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NMR water-proton spin-lattice relaxation time of human red blood cells and red blood cell suspensions

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NMR water-proton spin-lattice relaxation times were studied as probes of water structure in human red blood cells and red blood cell suspensions. Normal saline had a relaxation time of about 3000 ms while packed red blood cells had a relaxation time of about 500 ms. The relaxation time of a red cell suspension at 50% hematocrit was about 750 ms showing that surface charges and polar groups of the red cell membrane effectively structure extracellular water. Incubation of red cells in hypotonic saline increases relaxation time whereas hypertonic saline decreases relaxation time. Relaxation times varied independently of mean corpuscular volume and mean corpuscular hemoglobin concentration in a sample population. Studies with lysates and resealed membrane ghosts show that hemoglobin is very effective in lowering water-proton relaxation time whereas resealed membrane ghosts in the absence of hemoglobin are less effective than intact red cells.

NMR; Spin-lattice relaxation time; (Red blood cell)

1. INTRODUCTION

This paper presents studies of NMR water-proton spin-lattice relaxation times in human red blood cells. Water makes up about 50% by weight of the red blood cell and its interaction with red cell components is important for maintenance of red cell structure and metabolism. Extracellular plasma water is also influenced by red cells and the flow properties of plasma depend partly on this interaction.

The water-proton relaxation time is partly a measure of rotation freedom of water molecules in the measured sample [1]. Lower relaxation times suggest less rotational freedom of water in the sample. The ability of red cells to lower water-proton relaxation time may reflect their ability to structure and influence extracellular and intracellular water.

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Water-proton relaxation times have been shown to be of value in diagnosis of cancer tissue and potentially in mental disorders [1,2]. Specifically, Rosenthal et al. have shown that red cell relaxation time may be useful in studies of menstruation [3] and bipolar illness [4]. Red cell water-proton relaxation times may be useful in many other medical illnesses and also for the basic study of red cell biology.

In this paper we study the relationship of water-proton relaxation time to hematocrit, intracellular osmolality, red cell volume and hemoglobin concentration. We also study the effect of various red cell compartments (membrane, hemoglobin, cytoplasm) on relaxation times.

2. MATERIALS AND METHODS

2.1. *Red cell preparations and incubations*

Adult human blood was drawn daily into a test tube containing a small amount of 3.8% sodium citrate solution. After centrifugation, plasma and white cells were removed, and red cells were washed three times with 0.9% NaCl. Appropriate volumes of packed red blood cells were mixed with 0.9% NaCl to

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prepare suspensions of various hematocrits. Osmolality experiments were carried out by mixing 50% hematocrit suspensions with equal volumes of NaCl solutions at various NaCl concentrations. These suspensions were then incubated for 1 h at 37°C in a shaking water bath. After centrifugation, relaxation times of packed cells were measured.

Red cell lysates were prepared by adding 1 ml packed cells to 3 ml water; the resulting lysate was mechanically agitated to insure complete lysis of red cells; a small quantity of concentrated NaCl solution was then added back to make the final solution 154 mM NaCl. Red cell ghosts and lysates free of ghosts were prepared by centrifuging lysates after the mechanical agitation step. The supernate was centrifuged a second time to clear any remaining particulate matter and NaCl added to bring the solution to 154 mM; this sample was the red cell lysate free of ghosts. The centrifugation pellet consisting of red cell ghosts was resuspended in 3 ml water; the suspension was mechanically agitated followed by centrifugation and suspension of the final pellet in 4 ml 154 mM NaCl to form the red cell ghost suspension.

Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) of red blood cells were measured by the Beth Israel Medical Center hematology laboratory.

2.2. *Measurement of NMR water-proton spin-lattice relaxation times*

Measurements of NMR relaxation times were carried out using a Seimco MicroPulse NMR spectrometer (New Kensington, PA) operating at 12.75 MHz. Measurements were made at a sample temperature of 25°C on 0.2 ml sample volumes while using a Steady State Sequence [5] described by Freeman and Hill [6]. Samples were lysates, suspensions or packed cells depending on the experiment. Relaxation times (T_1) were calculated by determining the inverse negative slope of an $\ln(A_\infty - A_\tau)$ versus τ plot as described by Farrar and Becker [7]:

$$\ln(A_\infty - A_\tau) = \left(-\frac{1}{T_1}\right)\tau + C,$$

where A is the signal amplitude for a given τ and C is a constant.

