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DECREASED DEFORMABILITY IN AGING ERYTHROCYTES

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Deformability of bovine erythrocytes separated according to density (and age) was estimated by a modified Teitel's filterability test, the centrifugational test of Sirs, and viscosity measurements of cell suspensions. Both youngest and oldest erythrocytes were found to be less deformable than middle-aged cells, a result speaking against any chief role for deformability in the recognition of senescent erythrocytes and their removal from the circulation.

Introduction

Decreased deformability of in vivo aged erythrocytes has been demonstrated by many investigators [1,2] and hypothesized to be of critical importance for the removal of senescent red blood cells from the circulation [3,4]. It was argued that aged, less deformable erythrocytes pass microcapillaries with greater difficulty and can be trapped and eliminated in the spleen [4]. However, a recent report indicates that flexibility of the human erythrocyte membrane is not significantly altered in aged cells, suggesting that the red cell life span is not determined by factors related to membrane flexibility [5]. This conclusion is in agreement with the hypothesis of an immune mechanism of recognition of senescent red blood cells [6–8]. According to this theory, aging of red blood cells involves exposure of a specific 'senescent antigen' on the cell surface which binds IgG autoantibodies. Cells coated with the autoantibodies can be recognized and phagocyted by macrophages. Reduced deformability of aged erythrocytes is here not a prerequisite for their elimination from the circulation.

This study was aimed at a re-examination of the question of age-related changes in the erythrocyte deformability. As the majority of studies in this field have been performed on human erythrocytes, we chose another object, namely, bovine red blood cells. The rationale for this was that studies of the red cell aging in various mammalian species could allow for differentiation between fundamental and accidental events in this process.

Materials and Methods

Bovine blood at slaughter, and human and rabbit blood from venipuncture were taken into 3.3% sodium citrate (9 : 1, v/v). Erythrocytes were separated according to density (and age) by the method of Murphy [9]. Briefly, the method consists of ultracentrifugation of packed red cell suspensions in their own plasma (39600 × *g*, 1 h, 30°C). The stratified cells are divided into six equal-size fractions. Successful separation of erythrocytes according to age by this method is demonstrated, among others, by location of a vast majority of young ^{59}Fe-labeled erythrocytes in the top fraction (unpublished data) and progressive decrease of many enzymatic activities from top [1] to bottom [6] fractions [10,11]. Erythrocytes from

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successive age fractions were washed four times and resuspended in 0.5% bovine serum albumin (Biomed, Cracow, Poland) in phosphate-buffered saline (152 mM NaCl in 10 mM sodium phosphate, pH 7.4) at a hematocrit of 0.5. Deformability of erythrocytes was evaluated using three indirect tests:

(i) The filterability test of Teitel [12] in the modification of Stäubli and Straub [13] consisted of the determination of the ratio of the half-filtration time of a 50% (v/v) erythrocyte suspension (2 ml) to the time of filtration of 3/5 volume of cell-free medium (5 ml) through a filter paper (Filtrak 3 h, G.D.R.) mounted on a glass funnel.

(ii) The centrifugation test of estimation of red cell flexibility was adapted from Sirs [14]. The packing coefficient of the cells, p, was defined as

$$p = \frac{H_{ap} - H}{H},$$

where H_{ap} is the apparent hematocrit of a red cell suspension centrifuged at 1400 rpm in a Janetzki TH12 microhematocrit centrifuge ($208 \times g$) for 15 min, H is the hematocrit determined by subsequent 5-min centrifugation of the suspension under standard conditions (12000 rpm or $15250 \times g$).

(iii) Viscosity of 50% (v/v) red cell suspensions was measured in a LVT Brookfield cone-plate viscometer for shear-rate values in the range of 1.15–230 s^{-1}, at a temperature of 37°C.

The results are expressed as percentages of appropriate values found in the fraction of lightest (youngest) cells.

Results and Discussion

All the three methods of indirect estimation of red blood cell deformability employed gave consistent results demonstrating a relatively low deformability of cell fractions of lowest density (and mean age) increasing in the middle-age cell fractions and decreasing again in most dense (oldest) cell fractions (Fig. 1, Tables I and II).

We are not aware of any report stating non-monotonous changes in deformability during aging of circulating red blood cells. It is known that human reticulocytes have a low deformability, but it seems to be normalized soon in the circulation [15].

