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The effects of the extracellular manganese concentration and variation of the interpulse delay time in the CPMG sequence on water exchange time across erythrocyte membranes

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There has been broad disagreement in the literature regarding the dependence of water exchange times (T_e) across erythrocyte membranes studied by the ^{1}H-NMR Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence on extracellular Mn^{2+} concentration. While some workers saw no change in T_e with Mn^{2+}, others reported a 35–50% decrease in T_e with this extracellular paramagnetic relaxation agent. We present ^{1}H-NMR evidence that a 30–50% change in T_e can be produced by interdependence of the interpulse delay time of the CPMG pulse sequence and the external Mn^{2+} concentration. Such a large dependency is interpreted in terms of the diffusional effect as a major source. However, it is shown experimentally that if a large number of refocusing π pulses are used, the observed transverse relaxation times are unaffected by Mn^{2+}. Under these conditions excellent agreement of T_e obtained in our study (13.0 ± 0.64 ms ($N = 36$) at 21°C) and that of 12.8 ± 3.6 ms at 20–23°C reported by the radiotracer method was found. Our findings suggest new and important implications for evaluating the previous reports of the ^{1}H-NMR CPMG method concerning the [Mn^{2+}] effect in the decrease of T_e, and provide conditions where studies of water transport across erythrocyte membranes using this magnetic resonance method can be used with confidence.

Introduction

The Carr-Purcell-Meiboom-Gill (CPMG) spin echo method has been frequently used in the study of water transport across human erythrocyte membranes [1–9]. This ^{1}H-T_2-NMR method, originally used by Conlon and Outhred [1,5], involves the use of paramagnetic manganese as a relaxation agent, usually with an extracellular Mn^{2+} concentration of 5–30 mM [2–4]. Fortunately, Mn^{2+} is relatively impermeable to the membrane and the effect due to the penetration of this cation into the cells is negligible for a short period [10–12].

In spite of widespread use, the dependence of water exchange time (T_e) across human erythrocyte membranes on extracellular paramagnetic Mn^{2+} concentrations is not well understood. The reported T_e values regarding this dependence are controversial among four research groups: in the early studies, two groups reported that the T_e values were independent of Mn^{2+} concentrations in the 10–30 mM range [5,7]; later on, however, two other groups claimed that a decrease in T_e could be 35% or larger as Mn^{2+} concentrations varied over this range [6,13–16].

Various interpretations for the reported T_e decrease have been suggested by the latter two groups, including: a fitting error due to the kinetic equation used [6]; Mn^{2+} penetration into the cells [13,15]; the effects of packed cell volume in the cell suspension [17]; as well as the plasma effects or possible Rouleaux formation [3,16–17].

Since the origin concerning the dependence of T_e on the concentrations of the external paramagnetic species remains unclear [2–4], we thought that it was desirable to clarify the literature. In addition, in the course of this study, a new fundamental observation, previously unreported, was made regarding the interdependence of T_e on the interpulse delay time (the time between the refocusing π pulse, referred to as τ) and the external Mn^{2+} concentration.

Methods and Materials

Principles. There are several ways to measure water transport. Most often used are either the radioactive

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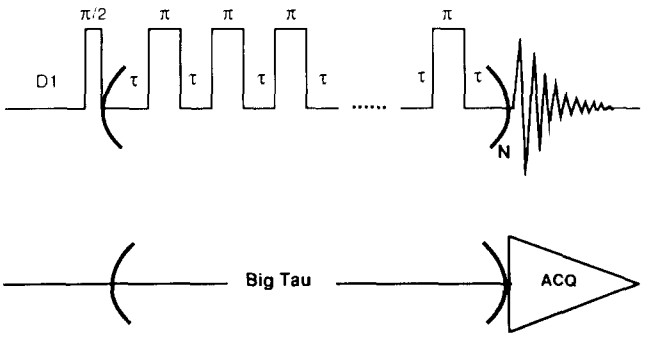

