4. M. B. Cochrane and D. Koffer, Adv. Immunol., 16, 185 (1973).
5. A. P. Kaplan, Bull. N.Y. Acad. Med., 57, 638 (1981).
6. M. E. Medof, G. N. Prince, and J. J. Oger, Clin. Exp. Immunol., 48, 715 (1982).
7. M. B. Oldstone, Prog. Med. Virol., 19, 84 (1975).
8. L. H. Perrin and M. B. Oldstone, J. Immunol., 118, 316 (1977).
9. E. Rajnovölgye, G. Füst, J. Kulics, et al., Immunochemistry, 15, 887 (1978).
10. T. Sakurai, T. Fujita, J. Kono, et al., Clin. Exp. Immunol., 48, 37 (1982).

CHANGES IN PROPERTIES OF HUMAN ERYTHROCYTE MEMBRANES IN THE PRESENCE OF ISOIMMUNE ANTIBODIES

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KEY WORDS: erythrocytes: antibodies; scattering of light; agglutination.

In the modern view cell membranes are complex quasicrystalline structures with a high degree of lability [5, 6, 8]. Several papers have been published on changes in cell membranes under the influence of certain physicochemical factors: temperature, γ-irradiation, and different substances [2]. The action of antibodies on the erythrocyte membrane has not been adequately studied [3, 4]. Little is still known on the pathogenetic importance of low-activity auto- or isoantibodies which may be present in human blood, but which are not detected by immunological methods usually adopted. Research in this direction may be promising from the standpoint of explaining the mechanism of development of antigen–antibody reactions, for injury to the erythrocyte membrane is an organic stage in this process.

The insufficient study paid to changes in the erythrocyte membrane under the influence of antibodies may perhaps be attributable to difficulties of a technical character. Phase structural transitions in artificial phospholipid membranes are usually studied by differential temperature analysis (scanning calorimeter [7, 9]), which is not sufficiently sensitive to study erythrocyte membranes of man and animals because of the high cholesterol content in the erythrocytes. In the present investigation it was therefore decided to use a light scattering technique, so that both the initial density of the erythrocyte membranes and changes in density during phase transitions could be recorded [5].

The aim of this investigation was to study some properties of the erythrocyte membrane of human blood in the presence of isoimmune anti-rhesus antibodies.

EXPERIMENTAL METHOD

Experiments were carried out on 34 samples of rhesus-positive erythrocytes from group O(I) blood donors. Two tubes of blood were taken from each donor: In one tube the blood contained heparin as stabilizer in a dose of 125 U/ml, the other tube was without stabilizer. The same batch of anti-rhesus group O(I) serum with antibody titer of 1:128 was used. The erythrocyte agglutination test was conducted on the blood for investigation, with anti-rhesus serum, by the conglutination method with gelatin and by the indirect Coombs' test. The titer of anti-rhesus serum with the test erythrocytes from different donors varied from 1:16 to 1:256, in most cases from 1:64 to 1:128. To study phase transitions in the erythrocytes 9 parts of heparinized blood were added to 1 part of anti-rhesus serum diluted with 0.9% NaCl solution. In the control, blood from the same donor was treated with similar dilutions of

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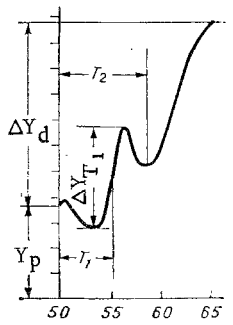

Fig. 1. Changes in light scattering depending on blood temperature. Abscissa, temperature (in °C); ordinate, I (in relative units).

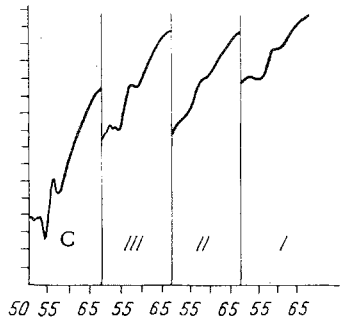

Fig. 2. Changes in light scattering from blood samples in presence of antibodies. C) Control, I) agglutination visible to the unaided eye; II) agglutination visible under the microscope; III) agglutination absent. Remainder of legend as in Fig. 1.

serum of blood group AB(IV), not containing antibodies. Anti-rhesus serum was added to the test blood in the following dilutions: evoking agglutination just visible with the unaided eye (I) or under the microscope (II), and in a dilution not leading to agglutination of the erythrocytes (III).

Experiments to study the effect of light scattering from the test blood samples were conducted on an apparatus built by one of us (A.E.G.) [1].

The intensity of light I_φ, scattered by a particle at an angle φ to the direction of the incident beam of light depends on the following parameters [9, 12]:

$$y_\varphi \cong \frac{(n-n_0)^2 \cdot N \cdot V^2}{R^2 \lambda^4} \cdot y_0 (1+\cos^2 \varphi),$$

where V is the volume of one particle, R the distance from the scattering volume to the observation point, λ the wavelength, I_0 the intensity of incident light, n and n_0 the refractive indices of the substance and medium respectively, and N the number of particles.

