METHODS

Comparison of the Blood Level of Endogenous Paramagnetics in Health and Pathology Using Proton Magnetic Relaxation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 120, № 11, pp. 557-560, November, 1995
Original article submitted November 5, 1994

An experimental study is made of the longitudinal proton relaxation time T_1 in human serum in health and pathology as a function of the protein concentration using partially lyophilized samples. A comparison of the results with those obtained with modeled aqueous protein solutions and extrapolation of the dependences to the zero concentration demonstrate that the concentration of paramagnetic admixtures is the same in groups of healthy subjects and patients with malignant neoplasms who have reliably different T_1 values in native samples. There is no difference in the longitudinal proton relaxation time of the nonaqueous component of the serum between these groups.

Key Words: *NMR relaxation; blood serum; pathology; paramagnetic centers; mass ratio*

More than 20 years have passed since it was found that nuclear magnetic resonance (NMR) relaxation times T_1 and T_2 are increased in tissues [7] and physiological fluids [4,9] in a number of pathological states, notably in carcinogenesis [5], and yet the nature of this phenomenon is still unclear. For this reason, an experimental study of native physiological fluids and appropriate models remains of current importance.

In the present study we investigated the longitudinal proton relaxation time T_1 of water and of the nonaqueous component of serum as a function of the protein concentration (mass ratio).

Our goal was to develop a method for evaluating such a dependence, and, if it worked well, to compare serum in health and pathology within the zero protein mass ratio. Possible differences are assumed to be attributed to the varying content of endogenous paramagnetic centers in the physiological fluid.

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MATERIALS AND METHODS

The serum was separated by centrifugation of fasting venous blood obtained either from essentially healthy subjects or from patients without documented oncological disease (gastritis, duodenal ulcer, pyelonephritis, n=15) and from primary patients with malignant neoplasms (stage II-IV) of the gastrointestinal tract or urogenital system (n=14). The serum was insulated from the atmosphere and all measurements were carried out not later than 6-9 hours after separation; the total protein concentration was measured by the biuret method [3]. The relaxation time was measured using the inversion-reversion method (180-t-90 pulse sequence, where t is the interval between the 180° and 90° pulses [1]

0007-4888/95/0011-1179$12.50

and the duration of the 90° pulse is about 1 μsec). The majority of the measurements were performed using a Minispec pc-20 NMR relaxometer (Bruker) at a working frequency of 20 MHz. Some measurements were carried out with a Pal'ma domestic relaxometer at a working frequency of 35 MHz. All measurements were performed at a constant temperature of 40°C, the error in the measurement and maintenance of the temperature being within 0.1°C. Prior to the measurements the samples were kept at this temperature for at least 15 min. Sixteen to 25 signal accumulations were usually enough for construction of the entire relaxation curve with an accuracy high enough for isolation of the nonaqueous component of the serum, so that the determination of the NMR parameters for each concentration lasted for 40-50 min.

Proteins in the samples were condensed by freezing and dewatering the frozen samples, the weight of samples being controlled with an accuracy of 0.5 mg. For each new concentration the relaxometer was readjusted before the measurements were carried out.

The amplitude of the NMR signal and the concentration curves were processed using regression analysis. If the coefficient of the paired correlation between the logarithm of amplitude and time (interval between probe pulses t>0.3 sec) for relaxation curves was less than 0.99995, these curves were excluded from the analysis, since the accuracy of separation of the nonaqueous component of the NMR signal was unacceptably low.

RESULTS

Relaxation times T_1 for the aqueous component in the initial samples from the control individuals (T_1= 1.65±0.08 sec) and oncological patients (T_1=1.90± ±0.13 sec) did not differ from the values reported earlier [2]. In these samples we also determined the longitudinal relaxation time T_{10} of the nonaqueous component in the representation of the total signal A_i from the sample in the formula [1]:

$$A_i=A_{10}[1-2\exp(-t_i/T_{10})]+A_{20}[1-2\exp(-t_i/T_1)], \quad (1)$$

where A_{10} and T_{10} are signal amplitude and relaxation time for a nonaqueous component, A_{20} and T_1 are the amplitude and relaxation time for water (T_1 for the studied groups is given above), and t_i is the interval between the 180° and 90° pulses. Since the ranges of T_{10} and T_1 are tens of milliseconds and seconds, respectively, the achieved signal/noise ratio usually allows for separation of the nonaqueous component and for determination of T_{10} and A_{10}. The mean values and standard deviations of these parameters for the studied groups are presented in Table 1.

