NUCLEAR MAGNETIC RELAXATION OF AQUEOUS SOLUTIONS OF PROTEINS, BLOOD PLASMA, ERYTHROCYTES, AND WHOLE BLOOD

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Interest in the study of nuclear magnetic relaxation (NMR) of blood and biological fluids [2, 11] is due to the wide opportunities presented by the method for studying the properties of blood and its rapid analysis. The parameters of NMR which can be measured, namely the spin-lattice (T_1) and spin-spin (T_2) relaxation times of the protons of water, in fact depend on the rheologic properties of the blood, dynamic changes in water in the membranes, diffusion of water molecules, protein hydration, the biochemical composition of the plasma, and the state of the erythrocytes.

It is thus possible in principle to carry out a comprehensive rapid analysis of whole blood and its components and, in addition, to record pathological changes associated with various diseases [7, 15]. It has been shown that the spin-lattice relaxation time of blood depends essentially on the frequency of NMR [12]. For instance, at frequencies of under 5 MHz and over 15 MHz, constancy of the relaxation rate is observed, and that at low frequencies its value is almost 4 times greater. Frequency correlations have been found between the measured properties and concentrations of individual blood components, namely for solutions of hemoglobin (Hb) [12, 15] depending on pH and temperature, including when pathological changes are present [13], and also for solutions of plasma and blood serum [10].

In our view, analysis of the blood ought to be based on a separate study of the relaxation relationships of serum, plasma, solutions of erythrocytes, and hemoglobin, and on the construction of a general model which will take account of changes in the relaxation time of water protons relative to all its components as a single physiological system. On that basis, the aim of the investigation was to study the components of blood, which could serve as the basis for construction of a relaxation model of whole blood.

EXPERIMENTAL METHOD

Serum was obtained by centrifuging blood at 1000*g* for 10 min; plasma was obtained by stabilizing blood with heparin; after centrifugation the erythrocytes were washed 3 times in Ringer's solution. Tests were carried out for 1 h after the blood was taken. Relaxation measurements were made on the Minispinekho instrument at 25°C and with a frequency of NMR of 5 MHz [1]. The T_1 relaxation time was measured by Khan's method, and T_2 by the method in [3]. Altogether 143 blood samples were tested. Blood serum was investigated in 22 healthy women and 12 pregnant women with toxemia. As the comparison solution we used physiological saline (0.9% NaCl solution), whose relaxation velocity $(T_{1,2})^{-1}$ was 0.42 sec (for bidistilled water 0.40 sec^{-1}). For human serum and a solution of human albumin, relaxation time was a linear function of concentration of the component in physiological saline. For whole erythrocytes, amplitude was a biexponential function of time, with short and long relaxation time (T_1, T_2); relaxation time, moreover, was a linear function of hemoglobin concentration. The measured values of relaxation time lay within the following limits:

for blood T_1 is 0.4-0.7 sec, T_2 0.05-0.4 sec;
for plasma, 0.7-1.1 and 0.05-0.5 sec respectively;
for erythrocytes 0.25-0.5 and 0.03-0.3 sec respectively.

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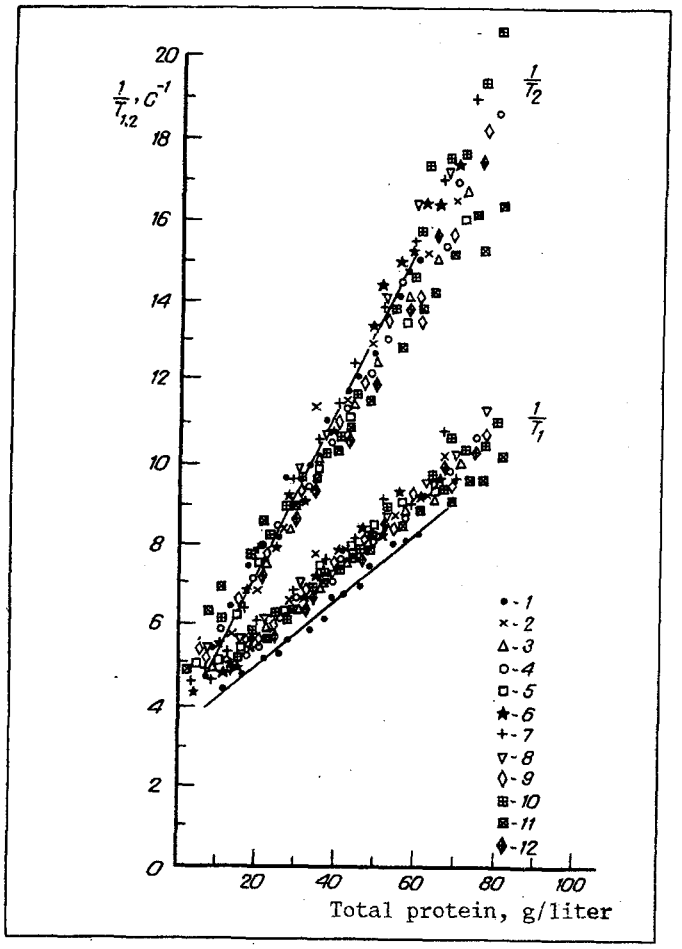

