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A Simple Technique of Measuring High Membrane Permeabilities of Human Erythrocytes

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Summary. A new way of measuring high diffusional membrane permeabilities of intact erythrocytes is presented using THO and ^{14}C-glycol as test solutes. The technique combines the theoretical approach used by Redwood, Rall and Perl (*J. Gen. Physiol.* **64**:706–729, 1974) and an experimental procedure introduced by Wang (*J. Am. Chem. Soc.* **73**:510–513, 1951), which greatly simplifies the performance of the experiments. Permeability coefficients obtained by the new technique compare well to data derived by the approaches hitherto available. In view of its simplicity our method may be appropriate for the serial experiments necessary to characterize the transport mechanisms of water and other highly permeable lipophilic nonelectrolytes and for studies on other single cell systems.

Key words erythrocyte membrane · water permeability · nonelectrolyte permeability · diffusion coefficient

Introduction

Membrane permeability of red blood cells has been measured by a variety of methods. In the classical type of flux experiments with radioactive tracer solutes at equilibrium, cell or extracellular space are loaded with radioactive solute at time $t=0$, and the decrease or increase of radioactivity in one of the two compartments is observed by collecting samples by centrifugation after certain time intervals. This method, though accurate and simple, fails for exchange times in the range of seconds or shorter, corresponding to a diffusional permeability coefficient P_d larger than about 10×10^{-5} cm sec^{-1}. Paganelli and Solomon (1957) therefore introduced the rapid flow technique into membrane flux measurements using the same principle, tracer flow at osmotic equilibrium. Red cell permeabilities to water (Barton & Brown, 1964; Vieira, Sha'afi & Solomon, 1970) and several fast permeating nonelectrolytes (Savitz & Solomon, 1971) have been determined by this technique. Yet, complexity of both equipment and operation make it inadequate for routine measurements.

A method to measure the kinetics of osmotic swelling was worked out and applied to water and several fast permeating nonelectrolytes by Sha'afi, Rich, Mickulecky and Solomon (1970) and Sha'afi, Gary-Bobo and Solomon (1971). The time course of the red cell volume change under an initial osmotic gradient was followed by means of a rapid reaction stop-flow apparatus. Apart from the complexity of equipment and operation, the basic disadvantage of this procedure is the requirement to measure solute flow at high extracellular solute concentrations (0.2–0.8 mol/liter), which is necessary to obtain measurable cell volume changes. Measurements in the low concentration range are thus excluded.

Fast permeation measurements by means of the NMR-technique were first reported by Conlon and Outhred (1972) and have been further elaborated since that time (Conlon & Outhred, 1978; Pirkle, Ashley & Goldstein, 1979). They require high concentrations of a solute detectable by NMR, so far confining the method to water as permeant.

Thus, all of the methods presented have certain limitations, precluding measurements of high permeability with respect to either accuracy, simplicity, or interesting solutes. Redwood, Rall and Perl (1974) presented an alternative method which was expected to close this gap. They proposed a procedure to deduce red cell membrane permeability to a radioactive solute from bulk diffusion measurements on concentrated cell suspensions. The basic idea is to enlarge the distance traversed and thus to slow down the permeating process by arranging a multitude of membranes in series. On the basis of these considerations we worked out an improved experimental procedure to measure high permeabilities.

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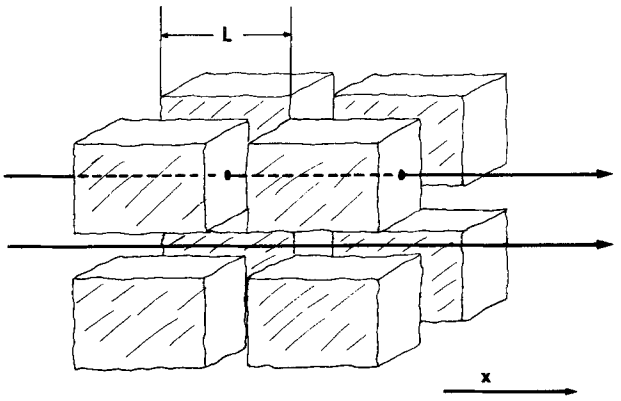

Fig. 1. Model of tightly packed red cells of length l in a column. The arrows symbolize the two main diffusion pathways along the column axis. For details *see text*

Table 1. Transport and geometrical parameters used in Eqs. (1) to (4)

P_d:	Membrane diffusional permeability coefficient
D_1:	Tracer diffusion coefficient referring to the extracellular fluid
D_2:	Tracer diffusion coefficient referring to the intracellular fluid (hemoglobin solution)
D_{ef}:	Bulk diffusion coefficient
A_1:	Total extracellular cross-sectional area
A_2:	Total intracellular cross-sectional area
A:	Total area perpendicular to the direction of diffusion flow
L:	Average erythrocyte length in the direction of the diffusional flow
S/A_2:	Lateral red cell membrane area related to end-face membrane area
V_1:	Volume of the extracellular space
V:	Total volume of the suspension of packed erythrocytes
L_1/L_2:	Tortuosity factor, i.e., length of extracellular diffusion path related to length of intracellular diffusion path

Theory

Model and Mathematics

The method reported in this work is based on the theoretical analysis of Redwood et al. (1974), but involves a different experimental procedure, which greatly simplifies permeability determinations. The theoretical analysis has been presented in detail by Redwood et al. (1974) and will be outlined only briefly in the following.