2.3. *Statistics*

Pearson's product moment correlation coefficients (r) for comparisons of relaxation times versus mean corpuscular volume and relaxation times versus mean corpuscular hemoglobin concentration were calculated as described by Bahn [8]. The t values and significance levels of these correlations were also determined as described by Bahn [8].

3. RESULTS

Fig.1 shows the water-proton relaxation times of red blood cell suspensions of various hematocrits. Normal saline in the absence of red cells had a relaxation time of 3000 ms. Packed red blood cells had a relaxation time of about 500 ms. Red cells in suspension caused the extracellular water to behave more like intracellular water so that by 50% hematocrit (0.5 ml RBC/ml suspension) the relaxation time had only increased to 750 ms.

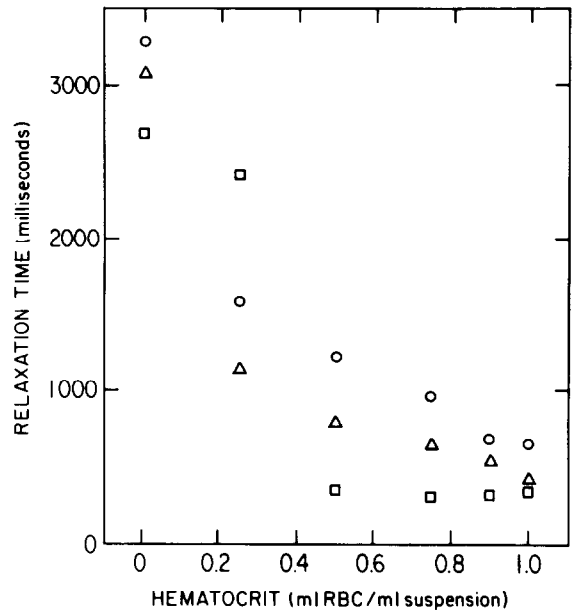

Fig.1. NMR water-proton spin-lattice relaxation times of red blood cell suspensions of various hematocrits in 154 mM NaCl. Expt 1 (□), expt 2 (○), expt 3 (△).

When the osmolality of intracellular water was altered by incubation of red cells in media of various NaCl concentrations, relaxation times

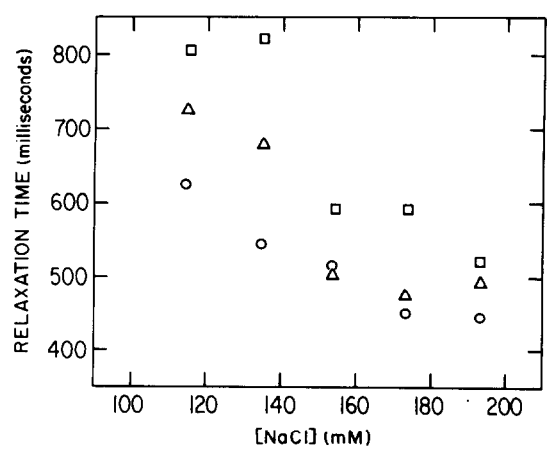

Fig.2. NMR water-proton spin-lattice relaxation times of packed red blood cells after 1 h incubation at 37°C in 25% hematocrit suspensions at various NaCl concentrations. Expt 1 (□), expt 2 (○), expt 3 (△).

were found to increase with decreasing NaCl concentration and decrease with increasing NaCl concentration (fig.2).

Since red cells behave as osmometers, swelling and shrinking with decreasing and increasing extracellular NaCl concentration, relaxation time changes may reflect changes in red cell volume or hemoglobin concentration. Water-proton relaxation was measured in 16 individual volunteers and compared to red cell volume (MCV) and hemoglobin concentration (MCHC) (fig.3). No correlation between relaxation time and either red cell volume or hemoglobin concentration was observed.

The decrease in water-proton relaxation time from 3000 ms with normal saline to 500 ms in packed red blood cells must be explained by the interaction of red cell intracellular and extracellular water with membrane, cytoskeleton, hemoglobin and cytoplasmic salts and organic compounds. Table 1 shows studies with red cell lysates and ghosts. All the samples had the same final NaCl concentration of 154 mM. Relaxation times of 25% red cell suspensions measured about 1500 ms. Complete lysis at this suspension concentration resulted in only a small increase in relaxation time. An equivalent suspension of resealed membrane ghosts alone, without hemoglobin or other cytoplasmic components, resulted in an increase of relaxation time to about 2400 ms. Hemoglobin and other cytoplasmic components without membrane ghosts were almost as effective as the complete lysate in lowering relaxation time.

