Electrokinetic Behavior of Rat Blood Erythrocytes: Dependence on Cell Volume

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The interrelationship between electrophoretic mobility and the volume of rat blood erythrocyte is described by a non-linear curve, where the deviation of cell volume from the physiological normal is accompanied by a decrease in their mean electrophoretic mobility. In some ranges, the deviations of erythrocyte volume do not affect their electrokinetic properties. It can be hypothesized that the simultaneous shifts of these parameters are caused only by some hematological abnormalities that are manifested in pronounced changes in cell homeostasis.

Key Words: *rat; blood; electrophoretic mobility; erythrocyte; volume; correlation*

The study of factors affecting erythrocyte electrophoretic mobility (EEPM), a key parameter of sedimentation resistance of the blood [8], is important for elucidation of the mechanisms responsible for homeostasis of the internal medium of the organism. We previously found correlations between EEPM and erythrocyte volume in rats [4], but only in stressed animals. However, it was not clear whether this correlation was only a statistical phenomenon or they are based on some functional relationship between EEPM and cell volume. Here we examined the causal relationships between the changes in EEPM and the volume of blood erythrocytes.

MATERIALS AND METHODS

The blood drawn from random-bred albino male rats weighing 160-200 g was examined under normal conditions (n=32) and after administration of CCl_4 and ionol. CCl_4 was administered intragastrically (6.2 g/kg, n=39). Ionol was injected intraperitoneally in a dose of 50 mg/kg 20 min before CCl_4 (n=29). In several experiments ionol was applied alone (n=30). The blood was taken after decapitation of the rats under ether narcosis.

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To measure EEPM, the blood (0.02 ml) was diluted 1:200 with 0.1 M phosphate buffer (pH 7.4). The measurements were performed automatically on a Parmoquant-2 setup at 25°C. The volume of red cells was determined on a Coulter hematological analyzer. The concentrations of Na^+ and K^+ in the plasma were measured on an OP-266 analyzer. The data were processed statistically using Student's t test and STATISTICA software.

RESULTS

Both chemicals (ionol and CCl_4) were used to widen the range of blood parameters. To reveal the correlation of the examined characteristics of erythrocytes in a wide range, mixed data samples were used, which ignored the particular cause inducing changes in EEPM and erythrocyte volume [9]. These two parameters are interrelated in a non-linear way, and the dependence of EEPM on erythrocyte volume can be fitted by a fourth degree polynomial (Fig. 1, *1*). Variations of cell volume from minimal to maximum values were accompanied by a decrease in the mean EEPM. Similar features were observed in intact rats (Fig. 1, *2*). These peculiarities explain why we revealed no linear correlations in our previous experiments [4].

The correlation between erythrocyte volume and mobility within the physiological range of these para-

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meters can be explained by the dependence of surface charge on cell size [8]. Similar effect of various factors on both parameters can be mediated by different mechanisms. It cannot be excluded that the correlation between erythrocyte volume and mobility in the examined range is determined by physiological age of circulating erythrocytes, since younger cells are larger than old cells and have higher electrokinetic potential [2]. This hypothesis is corroborated by simultaneous increase in the charge, surface area, and size of erythrocytes in animals during stimulation of hemopoiesis with erythropoietin [7].

The intensity of erythrocyte metabolism, in particular, functioning of ion transporting systems can be considered as the factors involved in autoregulation of erythrocyte volume and EEPM [5]. According to this view, increased mobility does not result from larger size of these cells, but is determined by higher intensity of metabolism and greater energy store. The correlation between EEPM and cell volume does not determine, but merely illustrates the metabolically caused dependence: probably, EEPM is not directly determined by erythrocyte volume. Both parameters can be related indirectly, because they reflect metabolic phenomena. It is possible, that the correlation between EEPM and cell volume does no involve the entire range of their variations, since the effect of metabolism on cell volume is insignificant if it is stable and/or does not surpass a certain level [6]. The correlation between cell volume and electrokinetic potential becomes more pronounced for larger cells: the increase in cell volume is accompanied by a decrease in EEPM.

Taking into consideration that erythrocyte in water solutions behaves as a natural osmometer, the contribution of plasma osmolarity disturbances into changes of EEPM and erythrocyte volume cannot be excluded *a priori* [3]. However, our study revealed no correlation between cell size and plasma concentration of Na^+ and K^+ under normal conditions. However, a weak, but significant correlation between the mean volume of erythrocytes and K^+ concentration in the plasma was revealed after administration of ionol (r=-0.4, p<0.05). By contrast, there was no correlation between mean EEPM and K^+ concentration in plasma. Thus, plasma osmolarity affects the volume of erythrocytes, but plays little role in EEPM regulation, because the increase in cell volume is not always accompanied by a decrease in cell mobility. In other words, pronounced increase in erythrocyte volume is not accompanied by a decrease in their electrokinetic potential. Similarly, the greater size of erythrocytes does not imply higher EEPM. Evidently, simultaneous and opposite changes in the volume and electrokinetic properties of erythrocytes are largely determined by disturbances in intracellular

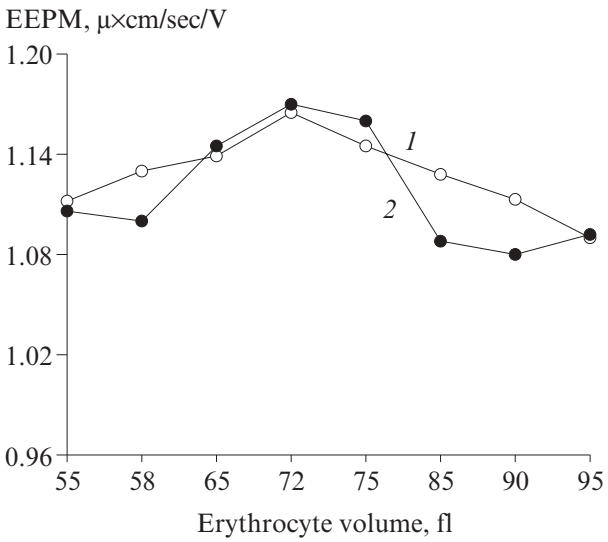

Fig. 1. Regression curves of the dependence of electrophoretic mobility (EEPM) on volume of erythrocyte taken from mixed samples of experimental (*1*) or intact rats (*2*). The R^2 coefficients are 0.95 and 0.93; approximation errors (ε) are 4.7 and 5.1%, correspondingly.

ion homeostasis (for example, depletion of energy store or activation of free radicals in erythrocyte membrane), while the effects of plasma factors are less pronounced.

Thus, the relationship between electrokinetic properties and volume of erythrocytes is only statistical, and the character of this correlation is ambiguous and non-linear. Variations in erythrocyte volume are not obligatorily reflected in cell mobility in electric field. It is likely that simultaneous shifts of both parameters can be caused only by pronounced disturbances in cell homeostasis, and under these conditions, EEPM is more stable than cell volume. Despite principal similarity of basic mechanisms of stabilization of electric charge and volume of erythrocytes via the regulation of ion balance in the cells [1], EEPM is more strictly regulated. The key role in stabilization of EEPM is probably played by local control of electrokinetic properties of erythrocytes by hormones and various modulators.

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