Tracer diffusion through a column of tightly packed erythrocytes may be phenomenologically characterized by a bulk diffusion coefficient D_{ef}. With a multitude of membrane barriers arranged in series as well as in parallel in this system, there should be a significant influence of membrane diffusional permeability on D_{ef}; vice-versa, it should be possible to derive P_d, the permeability coefficient, from the measured value of D_{ef} on the basis of an appropriate model.

In this model, the tightly packed erythrocyte suspension is assumed to resemble a well-ordered stack of equally sized bricks. There are two parallel diffusion pathways along the column axis (*see* Fig. 1); the extracellular pathway and the transcellular pathway. Solute exchange through the lateral membrane area is allowed, thus connecting the two diffusion pathways. In addition, a local steady state is assumed; i.e., the local transport along the cell axis is considered to be in a steady state, with the driving force – the concentration gradient – and the diffusion flux changing slowly with time.

As shown in the work of Redwood et al. (1974), these assumptions lead to the following set of equations relating P_d, the membrane diffusional permeability coefficient, to D_{ef}, the measured bulk diffusion coefficient, taking into account several other transport and geometrical parameters all specified in Table 1.

$$\frac{D_{ef}A}{D_2A_2}=\left(1+\frac{D_1A_1}{D_2A_2}\right)(1+Z)^{-1} \tag{1}$$

with

$$Z=2\left[\Pi\left(1+\frac{D_1A_1}{D_2A_2}\right)+\frac{D_1A_1}{D_2A_2}\,\alpha\coth\frac{\alpha}{2}\right]^{-1} \tag{2}$$

where

$$\alpha=\left[\Pi S/A_2\left(1+\frac{D_2A_2}{D_1A_1}\right)\right]^{1/2}. \tag{3}$$

Π is a dimensionless quantity related to P_d by

$$\Pi=P_dL/D_2. \tag{4}$$

Permeability is assumed to be the same on the front and lateral membrane area.

Equations (1) to (3) contain relative cross-sectional areas A/A_2 and A_1/A_2, which are connected by

$$A/A_2=1+A_1/A_2. \tag{5}$$

A/A_2 is inconvenient to measure. Redwood et al. (1974) therefore proposed to derive values of A/A_2 from an easily measurable quantity, the relative extracellular volume V_1/V, by an equation

$$\begin{aligned}A/A_2&=1+(V_1L_2)/(V_2L_1)\\&=1+(V_1/V)/(1-V_1/V)\cdot(L_1/L_2)^{-1}\end{aligned} \tag{6}$$

where L_1/L_2 is a tortuosity factor.

Determination of Diffusion Coefficients

Diffusion coefficients D_{ef} as well as D_1 and D_2 were determined alternatively by two methods both involving measurements of one-dimensional diffusion through a column of diffusion medium[1] under defined boundary conditions. The first one, which will be denoted the *differential method*, was applied in the work of Redwood et al. (1974) and will not be described further in this communication. A diffusion coefficient is derived from the concentration profile over a column of diffusion medium established at time t after deposition of an infinitely thin layer of solute at distance $x=0$ and time $t=0$.

The second method will be called the *integral method*. It was worked out by Wang (1951) for binary solutions and is introduced into permeability studies in this work. The basic idea is tracer diffusion from a homogeneously loaded capillary of length l into an infinite sink of solvent. The capillary initially contains diffusion medium equilibrated with a known amount n_0 of labeled solute. It is placed, at time $t=0$, into a large bath of diffusion medium free of labeled solute and incubated. The residual total amount of solute $n(t)$ at time $t>0$, is measured. Knowledge of three variables, $n(t)/n_0$, l and t, allows calculation of D by means of an equation given by Wang (1951):

$$n(t)/n_0 = 8/\pi^2 \sum_{n=0}^{\infty} (2n+1)^{-2} \cdot \exp\{-(2n+1)^2 \cdot \pi^2 \cdot Dt/4l^2\}. \quad (7)$$

Geometrical Parameters

The relative extracellular volume V_1/V was measured with extracellular marker (Redwood et al., 1974). In view of the random orientation of the erythrocytes in our integral experiments, the cells were assumed to be cube-shaped. The cube length L was taken to be 4.5 μm, calculated from the third root of the mean volume of the human erythrocyte (Chien, Dellenback, Gregersen & Gregersen, 1965; Rich, Sha'afi, Barton & Solomon, 1967). S/A_2 is 4 for a cube by definition. This value also corresponds to a cylinder of identical length and end-face diameter. According to the "brick model" for tightly packed erythrocytes, the tortuosity factor L_1/L_2 is close to unity. Deviations from the model arrangement should increase L_1/L_2. Yet, for the present experiments, the assumption $L_1/L_2=1$ led to the most satisfactory results. Values of L, S/A_2, and L_1/L_2 depend on the model assumed.

