separated by the method of Murphy[8] (to be published). No reticulocytes were found in any fraction, in agreement with the previous report on the absence of reticulocytes from the blood of adult cows[13].

Mean activities of glutathione peroxidase and catalase in the fraction of lightest (youngest) erythrocytes were 68.0±26.4 e.u./mg hemoglobin (n=8) and 13.5±3.5 Bergmeyer units/mg hemoglobin (n=7), respectively. When comparing enzyme activities in cell fractions of various ages, activity in the lightest fraction was assumed as 100% in each separation to eliminate differences in absolute values between different animals. Results shown in the table indicate a decrease in the activity of glutathione peroxidase and no definite changes in the activity of catalase during erythrocyte aging.

Although the catalase activity was reported to decrease in older human erythrocytes[14,15], the more recent study of Sass et al.[4] did not confirm this, revealing even a slight negative correlation between the activity of this enzyme and the activity of aspartate aminotransferase, an enzyme especially sensitive to erythrocyte age. The present data on bovine red blood cells would be in line with those of Sass et al.[4], demonstrating no significant decrease in the activity of catalase during erythrocyte aging in vivo. Results of the present study indicate a diminution in the capacity of aging red cells for dealing with endogenously produced and exogenous hydrogen peroxide; the more so because glutathione peroxidase has been demonstrated to have a lower K_m for H_2O_2 than catalase and to be more relevant in protection against physiological concentrations of this agent[16]. The results offer at least a partial explanation for the increased sensitivity of aging erythrocytes to oxidative stress mediated by hydrogen peroxide[17].

Glutathione peroxidase and catalase activities in different age fractions of bovine erythrocytes

Fraction No.	Relative activity (%) Glutathione peroxidase Mean	SD	Catalase Mean	SD
1	100		100	
2	92.5	6.5	110.1	13.4
3	87.7	8.1	106.9	15.3
4	88.6	7.0	107.5	8.4
5	79.4	9.3	98.1	16.3
6	77.8	9.7	102.2	11.3

Activities (per mg hemoglobin in hemolysates) are expressed as percent of activities found in the youngest cell fraction. Combined data from measurements on blood from 8 and 7 animals, respectively.

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Aging of the erythrocyte. III. Cation content

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Summary. Studies on the main cation content of density-separated bovine erythrocytes showed a progressive decrease in the levels of K^+ and Mg^{2+} with increasing cell density (and age) accompanied by an increase in the level of Na^+. The magnitude of net cation loss corresponded to that of red cell volume decrease, but could not account for the total increase in the microviscosity of the erythrocyte interior.

Changes in intracellular cation concentrations are a known characteristic of in vivo erythrocyte aging in several mammalian species[1-6]. These changes result in a net cation loss and this could be the main mechanism responsible for the shrinking of senescent red blood cells. In a previous report[7] we demonstrated that the increase in density accompanying the intravascular aging of bovine erythrocytes is related to an increase in the microviscosity of their interior. The aim of this study was to examine whether the red cell microviscosity increase during in vivo aging can be accounted for by electrolyte loss. With this goal in mind, cell density- (and age-)related alterations in main cellular cations were measured in bovine erythrocytes.

Methods. Separation of erythrocytes according to density (and age), and estimation of hemoglobin, were carried out as described in the accompanying paper. Sodium and potassium were estimated by atomic emission photometry, and magnesium by atomic absorption photometry in an AAS-2 atomic absorption spectrophotometer (Karl Zeiss, GDR).

Results and discussion. As in the case of human red cells[8], the method of Murphy[9] permits an excellent separation of bovine erythrocytes according to density and a reasonable separation according to age (to be published). Although it is hardly possible to extract exact quantitative data on rates of in vivo change from studies of various fractions of red

cells separated by this method, clear-cut information can be obtained in this way about the directions of age-related changes in the erythrocytes. Moreover, valid comparisons can be made between various sets of experiments performed under standard conditions of cell fractionation.

The levels of the main cations in different density fractions of bovine erythrocytes are shown in the table. As in other mammalian species[1-6], the K^+ content decreased gradually with increasing cell density and the Na^+ content showed an increase, though of smaller magnitude than the K^+ loss. In contrast to results obtained for human erythrocytes fractionated according to Murphy[8] (but not by other means[10]) the Mg^{2+} content was found to decrease progressively. The K^+ loss shown by these data is apparently reminiscent of the phenomenon of K^+ loss induced by oxidative stress[11], and may be due either to an increased passive K^+ permeability of the erythrocyte membrane, or the cell age-related modifications in ATPase properties.

