HIGH FREQUENCY ELECTROSPECTROSCOPY OF WHOLE HUMAN BLOOD DIFFERING IN THE DEGREE OF ITS OXYGEN SATURATION

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Studies of high-frequency spectra of dielectric permeability and specific electrical conductance of human blood have shown that normal blood, within the radio-frequency band, has a single region of dispersion (known as β-dispersion) with the characteristic frequency lying in the region of 1 MHz [2-4, 9]. Investigations also have revealed the mechanism of β-dispersion and the effect of hydrodynamic mixing of the blood has been described [2-4]. Allowing for this effect, when electrical characteristics of whole blood are measured, two different states of the blood must be distinguished: mixed blood (erythrocytes separated from one another) and sedimented blood (the erythrocytes aggregated into rouleaux). High-frequency electrospectroscopy of an aqueous solution of hemoglobin, saturated to different degrees with oxygen, has been described [5, 10-12]. We know that the mechanisms responsible for dispersion of the electrical characteristics of a solution of hemoglobin molecules and of whole blood are different [4], and for that reason the study of the effect of oxygen on β-dispersion of blood is of definite interest, especially in connection with elucidation of the effect of oxygenation on structural changes in the hemoglobin-erythrocyte system.

In the investigation described below dispersion of electrical conductance and of dielectric permeability of normal sedimented human blood, saturated to different degrees with oxygen, was studied and dependence of the relaxation time of β-dispersion of mixed blood on the degree of its oxygenation also was investigated.

EXPERIMENTAL METHOD

To study the electrical spectra of blood within the frequency band from 0.1 to 20 MHz we used a high-frequency VM-432 admittance bridge (Czechoslovakia), on which a special cuvette was mounted. The accuracy of determination of capacitance was ±0.1 pF, and the accuracy of determination of conductance ±0.5 μmho. The generator frequency was stabilized with an accuracy of ±2%. A change of voltage in the cuvette from 10 to 150 mV/cm did not affect the results of measurement. The cuvette was a closed vessel measuring 2 × 5 × 10 mm, made of plexiglas. Platinum electrodes with an area of 1 × 4 mm^2 were arranged parallel to each other and 10 mm apart. The electrical characteristics of blood were measured at 20°C in an electric field with an intensity of 50 mV/cm. The partial pressure of oxygen was measured with a Clark electrode on a micro-Astrup apparatus. The pH and pCO_2 of the blood and the degree of oxygenation of the hemoglobin (by Van Slyke's equation) were determined on the same apparatus. The hemoglobin concentration and also the duplicating degree of blood oxygenation were determined on an S-202 spectrophotometer. The hematocrit index of the erythrocytes was measured on a Clay-Adams microcentrifuge. The error of determination of the blood parameters measured was not more than 5%. Fresh heparinized blood was used for the investigation. The measurements were made on blood taken immediately from individuals with different degrees of oxygenation (from the heart during catheterization), and also on blood obtained from healthy donors, deoxygenated by bubbling a mixture of nitrogen and CO_2 through it. Blood from a sample with a known degree of oxygenation was well mixed (to separate the erythrocytes, which were aggregated into rouleaux) and injected by means of a syringe into a measuring cuvette, which had previously been rinsed out with heparin. To calculate the relaxation time of the mixed blood, capacitance and conductance were measured at three frequencies immediately after introduction of the blood into the cuvette, for a period

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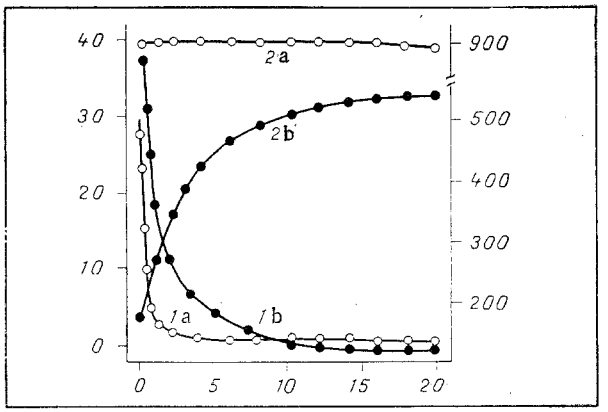

Fig. 1. Dispersion of capacitance (1a, 1b) and electrical conductance (2a, 2b): a) plasma, b) sedimented blood. Abscissa, frequency (in MHz); ordinate: on left, capacitance (in pF), on right, conductance.