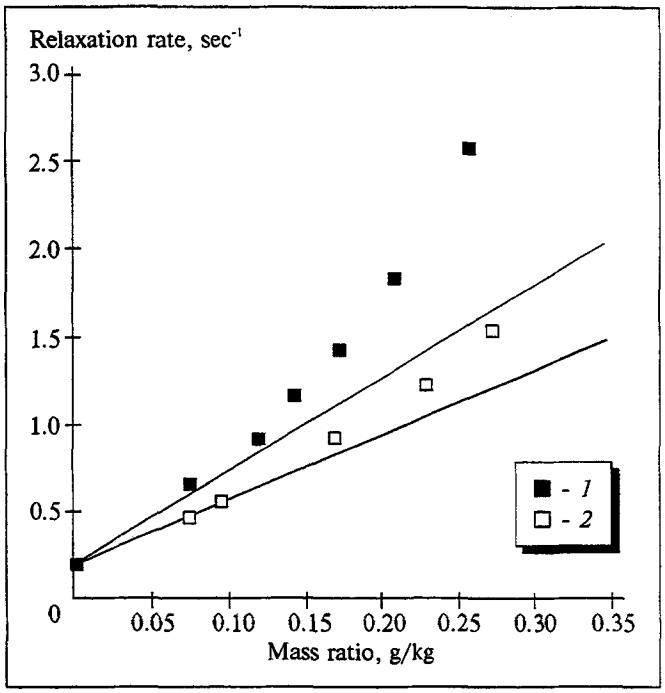

Fig. 1. NMR relaxation rate ($1/T_1$) of water protons as a function of mass ratio in health (*1*) and in stomach cancer (*2*).

The data suggest that the groups in question differ reliably in only one parameter, i.e., the longitudinal relaxation time of water T_1.

As an illustration, Fig. 1 shows two characteristic concentration-relaxation rate curves for the samples from patients with stage III stomach cancer, T_1=2.03 sec ($1/T_1$=0.49 sec^{-1}, C_0=78 g/kg), and from nononcological patients (duodenal ulcer in remission), T_1=1.61 sec ($1/T_1$=0.62 sec^{-1}, C_0=75 g/kg). C_0 is the mass ratio of proteins in the initial serum samples.

Figure 1 depicts an approximation of the dependence by the expression:

$$R=R_0(1-C\times k)+C\times k\times B, \quad (2)$$

where R_0 is the relaxation rate in pure water, C is the mass ratio, k is the C-independent proportion-

TABLE 1. NMR Parameters and Mass Ratio in Native Serum in Cancer and Noncancer Patients

Group	T_1, sec	T_{10}, msec	$A_{10}/(A_{10}+A_{20})$, %	C_0, g/kg
Cancer patients	1.9±0.1	32±6	4.2±1.0	78±10
Noncancer patients	1.65±0.08	35±6	4.0±1.0	74±6

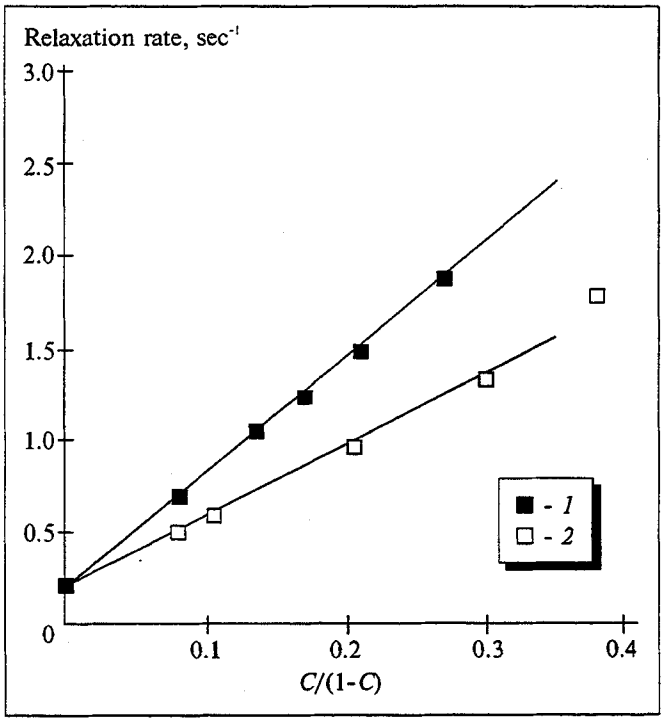

Fig. 2. Data from Fig. 1 replotted in coordinates $C/(1-C)$ versus relaxation rate.

