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Effects of temperature on water diffusion in human erythrocytes and ghosts – nuclear magnetic resonance studies

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The temperature-dependence of water diffusion across human erythrocyte membrane was studied on isolated erythrocytes and resealed ghosts by a doping nuclear magnetic resonance technique. The conclusions are the following: (1) The storage of suspended erythrocytes at 2°C up to 24 h or at 37°C for 30 min did not change the water exchange time significantly, even if Mn^{2+} was present in the medium. This indicates that no significant penetration of Mn^{2+} is taking place under such conditions. (2) In case of cells previously incubated at 37°C for longer than 30 min with concentrations of *p*-chloromercuribenzene sulfonate (PCMBS) greater than 0.5 mM, the water-exchange time gradually decreased if the cells were stored in the presence of Mn^{2+} for more than 10 min at 37°C. (3) When the Arrhenius plot of the water-exchange time was calculated on the basis of measurements performed in such a way as to avoid a prolonged exposure of erythrocytes to Mn^{2+} no discontinuity occurred, regardless of the treatment with PCMBS. (4) No significant differences between erythrocytes and resealed ghosts regarding their permeability and the activation energy of water diffusion ($E_{a,d}$) were noticed. The mean value of $E_{a,d}$ obtained on erythrocytes from 35 donors was 24.5 kJ/mol. (5) The value of $E_{a,d}$ increased after treatment with PCMBS, in parallel with the percentage inhibition of water diffusion. A mean value of 41.3 kJ/mol was obtained for $E_{a,d}$ of erythrocytes incubated with 1 mM PCMBS for 60 min at 37°C and 28.3 kJ/mol for ghosts incubated with 0.1 mM PCMBS for 15 min, the values of inhibition being 46% and 21%, respectively.

Introduction

There are several reasons for studying the temperature-dependence of water permeability in various cells. The most important reason is to gain insight into the mechanism of water transport. Additionally, such studies can be useful in comparing the permeability of different cells or tissues measured at different temperatures. Furthermore, to interpret most transport studies with tissues and organs it is necessary to make assumptions about the permeability characteristics of the cell

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membrane based on data available for isolated cells. However, most of the studies of the permeability characteristics of isolated cells have been conducted at 20 °C, while in vivo organ studies are conducted at 37 °C, the average body temperature for mammals [1].

Because of its simple structure, lacking internal membrane systems, the red blood cell has been a favorite object for investigating water permeability (for recent reviews see Refs. 2–5). Studies on the effects of temperature on water permeability can contribute to our understanding of molecular mechanisms of water transport in red blood cells. The apparent activation energy of water permeation across fluid lipid bilayers is around 40 kJ/mol [6,7]. The activation energy of the water exchange through erythrocyte membrane is much lower [3,8–10]. This shows that the mechanisms of water permeation which operate in the case of the erythrocyte are different from those in lipid bilayers, i.e., that a major role in red blood cell water transport is played by hydrophilic channels in proteins.

On the other hand, there are conflicting data regarding the occurrence of significant deflections of the activation energy at a certain temperature, i.e., of 'break' points or discontinuities in the Arrhenius plots of water-exchange parameters. While in some investigations a linear temperature-dependence of water exchange was found, other studies revealed breaks in the Arrhenius plot of water exchange in human red blood cells measured by the doping NMR method [12,13]. Morariu et al. [14,15] have recently shown that apparent thermal transitions are expected to occur when the NMR manganese doping technique is used because of a shift from intermediate exchange rates, at which water diffusion through the membrane is dominant, to either fast or slow exchange rates, at which proton relaxation is the controlling process. This conclusion might cast some doubt on the usefulness of NMR method for measuring water diffusion, particularly for those unfamiliar with the technique.

The aims of our paper are: (a) to clarify the conflicting reports regarding the discontinuities in the Arrhenius plot of water-exchange parameters; (b) to determine the values of the activation energy of water diffusion ($E_{a,d}$) across the red blood cell membrane in a large number of subjects; (c) to compare the values of the activation energy of water diffusion in erythrocytes and ghosts, and also under the influence of sulfhydryl group (SH) reagents.