Fig. 1. Schematic drawing of the Carr-Purcell-Meiboom-Gill (CPMG) spin-echo pulse sequence used. D1, the first time delay, was set to at least 5-times the longest T_1 of the system under study. The interpulse delay time, τ, ranged from 0.2 to 1.0 ms; N is the number of π refocusing pulses. The $\pi/2$ pulse was found to be 8.2 μs for suspended cells. An echo is formed at the time of $2N\tau$ to retain coherence of the magnetization. After the desired evolution time, Big Tau (BT), the acquisition (ACQ) is initiated and the FID is collected. The peak intensity as a function of variable BT will provide the decay information for the calculation of T_2. See text.

tracer method [18,19], or the ^{1}H-T_2-NMR technique using the CPMG sequence in the presence of a membrane-impermeable paramagnetic relaxation agent. The principles and advantages of ^{1}H-T_2-NMR method in the study of water transport across the cell membranes have been extensively described elsewhere [1–9]. In the ^{1}H-T_2-NMR method a paramagnetic relaxation agent (usually Mn^{2+}) is added to the external side of the membrane. The CPMG pulse sequence (Fig. 1) is then used to obtain the transverse relaxation time, T_2, from which the water exchange time, T_e, can be determined. In this study we use a simplified expression to obtain T_e, based on the work of Conlon and Outhred:

$$1/T_e = 1/T_2 - 1/T_{2a} \quad (1)$$

where T_2 and T_{2a} are the observed transverse relaxation time of water-protons and the natural T_2 decay of intracellular water, respectively. The observed echo amplitudes were fit by using a mono-exponential decay expression with three parameters to obtain the observed T_2:

$$I_t = I_\infty + (I_0 - I_\infty)\, e^{-t/T_2} \quad (2)$$

where I_0, I_t, and I_∞, are the relative echo intensity measured by the CPMG sequence at time = 0, t, and infinity, respectively. As has been discussed by Craik et al. [20,21], the advantages of the three parameter fitting are more precision and smaller standard errors. Another fitting procedure has been suggested in which a biexponential analysis was needed [6,17], but the latest results reported by the same group suggested that if extracellular Mn^{2+} concentration is greater than a certain threshold value, such as about 4.4 mM [3,22], the differences in water exchange time due to mono- or biexponential fitting methods are extremely small. Under our experimental conditions (5–30 mM Mn^{2+}), a mono-exponential analysis, Eqn. 2, will thus suffice.

NMR parameters. The measurement of T_2 was performed using a Varian XL-200 MHz instrument with a programmed CPMG sequence (Varian, Publication number 87-146-006, Palo Alto, CA) in which four CPMG transients were used. The $\pi/2$ pulse length for suspended cells was calibrated as 8.2 μs and that of packed cells was 7.2 μs, both at 21°C. Interpulse delay times, τ, were varied from 0.2 to 1.0 ms which gives the rate in number of echoes/s ($1/2\ \tau$) as 2500 to 500 s^{-1}. The 0.2 ms spacing, which included the receiver dead time of 10 μs, is the upper limit of the instrument and this value corresponds to the maximum digitization rate of the ADC. The evolution time, Big Tau (BT) (Fig. 1), was set from about 0.6 T_2 to 4 T_2. It should be noted that if BT were set at less than 5 ms, a biphasic spectrum would have been detected due to mixing of decay signals between the intra- and extracellular water. Thus, BT was set so that at least 5-times the extracellular water T_2 passed before acquisition, and ranged from 6 to 36 ms for the suspended cells. D1 (Fig. 1) was set to 10 s for suspended cells and packed cells. It was determined that the intracellular water proton T_1 value of packed cells was 1.18 ± 0.05 s ($N = 3$).

Shimming. The field homogeneity was checked by detecting traces of protons in the D_2O solvent, with $\Delta\nu_{1/2}$ not more than 1.5 Hz, which remained unshifted for several hours. However, no spin or lock was used for either shimming or measuring for T_2 values. Rather, shimming was performed based on an oscilloscope for a FID [23].

