

Osmotic Properties of Bovine Erythrocytes Aged in Vivo

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Abstract. The osmotic properties of bovine erythrocytes aged in vivo were studied by the modified microhematocrit method. The osmotic fragility of older red cells decreases due to their larger relative osmotically non-active volume. Relative critical cell volume of bovine erythrocytes does not alter significantly with cell age. The age dependent change in the osmotic fragility of human red blood cells, the reverse of that found for bovine erythrocytes, is due to a different alteration of the critical cell volume during intravascular erythrocyte aging.

Key words: Erythrocyte aging — Osmotic fragility — Critical cell volume — Osmotically non-active volume — Microhematocrit method

Introduction

Despite numerous studies on both human (Van der Vegt et al. 1985) and bovine red blood cells (Bartosz 1981b), the relationship between osmotic fragility and erythrocyte aging has not yet been fully explained. The results of serial osmotic hemolysis of human erythrocytes have shown young red cells to be osmotically less fragile (Simon and Topper 1957). However, experiments on cells of different densities separated by centrifugation indicate that cellular aging does not produce a progressive slow increase in osmotic fragility (Rifkind et al. 1983). The relationship between osmotic fragility and cell age is not linear for bovine erythrocytes (Bartosz 1981b). The age dependence of the osmotic fragility of bovine erythrocytes is the reverse of that for human red cells (Mosior et al. 1984). The osmotic fragility of erythrocytes depends on the ratio of the surface area of membrane to the isoosmotic volume of the cells (Dick 1959). Both the internal solute content and the lipid content of the erythrocyte decrease during cell aging for both human (Cohen et al. 1976) and bovine red blood cells (Bartosz 1981a; Bartosz et al. 1981), which produces alterations in osmotic fragility. In order to explain the observed difference in the dependence of osmotic fragility on erythrocyte age between the two species investigated the

osmotic properties of bovine erythrocytes aged in vivo have been measured and compared with the data obtained for human red blood cells (Cohen et al. 1976; Nash and Wyard 1982; Shiga et al. 1979).

Materials and Methods

Fresh, heparinized bovine blood was obtained from a local slaughterhouse. The analytical grade reagents used in the experiments were available commercially.

Separation of erythrocytes by age

Blood was centrifuged at $2700 \times g$ for 10 min at 4°C , the plasma and buffy coat removed by aspiration, and the red cells were suspended in plasma. This procedure was repeated twice. Erythrocytes were separated by age using the method of Murphy (1973), which was also proved to be effective for bovine red cells (Bartosz and Bartkowiak 1981). A highly concentrated erythrocyte suspension (hematocrit (80–90)%) was centrifuged at $38,500 \times g$ (max.) for 60 min at $(30 \pm 2)^{\circ}\text{C}$ (Janetzki VAC 602 ultracentrifuge, angular rotor $8 \times 11 \text{ cm}^3$). The top cell fraction (15%) was carefully collected in a syringe. The middle 70% of cells were discarded. The bottom layer (15%) was washed with plasma. Both samples were then washed three times in the following solution: 138 mmol/l NaCl, 5 mmol/l KCl, 1.5 mmol/l MgCl_2 , 5 mmol/l Tris/HCl, 10 mmol/l glucose, pH 7.4.

Evaluation of osmotic parameters

The critical volume of red cells, V_c , was calculated from van't Hoff's law modified for erythrocytes (Dick 1959):

$$V_c = \frac{c}{\pi_h} + b$$

where: c is the sum of the products of osmotic coefficients and the amounts of internal solutes, π_h is the cells' osmotic fragility, b is the volume of the osmotically nonactive part of the erythrocyte. The parameters c and b were evaluated by the linear regression method from van't Hoff's law modified for erythrocytes. The microhematocrit method (Savitz et al. 1964) with a mathematical modification (Mosior and Gomul'kiewicz 1985) was used to obtain the relationship between cell volume and inverse osmolarity. The procedures were as follows: six NaCl solutions with relative osmolarities of 0.60–1.84 (unity corresponds with 310 mosmol/l) were mixed in the ratio 2:3 with an isoosmotic erythrocyte suspension of hematocrit 75%. The final osmolarities were calculated using the hematocrit of the isoosmotic cell suspension, the final hematocrits of cell suspensions of varying osmolarity and the volume of solution trapped between the erythrocytes, (after Takano 1975). The results are not significantly different even if there is a 100% difference in the volumes of extracellular solution trapped between young and old erythrocytes. All the hematocrits were measured after centrifugation at $15,000 \times g$ for 5 min. In order to calculate the final osmolarity it is necessary to know the relative volume of the intracellular solution. This last value was determined by an application of the Brouwer theorem on the fixed point of representation (Musielak 1976) to a modified linear regression operator, that also included the evaluation of the osmolarity of the cell suspensions. The volume of the osmotically active water, V_w , was found by iteration: an arbitrary value of V_w , from the range 0–1, was used for the first calculation. The result obtained was then used for the next calculation instead of the initial V_w . The procedure was terminated when the new result did not improve the previous value by more than 0.02%.