[1] Any solution or suspension (packed erythrocytes, isotonic saline, etc.) used for diffusion measurements will be denoted a "diffusion medium" in this work.

Materials and Methods

Human blood was collected by venipuncture, heparinized, and used for diffusion experiments on the same or the next day after storage at 4 °C. Red cells were isolated by centrifugation (6,000 × g, 5 min) and were washed 3 times in a solution composed of 95 volumes isotonic saline (154 mmol/liter NaCl) and 5 volumes isotonic phosphate buffer, pH = 7.4. For permeating test solutes other than water this solution additionally contained the solute at the concentration required.

Concentrated cyan-met-hemoglobin solutions were prepared by lysing washed erythrocytes with distilled water, sedimenting the membranes at 45,000 × g, adding $K_3[Fe(CN)_6]$ and KCN, subsequent dialysis and final reconcentration by ultrafiltration (Amicon, PM 10).

The Differential Method

We first adapted the procedure of Redwood et al. (1974) in all details extractable from the authors' description. However, a number of problems arose in the course of this attempt, which we found difficult to overcome (Osberghaus, 1980).

Firstly, the experimental starting conditions were hard to reproduce. Unavoidable and irreproducible mixing and creeping effects following the deposition of the diffusing labeled solute on the column at time $t=0$ made it difficult to localize the origin $x=0$ on the distance axis which is mandatory for the evaluation of a diffusion experiment.

Secondly, solute absorption by or adsorption to the capillary walls turned out to be another complication. We established this effect using a 16 mM solution of ^{14}C-hexanol in isotonic saline which we injected into the polyethylene tubing. We found ^{14}C-hexanol to leave the aqueous phase rapidly to an extent of, e.g., 75 % after a period of 1.5 hr. Recently reported diffusion and permeability coefficients for higher aliphatic alcohols may suffer from such absorption effects (Garrick & Redwood, 1977; Garrick, Patel & Chinard, 1980).

Thirdly, we found a nonreproducible decrease of the relative extracellular volume along the packed cell column by 10 to 20 % over a distance of about 1 cm. Additionally, many solutes were not available at sufficiently high specific radioactivities, which further restricts the applicability of the differential method.

The Integral Method

Based on suggestions of Saraf et al. (1963) and Witherspoon and Saraf (1965) microliter syringes were used to obtain capillaries of variable length l. We used syringes (Unimetrics Corp., type 4025) with glass capillaries with an internal diameter of 0.73 mm. The capillaries had been ground flat at the mouth end normally bearing the needle. A gas tight Teflon plunger was inserted into the other end; the capillary length could thus be varied over one decade from approximately 5 to 60 mm, making possible measurements of diffusion coefficients over two decades according to Eq. (7) within the same period of time. Diffusion periods were in the range of hours. The length of the capillary was fixed by means of a guide rod attached to the capillary, and a stopping device. Up to 8 capillaries thus prepared were mounted on a rack which could be moved in vertical direction (Fig. 2).

The capillaries were then filled with diffusion medium containing a known amount of radioactive solute using a capillary syringe of the same type equipped with a needle. An excess droplet of medium was left on the flat mouth end of each capil-

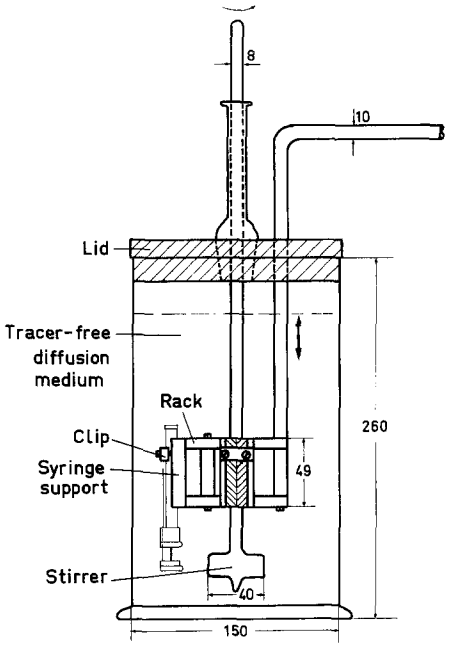

Fig. 2. The device used to measure diffusion coefficients according to the integral method described in the text. Only two syringe supports are shown for reasons of clearness

lary, and the rack carefully immersed into a large (3 liter) thermostatized (20.00±0.04 °C) bath of tracer-free diffusion medium under gentle stirring. After an appropriate period ranging from about 1 to 20 hr the rack was carefully lifted out of the bath. The capillary contents were pushed out by means of the Teflon plunger.