Preparation of suspended cells for measuring T_2. This procedure has been previously described. Blood was collected by venipuncture from healthy individuals aged 19 to 30 in preheparanized vacuum tubes and placed on ice. The whole blood was processed within 15 min after the collection. Fresh cells were washed three times with 5 mM Hepes (pH = 7.4), 150 mM NaCl (buffer A) and centrifuged at 500 × g for 5 min. Washed cells were resuspended in the buffer A containing 0.5% human serum albumin (HSA) and the hematocrit was adjusted to 45%. The suspension was stored at 2–4°C until use, but in no case longer than 8 h. All hematocrits were determined by the standard microhematocrit technique (Clay-Adams Readacrit) at room temperature.

The cold cell suspension was allowed to stand at room temperature for a few minutes and then the NMR sample was prepared by mixing the 0.3 ml cell suspension with 0.6 ml isotonic (300 mosM) of stock Mn^{2+} solution at various concentrations. The mixing process was performed in a small test tube by gently inverting the suspension several times and then the mixture (which was 15% hematocrit) was carefully pipetted into a 5 mm o.d. NMR tube. The T_2 measurement, which was per-

formed at room temperature, was completed within 5 min after mixing. For the samples measured at other temperatures, an additional 8–10 min was given to allow the samples to reach thermal equilibrium. The temperature was controlled in the NMR probe; the error in temperature was never greater than ± 0.2°C.

Many researchers often mix isotonic Mn^{2+} solution with blood based on a certain volume ratio for convenience: about two ml isotonic Mn^{2+} solution to one ml blood was recommended [5]. However, among many reports the free extracellular Mn^{2+} concentration had not been explicitly specified [3,8]. In order to control experimental variables and observe the Mn^{2+} effect, we define the final free extracellular Mn^{2+} concentration, $[Mn]_f$, as follows:

$$[Mn]_f = \frac{[Mn]'}{V_{eff}} = \frac{2[Mn]'}{V_{tot} - V_{ht}} \quad (3)$$

where $[Mn]'$ is the isotonic stock Mn^{2+} concentration before mixing. The V_{tot} is the final volume after mixing and V_{ht} is the volume of the packed cells themselves. Thus, $V_{eff} = V_{tot} - V_{ht}$ is equal to the total volume for the suspension doped with Mn^{2+}, minus the cells own volume. Equation 3 emphasizes the free extracellular volume which was actually available to Mn^{2+}, and such a correction is particularly pronounced if the hematocrit is high. The detailed results are given in Table I.

Preparation of the packed cells for measuring T_{2a}. The cells, which were washed three times, were resuspended in buffer A without adding HSA. 2 ml of 45% hematocrit cell suspension were directly pipetted into a 5 mm o.d. NMR tube and this tube was centrifuged at $1000 \times g$ for 30 min at 4°C. The remaining top buffer-saline layer, which was about 1.02 to 1.06 ml, was removed. From a knowledge of the hematocrit before centrifugation and the volume of aqueous layer removed afterwards, the hematocrit, which ranged from 92–96%, could be estimated without further calibration. The estimation was based on $H_1V_1 = H_2V_2$, where H and V are the hematocrits and volume of the suspension, and the subscripts 1 and 2 are the referred quantities before and after the centrifugation, respectively. In this case, $H_1 = 0.45$, $V_1 = 2.0$ ml, and if $V_2 = 0.94$ ml, then H_2 is readily calculated to be 0.96, or a hematocrit of 96%. It should be noted that the hematocrit of packed cells depends on the centrifugation time and the relative centrifugal force used. To judge the accuracy of our results, we noted that under similar conditions ($1000 \times g$ for 30 min) an average hematocrit of 97%, which was not measured in the NMR tube, has been reported [24].

