Journal of Engineering Physics and Thermophysics, Vol. 76, No. 3, 2003

FEATURES OF THE MICRORHEOLOGICAL BEHAVIOR OF BLOOD IN CHILDREN

Yu. V. Rumyantseva,[a] **A. Yu. Ostroumova,**[b] **N. S. Smetanina,**[a] **and N. N. Firsov**[b]

UDC 577.353:567.322:612.766

The blood of healthy children aged 2–14 was investigated. No differences between the aggregation parameters depending on the sex were revealed. In the children under the age of five, the amplitude of aggregation and the time of formation of linear aggregates were reliably lower and the strength of the largest of them and their total strength were decreased in comparison with the other age groups. An inverse correlation between the strength of the aggregates and the number of reticulocytes and leukocytes and HbA_2 in the blood has been revealed.

Standardization of the microrheological indices of blood is a topical problem in modern hemorheology. This is explained by the fact that it is necessary to know the bounds of the normal indices to estimate the heaviness of the hemorheological disturbances and compare them with the clinical picture of the disease. Such an investigation has been carried out earlier on the donor blood of adults [1]. The present work is devoted to investigating the normal microrheological indices in children.

Materials and Methods. We investigated the blood of clinically examined healthy children (girls and boys). The investigation involved physical examination, total blood count with counting reticulocytes, calculation of their mean diameter, morphological estimation of erythrocytes, determination of the level of HbA_2, biochemical analysis of blood, ultrasonic investigation of the abdominal cavity organs, and investigation of the aggregation and disaggregation of erythrocytes by the nephelometry methods.

The samples of venous blood were placed in vacutainers with ethylenediaminetetraacetic acid (Becton Dickinson Vacutainer Systems, USA) and in sterile test tubes without an anticoagulant.

The total blood count was carried out on a Contraves Digicell-800 automatic haematological analyzer (Contraves, Hungary) with a subsequent morphological estimation of the peripheral blood cells colored according to Romanovskii.

The reticulocytes were counted in a smear after a supravital coloration with methylene—cresyl blue.

The morphometry of the erythrocytes was carried out by the unified microscopic method with the use of an ocular-micrometer and a telemedical system of input and processing of a morphological image on a personal computer. With the results of the morphometry we constructed a Price–Jones curve and calculated the spherical index (S) and the ovalocytosis index (OI) by the following formulas:

$$\mathrm{S} = \frac{3.14 D^3 A}{400 \mathrm{Hct}} ,$$

where D is the mean diameter of the erythrocytes, μm, A is the first two digits in the number of erythrocytes in 1 μl, and Hct is the packed cell volume, %;

$$\mathrm{OI} = \frac{\text{sum of minimum diameters}}{\text{sum of maximum diameters}} .$$

[a]Scientific-Research Institute of Children's Haematology, Ministry of Public Health of the Russian Federation, Moscow, Russia; [b]Russian State Medical University, Moscow, Russia. Translated from Inzhenerno-Fizicheskii Zhurnal, Vol. 76, No. 3, pp. 209–213, May–June, 2003. Original article submitted September 6, 2002.

The amount of HbA_2 and HbF was determined by scanning the foregrams after the electrophoresis with the use of a gel-agarose carrier at pH 8.6 and 6.0, respectively, on a Paragon Apprise device (Beckman, USA) in accordance with the recommendations of the manufacturer.

The kinetics of aggregation and disaggregation of erythrocytes was investigated in a specially designed coaxial-cylindrical erythroaggregometer by way of irradiation of the blood with a directed light beam formed by a light-emitting diode. The design of the device, the physical justification, and the methods of calculation of the aggregation data have been described in detail in [2, 3]. The method is based on recording the change in the intensity of the backscattering of light by a blood sample.

The blood of volume 2.4 ml was positioned in the rheological clearance of dimension 1 mm between two cylinders. The outer cylinder was rotated and the inner cylinder was fixed. At first the blood sample was automatically driven up to attain a high rate of shear — 500 sec^{-1}, providing complete disaggregation of the erythrocytes. At this instant the intensity of the backscattering of light was maximum, since, to a first approximation, the intensity of the reflected light is directly proportional to the number of scattering centers, which individual erythrocytes can be.

