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Morphological Changes in Red Cells Exposed to Perfluorodecalin and Oxygenation

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The hemoprotective effect of perfluorocarbons was studied by examining the surface architectonics of red cells during exposure of the blood to perfluorodecalin and contact oxygenation. Scanning electron microscopy revealed an appeciable increase in the percentage of damaged erythrocytes directly exposed to oxygen in a contact oxygenator. Interaction between blood and perfluorodecalin not only does not impair the morphology of the blood, but also prevents untoward effects of subsequent contact oxygenation. Fluorocarbon treatment of the blood gives rise to a number (up to 17-18%) of special cells, which morphologically represent somewhat altered normocytes with increased resistance, this reflecting a specific reaction of the blood to perfluorodecalin.

Key Words: *perfluorodecalin; blood; oxygenation; electron microscopy; morphological changes*

Organoperfluorine compounds (OPFC), characterized by chemical inertness and the ability to bind large amounts of oxygen and carbon dioxide, have been studied with a view toward being used in membrane oxygenators of a new, liquid, type [1,3,4, 8,9,11,12]. Effective gas exchange, the lack of direct oxygen-blood contact, and the constant self-regeneration of the liquid fluorocarbon membrane in OPFC-based oxygenators create more favorable conditions for extrapulmonary gas exchange in comparison with contact (bubble) and membrane (ordinary) oxygenators [1,2,13].

The fact that blood cells are damaged less during prolonged operation of liquid oxygenators may be due not only to the prevention of unfavorable effect of gaseous oxygen on the blood, but also to the enhanced red cell resistance to this and other unfavorable factors of artificial circulation upon exposure of the blood to OPFC. Data on the blood-protective action of OPFC are extremely scant [6,7,11].

This study continues the investigations of the effects of OPFC on the blood. Our goal was to study the morphological changes of blood exposed to the organoperfluoride compound perfluorodecalin (PFD) and subsequent oxygenation in a bubble (contact) oxygenator.

MATERIALS AND METHODS

Fresh (or stored for up to 2 days) preserved (with glugicir in a 4:1 ratio) donor blood was used in the trials. It was used in 4 series of experiments, each consisting of 7 observations: in the first series intact blood was used (control); in the second series blood was treated with PFD for 1 h in two connected chambers, in one of which the liquids were mixed on rotor disks and in the other PFD and the blood were completely and reliably separated; in the third series blood was exposed to oxygen in a con-

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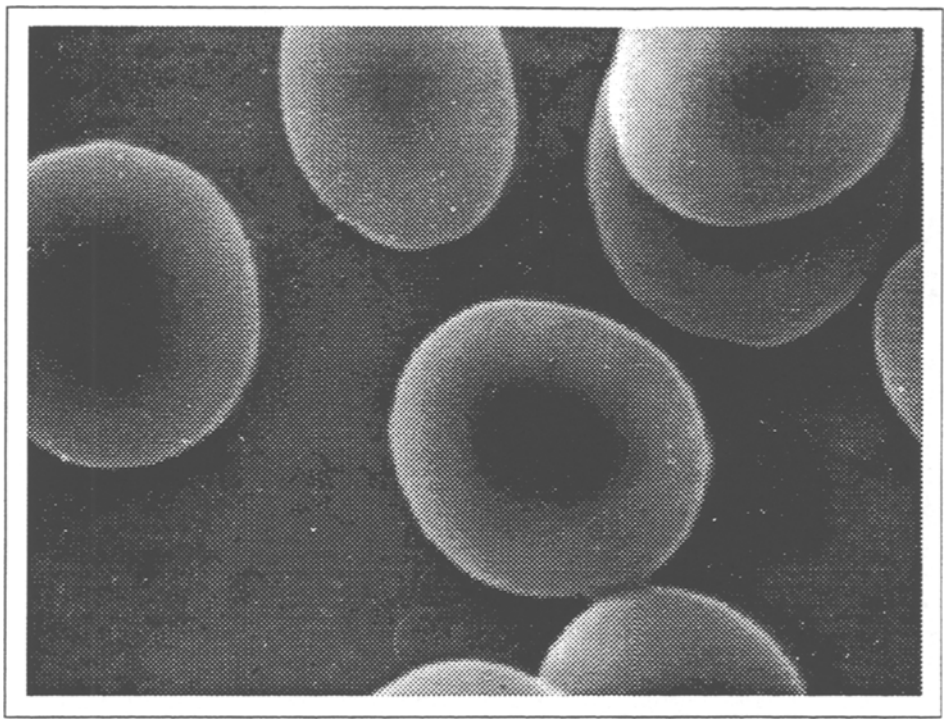