Fig. 1. Dependence of $(T_1)^{-1}$ and $(T_2)^{-1}$ on concentration of albumin (1) and total protein in blood serum C_p (in g/liter)/α_{alb}: 68.5/0.61 (2); 70.6/0.72 (3); 70.0/0.55 (4); 70.6/0.71 (5); 70.1/0.68 (6); 76.0/0.52 (7); 70.0/0.50 (8); 77.0/0.68 (9); 80.0/0.35 (10); 81.2/0.61 (11); 81.4/0.48 (12).

EXPERIMENTAL RESULTS

The principle of the technique of construction of a model reflecting dependence of relaxation velocities $(T_{1,2})^{-1}$ of protons of water molecules on the composition of the solution to be analyzed, is one of additiveness of the measured property for all sorts (types) of water protons, and allowing for their concentration. The effect of the different proteins and of other components of the blood on the relaxation characteristics of water molecules differs, and this is reflected in the change in the values of the parameters in response to a change in the qualitative and quantitative composition of the blood. Taking the above considerations into account, it is best to begin an investigation of the multicomponent system with model solutions, simulating changes in the concentration of the principal blood components. The effect of each component on the property measured is described by the equation:

$$(T_{1,2})^{-1} = \sum_i K^i_{e_{1,2}} \cdot C_i \cdot \alpha_i \qquad (1)$$

where $K^i_{e_{1,2}}$ is a coefficient of proportionality, characterizing the contribution of the i-th component to acceleration of relaxation of water protons; C_i and α_i denote its concentration and fraction respectively.

In accordance with the approach in [2], describing at least two sorts of water molecules ("bound" and "unbound"), a proportional relationship can be expected between the relaxation velocity and protein concentration. In fact, a linear relationship is observed not only for model solutions containing albumin (Fig. 1), but also for solutions obtained by diluting blood serum with different total protein concentrations and different relative proportions of the albumin and