Materials and Methods

Blood sample preparations

Human blood was obtained by venipuncture in heparinized tubes. The donors were healthy male or female subjects, 10–51 years old. The erythrocytes were isolated by centrifugation, and washed three times in 166 mM NaCl. For the preparation of resealed (pink) ghosts, the procedure of Bodemann and Passow, as described by Wood and Passow [16] has been used.

The incubations of erythrocytes or ghosts with SH reagents were performed after suspending the cells in medium A (150 mM NaCl/5.5 mM glucose/5 mM Hepes (pH 7.4) at a cytocrit of 25% in the case of treatment with *N*-ethylmaleimide and at a cytocrit of 10% in the case of treatment with PCMBS. The final concentrations of SH reagents and the durations of incubation are indicated in the legend to Fig. 1 and in the tables. After incubation, three washings of the cells in medium A, each followed by a centrifugation, were performed to remove the reagent. Finally, the erythrocyte or the ghosts were suspended in medium A supplemented with 0.5% bovine serum albumin at a cytocrit of 50%.

NMR measurements

Samples for NMR measurements were prepared by carefully mixing 0.2 ml erythrocyte or ghost suspensions and 0.1 ml doping solution (40 mM $MnCl_2$/100 mM NaCl). The water proton relaxation time of the cells (T'_{2a}) was evaluated by the spin-echo method [17], as previously described [11,18]. T'_{2a} is dominated by the exchange process through erythrocyte membrane and is related to the water diffusion exchange time (T_e) by the equation [17]:

$$\frac{1}{T_e} = \frac{1}{T'_{2a}} - \frac{1}{T_{2i}} \qquad (1)$$

where T_{2i} is the traverse relaxation time of the cell interior. T_{2i} was measured by the 90–180° method using the Carr-Purcell-Meibom-Gill sequence [19], on packed cells or ghosts from which the supernatant, with no added Mn^{2+}, had been removed by centrifugation at $50000 \times g$ for 60 min.

The membrane permeability for water diffusion, P, is related to $1/T_e$, the cell water volume, V, and the cell surface area, A, by:

$$P = \frac{V}{A} \cdot \frac{1}{T_e} \tag{2}$$

Since different authors have used different values of V and A, in order to compare our results with previous ones, we have used two sets of values. On one hand, we have taken a value of 65 μm^3 for the intracellular solvent volume of erythrocytes and 86 μm^3 for that of resealed ghosts and a value of $1.42 \cdot 10^{-6}$ cm^2 for the membrane area after Brahm [10]. These give V/A ratios of $4.58 \cdot 10^{-5}$ and $6.06 \cdot 10^{-5}$ cm for erythrocytes and ghosts, respectively. On the other hand, we have used a slightly higher V/A ratio, after Dix and Solomon [20], e.g., $5.33 \cdot 10^{-5}$ cm for erythrocytes and the corresponding value for ghosts.

The inhibition of water diffusion across human red blood cell membranes was calculated assuming that the permeability coefficient is inversely related to T'_{2a}, according to the formula:

$$\% \text{ inhibition} = \frac{\frac{1}{T'_{2a}\text{ (control)}} - \frac{1}{T'_{2a}\text{ (sample)}}}{\frac{1}{T'_{2a}\text{ (control)}}} \times 100 \tag{3}$$

The NMR measurements were performed with an AREMI-78 spectrometer (manufactured by the Institute of Physics and Nuclear Engineering Bucharest-Măgurele, Romania) at a frequency of 25 MHz. The temperature was controlled to ±0.2°C by air flow over an electrical resistance using the variable temperature unit attached to the spectrometer. The actual temperature in the sample was measured with a thermocouple connected to a microprocessor thermometer (Comark Electronics Limited, Rustington, Littlehampton, U.K.).

Other procedures

The hemoglobin content of resealed ghosts (reduced to 4–7% of that of erythrocytes) was estimated spectrophotometrically [21]. The calculations of the correlation coefficients of the lines obtained with the sets of data points in the Arrhenius plots have been performed with an HP-41 CV computer (Hewlett-Packard, U.S.A.).