Fig. 1. Normocytes (characteristic morphological picture of intact blood). ×2000.

tact bubble-type oxygenator for 8 and 12 h (oxygen consumption per 100 ml of blood 100 ml/min); and in the fourth series blood was treated with PFD and then exposed to oxygenation according to the described regimen. Morphological changes in the blood interacting with PFD and during its subsequent oxygenation in a contact oxygenator were examined by scanning electron microscopy of erythrocytes using a Hitachi H-300 microscope. The counts of different morphological forms of red cells (normocytes, ovalocytes, echinocytes, cupola-shaped cells, spherocytes, microcytes, and deformed erythrocytes according to an established classification [5,10] per 100 cells were estimated at an accelerating voltage 20 kV and 2000-5000 magnification. The results were statistically processed using Student's *t* test and Statgraphics software.

RESULTS

The large majority of erythrocytes in health are shaped like biconcave disks, that is, they are discoor normocytes [5,10]. If the blood is exposed to some adverse factors, normocytes can alter their shape, either reversibly or irreversibly, depending on the factor and on the duration of exposure to it.

TABLE 1. Ratio of Different Forms of Erythrocytes in Experimental Series in Percent ($M \pm m$, $n=7$)

Morphological types of cells	Series of examined blood					
	1st	2nd	3rd oxygenation		4th oxygenation	
			8 h	12 h	8 h	12 h
Normocytes	71.7±1.2	67.3±1.4	58.4±1.7	53.5±2.1	65.6±1.3	65.0±1.3
Ovalocytes	1.4±0.42	0.1±0.05	1.6±0.68	0.9±0.31	0.4±0.17	0.1±0.06
Echinocytes	2.3±0.67	5.2±0.34	2.3±0.47	6.4±0.84	4.5±0.53	4.0±0.39
Cupola-shaped cells	2.9±0.34	0.7±0.11	4.3±1.41	4.0±0.62	0.6±0.19	1.3±0.22
Microcytes	0.6±0.12	0.3±0.08	0.9±0.21	0.6±0.29	0.3±0.09	0.3±0.08
Deformed cells	10.9±1.5	7.7±0.71	14.0±0.9	18.0±1.7	10.1±1.1	10.1±1.3
Spherocytes	1.6±0.36	0.4±0.11	4.0±0.79	7.7±0.59	0.4±0.08	0.4±0.10
Destroyed cells	0.4±0.11	0.1±0.06	0.4±0.19	1.2±0.39	0.1±0.06	0.5±0.13
Other forms	8.2±0.67	0.6±0.19	14.1±0.7	7.7±0.67	0.4±0.16	1.2±0.23
SC	-	17.6±1.4	-	-	17.6±1.8	17.1±1.6

Among such forms are ovalocytes, echinocytes, microcytes, deformed, and cupola-shaped cells. These last cells are at the prestage of development of spherocytes, which are a prelytic form of erythrocytes [10]. Hence, the number of normocytes and their ratio to other forms can be used as a gauge of the functional status of the blood and the maturity of its formed elements.

The results of assessing the quantitative ratio of different forms of red cells in different series of experiments are presented in Table 1. It is noteworthy that electron microscopy permits the detection of other forms, besides normocytes, whose share is only 71.7±1.2%, even in intact (control) portions of donor blood stored for no more than 2 days (Fig. 1). These forms more (cupola-shaped, deformed cells, and spherocytes) or less (ovalocytes and microcytes) approach weakly resistant physiologically impaired forms. It is quite natural for blood oxygenation in a contact oxygenator to have a negative impact on blood morphology. The counts of normocytes after 8- and 12-hour oxygenation (58.4±1.7 and 53.5±2.1%, respectively) are appreciably reduced in comparison with intact blood (by 18.6 and 25.4%, respectively). The number of altered forms of erythrocytes increases. Note the increase in the count of echinocytes (from 2.3±0.67% in intact blood to 6.4±0.84% after 12-hour oxygenation), deformed (10.9±1.5% in intact blood and 14.0±0.9 and 18.0±1.7% after 8- and 12-hour oxygenation, respectively), and destroyed cells (a threefold increase after 12-hour oxygenation in comparison with intact blood). The increase in the content of cupola-shaped erythrocytes and spherocytes as prelytic forms gives an idea of the extent of the direct effect of oxygen on the blood during its contact oxygenation (Fig. 2).