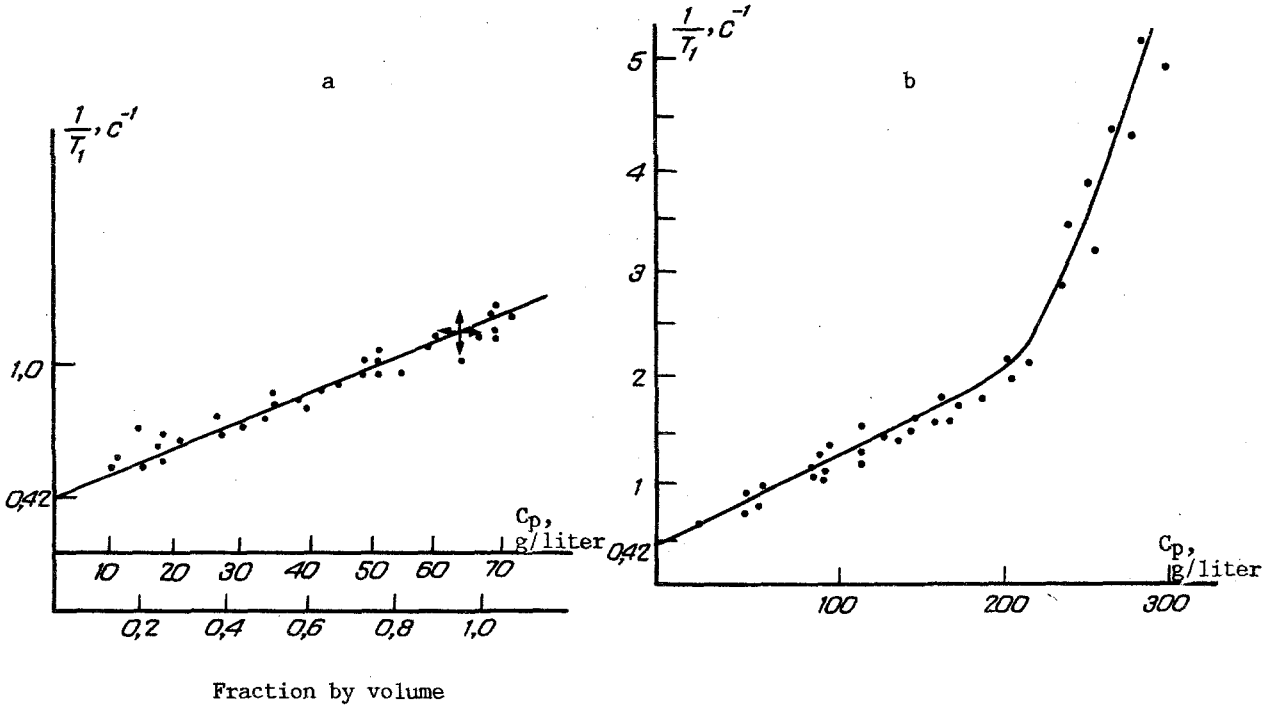

Fig. 2. Dependence of $(T_1)^{-1}$ on fraction by volume and concentration of total protein in plasma (a) and on hemoglobin concentration in physiological saline (b).

globulin fractions. This character of the relationship indicates definite relaxation efficiency of proteins [5], and the ratio T_1/T_2 is approximately 2. Dependence of relaxation velocity on total protein concentration (C_p), with close correlation ($r > 0.9$) is in the form:

$$(T_1)^{-1} = 0.012C_p + 0.42, \tag{2}$$

$$(T_2)^{-1} = 0.024C_p + 0.42. \tag{3}$$

The observed scatter of the experimental data relative to a straight line, described in equations (2) and (3), may be due to various factors: the pH of the medium, the salt composition, and also differences in the ratio of albumins to globulins. Taking the latter into account, correlations ($r > 0.8$) were obtained relative to the percentage content of albumin in the blood serum ($\alpha_{alb} = C_{alb}$, %/C_p, % = 0-1):

$$(T_1)^{-1} = 1.03 - 0.23\alpha_{alb}, \tag{4}$$

$$(T_2)^{-1} = 1.62 - 1.80\alpha_{alb} \tag{5}$$

for the usual protein concentrations (60-80 g/liter).

With the relationships thus obtained it is possible to determine the total protein concentration and the ratio of albumins to globulins in solutions of blood serum. Relaxation relationships in solutions of plasma were studied in the same way (Fig. 2a). In this case also linear correlation is observed, but with a coefficient of relaxation efficiency rather greater ($K_{el} = 0.015$). This difference is evidently due to the effect of fibrinogen, which also reflects the possibility of its quantitative determination.

Correspondingly, for solutions containing only erythrocytes, relationships also were obtained between the measured property and the hemoglobin concentration in solution (Fig. 2b) in agreement with data in the literature [12, 15]. The appreciable change in relaxation velocity with an increase in hemoglobin concentration (over 220 g/liter) can evidently be explained not only by an increase in the number of water molecules bound with protein, but also by a change in viscosity of