When the above-indicated rate of shear was attained, we stopped mixing and observed spontaneous aggregation of erythrocytes for the next 2 min. As aggregates of erythrocytes were formed, the intensity of the reflected light gradually decreased and became minimum at the instant their complete aggregation was attained. It is suggested that this process consists of two stages: rapid, at which linear aggregates are formed, and slow, at which three-dimensional aggregates are formed. Each stage is characterized by its own time: T_1 for the rapid stage and T_2 for the slow one. The smaller the time of aggregation, the higher its rate. After completion of spontaneous aggregation, the reverse process — disaggregation — begins. In the case where the rate of shear is increased stepwise from 2.5 to 105 sec^{-1} by gradually driving up the outer cylinder, normal aggregates should break down rapidly and easily even at low rates of shear (2.5 sec^{-1}). At this instant the intensity of the backscattering should increase again. This does not take place at various pathological states; furthermore, the process of aggregation continues, which manifests itself as an increase in the amplitude of backscattering — I_a (2.5 sec^{-1}), which approaches (in percent) the amplitude of spontaneous aggregation. This is explained by the fact that erythrocytes are strongly linked to each other and continue to amalgamate under the action of the rate of shear. The parameter I_a is given the minus sign when the light scattering increases and the plus sign when it decreases.

The index characterizing the stability of the erythrocytic aggregates is their hydrodynamic strength β, which determines the rate of shear at which the intensity of the light scattering increases by a factor of 2.72 (by a factor of e) in comparison with the complete aggregation. At low rates of shear, the effect of increasing intensity of the light scattering correlates with β. Thus, the most sensitive parameters of spontaneous aggregation of erythrocytes are 1) the time of rapid aggregation T_1, characteristic time of formation of two-dimensional (linear) aggregates of erythrocytes; 2) the hydrodynamic strength of the erythrocytic aggregates β; and 3) the change in the level of light scattering at a rate of shear of 2.5 sec^{-1}, which is determined as a percentage of the complete amplitude of aggregation of erythrocytes at a maximum rate of shift — I_a.

Results and Discussion. The clinical examination has shown that the children had no acute or chronic diseases at the moment of investigation. The indices of the hemograms and the biochemical analysis of blood corresponded to the age norms. The morphological estimation revealed spherocytes in seven of the twenty children (35%) in amount of 1–3%. The mean diameter of the erythrocytes was 7.1 ± 0.06 μm at a sphericity index of 3.5 ± 0.07 and an ovalocytosis index of 1 ± 0.006. The hemoglobin had the following values: $HbA_2 = 2 \pm 0.12\%$ and $HbF = 2.9 \pm 0.3\%$.

The main microrheological indices calculated on the basis of the results obtained for children and adults are presented in Table 1. No significant differences are observed between the mean values of the indices, except for the slowered formation of three-dimensional aggregates.

It is seen in Table 2 that there are no differences between the microrheological indices depending on the gender.

A more detailed investigation has shown that the group examined is inhomogeneous and the group of children under the age of five should be separated (Table 3).

It is seen in Table 3 that there are reliable differences between the following indices (they are denoted by the asterisks, $p < 0.05$): T_1, the linear aggregates are formed more rapidly, I_a, the strength of the largest aggregates is de-

TABLE 1. Mean Values of Microrheological Indices for Adults and Children

Object of investigation	Parameters				
	T_1, sec	T_2, sec	I_a, %	β, sec^{-1}	Ampl.
Children	10.7±1.0	57.1±2.9	–20.0±2.6	22.4±2.7	76.1±4.4
Adults	10.0±0.4	49.0±0.7	–21.0±0.7	28.5±0.8	–

TABLE 2. Microrheological Parameters of Blood in the Control Group in Relation to Their Gender ($M \pm m$)

Parameter	Girls, n = 10	Boys, n = 10	p
Ampl., conv. units	83.4±6.96	78.8±4.69	0.590
T_1, sec	11.89±0.74	11.44±1.76	0.816
T_2, sec	59.08±3.51	56.1±4.69	0.617
I_a, %	–20.55±3.57	–22.5±3.82	0.714
β, sec^{-1}	20.02±2.09	17.08±2.24	0.350