Results

Since the determination of activation energy of water diffusion involves experiments which require about 2 h, we have tested the effect of storage conditions on T'_{2a} values. Washed erythrocytes suspended in the medium A were stored at various temperatures, in the presence or absence of Mn^{2+}. The storage of suspended erythrocytes at 2°C up to 24 h did not change the T'_{2a} significantly, even if Mn^{2+} was present in the medium (Table I). Brief storage (for about 30 min) at 20°C or 37°C also had a negligible effect on T'_{2a} (Fig. 1).

In case of cells treated with PCMBS, washed and suspended in medium A and then stored at 2°C for up 2 h the T'_{2a} did not change significantly (Table I). However, the effects of brief storage at 20°C or 37°C were dependent on the way in which the previous treatment with PCMBS was conducted. In case of erythrocytes incubated for a short time (15–30 min) with low concentrations (0.2–0.5 mM) of PCMBS, the T'_{2a} remained constant for a storage time of about 30 min at 37°C (Fig. 1). For erythrocytes incubated with concentrations of PCMBS greater than 0.5 mM and for times longer than 30 min, the T'_{2a} remained constant only for about 10 min at 37°C (Fig. 1) and then gradually decreased. This effect was dealt with by making the measurement quickly enough to obviate it. In case of PCMBS-treated cells, a sample prepared from erythrocytes suspended in medium A and doped with Mn^{2+} was used for a single measurement of T'_{2a}. As this can be performed in 3–4 min, no significant penetration of manganese takes place in this short time.

The origin of discontinuities in the Arrhenius plots of water-exchange time

It is clear from the experiments described above

TABLE I

THE EFFECT OF STORAGE CONDITIONS ON T'_{2a} OF ERYTHROCYTES

Samples for NMR measurements were prepared as described under Materials and Methods. After a first measurement of T'_{2a}, the samples were stored at 2 °C for 2 or 24 h and the measurements were repeated.

Donor No.	Sample	Storage conditions at 2 °C		T'_{2a} (ms)
		h	Mn^{2+} in medium	
1	control erythrocytes	0		7.46
		2	+	7.51
2	control erythrocytes	0		6.61
		2	−	6.82
		2	+	6.24
		24	−	7.05
		24	+	7.10
3	control erythrocytes	0		7.15
		2	+	7.17
	erythrocytes treated with:			
	0.2 mM PCMBS for 15 min	0		10.02
		2	+	10.11
	0.2 mM PCMBS for 30 min	0		12.21
		2	+	11.30
	0.5 mM PCMBS for 15 min	0		11.70
		2	+	11.08
4	control erythrocytes	0		7.55
		2	+	7.79
		24	+	7.38
	erythrocytes treated with:			
	1 mM PCMBS for 60 min	0		14.01
		2	+	14.70

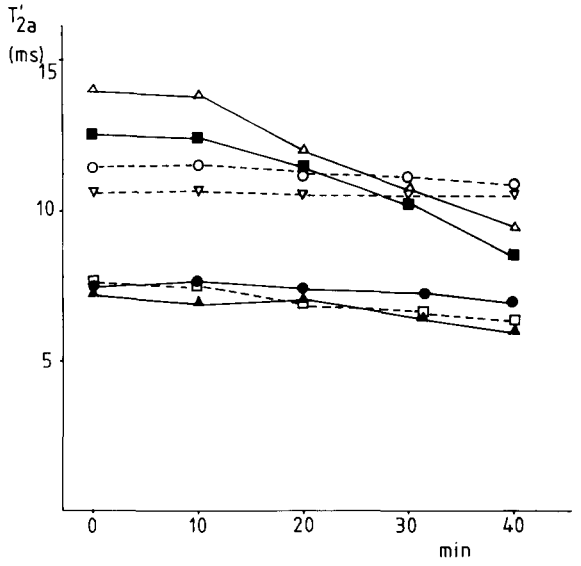

Fig. 1. The change of T'_{2a} for erythrocytes stored at 37 °C in the presence of 13.3 mM Mn^{2+}. Control erythrocytes were suspended in an equal volume of medium A, doped with manganese, stored at 2 °C for 0 (●———●), 2 (□— — —□) or 24 h (▲———▲), then brought to 37 °C and the T'_{2a} was measured at the times indicated. For samples marked by the symbols: △, ▽, ○, ■, the erythrocytes were diluted to a hematocrit of 10% with medium A containing PCMBS to give final concentrations of 0.2 mM (▽), 0.5 mM (○, ■) and 1 mM (△) and incubated at 37 °C for 15 min (○), 30 min (▽) or 60 min (△, ■). After incubation and three washings, the erythrocytes were suspended in an equal volume of medium A and doped with manganese, and the T'_{2a} was measured at 37 °C at the times indicated.