Chemicals. 4-(2-Hydroxyethyl)-2-piperazineethanesulfonic acid, Hepes (99%) and $MnCl_2 \cdot 4H_2O$ (99.99%)

TABLE I

Isotonic stock manganese doping solutions with the correction of cell volume [a]

$[Mn^{2+}]_f$ (mM)	$[Mn^{2+}]'$/[NaCl] (mM)	μ [b] (mM)	V_{eff} (ml)
5	6.38/140	160	0.765
10	12.8/131	169	0.765
20	25.5/112	188	0.765
30	38.2/92.7	208	0.765

[a] The 0.3 ml cell suspension with hematocrit of 45% was mixed with 0.6 ml of stock Mn^{2+} solution, which was made isotonic with NaCl (300 mosM), to give a total volume of 0.9 ml minus 0.135 ml of the cell's own volume, yielding an effective free volume of 0.765 ml in all cases. The final hematocrit was 15% and the final Mn^{2+} concentrations were as indicated. See Eqn. 3.

[b] μ, ionic strength.

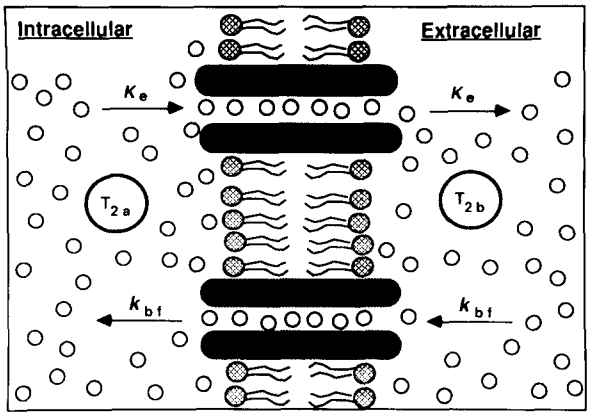

Fig. 2. Illustration of water transport across erythrocyte membrane between the intra- and extra-cellular compartments. Water molecules are represented by circles. T_{2a} and T_{2b} are the T_2 decays of intra- and extra-cellular water protons, and $k_e(k_e = 1/T_e)$ and k_{bf} are the rate constants of water transport across the membrane and water back flux, respectively. The putative protein water transporters are shown by ovals in the membrane lipid bilayer.

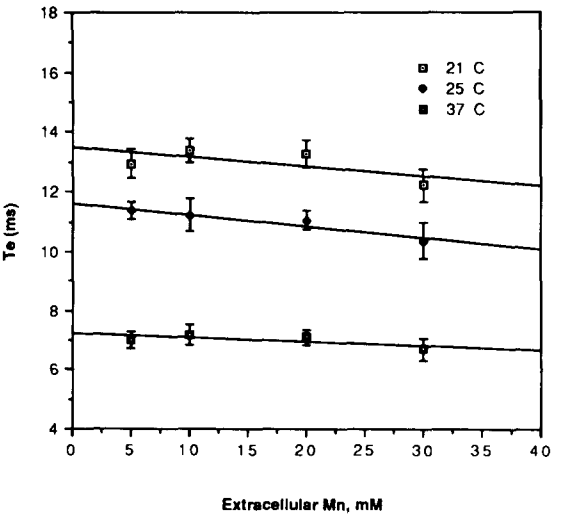

Fig. 3. The water exchange time, T_e, measured at various extracellular manganese concentrations and temperatures, as indicated in the figure. The bars are the standard deviation ($N \geq 9$). The hematocrit of the suspended cells is 15 ± 1%, HSA = 0.17%, (pH = 7.4) $\pm$ 0.1. The interpulse delay was 0.2 ms.

TABLE II

The observed transverse relaxation times at various extracellular manganese concentrations and temperatures [a]

Cell forms/ $[Mn^{2+}]_f$	21°C	N	25°C	N	37°C	N
Packed cells	73.5(2.23)	13	77.2(3.30)	7	90.3(2.3)	11
Suspd. cells/ 5 mM	10.9(0.36)	8	9.93(0.22)	10	6.50(0.24)	11
Suspd. cells/ 10 mM	11.3(0.29)	11	9.81(0.44)	16	6.66(0.30)	13
Suspd. cells/ 20 mM	11.2(0.32)	10	9.66(0.25)	12	6.58(0.22)	18
Suspd. cells/ 30 mM	10.5(0.39)	7	9.12(0.46)	10	6.29(0.15)	9

[a] The interpulse delay time was set to 0.2 ms and the observed relaxation times have a unit of ms. N is the number of samples studied. Standard deviations are given in parentheses.

were from Aldrich. Human serum albumin (HSA) was from Sigma (fatty acid content less than 0.005%).