Analysis of the ratio of different forms of red cells after the interaction between intact blood and PFD showed that the count of normocytes did not appreciably change (just a statistically unreliable decrease of their number, $p>0.05$, was observed). As

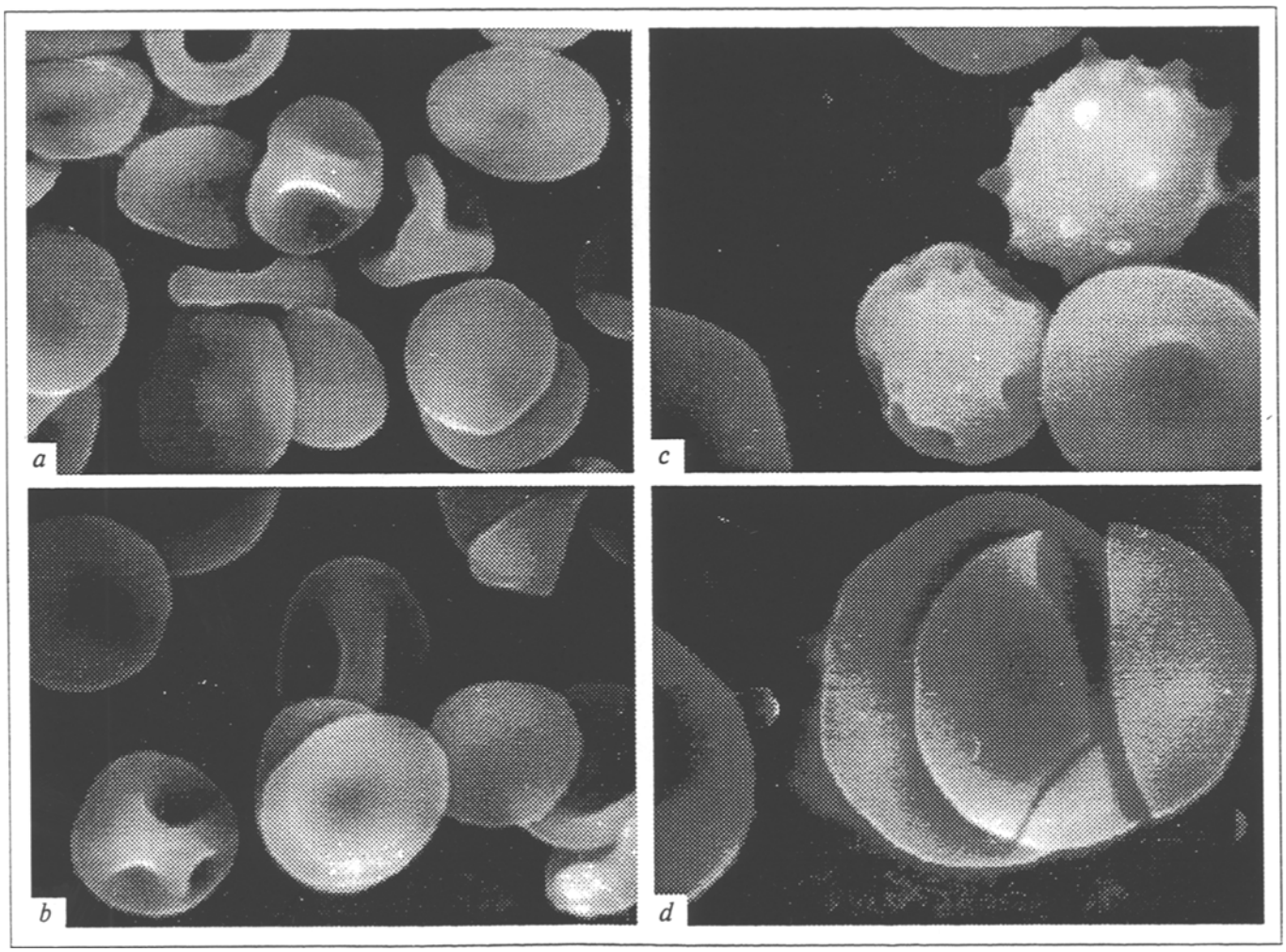

Fig. 2. Changes in the morphological picture of blood exposed to contact oxygenation. ×2000. *a*) increased number of deformed cells; *b*) prelytic forms of erythrocytes — spherocytes; *c*) echinocyte in a visual field; *d*) destroyed erythrocyte.

