Effect of Blood Reticulocyte Concentration on Electrophoretic Mobility of Erythrocytes in Rats

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In intact rats electrokinetic characteristics of erythrocytes weakly depended on the intensity of erythropoiesis. This dependence became significant when the degree of erythropoiesis approached the upper limit of variations. Electrophoretic mobility of erythrocytes progressively decreased with increasing blood reticulocyte count. Subpopulations of erythrocytes with low mobility in electric field accumulated in the blood. Our results indicate that erythrocytes whose electrokinetic potential is similar to the mean mobility of circulating cells migrate from the bone marrow into the circulation under normal conditions.

Key Words: *rats; blood; electrophoretic mobility of erythrocytes; reticulocyte count; interrelation*

The mechanisms for regulation of electrophoretic mobility of erythrocytes (EPME) attract much attention. Published data show that EPME determines suspension stability of the blood [6]. Mobility of erythrocytes in electric field is a function of their surface charge, which depends on the state of erythrocyte membranes and blood plasma. Possible systemic influences on EPME were not quantitatively assayed. Recent studies revealed a correlation between EPME and total content of circulating erythrocytes [5]. However, this relationship is not representative, because it depends on blood storage and cell elimination from the circulation. The state of reticulocytes is probably a more reliable criterion, since reticulocytosis serves as a marker of activation of erythropoiesis [1]. Here we studied the dependence of EPME on blood reticulocyte count in rats.

MATERIALS AND METHODS

Blood samples were taken from intact albino male rats (n=72, 160-200 g) and animals exposed to cold stress (n=293) [4]. Specific features of treatment are not described in the manuscript. This procedure increased the scatter of experimental data, which made our analysis more effective.

Blood smears were stained with azure I and microscopied for estimation of reticulocyte count (per 1000 erythrocytes) [2]. Rat blood was sampled from the caudal vein and dissolved with 0.1 M phosphate buffer (pH 7.4, 1:200). Automatic recording was performed on a Parmokvant-2 device at 25°C. The mean mobility of cells was evaluated. As of distribution coefficient of EPME in the blood from each rat and groups of animals was calculated after recording 300 values of erythrocyte mobility in the sample. The significance of differences between the means was estimated by Student's t test. Correlation and regression analyses were performed using Statistics software.

RESULTS

The role of erythropoiesis in the regulation of surface electrical properties of erythrocytes can be evaluated by the type of curves for the dependence of EPME on reticulocyte count in the vascular bed. In the combined sample of intact rats and animals exposed to cold stress, erythrocyte mobility in electric field progressively decreased with the increase in blood reticulocyte count.

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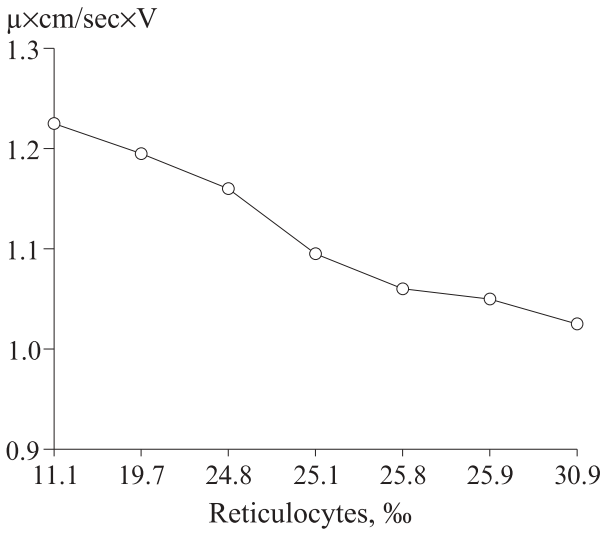

Fig. 1. Dependence of erythrocyte electrophoretic mobility on the count of peripheral blood reticulocytes in rats (combined sample).

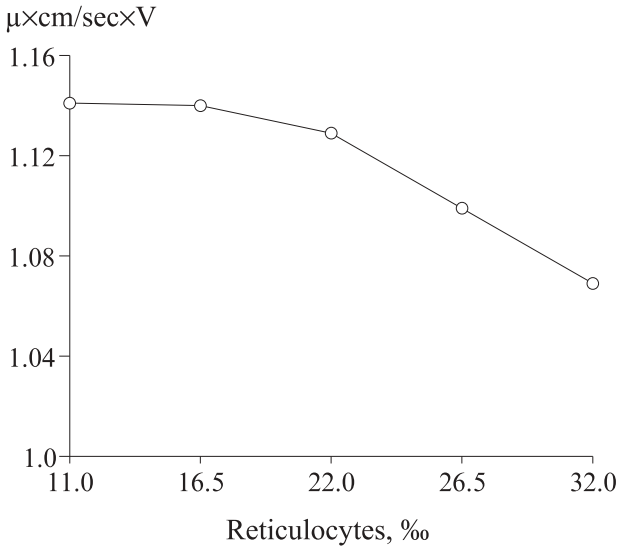

Fig. 2. Dependence of erythrocyte electrophoretic mobility on the count of peripheral blood reticulocytes in intact rats.