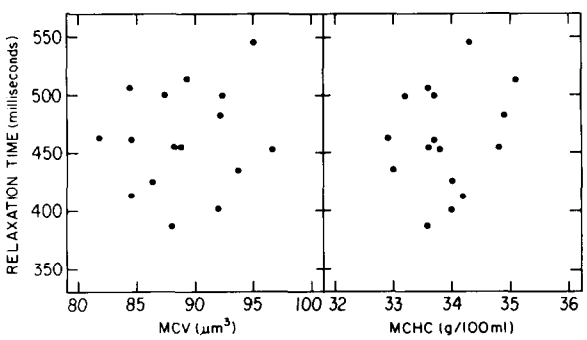

Fig.3. NMR water-proton spin-lattice relaxation times, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) of packed red blood cells of 16 male and female volunteers between the ages of 20 and 60. The correlation coefficient for relaxation time versus MCV is $r = 0.16$ with $t = 0.61$ and $P > 0.20$ (not significant); the correlation coefficient for relaxation time versus MCHC is $r = 0.20$ with $t = 0.77$ and $P > 0.02$ (not significant).

Table 1

NMR water-proton spin-lattice relaxation times of red blood cells, red cell ghosts and red cell lysates

Preparation	Relaxation time (ms)		
	Expt 1	Expt 2	Expt 3
Red cell suspensions	1759	1429	1429
Red cell lysates	2034	1515	1449
Red cell ghosts	2947	2083	2326
Lysates minus ghosts	1862	1754	1587

All preparations were 25% red blood cell suspensions in 154 mM NaCl or the equivalent red cell compartment (membranes and/or cytosol) in 154 mM NaCl

4. DISCUSSION

Studies of red cell suspensions of different hematocrits show that the surface of red cells effectively lowers the relaxation time of extracellular water. Normal saline has a relaxation time of 3000 ms while for packed red cells the relaxation time is 500 ms. An equal mixture of these two samples (50% hematocrit) has a relaxation time of 750 ms which demonstrates that the external surface of the red cell structures a volume of water nearly equivalent to the red cell volume. The shape of the plot in fig.1 relating relaxation time to hematocrit is similar to plots of the reciprocal viscosity versus hematocrit [9]. Water-proton relaxation time and viscosity of water are inversely related in red cell suspensions inasmuch as water-proton relaxation time decreases and viscosity increases with increasing ordered structure of extracellular water.

Incubating red cells in hypotonic media swells the cell with entry into the cell of free water. As expected this results in an increase in the relaxation time as this increased water would be expected to be less structured by red cell components. The decrease in intracellular salt concentration might also result in an increase in relaxation time. Hypertonic media cause shrinking of red cells by loss of free water. The remaining water would be expected to be more highly structured and the result is decreased relaxation time. The normal biological variation of red cell volume and hemoglobin con-

centration in the sample population is not correlated with water-proton relaxation time. This suggests that water structure in red cells is regulated and controlled by factors other than the purely physical relationship of water to the inner membrane surface, cytoskeleton and hemoglobin. Anion and cation flux across the membrane controlled by and coupled to red cell metabolism are probably involved in regulating water structure.

Experiments with lysates and ghosts suggest that hemoglobin in solution is about as effective as intact red cells in lowering water-proton relaxation time. Resealed ghost membranes were not as effective. In intact red cells suspended at 25% hematocrit, the cytoplasmic hemoglobin is relatively tightly packed in a viscous solution. This hemoglobin has access to 25% of the total water of the suspension and structures this water leading to decreased relaxation time (500 ms). The extracellular water is structured, albeit less effectively than the intracellular water, by the surface charges and polar groups of the red cell membrane as demonstrated in the hematocrit experiments. In lysates the hemoglobin has access to 100% of the sample water but at one quarter of the intracellular hemoglobin concentration. The decrease in water structure caused by dilution of the hemoglobin by cell lysis is almost compensated for by allowing hemoglobin access to 100% of the sample water.

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