Results

Water permeates the plasma membrane of the erythrocytes from both directions by two fundamental processes. In the dominant process, a protein facilitated water transporter, whose identity remains unknown [25–27], serves to move water across the membrane (Fig. 2). In the minor process, simple diffusion through the membrane is thought to occur.

We have used ^{1}H-NMR to measure water transport. Mn^{2+} was used as a relaxation agent that is reportedly membrane impermeable for several hours [1,9]. As can be seen in Table II, at different temperatures (21°C, 25°C and 37°C), the variations in the respective observed T_2 from low Mn^{2+} (5 mM) to high Mn^{2+} (30 mM) concentration were not more than 10% when τ is 0.2 ms. Water exchange times, calculated from the data in Table II based on Eqn. 1, are given in Fig. 3, which shows that T_e is approx. constant over the concentration range of 5–30 mM Mn^{2+} when τ equals 0.2 ms.

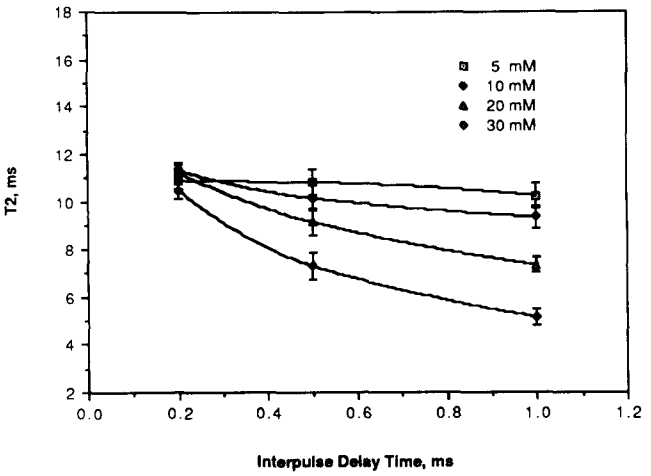

Fig. 4. The interdependence of observed T_2 for the suspended cells on the interpulse delay times and on various extracellular manganese concentrations measured by the CPMG sequence at 21°C. The bars are the standard deviation ($N \geq 6$).

As can be seen in Fig. 4, there was an obvious interdependence of the T_2 values on τ and various extracellular Mn^{2+} concentrations. As noted above, at $\tau = 0.2$ ms, there is little dependence of the T_2 values on varying Mn^{2+}; however, as the extracellular Mn^{2+} concentration increased, the observed T_2 is sensitive to the interpulse delay. When τ is 0.5 ms, the observed T_2 decreases by about 32%; the same pattern was found when τ is 1.0 ms, with a further decrease of about 50%. Interestingly, such a decrease over this percentage range is about the values reported by the two groups who suggested the effects of Mn^{2+} penetration and/or possible fitting errors were responsible for this observation [6,13–16].

Discussion

Surprisingly, in many reports regarding the effects of water exchange time on various Mn^{2+} concentrations studied by the CPMG sequence, none of these investigators has systematically examined the dependence of water exchange time on the interpulse delay time. Rather, various papers either report τ values ranging from 0.1 to 5.0 ms [3,4,8,28–29], or more commonly, the value is unspecified [5,9,10,14–15]. Thus, we thought it was important to assess the dependence of T_2 on the interpulse delay while trying to clear up some controversial points in the literature with respect to the effect of external Mn^{2+} concentration on T_e.