TABLE 3. Microrheological Parameters of Erythrocytes of Healthy Children in Relation to Their Age

Parameter	Age			p (dispersion analysis)
	under the age of five, n = 6	five–ten, n = 5	over the age of ten, n = 9	
Ampl., conv. units	59.33±3.29*	82.8±5.54	83.56±7.5	0.035
T_1, sec	6.25±0.54*	10.42±1.1	13.74±1.2	0.0001
T_2, sec	53.33±6.63*	55.82±6.38	60.3±3.32	0.593
I_a, %	–26.17±4.47*	–20.3±5.95	–15.78±3.51	0.041
β, sec^{-1}	13.67±2.79*	23.34±4.37	27.7±2.58	0.014

creased, β, the total hydrodynamic strength of the aggregates is sharply decreased, and Ampl. is lower than in the children over the age of five, i.e., the finite dimension of the aggregates is smaller.

The decrease in the strength of the aggregates can be due to the higher electronegativity of children's erythrocytes in comparison with the erythrocytes of adults, which was noted by many researchers. The electrophoretic mobility of erythrocytes increases with age.

In [4], the following data on the electrophoretic mobility of erythrocytes (μm·cm/(sec·V)) are presented:

Children	Adults
1.3–1.2	1.1–1.06
1.4–1.25	1.0–0.825
1.41	1.08

However, they are contradictory to the increase in the rate of formation of linear aggregates, since an increase in the electrokinetic charge is the main barrier to the approach of cells in the process of spontaneous aggregation of erythrocytes.

The investigation of the relation of the aggregation indices to the morphological composition of blood has given unexpected results.

It is seen in Fig. 1Aa that the index of strength of the largest aggregates I_a depends on the concentration of reticulocytes in the blood.

The small range of change in the concentration of reticulocytes casts doubt on the reliability of this relation. However, when the concentration of reticulocytes changes within a wide range, as in the case of hereditary spherocytosis, there also takes place the same inverse correlation with a close coefficient (Fig. 1Ab).

The total strength of the aggregates β is also related to the concentration of reticulocytes by an inverse correlation but with a higher correlation coefficient (Fig. 1B).

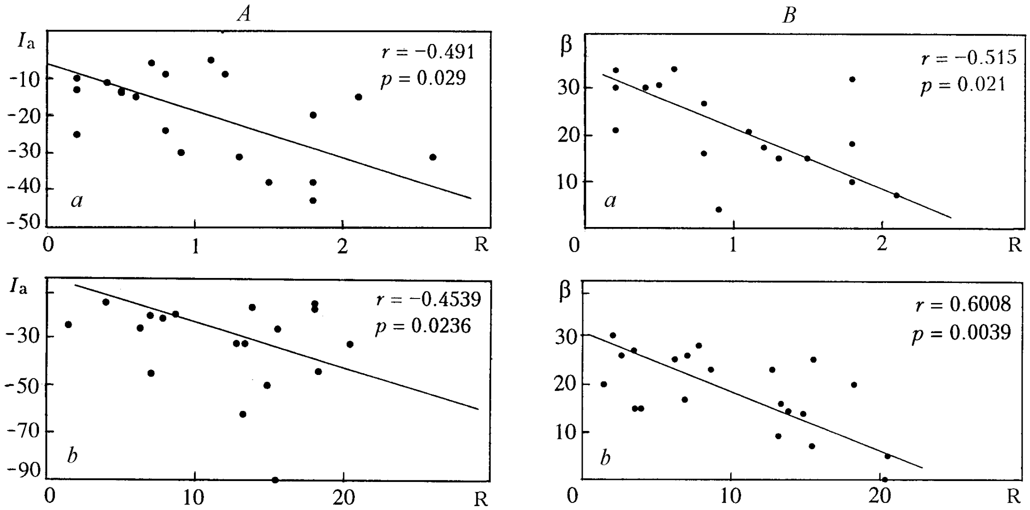

Fig. 1. Dependence of the strength of the large aggregates (A) and the total strength of the aggregates (B) on the number of reticulocytes in the control group (a) and in the group of patients with hereditary spherocytic hemolytic anemia (b).