Since the refractive index n of an erythrocyte is connected with the density of the erythrocyte, changes in density of the membrane can be assessed from the intensity of scatter (provided the number of cells per unit volume is constant). The source of light was an OI-19 lamp with red filter (λ = 0.6 μm). Scattering of light from the sample was recorded at an angle of 20° to the primary bundle of the FPF-78 photoresistor. During measurement of light scattering at an angle of 20° the contribution of the volume factor for such large particles as erythrocytes is virtually absent, and the change in intensity is connected mainly with the change in membrane density.

TABLE 1. Parameters of Curve of Light Scattering from Test Blood Samples after Addition of Different Concentrations of Antibodies

Group of experiments	Parameters of light scattering curve				
	in relative units			in °C	
	Y_ρ	ΔY_d	ΔY_{T_1}	T_1	T_2
I	83,6±7,5	40,7±4,6	30,7±3,6	56,4±0,2*	59,2±0,2*
II	84,4±9,6	50,1±7,4	32,8±4,5	56,3±0,1*	58,9±0,4*
III	77,2±7,6	47,6±3,8	31,2±2,8	56,2±0,1*	58,9±0,2*
Control	65,3±8,4	44,8±10,5	27,1±6,4	55,2±0,1	57,8±0,2

Legend. *P ≤ 0.05.

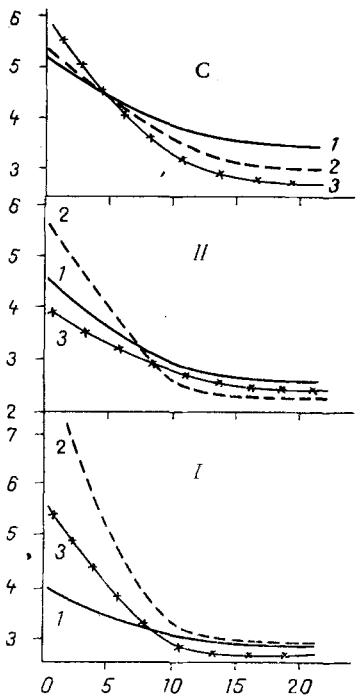

Fig. 3. Changes in viscosity of blood in presence of antibodies at 20°C (1), 37°C (2), and 42°C (3). Abscissa, time (in $10^2 \cdot sec^{-1}$); ordinate, viscosity (in relative units). Remainder of legend as in Fig. 2.

Blood for testing was poured into a special cuvette with a volume of 0.2 ml. The blood sample was heated with a nichrome electric coil, wound on the holder of the cuvette, at the rate of 4.5 deg/min. The temperature in the cuvette was measured by means of an MT-54 microthermistor. The viscosity of the blood was measured with a capillary viscosimeter within the range of shear velocities 10^2-10^4 sec^{-1} at temperatures of 20, 37, and 42°C.

EXPERIMENTAL RESULTS

One typical curve of changes in light scattering depending on temperature of the blood is given in Fig. 1; Y_ρ expresses the intensity of light scattering determined by the initial density of the erythrocyte membranes; T_1 and T_2 the phase transition temperatures; ΔY_{T_1} and ΔY_d denote changes in light scattering connected with changes in density of the membranes during phase transitions.

In most experiments (in 23 of 34) distinct changes in phase transition temperatures, in membrane density during phase transitions, and also in the initial density of the erythrocyte membranes were discovered. These changes increased with an increase in activity of anti-rhesus antibodies present in the test blood. The pooled experimental results (Table 1;

Fig. 2) reflect the uniformity of character of changes in the erythrocyte membranes under the influence of antierythrocytic antibodies. The fact will be noted that changes in the parameters of the curve of scattering of light from the test blood sample (Fig. 2) also take place in the presence of low concentrations of antibodies (III), not causing agglutination detectable by immunological methods of investigation. The increase in the parameter Y_ρ indicates an increase in density of the cell membranes during an increase in antibody concentration, whereas a rise of the phase transition temperatures T_1 and T_2 is evidence of a change in membrane structure.

The principal components of the erythrocyte membrane are lipids, phospholipids, and certain proteins [5]. The lipid matrix of the membrane evidently possesses some degree of flowability, and macromolecules buried in it can move relatively freely. Mobility of the proteins in the membrane depends on the lipids surrounding the given protein and on the degree of interaction between these lipids and other proteins [11].

Biophysical and biochemical tests show that structural transitions in biosystems have a functional role — the transition temperatures are determined by the lipid composition of the systems: With an increase in the ratio between unsaturated and saturated fatty acids the transition temperature falls and the temperature range of existence of the mesophase widens; with a decrease in this ratio the range of mesomorphism shifts into the region of higher temperatures, in some cases above physiological. Emergence of membrane lipids from the liquid-crystalline state is accompanied by disturbances of selective permeability and may lead to the development of pathological states and to death of the cell.

The results of this investigation show that proteins present in blood also are capable of exerting a strong action on structure of the membrane, producing changes in density and phase transition temperature. Since the packing density of molecules in the membranes is directly proportional to the relative impermeability of the membranes [11], the increase in density of the membranes which was observed can evidently disturb the normal rate of biochemical reactions and lead to a breakdown of regulation of biochemical processes strictly coordinated in time.