that artifacts expressed in a decrease of T_e as a result of manganese penetration can occur if the cells are incubated at temperatures above 20 °C for longer than 30 min. Consequently, we studied

the temperature-dependence of T_e in such a way as to avoid a prolonged exposure of erythrocytes to Mn^{2+}, especially at high temperatures and after incubation with PCMBS.

In case of cells that had not been incubated with PCMBS, three samples were prepared from the same batch of blood. The first was run at temperatures from 5 °C to 30 °C, the second from 30 to 37 °C and the third from 37 to 42 °C. In case of erythrocytes treated with PCMBS, after incubation and washing, several samples were prepared from erythrocytes of the same donor. Each sample was used for determination of T'_{2a} at a single value of temperature. A similar approach was used in case of resealed ghosts.

When the Arrhenius plot of T_e was calculated on the basis of measurements performed as described above, no discontinuity occurred. In contrast, when a single sample of cells or ghosts was run between 5 and 42 °C and then back to lower temperatures, a discontinuity in the Arrhenius plot could be seen around 25 °C, for the measurements performed from high to low temperatures, i.e., on samples that had been exposed to Mn^{2+} for a relatively long time at temperatures above 20 °C (Fig. 2). Under such circumstances a penetration of Mn^{2+} in the cells probably occurred. We conclude that the breaks in the Arrhenius plot of water-exchange time originate from lower values of T'_{2a} caused by a penetration of Mn^{2+} in the cells incubated at temperatures above 20 °C for a relatively long time.

In case of PCMBS-treated cells or ghosts, when each sample was used for a single NMR measurement the Arrhenius plot was always linear.

