Ageing of the erythrocyte. XVI. Free amino acid content

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Summary. The concentrations of some free amino acids, especially glycine, histidine, isoleucine, methionine, phenylalanine, serine and tyrosine decrease considerably during the intravascular ageing of bovine erythrocytes. They seem to provide as sensitive an indication of red cell age as creatine does.

It has been demonstrated that the creatine concentration drops significantly during intravascular red cell ageing[2,3] and can therefore be a useful marker of cell age in separated erythrocyte fractions[2], or of mean cell age in the circulating erythrocyte population[4]. Creatine is a product of amino acid metabolism and it seemed worthwhile to examine whether erythrocyte ageing also involves changes in the concentrations of free amino acids.

Materials and methods. Bovine erythrocytes were fractionated according to density (and age)[5] by the method of Murphy[6]. No leukocytes were found in smears of red cells from all fractions. Creatine was estimated by the diacetyl method[7]. Free amino acids were extracted from erythrocytes with ethanol[8] and analyzed according to Stein and Moore[9] in a JEOL JLC-6H automatic amino acid analyzer. Hematocrit was determined using a Janetzki TH12 (GDR) microhematocrit centrifuge.

Results and discussion. The concentration of creatine in red blood cells decreased with increasing cell density (table 1). In red blood cells separated according to density by the method employed, the concentration of creatine in the ⅙ heaviest cells was about 59% of that found in the ⅙ lightest cells. This confirms successful, though not optimal[5], separation of erythrocytes according to age. Absolute concentrations of free amino acids found in the lightest cell fraction are given in table 2. Comparison of amino acid concentrations in cell fractions obtained revealed progressive decreases in the concentrations of the majority of amino acids with increasing cell density. The most significant diminutions were found for glycine, histidine, isoleucine, methionine, phenylalanine, serine and tyrosine (fig. 1), whereas the concentrations of aspartate, glutamate, lysine, threonine and valine remained practically unchanged (not shown). The changes in concentrations of free amino acids

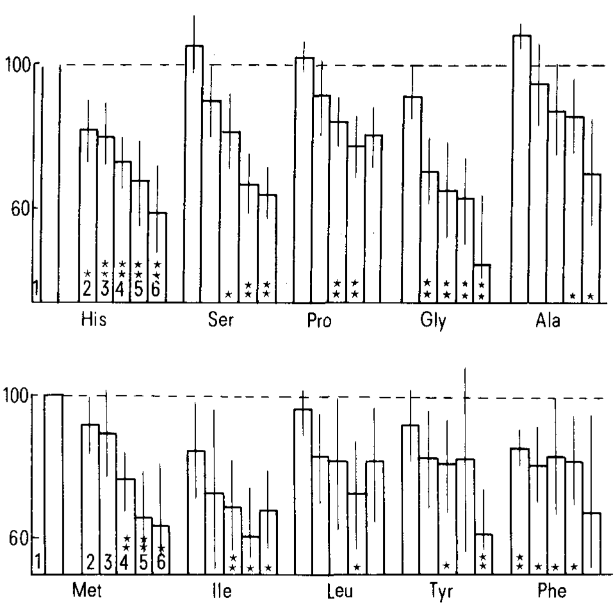

Figure 1. Changes in the concentrations of free amino acids in various density fractions of erythrocytes (values for fraction 1 set to 100%). SD indicated, n = 5 (different animals); * $p < 0.05$, ** $p < 0.01$ (1-tailed Student's t-test).

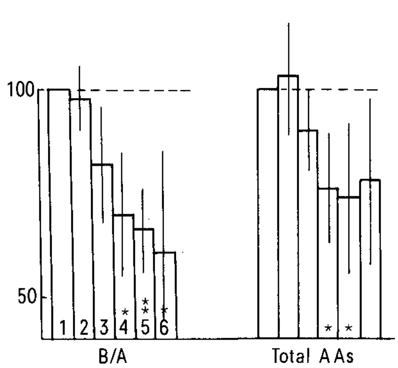

Figure 2. Changes in the ratio of basic to acidic amino acids (B/A) and total concentration of amino acids determined (total AAs) in erythrocyte fractions of different density.

Table 1. Creatine concentration in erythrocyte fractions of increasing density (and mean age). Mean ± SD, n = 6 (different animals). All differences significant at $p < 0.01$ (1-tailed Student's t-test)

Fraction No.	Creatine concentration
1 (lightest)	27.4 ± 5.5 µmoles/l cells = 100%
2	83.0 ± 6.5%
3	75.5 ± 9.8%
4	67.2 ± 6.3%
5	66.2 ± 10.8%
6	58.5 ± 8.5%

Table 2. Concentrations of free amino acids, ratio of basic to acidic amino acids (B/A) and total concentration of amino acids determined (total AAs) in the lightest erythrocyte fraction. Mean ± SD, n = 5 (different animals)