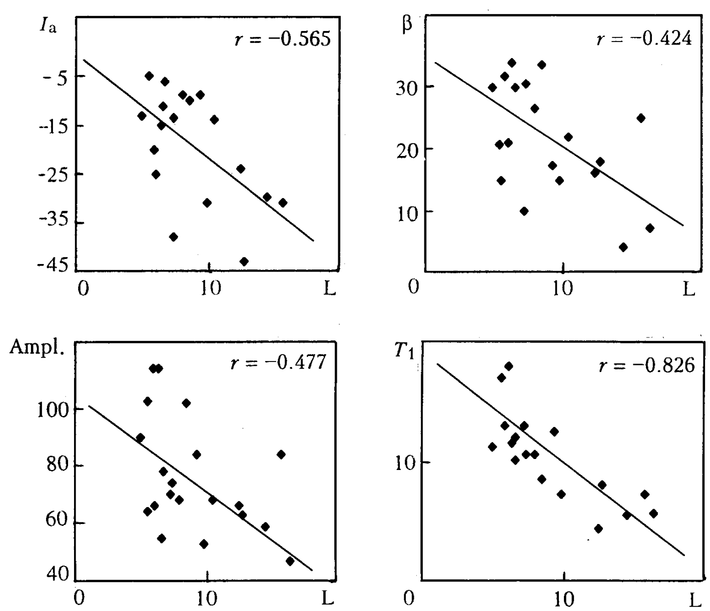

Fig. 2. Dependence of the aggregation parameters on the number of leukocytes in the control group.

The influence of different concentrations of leukocytes on the main indices of microrheology has the same negative correlation as in the case of reticulocytes (Fig. 2). The rate of formation of linear aggregates is most strongly dependent on the concentration of leukocytes.

Undoubtedly, such behavior of the parameters of aggregation of erythrocytes is explained by the electrokinetic properties of the cells, as in the case of reticulocytes, and by the morphology of the cells (their dimensions, shape, mobility, etc.) of the erythrocytes and leukocytes.

It is possible that the negative correlation obtained by us is not directly related to the reticulocytosis. In this case, reticulocytes serve as a marker of an increase in the concentration of the young forms of erythrocytes and a decrease in the concentration of the old ones. The young erythrocytes possess a higher electrokinetic charge and higher deformability.

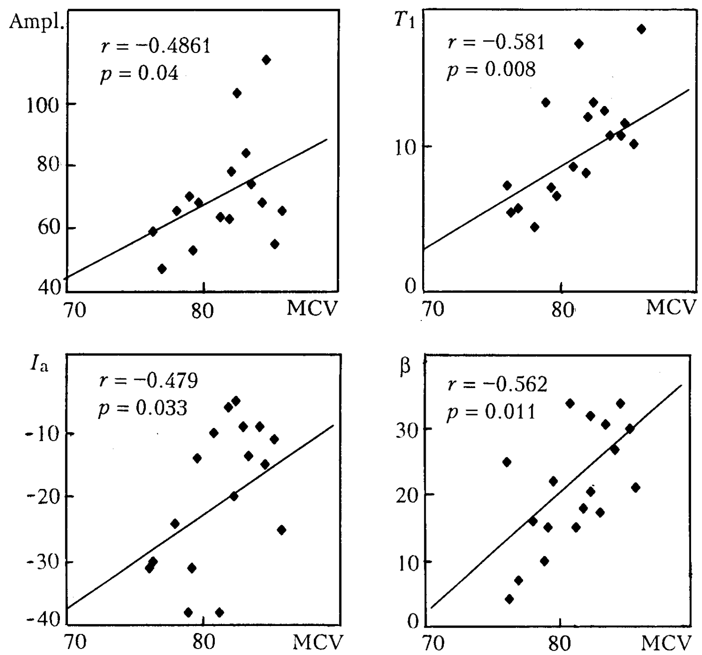

Fig. 3. Dependence of the aggregation parameters on the MCV (mean volume of erythrocytes) in the control group.