This lack of attention may have resulted from the observation [30] that in a homogeneous system lacking chemical exchange, the particular choice of the interpulse delay has no significant effect on the value of the observed transverse relaxation time. However, as pointed out by Luz and Meiboom [31], the observed T_2 is dependent on the interpulse delay if chemical exchange or other processes, such as diffusion [32,33], are involved. The large interdependence of the observed T_2 on τ and Mn^{2+} concentration (up to 30–50%) found in this study is the first such observation in red blood cells.

Many efforts have been made in trying to understand biological water T_2 in complex heterogeneous system [34–37]. However, in dealing with time-dependent magnetization decay for a heterogeneous system without linear and uniform field gradients, the explicit expression is not available [37–39]. Nevertheless, qualitatively, it is well known that the decrease in T_2 is due to the effect of molecular diffusion through a fluctuating local magnetic field [32,33]. In this case, the Carr and Purcell's echo amplitude, $A(t)$, is given by,

$$A(t) = A_0 \exp\{-(t/T_2) - [\gamma^2 D G^2 t^3/(12n^2)]\} \quad (4)$$

where: γ = the magnetogyric ratio; D = the diffusion constant; G = the field gradient(s); and n = the number of refocusing π pulses. The second term in the exponential in Eqn. 4 is generally known as the diffusion term. In the CPMG sequence, for the nth echo detected at time $t = 2n\ \tau$, $A(t)$ can be expressed as:

$$A(2n\tau) = A_0 \exp[-(2n\tau/T_2) - (2/3)n\gamma^2 DG^2\tau^3] \quad (5)$$

With these concepts in mind, the results in Fig. 4 suggest that the nonlinear dependence of T_2 on τ at different extracellular Mn^{2+} concentrations may result from the term $G^2\tau^3$. At fixed τ, the higher the Mn^{2+} concentration, the larger G will be, which presumably causes the decrease in the apparent T_2, and therefore, T_e (see Fig. 4). At fixed Mn^{2+}, the results of the two extremes in Mn^{2+} concentration are different: (1) if $Mn^{2+} = 5$ mM, there is essentially not much change in T_2 with τ, i.e., less than 10%; and (2) for 30 mM Mn^{2+}, the decrease in T_2 can be up to 50% as τ is increased (Fig. 4). Thus, in the latter case, we suggest that molecular diffusion may cause the decrease in the apparent T_2: high Mn^{2+} enlarges the field gradient, while the longer τ further enhances the diffusional contribution to the decay rate of M_y. The variation with the cube of τ exacerbates this effect and suggests that this parameter can no longer be ignored, especially at high Mn^{2+} concentration. It is noticed that in the first case with low Mn^{2+} (5 mM) the apparent T_2 values do not vary much with τ. This observation suggests that the dependence of transverse relaxation time on τ is not due to instrumental problems [40,41], baseline errors [42] or fitting artifacts [6,17].

Regarding using quite low Mn^{2+} concentration (not more than 2 mM) in the study of water transport, we feel that another problem may result: extracellular water may not be magnetically well relaxed; consequently, the contribution of these unrelaxed water protons to the subsequent intensities may dramatically alter the value of observed T_2. This caution is supported by the following reports in the literature (Refs. 6 and 10): when the range of Mn^{2+} employed was between 1.7 and 2.0 mM, the reported T_e values are 21 ms (23°C) and 16.4 ms (37°C), respectively. These values are far beyond the mean reported using the tracer method, which averages 12.8 ± 3.6 ms at 20–23°C [18,19,43–45]. In contrast, our result, 13.0 ± 0.64 ($N = 36$), calculated by Eqn. 1 from the data in Table II at 21°C and $\tau = 0.2$ ms, is in good agreement with the mean obtained by the tracer method.

