Role of Hemoglobin in Regulation of Membrane Electrical Properties and Erythrocyte Volume

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Erythrocyte volume in neonates is 25.8% greater than in women. Electrophoretic erythrocyte mobility was the same in neonates and in parturient women. The results obtained indicate that the more heterogeneous the hemoglobin system, the more heterogeneous are erythrocyte membrane electrical characteristics.

Key Words: *electrophoretic erythrocyte mobility; erythrocyte volume; neonates*

Instability of the erythrocyte volume and membrane electrical characteristics may lead to microcirculatory disturbances [5]. Decreased electrophoretic erythrocyte mobility (EPEM) reflecting the variations in the membrane surface charge is accompanied by enhanced intravascular erythrocyte aggregation and reduced erythrocyte deformability. The role of hemoglobin in the regulation of electrical properties of the erythrocyte plasma membrane (EPM) under pathological conditions has been studied [3,6]. The possible ways of this regulation are associated with variations of the charge of intracellular hemoglobin that determines the erythrocyte transmembrane potential [7] and with lateral conformational rearrangements in the glycocalyx or diffuse layer. These rearrangements may arise due to oxygenation resulting from phospholipid translocations in the EPM.

Study of the relationship between the parameters of hemoglobin and those of erythrocytes in neonates may be useful for specifying the mechanisms by which hemoglobin affects physicochemical properties of erythrocytes. There are several advantages in this approach:

1) The role of respiratory pigment at birth is played by fetal hemoglobin. In contrast to an adult form of hemoglobin, it is characterized by high heterogeneity of forms, and, therefore, manifests a variety of interactions with the EPM. Oxygenated derivatives of hemoglobin A_2 are known to react more actively with band 3 integral protein (associated with an anion carrier) [8].

2) At the early stages of ontogenesis, endogenous factors predominate in the regulation processes, while exogenous factors predominate at the later stages.

3) During the early postnatal period, the erythrocyte characteristics are determined mainly by specific interactions between the fetal and maternal organisms formed in the prenatal ontogenesis. This correlation may be revealed by statistics.

Our aim was to compare the erythrocyte volume and electrical properties in neonates and in women.

MATERIALS AND METHODS

Blood from 22 healthy newborns (10 male and 12 female, Apgar score: 8-9) and 22 women was studied. Peripheral blood from was collected from the heel in newborns and from the finger in women.

EPEM was measured at 25°C with a Parmokvant-2 apparatus (Carl-Zeiss Iena). For each test 0.01 ml blood was diluted with 10 ml physiological saline buffered at pH 7.4. EPEM was determined for 100 or more erythrocytes in each specimen.

Erythrocyte count, volume, heterogeneity by volume, and mean concentration and mean cellular concentration of hemoglobin were measured in a Symex apparatus. Multivariable correlation analysis

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and description statistics and plots were performed using Statistika software. In addition to mean values, the distribution asymmetry and excess were estimated with the help of erythrograms.

RESULTS

Since there were no sex-related differences in volume and membrane electrical properties, the data were pooled (Table 1).

A weak correlation was established between erythrocyte volumes in newborns and in women (r=0.35, p<0.05). There was no correlation between the electrical properties of EPM. This suggests that the erythrocytes of newborns contain a specific factor modifying these parameters. Of special interest is the shape of neonate electrokinetic erythrogram, which is symmetrical and is more than an order of magnitude greater than in women. Therefore, the neonate erythrocyte pool is homogenenous by bioelectrical properties and heterogeneous by volume.

A weak negative correlation was established between the EPEM and the leukocyte count in neonates (r=-0.45) and between the hemoglobin concentration in erythrocytes and the distribution excess (r=-0.58). In adults, electrokinetic characteristics vary independently of these parameters.

The differences in the erythrocyte properties are probably associated with specific features of the intracellular contents, in particular, with fetal hemoglobin forms. This suggestion is confirmed by the fact that in premature infants the volume of erythrocytes containing different forms of fetal hemoglobin is increased (140.4 fl) [4].

Erythrocyte volume in neonates depends predominantly on the intracellular concentration of fetal hemoglobin (r=-0.65, p<0.05), while in women, where the respiratory pigment system is formed, erythrocyte volume correlates with the mean hemoglobin concentration (r=-0.71, p<0.05). Figure 1 shows the dependence of the erythrocyte volume on the nature of intracellular hemoglobin.