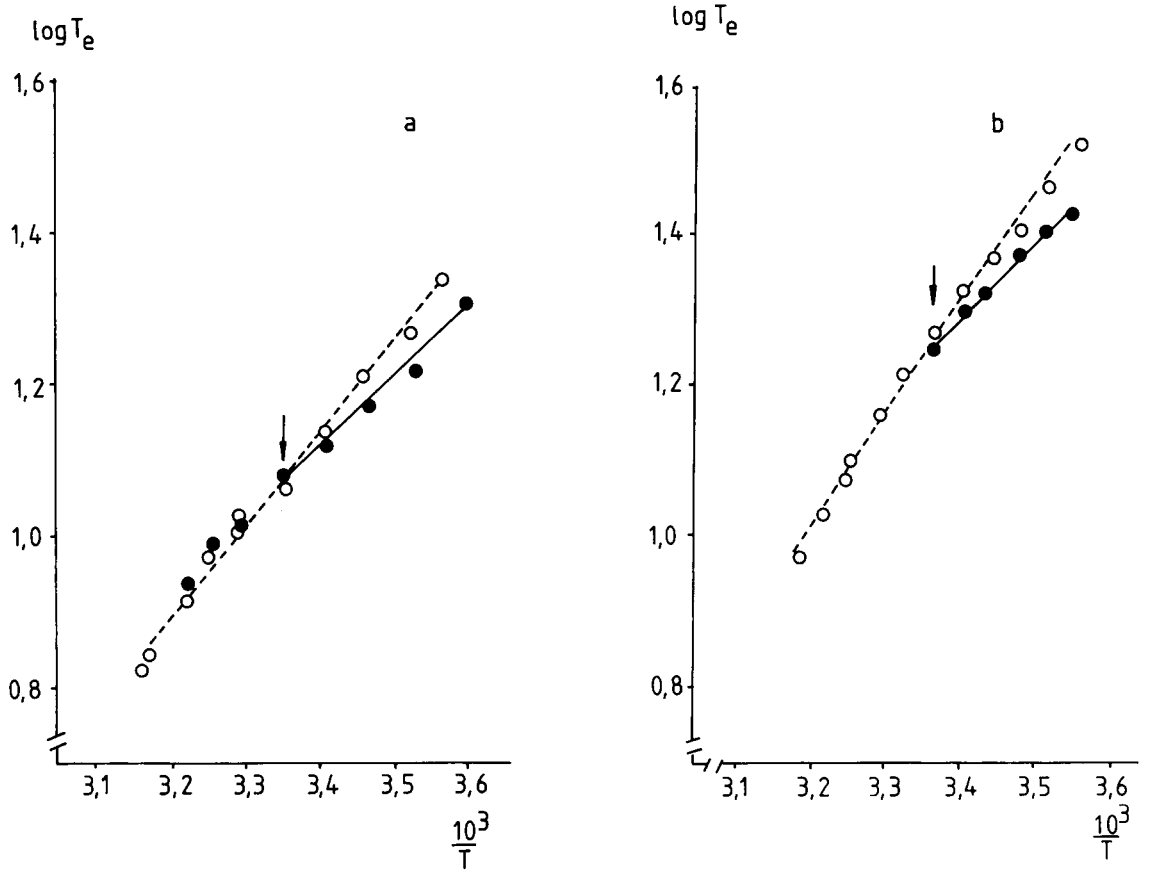

Fig. 2. Arrhenius plots of the water-exchange time (T_e) in erythrocytes (a) and resealed ghosts (b). The preparation of the samples was performed as described under Materials and Methods. The samples were run from 5 to 42 °C (○— — —○) and then back to lower temperatures (●———●). Several minutes were allowed for equilibration and NMR measurement at each temperature.

The activation energy of water diffusion in red blood cells and ghosts

The value of the water diffusional permeability and of the activation energy of this transport process in erythrocytes and resealed ghosts prepared from the blood of the same donor are listed in Table II. It is obvious that there are no significant differences between erythrocytes and ghosts regarding these parameters. This is in agreement with our previous studies showing that resealed ghosts have a permeability similar to that of erythrocytes [22].

The effects of sulfhydryl group reagents

The SH reagents can be divided in two groups as far as their effects on water diffusion are concerned. Mercurials are known as powerful inhibitors of water diffusion, while other SH reagents have no effect. We selected PCMBS and *N*-ethylmaleimide as typical for the two groups of SH reagents. As Macey et al. [23] pointed out, the measurement of the activation energy for a permeation process in the presence of a reagent is not simple. The measurement necessarily includes at least two factors: (a) the temperature-dependence of the permeation process; and (b) the temperature-dependence of the binding of PCMBS to the membrane. Taking into account the above-mentioned factors in our experiments, the cells were incubated with *N*-ethylmaleimide or PCMBS at a single temperature. After the incubation and washings of the cells, several samples were prepared from a batch of cells and each sample was used for a single NMR measurement of T'_{2a}. Then the Arrhenius plot of T_e was calculated from the individual measurements.

As shown in Table III, *N*-ethylmaleimide did not change the value of E_a. On the contrary, after incubation with PCMBS the activation energy of water diffusion was significantly increased. However, it appeared that the value of $E_{a,d}$ is dependent upon the concentration of PCMBS and the duration of incubation. For lower concentrations of PCMBS and shorter incubation times the value of $E_{a,d}$ was smaller, in parallel with smaller values of the inhibition of water diffusion. A high value of $E_{a,d}$ (over 40 kJ/mol) was noticed after an incubation of 60 min at 37 °C with 1 mM PCMBS

TABLE II

COMPARISON BETWEEN THE PARAMETERS CHARACTERIZING WATER DIFFUSION IN HUMAN ERYTHROCYTES AND RESEALED GHOSTS PREPARED FROM THE BLOOD OF THE SAME DONOR

The preparation of samples and the measurements were performed as described in the text. The permeability was calculated from T_e using a ratio of $4.58 \cdot 10^{-5}$ cm for erythrocytes and $6.06 \cdot 10^{-5}$ cm for ghosts as given by Brahm [10] in column P_I, and the slightly higher value for the V/A ratio (e.g., $5.33 \cdot 10^{-5}$ cm) for erythrocytes given by Dix and Solomon [20] in column P_{II}. For all three parameters, the difference between erythrocytes and resealed ghosts was not statistically significant: $P > 0.05$. The P value was obtained using the paired Student's t-test.