The possible effect due to the Mn^{2+} penetration into the cell as claimed by some [13–15] should be addressed. Recall that the ^{1}H-T_2 doping method ideally requires Mn^{2+} not to permeate into the interior of the cells [1–9]. Fortunately, this is true for short periods, since Mn^{2+} uptake is very slow [11–12]. Atomic absorption studies [9,10] suggested that no significant differences in intracellular Mn^{2+} were found between controls and the cells treated with Mn^{2+}, up to the effective $[Mn]_f$ concentration of 20 mM. In our experiments, which were normally completed in 5–10 min at room temperature after mixing, the measured relaxation time is reproducible for at least 1 h, even for the upper limits of 30 mM Mn^{2+} concentration, as long as the same τ is used for a repeated measurement. This observation clearly supports the view that Mn^{2+} uptake is the least factor which can be invoked to explain the dependence of T_e on the Mn^{2+} concentrations [5,13]. The close agreement between our findings and the findings using radiotracer method (which of course does not employ Mn^{2+}) further supports the suggestion that the effects due to Mn^{2+} penetration will not play a significant role under our experimental conditions.

One of the aims of this study was to more precisely define the best experimental conditions for performing water transport studies employing ^{1}H-T_2-NMR methods and extracellular Mn^{2+}. At $\tau = 0.2$ ms, for various extracellular Mn^{2+} concentrations studied, the observed T_2 approaches a limiting value (Fig. 4) and we suggest that this value of the interpulse delay is the one to use for the investigation of water exchange time.

Conclusion

(1) Our results from systematic examinations of the dependence of T_e on the external Mn^{2+} suggested that if a proper τ is used, no effects of the doping agent were observed. Nevertheless, to minimize any other possible external interferences, high extracellular Mn^{2+} concentrations (not less than 20 mM) are not recommended in the ^{1}H-T_2-NMR doping method, and neither are concentrations less than 5 mM.

(2) The origin of 30–50% interdependence of T_2 on the various Mn^{2+} and the interpulse delay time may possibly be attributed to the effects of diffusion that cause artifacts regarding the resulting water exchange time. Further study of such effects is currently in progress in our laboratory. However, the problem can be avoided experimentally by choosing a small τ (approx. 0.2 ms).

(3) The results of water exchange time reported in this study were in excellent agreement with the radioactive tracer method, and thus, the ^{1}H-T_2-NMR doping method can be viewed as a simple and reliable technique in the study of water transport across human erythrocyte membrane in health and disease. Such applications are currently in progress in our laboratory.