In order to compare the results obtained for young and old red cells, the values corresponding to the old cells were multiplied by the appropriate ratio of the cell isoosmotic volumes. The relative erythrocyte volume in isoosmotic solution was calculated from the concentration of hemoglobin released from erythrocytes in suspensions with identical hematocrits. The relative volume of the trapped extracellular solution, (after Takano 1975), has also been allowed for.

Osmotic fragility

The osmotic fragility was determined by a static method. Equal volumes of erythrocyte suspensions were added to phosphate buffered, pH 7.4, hypotonic NaCl solutions. After 15 min the suspensions were centrifuged at $2700 \times g$ for 10 min and the absorbance of the solution of released hemoglobin was measured at $\lambda = 540$ nm. The osmotic fragility was expressed as the mean osmolarity found numerically from the hemolytic curves. The osmolarities of the solutions were calculated as for ideal solutions, taking into account the dissociation constant of the phosphate buffer. The number of experimental points (20) assured the reproducibility of the results up to 0.2% of the ideal isoosmotic osmolarity (310 mosmol/l).

Results

The osmotic parameters of bovine erythrocytes separated by centrifugation are presented in Table 1. Parameter c for young erythrocytes is equal to the relative volume of the osmotically active water, since both the isoosmotic cell volume and the isoosmotic osmolarity were assumed to equal unity. The mean values shown in Table 1 cover the overlapping range of mean values due to the

Table 1. Osmotic properties of bovine erythrocytes aged in vivo, separated by the method of Murphy (1973). The values corresponding to the old erythrocytes were normalized to the values of the young cells (see Methods). The data are given as means \pm SD, sample number $n = 5$.

	Erythrocytes	
	young	old
Intracellular solute amount c (arbitrary units)	0.586 ± 0.024	0.518 ± 0.007
Osmotically non-active volume b (fraction of total cell volume)	0.414 ± 0.024	0.438 ± 0.008
Osmotic fragility π_h (per cent of isotonic osmolarity)	57.3 ± 3.5	55.2 ± 4.0
Critical cell volume V_c (per cent of isoosmotic volume)	143.9 ± 5.7	137.2 ± 6.6

Table 2. Osmotic properties of old erythrocytes relative to those of young cells. The numbers shown are confidence limits ($1 - p = 0.95$, sample number $n = 5$). All values are in per cent.

Isoosmotic cell volume V_0	94.9 ± 0.6
Intracellular solute amount c	88.2 ± 3.6
Osmotically non-active volume b	105.0 ± 5.1
Osmotic fragility π_h	96.4 ± 1.2
Critical cell volume V_c	95.3 ± 2.6

distribution of the measured parameters between the different individuals whose blood was studied. However, the differences between the values for the young and old erythrocytes are statistically significant ($p < 0.05$), Table 2.

A decrease in internal solute content is very evident (Tables 1 and 2). This is not surprising since the potassium content of the densest bovine erythrocytes decreases considerably more than the sodium content increases (Bartosz et al. 1981). The decrease in critical cell volume of old red cells equals the decrease in the isoosmotic cell volume (Table 2). In fact, the change in that initial value may be somewhat lower since the surface area extensibility of the erythrocyte membrane, responsible for 4.0–8.5% of the calculated critical cell volume (Evans

