Aging of the erythrocyte. XIV. ATP content does decrease

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Summary. ATP content shows a cell-age dependent decrease in bovine erythrocytes separated according to Murphy. An important point in the evaluation of results lies in considering differences in the ATP consumption between cells of various age during separation. The decrease in ATP occurs in spite of diminished ATPase activity in older cells.

The diminution in the ATP level in senescent erythrocytes has long been acknowledged as one of the most important processes in the in vivo aging of these cells[2-4]. However, it has been reported recently[5] that the ATP concentration in the most dense (and presumably oldest) human erythrocytes is not decreased. This prompted us to re-examine this question by estimation of the ATP content of cell fractions obtained by separation of bovine erythrocytes according to density, which have not yet been studied in this respect. In order to gain some insight into factors governing the ATP level, ATPase activity was also estimated in membranes of different fractions of erythrocytes.

Material and methods. Bovine erythrocytes were separated according to density by the method of Murphy[6]. The cell column obtained was divided into 6 equal-size fractions. ATP concentration was estimated by the method of Bücher[7] using reagents from Boehringer. Erythrocyte membranes were prepared according to Dodge et al.[8] but using Tris-HCl buffer. (Mg-Na-K)-ATPase was estimated by the method of Ellory and Carleton[9] employing the phosphate assay of Ames[10]. Ouabain-insensitive ATPase was estimated in the presence of 1 mM ouabain. Protein was determined by the method of Lowry et al.[11] in a modification[12] required for the presence of lipids.

Results and discussion. The results of Kirkpatrick et al.[5] on the lack of ATP decline in the most dense human red blood cells imply one of the following possibilities: a) the content of ATP does not decrease during intravascular erythrocyte aging, b) the oldest cells are not the most dense as they may swell before elimination from the circulation, and c) the ATP level may change during the separation procedure itself in a different manner in various cell fractions. Apart from the b) alternative discussed[5], the last one should be taken into account. It has been reported that the rate of ATP decay is identical for young and old human erythrocytes incubated in their own plasma at 0 °C[13]. However, the separation method of Murphy[6] requires at least 1 h of handling of erythrocytes at a temperature of 30 °C. Sass demonstrated that in a glucose-free medium at 37 °C ATP decreases more rapidly in young than in old erythrocytes[14]. We determined the rate of ATP decrease in various cell fractions incubated at a high hematocrit (80%, to approximate separation conditions) in their own plasma at 30 °C. There were significant differences between various age fractions of cells, the rate of ATP decrease being inversely correlated with cell density (age). During a 2-h incubation, the level of ATP decreased by 36% in about 17% youngest cells and only by 10% in about 17% oldest cells (mean values from 4 experiments). As, in this study, the whole period of handling erythrocytes at 30 °C was about 2 h (including preincubation, centrifuge stopping etc.), values of ATP decrease for individual fractions obtained in these experiments were taken as an estimate for corrections of the ATP content obtained by assay immediately after separation.

The corrected values of the ATP content shown in table 1 demonstrate a progressive ATP decline during aging of bovine red blood cells. The age-related differences in the rate of ATP decrease during separation contribute significantly to the calculated differences in the ATP content between cell fractions and may mask them if they are not considered. Addition of adenosine (final concentration of 3 mM) and glucose (15 mM) to the erythrocyte suspensions before ultracentrifugation did not significantly affect the ATP levels in cell fractions. (Mg-Na-K)-ATPase activity (both ouabain-sensitive and ouabain-insensitive) of erythrocyte membranes decreased with increasing cell age (table 2). This may contribute to the decreased ATP utilization by older cells in a glucose-free medium. On the other hand, the decreased ATPase activity suggests that the cell-age dependent diminution in the ATP level is due mainly to inhibition of ATP-generating processes[2].

Table 1. Corrected ATP concentrations in different density (age) fractions of bovine erythrocytes (mean ± SD, n = 6, different animals)

Fraction No.	ATP
1 (lightest)	436 ± 101 μmoles/l = 100%
2	98 ± 9%
3	95 ± 11%
4	92 ± 9%
5	87 ± 7%
6 (heaviest)	81 ± 10%

Table 2. (Ma-Na-K)-ATPase activity in membranes from different density (age) fractions of bovine erythrocytes; n = 6

Fraction No.	ATPase activity Total	Ouabain-sensitive
1	22.9 ± 3.1 nmoles/mg membrane protein · h = 100%	6.4 ± 1.7 nmoles/mg membrane protein · h = 100%
2	94 ± 5%	99 ± 8%
3	86 ± 12%	99 ± 10%
4	79 ± 18%	93 ± 7%
5	79 ± 15%	90 ± 10%
6	76 ± 12%	85 ± 13%

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