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References

1 Conlon, T. and Outhred, R. (1972) Biochim. Biophys. Acta 288, 354–361.
2 Benga, G. (1988) Prog. Biophys. Mol. Biol. 51, 193–245.
3 Herbst, M.D. and Goldstein, J.H. (1989) Am. J. Physiol. 256, C1097–C1104.
4 Chao, L.N., Shaffer, L.M. and Butterfield, D.A. (1989) Prog. Clin. Biol. Res. 292, 41–45.
5 Conlon, T. and Outhred, R. (1978) Biochim. Biophys. Acta 511, 408–418.
6 Pirkle, J.L., Ashley, D.L. and Goldstein, J.H. (1979) Biophys. J. 25, 389–406.
7 Chien, D.Y. and Macey, R.I. (1977) Biochim. Biophys. Acta 464, 45–52.
8 Fabry, M.E. and Eisenstadt, M. (1978) J. Membr. Biol. 42, 375–398.
9 Morariu, V.V. and Benga, G. (1977) Biochim. Biophys. Acta 469, 301–310.
10 Fung, L.W.M., Narashimhan, C., Lu, H.Z. and Westerman, M.P. (1988) Biochim. Biophys. Acta 982, 167–172.
11 Weed, R.I. and Rothstein, A. (1960) J. Gen. Physiol. 44, 301–314.
12 Serbu, A.M., Marian, A., Pop, V.I., Borza, V., Benga, I. and Benga, G. (1986) Muscle Nerve 9, 243–247.
13 Morariu, V.V., Ionescu, M.S., Frangopol, M., Grosescu, R., Lupu, M. and Frangopol, P.T. (1986) Biochim. Biophys. Acta 860, 155–158.
14 Morariu, V.V., Pop, V.I., Popescu, O. and Benga, G. (1981) J. Membr. Biol. 62, 1–5.
15 Morariu, V.V., Ionescu, M.S., Frangopol, M., Grosescu, R., Lupu, M. and Frangopol, P.T. (1985) Biochim. Biophys. Acta 815, 189–195.
16 Herbst, M.D. and Goldstein, J.H. (1984) in Kinetics of Aggregation and Gelation (Family, F. and Landau, D.P., eds.), pp. 53–55, Elsevier, Amsterdam.
17 Herbst, M.D. and Goldstein, J.H. (1984) J. Magn. Reson. 60, 299–306.
18 Paganelli, C.V. and Solomon, A.K. (1957) J. Gen. Physiol. 41, 259–277.
19 Brahm, J. (1982) J. Gen. Physiol. 79, 791–819.
20 Craik, D.J. and Levy, G.C. (1984) in Topics in Carbon-13 NMR Spectroscopy, Vol. 4, p. 241, Wiley, New York.
21 Dybowski, C. and Lichter, R.L. (eds.), (1987) NMR Spectroscopy Techniques, p. 81, Marcel Dekker, New York.
22 Caines, G.H., Morgan, C.F., Long, R.C. and Goldstein, J.H. (1987) Magn. Reson. Med. 5, 269–277.
23 Derome, A.E. (1987) in Modern NMR Techniques for Chemistry Research, p. 49, Pergamon, Oxford.
24 Ponder, E. (1948) in Hemolysis and Related Phenomena, Grune & Stratton, p. 76, New York.
25 Macey, R.I. and Brahm, J. (1989) in Water Transport in Biological Systems, (Benga, G., Ed.), 25–39, CRC Press, Boca Raton, FL.
26 Ojicius, D.M. and Solomon, A.K. (1988) Biochim. Biophys. Acta 942, 73–82.
27 Jenning, M.L. (1989) Annu. Rev. Biophys. Chem. 18, 397–430.
28 Lahajnar, G., Macek, P., Anzur, M. and Lebez, D. (1986) Period. Biol. 88, 150–151.
29 Small, W.C. and Goldstein, J.H. (1981) Biochim. Biophys. Acta 640, 430–438.
30 Carver, J.P. and Richards, R.E. (1972) J. Magn. Reson. 6, 89–105.
31 Luz, Z. and Meiboom, S. (1963) J. Chem. Phys. 39, 366–370.
32 Carr, H.Y. and Purcell, E.M. (1954) Phys. Rev. 94, 630–638.
33 Meiboom, S. and Gill, D. (1958) Rev. Sci. Instrum. 29, 688–691.
34 Santry, G.E., Henkelman, R.M. and Bronskill, M.J. (1988) J. Magn. Reson. 79, 28–44.
35 Glasel, J.A. and Lee, K.H. (1974) J. Am. Chem. Soc. 96, 970–978.
36 Grucker, D., Mauss, Y., Steibel, J., Ponlet, P. and Chambron, J. (1986) Magn. Reson. Imag. 4, 441–443.
37 Kurland, R.J. and Ngo, Q.H. (1986) Magn. Reson. Med. 3, 425–431.
38 Ando, I. and Webb, G.A. (1983) in Theory of NMR Parameters, Ch. 1, Academic Press, New York.
39 Slichter, C.P. (1980) in Principles of Magnetic Resonance, 2nd edn., p. 182, Springer, New York.
40 Putte, K.V. (1970) J. Magn. Reson. 2, 174–180.
41 Bull, T.E. (1974) Rev. Sci. Instrum. 45, 232–242.
42 Hughes, D.G. (1977) J. Magn. Reson. 26, 481–489.
43 Viera, F.L., Sha'Afi, R.I. and Solomon, A.K. (1970) J. Gen. Physiol. 55, 451–466.
44 Osberghaus, V., Schonert, H. and Deuticke, B. (1982) J. Membr. Biol. 68, 29–35.
45 Barton, T.C. and Brown, D.A.J. (1976) J. Gen. Physiol. 428, 304–311.