Donor No.	Erythrocytes			Resealed ghosts		
	P_I ($cm \cdot s^{-1} \times 10^3$)	P_{II} ($cm \cdot s^{-1} \times 10^3$)	$E_{a,d}$ (kJ/mol)	P_I ($cm \cdot s^{-1} \times 10^3$)	P_{II} ($cm \cdot s^{-1} \times 10^3$)	$E_{a,d}$ (kJ/mol)
1	5.38	6.26	23.4	5.64	6.68	26.1
2	5.87	6.83	24.3	6.25	7.23	25.9
3	5.59	6.48	26.4	4.62	5.35	26.1
4	6.11	7.11	22.1	6.52	7.54	26.1
5	6.62	7.66	24.8	6.64	7.72	26.9
6	7.05	8.20	24.0	5.09	5.89	24.5
7	6.56	7.64	24.3	6.47	7.48	22.2
8	6.28	7.31	22.7	5.77	6.66	25.5
9	5.87	6.83	23.5	6.38	7.38	22.6
10	4.82	5.61	26.1	6.25	7.23	25.7
11	5.73	6.66	26.3	6.38	7.38	26.8
12	4.98	5.79	22.9	6.06	7.01	25.6
Mean:	5.911	6.875	24.2	6.016	6.96	25.3
S.E.:	0.18	0.21	0.40	0.17	0.19	0.41

TABLE III

EFFECT OF SULFHYDRYL GROUP REAGENTS ON THE ACTIVATION ENERGY OF WATER DIFFUSION IN HUMAN ERYTHROCYTES

The measurements of T'_{2a} were performed as described in the text. The results represent the mean ± S.D. The statistical significance of the difference between samples incubated with *N*-ethylmaleimide (NEM) or PCMBS compared to control samples was obtained using the unpaired Student's *t*-test.

Sample	Conditions of incubation			Inhibition (%)	No. of donors	$E_{a,d}$ (kcal/mol)	Statistical significance
	concn. (mM)	time (min)	temp. (° C)				
Erythrocytes							
Control					35	24.5 ± 1.6	
NEM	2	60	25		5	25.5 ± 0.4	$P > 0.1$
PCMBS	0.2	30	37	25	1	30.0	
	0.5	30	37	33	3	32.3	
	1.0	30	37	41	5	35.6 ± 1.6	$P < 0.001$
	1.0	60	37	46	5	41.3 ± 3.9	$P < 0.001$
Resealed ghosts							
Control					4	23.0 ± 1.2	
NEM	2	60	25		1	23.9	
PCMBS	0.1	15	37	22	4	28.1 ± 1.5	$P < 0.01$

when the value of inhibition was above 45%. The effect of PCMBS on $E_{a,d}$ could also be noticed with ghosts, where the value of $E_{a,d}$ corresponded to that obtained in erythrocytes for the same degree of inhibition.

Discussion

The measurement of water transport across the red blood cell membrane by the doping NMR technique employs manganese as a relaxation agent to distinguish between water in the two compartments undergoing exchange. Any penetration inside the cell prevents distinction between intra- and extracellular water. As described above we found the conditions to avoid the penetration of manganese as a source of errors in the determination of T'_{2a}, even for the red blood cells exposed at temperatures higher than 20 ° C and in the presence of PCMBS.

As documented by the data in Table I and Fig. 1, no decrease in T'_{2a} values occurred at 37 ° C in 30 min for controls ampels doped with 13.3 mM Mn^{2+}, even if the samples had been previously stored at 2 ° C for several hours in the presence of this ion. This indicates that no significant penetration of Mn^{2+} is taking place under such conditions. This conclusion is in agreement with previous reports; Weed and Rothstein [24] showed that Mn^{2+} enters the red cells very slowly by passive diffusion, while other authors could not detect a significant penetration of Mn^{2+} after a short exposure of red cells to $MnCl_2$ at 20 ° C [11] or at 37 ° C [25].