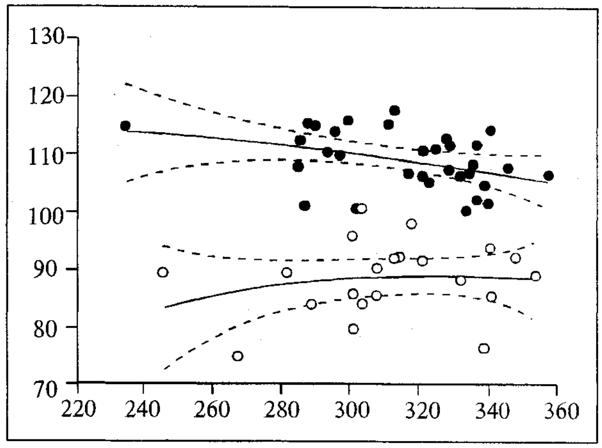

Fig 1. Erythrocyte volume as a function of the mean intracellular concentration of hemoglobin. *Ordinate*: erythrocyte volume, fl; *abscissa*: mean cellular concentration of hemoglobin, g/liter. White circles: women; black circles: neonates.

The higher the intracellular concentration of fetal hemoglobin the smaller the erythrocyte volume. By contrast, in adults the higher the concentration of hemoglobin the larger the erythrocytes. This may be due to the peculiarities of fetal hemoglobin, specifically, by its low resistance to oxidation and, consequently, increased content of met-hemoglobin. When hemoglobin concentration reaches the maximum, crystallization and formation of insoluble aggregates begins [1]. The aggregates precipitate on the plasma and reduce the effectiveness of the anion carrier and other transport systems. It is likely that

Table 1. Blood Parameters in Infants and Women

Parameters	Boys	Girls	Women
EPEM, 10^{-8} m^2/V×s	1.063±0.020	1.076±0.015	1.079±0.015
Asymmetry, 10^{-8} m^2/V×s	0.068±0.07*	-0.078±0.07*	-0.67±0.15
Distribution excess, 10^{-8} m^2/V×s	3.180±0.31*	3.08±0.18*	0.37±0.12
Leukocytes, 10^9/liter	22.4±2.28*	26.2±2.21*	13.8±0.9
Mean hemoglobin concentration, pg	33.5±0.60*	34.2±0.69*	29.5±0.7
Hemoglobin cellular concentration, g/100 ml	299.4±4.9	301.3±5.6	313±5.4
Erythrocytes, 10^{12}/liter	8.9±0.2*	6.7±0.21*	4.05±0.7
Erythrocyte volume, fl	112.6±2.0*	112.6±1.9*	89.5±1.6
Erythrocyte volume heterogeneity, fl	63.9±1.6*	66.2±1.5*	46.9±0.5
Na^+, mM/l	137.5±0.9	137.5±0.9	137.2±1.2
K^+, mM/l	5.63±0.4*	5.63±0.4*	4.62±0.4
Protein, g/l	55.4±1.2*	59.1±1.2*	66.0±1.4

Note. *p<0.05 compared with parameters in women.

the complexes of fetal hemoglobin derivatives with band 3 integral protein are responsible for low permeability of EPM to HCO_3, Cl^-, and H_2O [4]. The rise of intracellular concentration of fetal hemoglobin to 320 g/liter is followed by hemoglobin crystallization and oxidation, which increases the heterogeneity of bioelectrical characteristics of erythrocytes, statistically manifested as a reduction in distribution excess.

Our results suggest that fetal hemoglobin is involved in the maintenance of transmembrane ionic gradient, providing stable density of the surface charge on the EPM during hyperkalemia in the neonates.

It is noteworthy that EPEM correlated with the leukocyte count in neonates; such a correlation was found previously in adults in some pathological states [6]. The physiological significance of correlation between the number of cells with low electrophoretic mobility and high leukopoiesis is that pathological hemoglobin derivatives not only reduce the cell charge but also induce detachment vesicles containing complexes of oxygenated hemoglobin derivatives and EPM proteins, which activates macrophagal system and hemopoiesis. This correlation does not exist in healthy women seemingly due to higher resistance of hemoglobin system to damaging factors.

The phenomenon of electrokinetic erythrogram asymmetry in women may be explained in a similar way. In adults, hemoglobin at semisaturation consists of 40% completely oxygenated and 30% completely deoxygenated tetramers. This provides the cell with an extra amount of oxygen. Predomination of the erythrocyte pool of cells with a greater extent of oxygenation in women results in a distribution shift to the right or negative values of asymmetry.

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