ality factor, and B is the relaxation rate of protein-bound water. Expression (2) is characteristic for relaxation in a two-phase system in a rapid exchange approximation [8] at low protein concentrations (no more than a few weight percents). It may be assumed that $C \times k$ represents the relative fraction of bound water protons. On Fig. 2 the same data are replotted in coordinates R versus $C/(1-C)$, i.e., for the relation:

$$R = R_o(1 - C \times k/(1-C) + C \times k \times B/(1-C), \quad (3)$$

Equation (3) is derived from (2) on the assumption that protein globules occupy a limited volume in the sample and the amount of bound water on their surface still does not depend on the mass ratio C.

An analysis of Figs. 1 and 2 invites a number of conclusions. The approximation of experimental points by expression (3) on Fig. 2 suggests that the model of rapid exchange between so-called free and bound water with allowance for the volume of macromolecules proper adequately describes proton relaxation up to high values of mass ratio (about 300 g/kg) in spite of the fact that it is too simplified for serum. In particular, this model ignores the presence of the main serum fractions and their potential changes in pathology, changes in the ionic strength and concentration of salts produced by partial lyophilization, changes in the dissolved oxygen concentration due to dewatering, etc.

Figs. 1 and 2 represent only two typical dependences, but for both groups of patients similar measurements with a paired correlation coefficient of no less than 0.997 in coordinates R, $C \times k/(1-C \times k)$ are described by lines with individual slopes but with the same free term $R_o = 0.20 \pm 0.04$ sec^{-1}, which is in accordance with published data on the relaxation rate in pure degassed water at the same temperature [1]. This suggests that the serum concentration of paramagnetic centers is the same in health and disease and cannot be responsible for the difference between T_1 values in native serum in cancer and in health.

Table 2 presents a single concentration dependence of NMR parameters. As follows from these data, within the achieved accuracy of separation of the nonaqueous component our results imply that, first, the bound/free water ratio tends to increase with an increasing concentration (in comparison with measurement of the concentration by the weight method) and, second, the effective relaxation time T_{10} is independent of the mass ratio (the same results except for the T_1 value were also obtained for samples from the noncancer group).

As is seen from the above data, the rapid-exchange model, with an allowance for the individual volume of macromolecules and assuming a constant amount of bound water per macromolecule, satisfactory describes the experimental data up to high protein concentrations far surpassing the physiological range. Only when the mass ratio exceeds 250-300 g/kg does the relaxation rate increase more rapidly than is predicted by equation (3).

In our study we purposely did not use the method of diluting native serum with physiological saline or any other buffer solution [6], since in this

TABLE 2. Experimental Dependence for Patient Ch. (Stomach Cancer, $T_1 = 1.93$ sec)

Weight of sample, mg	Relaxation rate, sec^{-1}	C_0	T_{10}, msec	$A_{10}/(A_{10}+A_{20})$, %
600.2	0.52	75.0	32	3.7
503.4	0.56	89.2	36	5.7
375.4	0.69	119.5	34	6.1
261.0	0.96	169.0	38	7.4
196.8	1.22	229.0	35	9.2
160.2	1.46	281.2	29	9.8

case it is difficult to control the amount of paramagnetic admixtures and to judge the content of endogenous paramagnetic centers.

Even though the conventional formulas expressing the relaxation time T_1 through the so-called correlation times of molecular movements [8] can apparently not be applied directly for such complex systems, we can attempt to assess the possible effect of the experimental parameters on T_1.

The relaxation time T_{10} is directly determined by some effective time of Brownian rotational motion of a protein globule [8], and if in the zeroth approximation we ignore the presence of the main protein fractions of different weight and geometry (hence the use of the term "effective"), the fact that T_{10} is independent of the state of the patient and of the protein concentration in the samples (Tables 1 and 2) may suggest that, among the possible mechanisms underlying the increase of T_1, notably in cancer, some variation in the dynamic behavior of protein globules as well as possible variations in the content of paramagnetic centers are not crucial factors.

The use of the method of partial lyophilization of native serum to analyze the effect of carcinogenesis on NMR relaxation times seems to be justified. First, it allows for easy experimental evaluation of such an important parameter as the level of endogenous paramagnetics in a biological fluid. Second, this method is quite "gentle" in that it does not cause gross disturbances in the native dynamic structure of the serum and may be applied in studies of the effects of other pathological processes such as burns, intoxications of different genesis, myocardial infarction, crush syndrome, etc. on biological fluids.

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