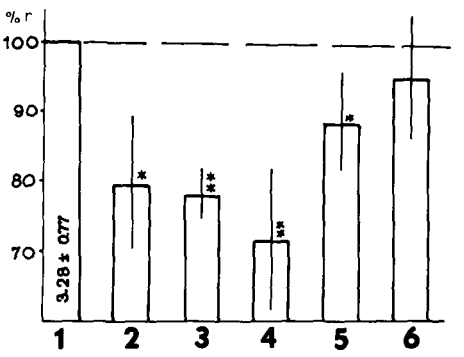

Fig. 1. Relative filtration half-time (r) of bovine erythrocyte suspension of various age fractions. Bars indicate S.D. $n = 5$ (different animals). Absolute value of 'r' indicated for fraction 1. * $P < 0.05$, ** $P < 0.01$ (one-tailed Student's t-test).

To exclude a possibility that a similar dependence in erythrocytes of other species escaped detection in studies employing a simple comparison of oldest and youngest erythrocytes, we determined the packing coefficient of human and rabbit erythrocytes separated according to age by the same method. In contrast to bovine red cells, a progressive increase in the value of p with increasing red cell age was found (Table III).

It seems, therefore, that the type of age-related changes in erythrocyte deformability observed in bovine erythrocytes is due to a species specificity of the process of red cell maturation and aging. No reticulocytes are detectable in blood smears of adult cows [11,16]. It can be hypothesized that the

TABLE I

PACKING COEFFICIENT OF BOVINE ERYTHROCYTES OF VARIOUS AGE FRACTIONS

Mean ± S.D., $n = 5$, $P < 0.01$ (one-tailed Student's t-test). The value of p taken as 100% is 0.64 ± 0.03.

Fraction No.	p (%)
1 (youngest)	100
2	91.4 ± 1.8
3	88.9 ± 4.7
4	90.2 ± 3.4
5	95.5 ± 6.1
6 (oldest)	98.4 ± 5.0

TABLE II

VISCOSITY OF BOVINE ERYTHROCYTE SUSPENSIONS FROM DIFFERENT AGE FRACTIONS AT VARIOUS SHEAR RATES

Values for fractions 2–6 are expressed as percentages of the corresponding fraction 1 value, x. $n = 5$, [a] $P < 0.05$, [b] $P < 0.01$.

V (s^{-1})	Cell fraction 1 (η in cP) ($x = 100\%$)	Cell fraction				
		2	3	4	5	6
230	4.1 ± 0.4	98.6 ± 4.5	97.3 ± 3.1	96.5 ± 4.0	98.9 ± 3.7	101.4 ± 0.8
115	4.3 ± 0.3	99.3 ± 5.3	91.2 ± 4.4 [a]	95.2 ± 7.6	101.1 ± 6.1	105.3 ± 7.2
46	5.9 ± 0.7	96.1 ± 6.2	89.3 ± 7.3 [a]	91.7 ± 9.0	96.3 ± 4.8	103.1 ± 3.1
23	7.9 ± 1.1	95.4 ± 3.8	75.4 ± 8.2 [b]	87.4 ± 8.5 [a]	98.2 ± 9.2	105.0 ± 7.6
11.5	11.3 ± 0.9	93.2 ± 4.9	70.9 ± 12 [b]	70.5 ± 18 [a]	92.8 ± 5.3	107.8 ± 10
5.75	13.0 ± 1.9	87.4 ± 7.6	65.3 ± 9.9 [b]	73.1 ± 14 [a]	87.2 ± 16	99.3 ± 12
2.35	16.5 ± 1.5	90.1 ± 9.2	60.8 ± 8.9 [b]	61.3 ± 10.9 [b]	81.4 ± 9.7 [a]	105.6 ± 8.9
1.15	25.0 ± 2.4	88.2 ± 7.0	51.2 ± 11 [b]	58.3 ± 13 [b]	76.3 ± 14.5	114.1 ± 13

TABLE III

PACKING COEFFICIENT OF HUMAN AND RABBIT ERYTHROCYTES OF VARIOUS AGE FRACTIONS

Mean values from triplicate experiments. 100% is 0.11 for human and 0.072 for rabbit.

Fraction No.	p (%)	
	human	rabbit
1 (youngest)	100	100
2	156	189
3	204	228
4	199	368
5	253	465
6 (oldest)	297	411

pattern of reticulocyte maturation differs between the human and bovine species. In the latter case the process of membrane maturation (normalization of deformability) may be retarded with respect to human cells. On the other hand, disappearance of cytoplasmic structure responsible for the reticulocyte staining in blood smears may proceed at a higher rate in bovine cells.

The data obtained speak against any significant role of a reduction in erythrocyte deformability for the removal of senescent mammalian erythrocytes. Though the existence of a very sensitive mechanism able to differentiate between youngest bovine erythrocytes of low deformability and still less deformable oldest red blood cells cannot be definitely excluded, it seems more probable that another type of recognition, arising from the circulation and independent of cellular deformability, is responsible.

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