocytes was appreciably reduced (down to 52%). The number of modified cells increased correspondingly: both reversible forms of spherostomatocytes of the 1st order (15%) and irreversibly altered forms — spherostomatocytes of the 2nd order (10%) and spherocytes (15%). Spheroid transformation of erythrocytes is demonstrated in Fig. 3b: dome-shaped, unilaterally convex cells and biconvex cells of spherical type. An echinocyte also can be seen, although

TABLE 2. Changes in Erythrocyte Morphology in Rats after Burning (M ± m)

Group of animals	Shape of erythrocytes				
	normocyte (diskocyte)	spherostomatocyte		spherocyte	microcyte
		1st order	2nd order		
Control rats	86,0±6	6,0±1,0	4,0±0,5	3,0±0,2	1,0±0,1
Burned rats time after burning:					
15 min	52,0±5,0	15,0±2	10,0±0,8	15,0±0,8	8,0±0,5
60 min	61,0±5,0	12,0±2,0	10,0±0,9	11,0±1,0	6,0±0,5

it has to be pointed out that echinocytes were very few in number. The character and intensity of changes in the shape of the erythrocytes 30 and 60 min after burning remained the same as after 15 min. Many dome-shaped erythrocytes and spherical, regularly shaped erythrocytes (known as "deflated balls") can be seen in Fig. 3c (60 min after burning), evidence of irreversible transformation. Spheriod erythrocytes with an unusually small diameter (microcytes) can also be seen, and they accounted for 6-8% of the total. An erythrocyte with a rough, crinkled membrane and an opening in it, can be seen in Fig. 3d.

Thus in the early stages after burning (15-30 min) both a decrease in the resistance of the erythrocyte membrane to peroxide hemolysis, synchronized with changes in the electrical properties of the erythrocytes, and change in their morphology took place. The latter can evidently be explained, first, by the action of membrane-damaging factors, formed soon after burning and circulating in the blood [11, 13], on the erythrocyte membane, but later, by a compensatory reaction of the bone marrow, producing abnormal, immature erythrocytes of the type of the microcytes which we observed.

The investigation shows that the structural and functional properties of the erythrocyte membrane and of erythrocytes themselves undergo sharp changes during the first few minutes after severe thermal burns.

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