Structural transitions of the gel—liquid crystal type in membranes are a trigger mechanism switching the cell from one metabolic state into another. Consequently, the effectiveness of adaptation during the action of external factors is determined by "structural mobility" of the membranes, i.e., the phase composition, ability to exhibit polymesomorphism with the given composition and at a physiological temperature [2]. It is possible, therefore, that a rise of phase transition temperature in the presence of isoimmune antibodies modifies the sensitivity of the membrane to the action of signal substances and affects the performance of the regulatory and informational role of erythrocyte membranes in cell metabolism.

An increase in density of erythrocyte membranes may change other properties of the blood and, in particular, viscosity [10]. To test this hypothesis 12 experiments were carried out to measure the viscosity of the blood. The results of one such experiment are given in Fig. 3. An increase in antibody concentration was found not only to increase the viscosity of the blood at low shear velocities (37°C), but also to alter the temperature dependence of viscosity abruptly: At 20°C viscosity falls, at 37°C it rises. Circulatory disturbances in some patients in cases of incompatibility of transfused blood may perhaps be connected with this fact.

Changes in erythrocyte membranes under the influence of low-activity isoimmune anti-rhesus antibodies, not detectable by the methods of investigation usually used, can thus be detected by the light scattering method. The degree of changes in the membranes increases with an increase in antibody concentration, but the character of the disturbances reflects structural transitions of the cell membrane. At a certain level of antibody activity, changes in the erythrocyte membrane may affect the course of various metabolic processes in the intact organism.

LITERATURE CITED

1. A. E. Gromov, Lab. Delo, No. 11, 693 (1981).
2. R. I. Mints and E. V. Kononenko, in: Progress in Science and Technology, Series "Biophysics" [in Russian], Vol. 13, Moscow (1982), p. 28.
3. G. D. Nisenbaum, A. L. Kazantseva, and S. P. Aksentsev, in: Structural Lability of Membranes [in Russian], Minsk (1974), p. 63.

4. G. D. Nisenbaum, G. V. Lyakhovich, and S. V. Konev, Biofizika, No. 4, 641 (1978).
5. E. A. Chernitskii and A. V. Vorobei, Structure and Functions of Erythrocyte Membranes [in Russian], Minsk (1981).
6. Biological Membranes [in Russian], Moscow (1978), pp. 38-61
7. Biochemical Thermodynamics [in Russian], Moscow (1982), pp. 210-251.
8. G. H. Brown and J. J. Wolken, Liquid Crystals and Biological Structures [Russian translation], Moscow (1982), pp. 165-171.
9. R. David, Introduction to Molecular Biophysics [Russian translation], Moscow (1982), pp. 124-128, 189-202.
10. C. G. Caro, T. J. Pedley, R. C. Schroter, et al., Mechanics of the Circulation, Oxford Univ. Press, Oxford (1978).
11. A. Kotyk and K. Janaček, Membrane Transport [Russian translation], Moscow (1980), pp. 17-161.
12. V. R. Williams and H. B. Williams, Basic Physical Chemistry for the Life Sciences, Freeman, San Fransicso (1973).

INTERACTION OF POLYANION MOLECULES WITH THE PLASMA MEMBRANE OF LYMPHOCYTES WITH DIFFERENT DENSITIES OF CHARGED GROUPS ON THE CELL SURFACE

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The writers showed previously that polyacrylic acid (PAA), a polyanionic mitogen, activates proliferation only of lymphocytes adherent to nylon wadding, but does not affect cells incapable of adhesion [3]. Cells not adherent to nylon wadding (mature T lymphocytes) are known to differ from adherent cells (mainly B lymphocytes, but also macrophages and T suppressor lymphocytes) in their high electrophoretic mobility, due to the larger number of negatively charged groups on the cell surface. These groups consist mainly of terminal N-acetylneuraminic acid and, to a much lesser degree, of terminal N-acetyl-glucosamine and N-acetylgalactosamine. In connection with the facts described above it has been suggested that interaction of the polyanionic polymer with the cell membrane of mature T cells may be impeded or blocked because of the high density of negatively charged groups on the surface of these cells.

To test this hypothesis the effect of PAA was studied on cell subpopulations enriched with B lymphocytes, with mature T cells or immature T lymphocytes, and also on mature T cells, treated beforehand with neuraminidase, in order to remove by enzyme action the terminal N-acetylneuraminic groups present on the cell surface.

EXPERIMENTAL METHOD

In vitro cultures of lymphocytes from mouse (CBA × C57BL)F_1 spleen and thymus were used. The conditions of induction of lymphocyte division with the aid of PAA and the technique of fractionation of the spleen cells based on ability to adhere to nylon wadding were described previously [3, 4]. The fraction of nonadherent cells was described as enriched with mature T cells, the fraction of adherent cells as enriched with B lymphocytes and with a small admixture of T cells [3]. A suspension of thymocytes was used as the source of immature T lymphocytes without any marked glycocalyx, i.e., a high density of charged groups on the cell surface.

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