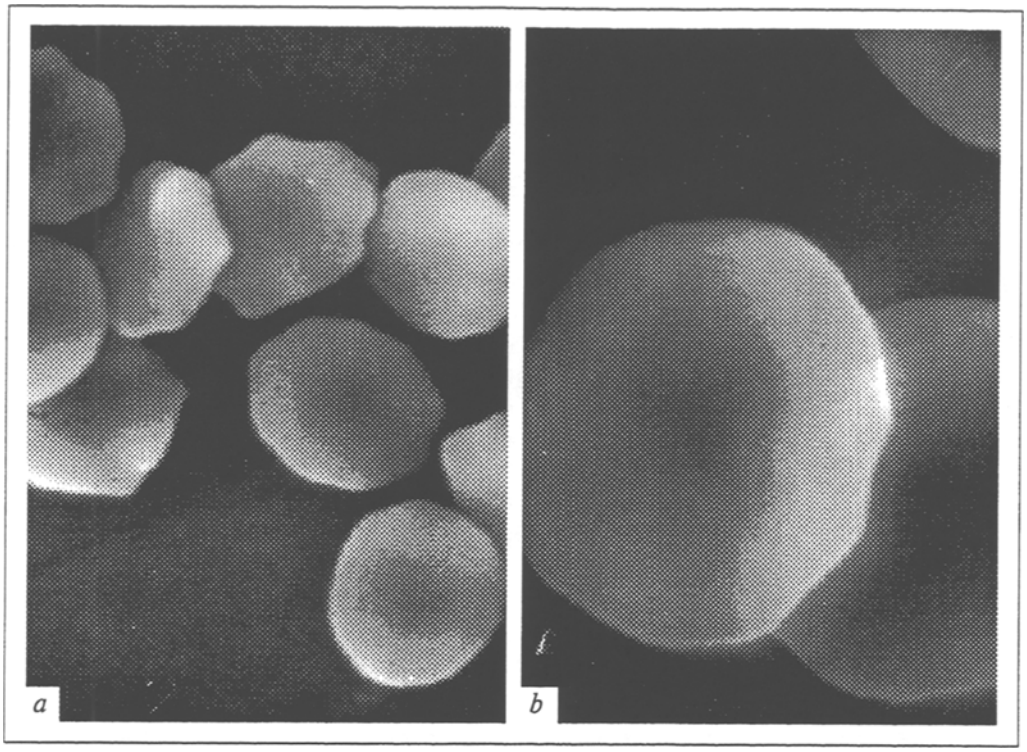

Fig. 3. Specific reaction of blood to PFD. *a*) SC appearing after PFD treatment of the blood. ×2000; *b*) SC. ×5000.

for the forms other than normocytes which we observed in intact blood (ovalocytes, microcytes, deformed and cupola-shaped cells, etc.), their number decreased, except for the echinocytes, whose number increased to account for 5.2±0.34%.

Peculiar cells, never heretofore described as pathological ones, were revealed in high amounts (17.6±1.4%) in intact blood exposed to PFD. We dubbed them special cells (SC) which make their appearance after PFD treatment of the blood. They represent a variety of normocytes, somewhat flattened with a not quite even, sometimes twisted, contour (Fig. 3). In shape these erythrocytes do not resemble the echinocytes with their characteristic sharp or cupola-shaped protrusions all over the surface, making them look like hedgehogs.

Treatment of intact blood with PFD demonstrated that, despite its well-known chemical inertness, this substance had a noticeable effect on the blood: the most stable forms (normocytes and SC) are retained, whereas the number of deranged cells, notably the cupola-shaped ones and spherocytes, is reduced almost twofold.

This reaction of the blood to PFD may be considered specific: first manifested during exposure to this substance, it is retained later as well, probably due to changes in the membrane structures of red cells.

During subsequent oxygenation of PFD-treated blood, which is freed of fluorocarbon in the separating device, not only is the count of echinocytes lowered, this proving the reversibility of the induced shifts, but a stabilizing effect of fluorocarbon treatment is also observed: the proportion of echinocytes after 8- and 12-hour oxygenation is 4.5±0.53 and 4.0±0.39%, respectively. A study of the content of other altered forms of red cells in group 4 confirmed the protective effect of PFD on the blood during its subsequent contact oxygenation. Eight-hour oxygenation did not lead to an increase in the numbers of ovalocytes, microcytes, spherocytes, or deformed and destroyed cells, etc.; moreover, their counts stabilized upon a longer exposure of the blood to oxygen.

Since the resistance of erythrocytes to unfavorable factors, their deformability, and their capacity for effective gas exchange are determined by the high ratio of their surface area to volume [10], it is possible that the acquisition of a flattened shape with twisted edges improves the functional properties of an SC, because such a transformation has the tendency to increase this ratio. The low number of cupola-shaped erythrocytes and spherocytes in blood exposed to fluorocarbon confirms this. The ratio of surface area to volume diminishes due to an increased volume of cells in the forms representing prelytic stages of erythrocytes.

Hence, electron microscopic study of blood exposed to PFD and subsequent oxygenation revealed the structural basis of the previously established (by functional data) blood-protecting effect of perfluorocarbons.

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