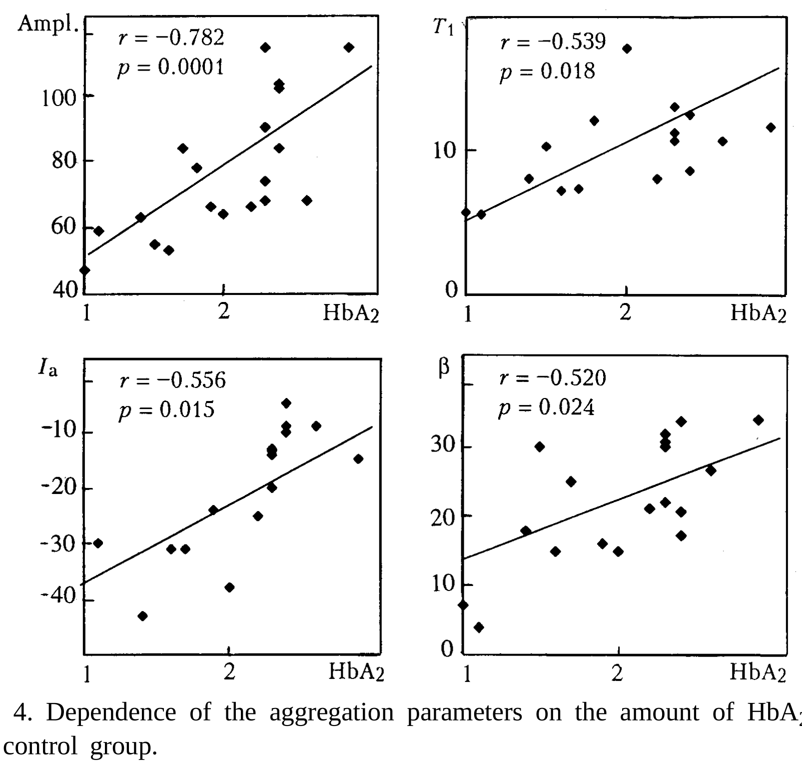

Fig. 4. Dependence of the aggregation parameters on the amount of HbA_2 in the control group.

The investigation of the correlation relations to the mean volume of erythrocytes (Fig. 3) underlines the importance of the morphological changes in the cells. An increase in the volume is associated with an increase in the area of the erythrocytes and consequently an increase in the total number of bridges of macromolecules connecting cells into "rouleaux".

It is difficult to explain the revealed correlation dependence of the main indices of aggregation on the amount of HbA_2 in the erythrocytes of children from the viewpoint of the existing theories of aggregation of erythrocytes (Fig. 4).

The way the replacement of the β-chains for the δ-chains in the hemoglobin molecule influences the dimension and strength of the aggregates should be revealed by biophysical methods. At most, this fact is evidence of the participation of hemoglobin molecules in the intermembrane interactions of erythrocytes.

Thus, there is a need to investigate the micro- and macrorheological properties of normal blood in children of different age in more detail on more representative samples with an interval of one year from the day of birth. To adequately estimate the microrheological indices it is necessary to take into account the morphological composition of blood and the hemoglobin composition.

NOTATION

T_1 and T_2, characteristic times of formation of linear and three-dimensional aggregates, sec; Ampl., total amplitude of aggregation: difference between the intensities of the backscattering in the case of complete aggregation and disaggregation of erythrocytes (in conv. units), is proportional to the finite dimension of the aggregates; I_a (2.5), index of strength of the largest aggregates at a rate of shear of 2.5 sec^{-1}, measurements of backscattering as a percentage of the total amplitude of aggregation; β, total hydrodynamic strength of the aggregates, sec^{-1}; HbA_2, minor hemoglobin of an adult with the chain composition $\alpha_2\delta_2$ as distinguished from the main hemoglobin HbA with the chain composition $\alpha_2\beta_2$, %; HbF, fetal hemoglobin (intrauterine); EDTA, ethylenediaminetetraacetic acid; S, spherical index of erythrocytes, characterizes the deviations from the diskoidity of the cells; OI, ovalocytosis index, characterizes the ellipsoidicity of the cells; *M*, mean value of a quantity; *m*, error of the mean value; *n*, number of examined children; *r*, coefficient of linear correlation; *p*, significance level, confidence coefficient; e, base of natural logarithm; MCV, mean volume of erythrocytes, μm^3; R, concentration of reticulocytes, %; L, concentration of leukocytes, 10^3 in 1 μl.

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