It was closely approximated by a fourth-order polynomial equation (R^2=0.99, Fig. 1). The study with combined samples is an adequate approach to generate allometric equations under these experimental conditions [7]. We evaluated the general tendency of variations in test parameters without concerning specific conditions.

The test parameters in intact animals underwent similar changes and were described by curves with slightly differing shape (R^2=0.99, Fig. 2). Smooth curve corresponded to values below 20 reticulocytes per 1000 erythrocytes, but EPME sharply decreased with increasing the ratio of circulating reticulocytes. In intact rats with low content of blood cells the intensity of erythropoiesis has little effect on EPME (judging from reticulocyte indexes). This effect became significant when blood reticulocyte count approached the upper limit of variations. It can be hypothesized that erythrocytes whose electrokinetic potential is similar to the mean mobility of circulating cells migrate from the bone marrow into the circulation under normal conditions.

Analysis of the relationship between total erythrocyte count and ratio of subpopulations with different electrophoretic mobility (EPM) in the vascular bed revealed a weak, but significant correlation between cell count and As of distribution of erythrocytes by EPM (r=0.35, p<0.05). These data do not indicate that covariance of variables is of low significance. It concerns only linear relations and does not exclude a more significant curvilinear dependence. For combined sample this dependence is described by a fourth-order polynomial equation (R^2=0.98, Fig. 3). Subpopulations of cells with high EPM prevailed at low content of peripheral blood erythrocytes in rats (negative As). An increase in the ratio of circulating erythrocytes above the normal mean value (5.80±0.27×10^{12} cells/liter) was followed by normalization of the distribution and sharp increase in the count of cells with low mobility (compared to intact animals, 1.10±0.01 μ×cm/sec×V). The subpopulation of rat blood erythrocytes underwent similar changes with variations in reticulocyte count. The ratio of erythrocytes with the mean-group mobility was high at low number of circulating reticulocytes. The number of erythrocytes with low EPM increased at high content of reticulocytes (more than 3.5%, positive As).

The observed changes indicate that cells with high EPM are presented by young erythrocytes migrating into the vascular bed during activation of erythro-

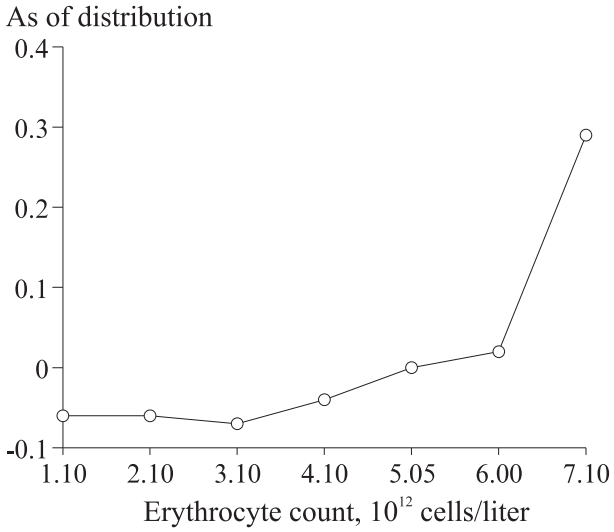

Fig. 3. As distribution of erythrocytes by electrophoretic mobility depending on blood erythrocyte count in rats (combined sample).

poiesis. Stimulation of erythropoiesis is accompanied by the release of metabolically and physiologically defective and rapidly aging erythrocytes into the vascular bed. Old erythrocytes enter the subpopulation of cells with low mobility. The electrokinetic potential of erythrocytes decreases with the increase in their number in the peripheral blood, which is probably related to the release of abnormal cells into the circulation.

Accumulation of erythrocytes with low EPM in the blood against the background of their increased count can be associated not only with accelerated aging of cells during stimulation of erythropoiesis, but also with migration of functionally abnormal cells from blood depots into the circulation. The stress-induced polycythemia is accompanied by a decrease in Na^+,K^+-ATPase activity of the erythrocyte membrane [3], which affects the transmembrane potential. It cannot be excluded that the reduced value of EPME at relatively high count of erythrocytes in the blood reflects decelerated elimination of old cells with low surface charge. All these possibilities can be realized. Electrokinetic changes reflect the interaction between these factors, which is determined by other mechanisms for regulation of APME in the vascular bed.

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