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THE APPLICATION OF DEXTRAN-MAGNETITE AS A RELAXATION AGENT IN THE MEASUREMENT OF ERYTHROCYTE WATER EXCHANGE USING PULSED NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

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SUMMARY: The rate of water exchange across the erythrocyte membrane was measured using dextran-magnetite as a paramagnetic relaxation agent. The mean residence time of water within the cell was found to be 21.4 ± 0.5(SE) at 22°C. This agreed extremely well with the value measured using manganese. The iron complex was found to not move across the erythrocyte membrane for at least 8 hours, making possible experiments which could not be done using manganese. The activation energy of water transport was measured and this value agreed within 1% of the value using manganese. This suggests that neither reagent induces a substantial perturbation on experimentally determined values of water transport rates.

INTRODUCTION

The measurement of water transport rates across biological membranes by non-gradient pulsed nuclear magnetic resonance employs paramagnetic doping to distinguish between water in the two compartments undergoing exchange. To this date, manganese has been the substance most often chosen to bring about shortening of the transverse relaxation rate constant in the extracellular compartment. Although results of water transport rate studies measured using manganese have been shown to be consistent with NMR studies employing no paramagnetic relaxation reagents (1,2), there are certain applications which cannot be performed with manganese. These include experiments which require over 5 hours and which test the effects of drugs which are capable of increasing divalent cation transport. In both cases, equilibration of manganese between the two compartments prevents distinction between intra- and extracellular water.

Dextran-magnetite, a suspension of dextran-coated ferromagnetic iron oxide, has recently been used as a water proton relaxation agent and its relaxation

Abbreviations: NMR, nuclear magnetic resonance; USP, United States Pharmacopeia; PAN, 1-(2-pyridyl-azo)-2-naphthol; τ_a, mean resonance time; CPMG, Carr-Purcell-Meiboom-Gill; SE, standard error; T_2, transverse relaxation time constant.

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properties have been compared to that of manganese (3). Because of its larger size, which prevents membrane permeation, and increased relaxation ability, dextran-magnetite may be a feasible substitute for manganese when the use of the latter is prevented by its penetration. However, dextran-magnetite appears not to have been previously used as a relaxation agent for biological systems.

We report the use of dextran-magnetite as a paramagnetic relaxation reagent applied to the measurement of erythrocyte water transport rates and the activation energy of the permeation process. In addition, the results are compared to measurements on the same blood samples using manganese as the relaxation reagent.

MATERIALS AND METHODS

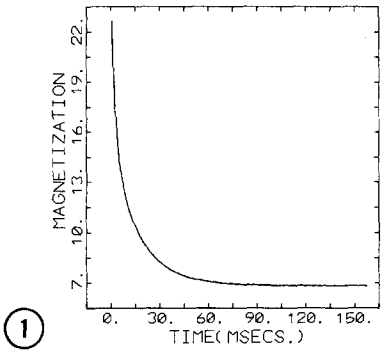
Blood was obtained from adult donors (6 men and 4 women) with no known hematological disorders, by venipuncture into Vacutainers with heparin used as anticoagulant. The final heparin concentration was 10 USP units/ml. All samples were used within 6 hours of collection - except for the timed run - and kept constantly mixed to prevent settling.

The procedure for the measurement of the mean residence time and the activation energy of water transport across the erythrocyte membrane using manganese as a paramagnetic relaxation reagent has been described in detail previously (1). The measurement of these values employing dextran-magnetite differs from this procedure only in the substitution of this iron-dextran complex for $MnCl_2$. Dextran-magnetite was obtained as a highly concentrated suspension from Meito-Sangyo (Nagoya, Japan). This suspension was diluted and centrifuged at 1000 g for 30 minutes to remove heavy aggregated particles. The dextran-magnetite was added directly to the blood to give an extracellular transverse relaxation rate of approximately 4 msecs. EDTA titration, using Cu-PAN as indicator (3,4), was employed to determine the concentration of dextran-magnetite in moles/liter iron. This titration was performed following the conversion of all Fe to Fe^{+3} by warming in 6 N HCl and after partial neutralization. The final concentration of dextran-magnetite in whole blood for mean residence time measurements was $1.3 \pm .2$ mM.

The mean residence time is determined by signal averaging of the output from a Spin-Lock CPS-2 pulsed spectrometer (Spin-Lock Ltd., Port Credit, Ontario, Canada) employing the Carr-Purcell-Meiboom-Gill pulse sequence (5,6), with only the tops of the peaks sampled by a home-built accessory. This magnetization decay is fit by non-linear regression analysis to the two-site exchange equations given by Hazelwood, et al. (7). The Arrhenius activation energy is determined by measurement of mean residence time over the temperature range 4°-37°C and linear regression analysis of the plot of $\ln(1/\tau_a)$ vs. 1/temperature.

RESULTS

The results of a typical CPMG magnetization decay employing dextran-magnetite as relaxation agent are shown in Figure 1. The figure includes the best-



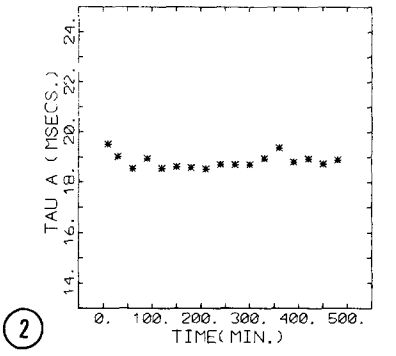


Figure 1. CPMG decay of whole blood with 1.3 mM dextran-magnetite with the best fit curve from nonlinear regression analysis.