The changes in cation content between the heaviest and lightest cell fractions [6]-[1] were: -2.2 moles/moles hemoglobin for K^+, -0.09 moles/moles hemoglobin for Mg^{2+} and $+0.7$ moles/moles hemoglobin for Na^+, equivalent to a net cation loss of 1.6 moles/moles hemoglobin. This cation loss should be accompanied by an equivalent anion loss to secure electroneutrality of the cell interior. Therefore the net decrease in the electrolyte content, approximated by changes in the concentrations of 3 main cell cations from fraction [1] to [6]: $((K^+ + Na^+ + Mg^{2+})_{[1]} - (K^+ + Na^+ + Mg^{2+})_{[6]})/(K^+ + Na^+ + Mg^{2+})_{[1]}$ would be $5.5 \pm 0.9\%$ of the cation content of fraction 1. In this set of experiments, increase in the mean cell hemoglobin concentration between fractions 1 and 6 was $6.2 \pm 1.3\%$. It thus seems that the shrinking of senescent bovine erythrocytes can be accounted for by the decrease in the electrolyte content. On the other hand, if one were to assume that the increase in microviscosity of the bovine erythrocytes interior, estimated[7] with the Tempamine spin probe, from fraction [1] to [6], is due only to concentration changes of intracellular solutes, the increase should be about 8.7%. (The separation efficiency did not differ between both sets of experiments, as judged from the magnitude of increase in the mean cell hemoglobin concentration.) Therefore, other factors apart from the electrolyte loss must contribute to the increased microviscosity of senescent erythrocytes. This is understandable, taking into account the progressive elevation in the microviscosity of the cell interior during incubation of extravasated erythrocytes which is not accompanied by significant changes in the electrolyte content.

Relative cation content of different density (age) fractions of bovine erythrocytes (percent of values found in the fraction of lightest cells)

Fraction No.	K^+	Na^+	Mg^{2+}
1	100	100	100
2	$89.5 \pm 6.8\%$	$101.1 \pm 1.5\%$	$90.6 \pm 5.5\%$
3	$83.5 \pm 6.9\%$	$102.0 \pm 1.6\%$	$87.0 \pm 7.0\%$
4	$76.9 \pm 8.8\%$	$101.8 \pm 1.1\%$	$82.8 \pm 5.8\%$
5	$74.9 \pm 7.3\%$	$103.0 \pm 3.5\%$	$77.4 \pm 7.1\%$
6	$67.5 \pm 8.8\%$	$103.2 \pm 1.3\%$	$73.3 \pm 7.4\%$

Absolute values for fraction 1: $K^+ - 6.7 \pm 0.6$; $Na^+ - 22.1 \pm 1.6$; $Mg^{2+} - 0.35 \pm 0.09$ moles/moles hemoglobin (mean $\pm$ SD; $n = 6$).

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A follow-up electrophysiological study of rats with poor intrauterine fetal growth: the development of visual evoked responses (VERs)[1]

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Summary. The development of some electrophysiological activities of the visual system (VERs) was compared in control rats and in young rats with poor intrauterine fetal growth caused by an electrolytic lesion of the placenta. Treated rats showed a delayed development of the electrophysiological functions considered, thus confirming the postnatal effect of poor intrauterine fetal growth.

The influence of nutrition on the development of the brain in the rat before and after birth has been studied by quite a large number of researchers. What we propose in the present paper is an investigation of this problem performed in young rats born after inducing placental insufficiency in dams by an electrolytic lesion.

Other authors have reported data about the changes in VERs of developing rats following starvation and other restricted conditions; this technique appeared to be suitable for the investigation of the effects of poor intrauterine fetal growth on the development of the visual system[3-5]. When investigating the visual system, most studies are on VERs to low frequency stimulation (transient VER), fewer deal with high frequency stimulation (steady-state VER).

The transient VER, by averaging, supplies some basic data which are the latency of the response and the main features of the response itself. Both are age-related. The latency decreases in the first 5-6 weeks and the shape of the response becomes more and more complete in the 1st month of life. These changes take place at fixed ages in Sprague-Dawley rats. The development of the transient VER, moreover, has been demonstrated to be related to the critical periods of intense protein and DNA synthesis and largely dependent on myelination[6].