In case of cells incubated with PCMBS, the decrease in T'_{2a} values can be explained by a penetration of Mn^{2+} in the cells. Weed and Rothstein [24] showed that PCMBS makes the red cell permeable to Mn^{2+}, whereas Dix and Solomon [20] found a significant increase in the intracellular concentration of Mn^{2+} only at times greater than 30 min after addition of 2.0 mM PCMBS to a suspension of erythrocytes at 28 ° C. The present data (Fig. 1) are in agreement with the results mentioned above, since a decrease of T'_{2a} occurred only for samples (prepared from PCMBS-treated cells) stored with Mn^{2+} for more than 10 min. Moreover, since a single measurement of T'_{2a} can be completed in 3–4 min the artifactual decrease of T'_{2a} caused by the penetration of Mn^{2+} can be avoided, even for cells previously incubated with PCMBS.

When the temperature dependence of water diffusion was followed under conditions avoiding the penetration of manganese, no discontinuity in the Arrhenius plot of T_c occurred. This shows that

the 'break' found in some investigations by the doping NMR technique [12,13] is an artifact due to the penetration of manganese. It can be obviated if the measurements of T_{2a}' are performed as described in this paper, i.e., by avoiding a prolonged incubation of erythrocytes with manganese at temperatures higher than 20°, especially after incubation with PCMBS. It appears that NMR and radiotracer measurements [10] are in agreement regarding the lack of any break in the Arrhenius plot of water-exchange time of human erythrocytes. However, it is possible that discontinuities in the Arrhenius plots of water exchange in some cells are real [26,27]. It has been suggested that such discontinuities may reflect the complexity of the permeation process [1]. On the other hand, nonlinearity of the Arrhenius plot for water permeation, either diffusional or osmotic, has also been observed in lipid bilayers [28–30] as a result of lipid phase changes.

The values of $E_{a,d}$ in red blood cells and resealed ghosts found in the present work do not differ significantly (Table II). This conclusion is slightly different from the data of Brahm [10], who reported a higher $E_{a,d}$ for ghosts (about 30 kJ/mol) compared to erythrocytes (about 21 kJ/mol). There is a very good agreement between the values of $E_{a,d}$ in erythrocytes obtained on the basis of NMR measurements in the present investigations and the data of other authors, who have used NMR or other methods to estimate water permeability (Table IV). Compared to other studies, the present investigation appears to have been performed on the greatest number of donors. The reasons that the value found by Shporer and Civan [9] lies outside the range of values reported by other groups is not known, although the ^{17}O-NMR measurements are subject to large errors because the intrinsic decay of water ^{17}O is similar to the exchange times [20].

TABLE IV

ACTIVATION ENERGY OF HUMAN RED BLOOD CELL WATER TRANSPORT

Type of permeability cell sample	Method	Temperature or range (°C)	E_a (kJ/mol)	Ref.
Diffusional permeability				
Erythrocytes	radiotracer	7, 22, 37	25 ±0.8	8
	radiotracer	3–37	21.1 ± 1.7	10
	NMR, doping (15.4 or 30.8 mM $MnCl_2$)	35–41	22.0 ± 0.6	13
	NMR, doping (13.3 mm $MnCl_2$)	25–37	25.1	11
	NMR, doping (13.3 mM $MnCl_2$)	25–37	23.8 ± 1.7	12
	NMR, doping (13.3 mM $MnCl_2$)	5–42	24.5 ± 1.6	– [a]
	NMR, doping (1.7 mM $MnCl_2$)	3–37	22.5	31
	NMR, $H_2^{17}O$	23–37	36.4	9
PCMBS-treated erythrocytes	NMR, doping (15.4 or 30.8 mM $MnCl_2$)	35–41	40.0	13
	NMR, doping (13.3 mM $MnCl_2$, 1 mM PCMBS)	5–42	41.3 ± 3.9	– [a]
Erythrocyte ghosts	$H_2O/^2H_2O$ exchange	3–37	23.5	32
	radiotracer	3–37	29.6 ± 2.4	10
	NMR, doping (13.3 mM $MnCl_2$)	5–42	23.9 ± 1.2	– [a]
PCMBS-treated ghosts	radiotracer (1 mM PCMBS)	3–37	60.0 ± 0.5	10
	NMR, doping (13.3 mM $MnCl_2$, 0.1 mM PCMBS)	5–42	28.1 ± 1.5	– [a]
Osmotic permeability				
Erythrocytes	stop flow	7–37	13.8 ± 1.7	8
	rapid injection	10–37	20.0	23
PCMBS-treated erythrocytes	rapid injection	10–31	48.0	23

[a] This paper.