Figure 2. Mean residence time vs. time after exposure to dextran-magnetite.

fit curve through the data and shows that the non-linear fit is indistinguishable from the experimental values.

The mean residence time (which is indirectly proportional to the diffusional permeability under conditions of constant volume and surface area) was measured over an 8 hour period after the exposure to dextran-magnetite. These results are shown in Figure 2. It is important to note that, although there is some variability over the period, there is no tendency toward an increase or decrease in τ_a. This constancy is important if measurements must be taken over a long time period. Independent measurements showed that dextran-magnetite did not increase the relaxation rate of intracellular constituents of the blood over a 16 hour period, whereas manganese had a noticeable effect under the same conditions.

Blood was collected from 10 donors and the mean residence time measured on these samples after exposure to either 1.7 mM $MnCl_2$ or 1.3 mM dextran-magnetite. The results shown in Table 1 are the average of 4 measurements made on each sample. It is evident from this table that there is no significant difference between measurements of mean residence time at 22°C using the two relaxation agents. The Student's t-test p value demonstrates this agreement.

Figure 3 shows a typical Arrhenius plot of $\ln(1/\tau_a)$ vs. reciprocal temperature along with the best fit straight line for both manganese and dextran-

TABLE 1

MEAN RESIDENCE TIME RESULTS

Relaxation Agent	No. of Samples	τ_a (msecs)
1.7 mM $MnCl_2$	10	21.836 ± .455 (SE)
1.3 mM Dextran-magnetite	10	21.354 ± .483 (SE)

P from Student's t-test = .492

magnetite as relaxation agent. This graph demonstrates that the curve can be well characterized by a single straight line. It also shows that the activation energies in the two cases are approximately the same. The results of activation energy measurements on blood from one donor with each relaxation agent is shown in Table 2. The activation energies measured in the two cases do not show a significant difference within experimental error.

DISCUSSION

The results of this study have demonstrated the applicability of dextran-magnetite as a relaxation agent for nuclear magnetic resonance measurements in biological systems. The results in Figure 1 and Table 1 indicate that dextran-magnetite can indeed achieve the desired decrease in the extracellular water

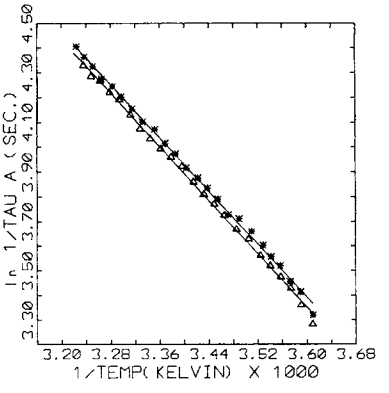


Figure 3. Arrhenius plot of ln ($1/\tau_a$) vs. reciprocal temperature. Relaxation reagents are denoted: 1.3 mM dextran-magnetite as asterisks, 1.7 mM $MnCl_2$ as triangles.

TABLE 2

ACTIVATION ENERGY MEASUREMENTS

Relaxation Agent	No. of Points	E_A (kcal/mole)
1.7 mM $MnCl_2$	23	5.38
1.3 mM Dextran-magnetite	24	5.33

transverse relaxation time constant which is needed to make the CPMG magnetization decay sensitive to the mean residence time (1). The results also demonstrate that the measurements are precise and reproducible - approximately 2.5% (SE) in this study. This reproducibility is important when the variation of permeability in certain disease states or after drug treatment are examined.

The dextran-magnetite complex has been estimated to have a diameter of 13 nm (8). Because of its large size, this species has been used to measure the accessibility of certain biological structures to extracellular macromolecules (9). Due to the fact that large macromolecules are not normally exchanged across the red cell membrane, it has been suggested that dextran-magnetite should not traverse the erythrocyte membrane barrier (3). The results shown in Figure 2 are in agreement with this idea. Any slow movement of the agent into the cell would decrease the intracellular T_2 and this alteration would be observed in the CPMG magnetization decay. Because this decay is constant over an 8 hour period, it is reasonable to conclude that the complex does not enter the cell.

The results in Table 1 show that there are no major differences between water transport measurements at 22°C using manganese or dextran-magnetite as the relaxation agent. Because the two species have notably different physical structures, the agreement of mean residence time values suggests that neither agent has a substantial effect on water transport rates.

The activation energy of water transport is an indicator of the state of the water transport system. Any change in net membrane charge or any significant

membrane protein conformational change may have noticeable effects on this value. Previous examination indicates that although the activation energy only varies slightly for a single individual, measurements on blood from different donors show a possible range of about 40%. All of the factors contributing to this difference are not yet known. Because the activation energy measurements using the different relaxation agents show no significant difference, it is suggested that these two reagents do not induce a noticeable perturbation on the erythrocyte membrane water transport system which is detectable by activation energy measurements.

The possibility of an increased propensity for rouleaux formation from exposure to dextran was also examined microscopically and there was no notable increase in the number of cell clusters after dextran exposure. This is not surprising due to the small dextran concentration to which the cells were exposed (10).

It should also be noted that there was some variation between donors in the concentration of dextran-magnetite needed to decrease the extracellular T_2 to a value less than 5 msecs. This variation is currently under examination and may contribute to understanding the nature of the complex in plasma.

Dextran-magnetite has been demonstrated to be a plausible substitute for manganese as a nuclear magnetic resonance paramagnetic relaxation agent. Its large size prevents appreciable movement across the red cell membrane so that experiments are made possible which could not be performed using manganese. The agreement of mean residence time measurements indicates that this relaxation agent does not induce a substantial change in the water transport rates and serves to help confirm measurements using manganese.

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