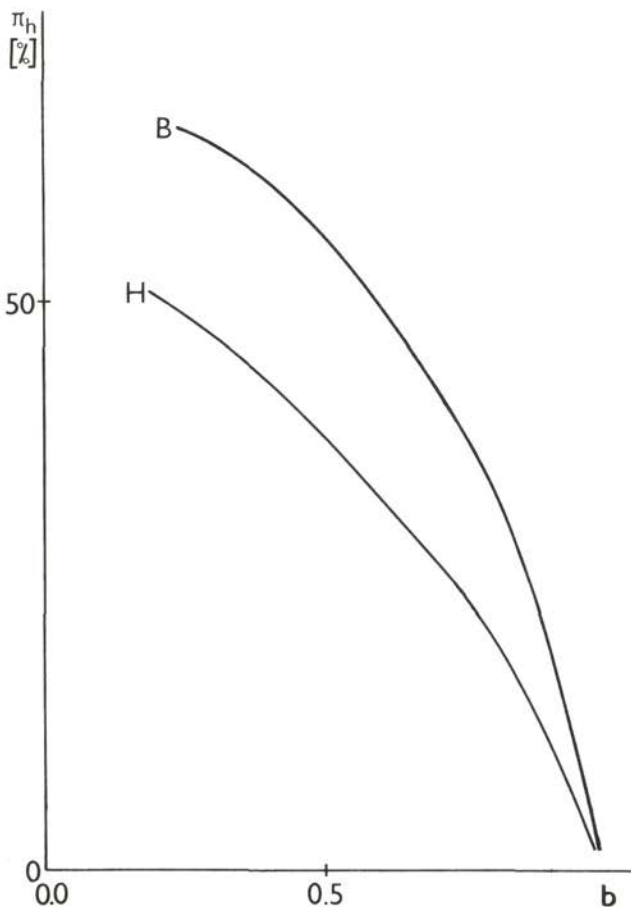


Fig. 1. Dependence of the osmotic fragility π_h on the relative osmotically non-active volume b , on the assumption that critical cell volume remains constant, for human (H, $V_0 = 1.78 V_0$ (Seeman et al. 1969)) and bovine (B, $V_c = 1.44 V_0$ (Mosior and Gomułkiewicz 1985)) erythrocytes.

and Skalak 1980), may be higher for the young red cells. The change in the isoosmotically non-active volume is relatively large but statistically nonsignificant (Table 2). The hemoglobin content of bovine erythrocytes does not alter during cellular aging, thus the isoosmotically non-active volume probably remains constant.

Discussion

The osmotic fragility of erythrocytes depends mainly on the ratio of the membrane surface area to isoosmotic cell volume (Dick 1959). Even if the mechanical properties of erythrocyte membranes (Evans et al. 1976) and the prehemolytic potassium efflux (Seeman 1969) are negligible, it is necessary to consider an effect of the osmotically non-active volume on the osmotic fragility. The relationship (1) between the osmotic fragility π_h and the relative osmotically non-active volume b was plotted in Fig. 1 for two different relative critical cell volumes V_c .

$$\pi_h = \frac{1 - b}{V_c - b} \quad (1)$$

The relative critical cell volumes of old and young bovine erythrocytes are the same, however, the relative volume of the osmotically non-active part of old erythrocytes is larger than that of young cells, thus the latter should be more fragile. The calculated difference in osmotic fragilities between young and old erythrocytes, following from Fig. 1, is somewhat lower than that measured (Table 2). Nevertheless, the relative critical cell volume of young erythrocytes may in fact be lower, since the computed critical cell volume depends partly on membrane extensibility, which is probably greater for young red cells. According to the Evans-Skalak model of the erythrocyte membrane (Evans and Skalak 1980) the membrane of an old red cell should be less extensible because it is more rigid than that of a young cell (Shiga et al. 1979). The hemoglobin content of human erythrocytes does not alter during cell aging (Cohen et al. 1976). If the relative critical cell volume of human erythrocytes were constant for all age fractions of cells, the osmotic fragility distribution would be as for bovine red cells (Fig. 1). However, the sphericity of human erythrocytes is by about 2% greater for aged red cells than for younger cells (Nash and Wyard 1982). This means that the relative critical cell volume of old human red cells is 3% lower than that of young cells.

Therefore, despite the constant hemoglobin content (Cohen et al. 1976) and consequently larger relative osmotically non-active volume of old erythrocytes, the osmotic fragility of human red cells should remain constant during aging.

Old human red cells separated by centrifugation are, however, more fragile (Rifkind et al. 1983).

Recently the dependence of the critical cell volume on the state of glycolysis, probably due to changes in membrane extensibility, has been established (Mosior and Gomul'kiewicz 1985). The rate of glycolysis of old human erythrocytes is lower than that of young cells (Mangani et al. 1983), therefore the membrane of the latter is probably more extensible. The observed changes in osmotic fragility of both human and bovine erythrocytes during cellular aging result effectively from three factors: (i) the increase in relative isoosmotically active volume of the cell, (ii) alterations in relative critical cell volume, (iii) the decrease in membrane extensibility. The relative critical cell volume of bovine red blood cells increases slightly during cell aging, contrary to that of human erythrocytes, which produces the observed differences in the dependence of osmotic fragility on cellular aging.

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Final version accepted April 14, 1987