The best estimate of $E_{a,d}$ for human red blood cell membrane is possibly an average of the values in Table IV for cells and ghosts, reported by all authors, except the value given by Shporer and Civan [9]. The average value, 24.1 kJ/mol, coincides with the average of the values for cells and ghosts found in the present investigation.

A comparison of this value with the activation energy of water transport in other systems is interesting (Table V). The $E_{a,d}$ is somewhat higher than the activation energy for osmotic water transport in red blood cells, which does not differ from the activation energy of self-diffusion of water. The magnitude of $E_{a,}$ for erythrocytes is similar to the activation energy of diffusion of water adsorbed to SiO_2. This suggests that the mobility of water molecules that diffuse across the membrane is changed as a result of interaction with the channel.

On the other hand, the value of the activation energy for water transport across the red blood cell membrane is much lower than the value for the lipid bilayer. This is in agreement with the idea that the major role in red blood cell water transport is played by hydrophilic channels in proteins [2,202,23]. This might not be the case for some cells where high values of $E_{a,d}$ have been reported.

TABLE V

ACTIVATION ENERGY OF WATER DIFFUSION IN DIFFERENT SYSTEMS

System	$E_{a,d}$ (kJ/mol)	Ref.
Water	19.2	33
H_2O/SiO_2	25.0	34
Planar lipid bilayer	28.4	35
Liposomes (dipalmitoylphosphatidylcholine)	30.1	30
Liposomes (dipalmitoylphosphatidylcholine/egg phosphatidic acid (PA), 92:8)	29.3	7
Liposomes (egg lecithin/egg PA, 92:8)	38.5	7
Liposomes (egg lecithin/egg PA, 96:4)	27.6	7
Liposomes (egg lecithin)	43.9	30
Human erythrocytes	24.1	– [a]
Dog erythrocytes	20.5 ± 1.2	8
Dog erythrocytes	22.2	26
Bovine erythrocyte ghosts	24.5	32
Dog lung alveolar macrophages	20.9	1
Dog lung cells (alveolar epithelial and endothelial cells)	50.2	1
Chinese hamster lung fibroblasts	29.3 ± 6.7 (above 21° C)	27

[a] Average of values in Table IV.

As the major part of water permeates the membrane across channels located in proteins, it is understandable why $E_{a,d}$ is markedly increased after incubation of cells with PCMBS. There is a general agreement that PCMBS inhibition reflects the closure of water channels in proteins. The inhibitory effects of mercurials on red-cell water permeability, discovered by Macey and Farmer [36] have been studied by several groups [10,18,20,36–39]. This allowed us to identify the proteins in human red-cell membrane involved in water permeability [41,42]. Macey et al. [23] considered that PCMBS-treated red cells are indistinguishable from lipid bilayers as far as the activation energies are concerned. The values of $E_{a,d}$ for PCMBS-treated red blood cells found in the present work are indeed similar to the $E_{a,d}$ for water permeation through lipid bilayers. At a first sight this could be interpreted as resulting from water entering these cells primarily by diffusion across the lipid areas of the membrane, i.e., across a 'lipid path' [40]. This view was, however, questioned recently by Dix and Solomon [20], who concluded that the primary route for water diffusion in PCMBS-treated cells is not through the membrane lipids, but rather through a membrane protein channel. Therefore, we agree with the designation [40] of the 'lipid path' as the 'PCMBS-insensitive pathway'. As shown by Moura et al. [40], while the PCMBS-sensitive pathway has characteristics commonly attributed to channels, the PCMBS-insensitive path does not.

In conclusion, the NMR for the study of water exchange based on doping with Mn^{2+} having the advantages of relative technical simplicity, speed of data collection, and reproducible results, allows an almost unrestrained number of measurements. Consequently, this method is very appropriate for evaluation of the activation energy of water diffusion in red blood cells. Such studies may be useful for further characterization of this transport process, including pathological conditions where abnormal erythrocyte water permeability have been reported [43–45].

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