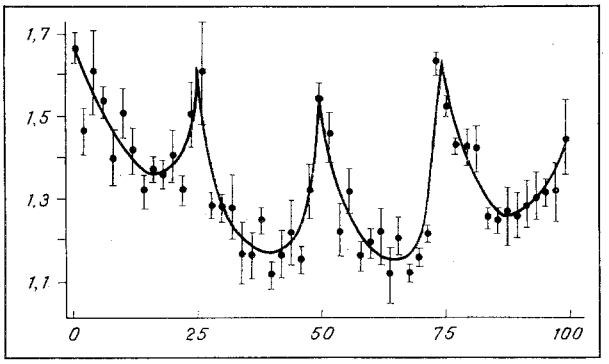

Fig. 2. Dependence of relaxation time of mixed blood on its degree of oxygen saturation. Abscissa, oxygenation (in per cent); ordinate, relaxation time $\times 10^{-7}$ (in sec).

of 1-2 min. Dispersion of permittivity and of electrical conductance of the sedimented blood was measured 5-10 min after introduction of the blood into the cuvette (when the effect of hydrodynamic mixing of the blood had disappeared [3]). The relaxation time of the mixed blood (T) was calculated as the geometric mean of the relaxation time of dispersion of permittivity (T_1) and of electrical conductance (T_2) [1, 7]:

$$T = \sqrt{T_1 T_2}, \tag{1}$$

where, according to the theory of relaxation and the 3 points method,

$$T_1 = \sqrt{\frac{A - E}{E\omega_2^2 - A\omega_1^2}}; \quad T_2 = \sqrt{\frac{B - E}{E\omega_2^2 - B\omega_1^2}}. \tag{2}$$

Here

$$A = \frac{C(\omega_1) - C(\omega_3)}{C(\omega_2) - C(\omega_3)}; \quad B = \frac{G(\omega_1) - G(\omega_3)}{G(\omega_2) - G(\omega_3)};$$

$$E = (\omega_3^2 - \omega_1^2)(\omega_3^2 - \omega_2^2);$$

$C(\omega_i)$, $G(\omega_i)$ denote the capacitance and conductance of blood in the measuring cuvette at the ω_i-th frequency.

EXPERIMENTAL RESULTS

Measurements of the electrical characteristics of normal human blood in a state of aggregation of the erythrocytes, within the frequency band from 0.1 to 20 MHz, showed that neither dispersion of permittivity nor dispersion of electrical conductance of the blood within the limits of experimental error is independent on the degree of its oxygenation.

Dependence of capacitance and conductance of sedimented human blood and its plasma on the frequency of the electric field is shown in Fig. 1. It will be clear from Fig. 1 that in the region of 1 MHz there is a sharp decrease in permittivity of both blood and plasma. As a result of the low plasma protein concentration, dispersion of electrical conductance of plasma is absent. The relaxation time of the sedimented blood, calculated by the clock diagram method, was $(2.08 \pm 0.14) \times 10^{-7}$ sec.

As the three reference points for calculating the relaxation time of nonequilibrium blood frequencies of 1, 1.2, and 1.4 MHz were chosen, i.e., frequencies at which the greatest changes in the electrical characteristics of whole blood were observed compared with changes in the characteristics of plasma (Fig. 1). From the measured values of capacitance and conductance of the mixed blood at the 3 frequencies, time constants of relaxation were calculated by equations (1) and (2) by computer, and these were subsequently averaged by the sliding summation method. The result (dependence of relaxation time of mixed blood on its degree of oxygen saturation) is shown in Fig. 2. The absolute accuracy of determination of the relaxation time of the mixed blood by the 3 points method was about 8%.

It has been shown [4] that the mechanism causing β-dispersion of blood is linked mainly with the existence of a "time constant" (relaxation time) of the erythrocyte membrane. For separate erythrocytes of average radius (R), with a membrane capacitance of (C), the relaxation time of blood was calculated by the equation $T = kRC$, where k is a constant, depending on the conductance of plasma and of the intraerythrocytic medium [8]. If the value of C is taken to be independent of oxygenation, it follows from the data in Fig. 2 that the degree of saturation of normal human erythrocytes has a variable and relatively weak influence on their average radius (membrane configuration). Incidentally, the shape of the sickle-cell erythrocytes is highly dependent on the degree of their oxygen saturation: a change in configuration of the membrane of these erythrocytes can be observed under the ordinary microscope [6]. Accordingly, it is interesting to carry out similar experiments using blood containing sickle-cell erythrocytes.

The formula $T \sim RC$ is applicable also for erythrocytes aggregated into rouleaux [4]. In this case, a certain general radius of the aggregate must be taken for R, and the capacitance of the outer membranes of the erythrocytes forming an aggregate like one large cell, for C. It follows from independence of the relaxation time of the sedimented blood on its degree of oxygen saturation that oxygenation does not affect the general radius of the aggregate.

In conclusion, it can be pointed out that the curve illustrated in Fig. 2, whatever the mechanisms producing it, is ultimately connected with the ligand states of the hemoglobin molecule — with their degree of oxygenation. It will be clear from Fig. 2 that this dependence is wave-like with maxima at the points of 0, 25, 50, 75, and 100% oxygen saturation of the hemoglobin. The existence of these maxima is evidence of the presence of collective effects in the system of hemoglobin molecules (which, at the initial period of action of the high-frequency electric field on dipole macromolecules, are in conformationally nonequilibrium states).

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