Amino acid	Concentration
Lys	235 ± 59 µmoles/l
His	211 ± 92 µmoles/l
Asp	195 ± 90 µmoles/l
Tre	108 ± 17 µmoles/l
Ser	107 ± 18 µmoles/l
Glu	168 ± 20 µmoles/l
Pro	109 ± 11 µmoles/l
Gly	338 ± 136 µmoles/l
Ala	185 ± 25 µmoles/l
Val	119 ± 42 µmoles/l
Met	49 ± 32 µmoles/l
Ile	74 ± 35 µmoles/l
Leu	167 ± 55 µmoles/l
Tyr	61 ± 2 µmoles/l
Phe	54 ± 16 µmoles/l
B/A	1.32 ± 0.52
Total AAs	2.18 ± 0.09 mmoles/l

in older cells are probably due to alterations in the rates of transport of these compounds, and differences in the patterns of age-related changes of various amino acids may reflect different rates of inactivation of individual amino acid carriers. Another explanation might involve a more intensive proteolysis in young than in mature erythrocytes[10]. The decrease in the total concentration of amino acids determined during intravascular erythrocyte ageing (fig. 2) can contribute to the diminution in the volume of old erythrocytes via osmotic water efflux from the cells. Since the magnitude of the decreases in the concentrations of some amino acids was comparable to that of creatine, they could also serve as sensitive indices of red cell age when using less elaborate methods of estimation.

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Effects of DL-propranolol on exercise heart rate and maximal rates of oxygen consumption in *Scaphiopus intermontanus*[1]

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Summary. Lymphatic injection of propranolol (0.2–10 µg) into toads decreased exercise heart rate in a dose-dependent manner. There was a significant linear correlation between exercise heart rate and maximal oxygen consumption rates ($\dot{V}O_2$ max). These data are consistent with the hypothesis that blood oxygen transport is the limiting process for $\dot{V}O_2$ max in anuran amphibians.

The quantity of oxygen transported to tissues per unit time (ml $O_2 min^{-1}$) is, by Fick Principle, the product of blood oxygen content (ml $O_2 \cdot$ ml $blood^{-1}$) and blood flow rate (ml blood $\cdot$ min^{-1}). If the maximal rate of oxygen consumption ($\dot{V}O_2$ max) is limited by blood oxygen transport, then $\dot{V}O_2$ max should be influenced by either changing the blood oxygen content and/or the blood flow rate. Evidence that intraspecific differences in $\dot{V}O_2$ max for an anuran amphibian was proportional to blood oxygen content has been previously presented[2]. The purpose of these experiments was to evaluate the proportionality of $\dot{V}O_2$ max and maximal heart rate in the anuran amphibian *Scaphiopus intermontanus*. By using the β-adrenergic blocker propranolol maximal heart rate during activity could be reduced. Since maximal heart rate is reduced so presumably is blood flow rate since blood flow rate is the product of heart rate and stroke volume. The relationship of $\dot{V}O_2$ max and maximal heart rate should provide a key test of the hypothesis that blood oxygen transport limits $\dot{V}O_2$ max in amphibians.

Materials and methods. Great Basin Spadefoot toads, *Scaphiopus intermontanus*, were collected in Central Oregon (Oregon scientific collecting permit 9, 1981). Experiments were performed in the spring 1–2 weeks after collection of the toads. Toads were not fed during the experiments and were maintained in containers with access to both water and dry areas. Only males were used with a mass of 18.2 ± 0.6 ($\bar{X} \pm SE$). Maximal oxygen uptake rates were determined as previously described[3]. The procedure consisted essentially of forcing the animal to right itself constantly in a closed container for 3 min of activity, then a gas sample withdrawn and analyzed polarigraphically (Beck-

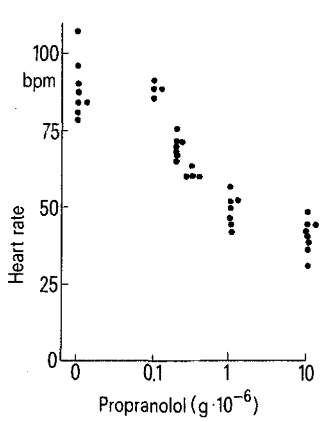

Figure 1. The effect of various doses of DL-propranolol on heart rates immediately post exercise. Points represent individual toads.

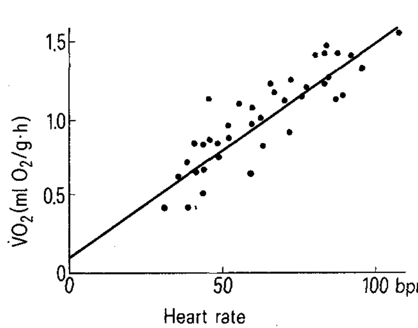

Figure 2. The relationship between post exercise heart rate and $\dot{V}O_2$max. The line of the regression equation relating these 2 variables is drawn in ($\dot{V}O_2 max = (0.014 \times$ heart rate$) + 0.1$).