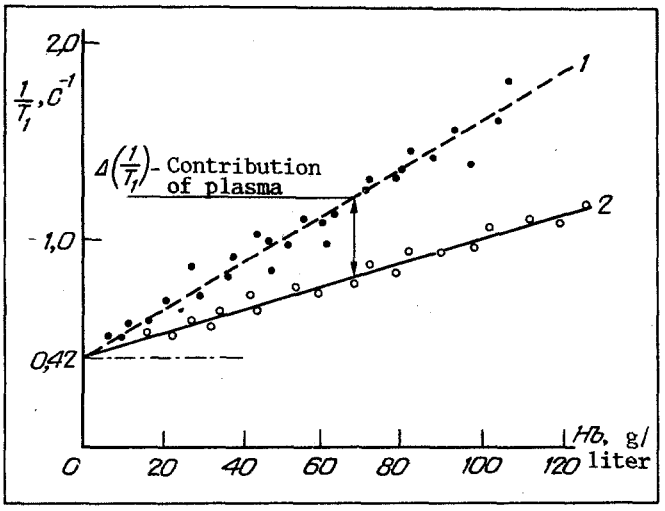

Fig. 3. $(T_1)^{-1}$ as a function of hemoglobin concentration for solutions of whole blood (1) and for erythrocyte suspension in physiological saline (2).

the solution due to the increase in concentration of erythrocytes. We know [6] that hemoglobin concentration is a linear function of hematocrit (H), through the color index (0.8-1.1), an abnormality of which is determined by pathology. We know that $C_{Hb} = 30 \cdot H + 17.0$. It is thus possible to use the change in $(1/T_1)$ to estimate not only C_{Hb} in the blood, but also the hematocrit. The relationship of viscosity (η) to hematocrit [8] is similar in form to that of $1/T_1$ on C_{Hb}. The reason is that $(T_1)^{-1} \sim \eta$ [3]. Scatter of the experimental data is usually observed in dependences of viscosity and relaxation velocities on H, and C_{Hb} for whole blood, due to the contribution of erythrocyte aggregation (EA) [8] and pH [12] In fact, definite correlation between EA and H also was found on the basis of our experimental data. Thus correlation between the rheologic parameters of the blood and relaxation velocity of protons of water molecules in the blood was confirmed experimentally. Dependences of relaxation velocity on content of whole blood in physiological saline by volume, confirming the effect of concentration of the separate components on the relaxation efficiency of the blood, are shown in Fig 3. Thus the equation for correlation of the relaxation data with the results of clinical analysis has the form:

$$(T_1)^{-1}_{serum} = 0{,}0063\ C_{Hb} + 0{,}015\ C_p + 0{,}42. \tag{7}$$

Subsequent modeling of the equation of reaction velocity in blood, allowing for rheologic properties and the effect of salt composition and the hydration properties of the proteins [12], on the basis of experimental data, yielded the following equation ($r > 0.92$):

$$T_1)^{-1}_{serum} = [0{,}004 \cdot A\ni + 0{,}0018(1 - EA)] \cdot C_{Hb} + 0{,}015\ C_p \cdot (1 + \alpha' K)^2 + 0{,}42, \tag{8}$$

where the first two terms take account of aggregation of erythrocytes (aggregated and nonaggregated respectively), but the contribution of total protein in plasma with fibrin was corrected by a conformation coefficient (α'_K), taking account of the effect of Na^+ concentration on the hydration state of the protein in the plasma, and equal to

$$\alpha'_K = (C_{Na^+} + 2{,}8) \cdot 1{,}7, \tag{9}$$

where 2.8 g/liter is the normal Na^+ concentration in the plasma and 1.7 is a coefficient for converting the experimental data into relative deviation.

In diagnosis it is best to study solutions of erythrocytes, plasma, and blood serum separately, for it facilitates interpretation of the results. For applied purposes it is very interesting to study the effect of paramagnetic metallic ions ("paramagnetic doping") on a ("paramagnetic doping") on a ("paramagnetic doping") on a substantial change in relaxation time [4]. In cases when ions of metals do not penetrate erythrocytes, the effect of intracellular water on the relaxation velocity will be small, but the characteristics of the water of blood plasma are very considerably changed. In the presence of paramagnetic doping, the curve of amplitude as a function of time in relaxation will be biexponential in character; moreover, $(A_{cw}/A_0) \cdot 100\% = H$, where A_0 and A_{cw} denote the initial amplitude of the spin echo signal and the amplitude of

protons inside the cellular water respectively. As paramagnetic probe to assess the content of extracellular water, it is suggested that manganese salts be used [14] in very small amounts, since the relaxation efficiency of manganese is one of the highest [9].

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