In case of diffusion medium containing hemoglobin of packed cells, the capillary contents were diluted into 1.4 ml distilled water and protein precipitated by addition of 20–30 µl 60 % $HClO_4$. Aliquots of the supernatant after centrifugation were counted for radioactivity in a liquid scintillation counter (Packard Corp., Illinois USA, model 3385 Tri-Carb). Values of $n(t)/n_o$, l, and t were inserted into Eq. (7) to determine the diffusion coefficient D by iteration. The terms up to $n=6$ out of the infinite series on the right-hand side were considered, the higher terms being negligible for $Dt/l^2 > 0.06$, the minimum value for the present experiments.

A possible systematic error in this type of diffusion measurement is the so-called "Δl-effect" (Wang, 1952) which means that the actual length of the diffusion path may differ from the geometric capillary length l owing to convection currents which may wash out ($\Delta l < 0$) or heap up ($\Delta l > 0$) material near the capillary mouth. A Δl-effect will lead to a dependency of diffusion coefficients on the capillary length. Table 2 presents diffusion coefficients for THO as solute and capillary lengths varying from 0.48 to 4.80 cm. Diffusion coefficients are remarkably stable with respect to capillary lengths down to 0.5 cm. Furthermore, our values are close to data given in the literature for tritiated water. Wang, Robinson and Edelman (1953) and Mills (1973) determined diffusion coefficients by means of the capillary method and the diaphragm cell method, respectively. They published values for various temperature, from which values given in Table 2 were derived by interpolation to 20 °C.

Tightly packed cells loaded with radioactive solute were prepared in the following way. Precentrifuged packed cells were thoroughly mixed with a small volume of tracer solution to reach a specific radioactivity of the order of <1 µCi/ml of packed cells,

Table 2. Diffusion coefficients of THO in water at 20 °C as obtained with different capillary lengths[a]

l (cm)	D (10^{-5} cm^2 sec^{-1})
4.80	2.14±0.10 (3)
2.40	2.15±0.18 (6)
1.44	2.22±0.10 (6)
0.96	2.20±0.08 (7)
0.72	2.22±0.22 (12)
0.48	2.25±0.16 (7)
	2.14[b]
	1.98[c]

[a] Errors are standard deviations (SD). Number of experiments in parentheses.
[b] Wang et al. (1953).
[c] Mills (1973).

incubated for a few minutes for reasons of equilibration, and centrifuged once for 15 min at 3,000 and 17,000 × g, respectively. The supernatant was removed with a Pasteur pipette, the sediment thoroughly but carefully mixed and used for integral diffusion experiments.

Fractional extracellular volumes of the tightly packed cells were determined with radiolabeled sucrose or inulin to be 0.0489 ±0.007 (SEM, $n=14$; 5 different blood samples) for 3,000 × g, and 0.0279±0.007 (SEM, $n=16$; 7 different blood samples) for 17,000 × g. Hemoglobin solutions and water were used for diffusion measurements without further processing except for being mixed with a small volume of tracer solution.

Materials

Chemicals used were of analytical grade whenever available. (1,2-^{14}C)-glycol (1–5 mCi/mmol) and ^3H_2O (25 mCi/g) were obtained from New England Nuclear, Dreieich, Germany; (U-^{14}C)-sucrose (350 mCi/mmol), ^{3}H-inulin (500 mCi/mmol) from Amersham Buchler, Braunschweig, Germany, and (1-^{14}C)-hexanol (5 mCi/mmol) from ICN Pharmaceuticals, Irvine, Calif.

Results and Discussion

Application of the Integral Method

The integral method was tested on two solutes with very different permeability coefficients. THO and (1,2-^{14}C)-glycol as test solutes were diluted with isotonic saline to an activity of 2.0 and 0.05 µCi/µl, respectively. Much lower specific activities may be used as well. Tables 2–5 compile diffusion data for both solutes. As for ^{14}C-glycol, diffusion media were pre-equilibrated with nonactive glycol at a concentration of 50 mmol/liter.

Bulk diffusion coefficients for tightly packed cells are shown in Table 3. Values for two different extracellular volumes were determined for THO to test the internal consistency of Eqs. (1) to (4). Errors are less than ±2 % of the mean, reflecting the reproducibility of the integral method. Diffusion coef-

Table 3. Bulk diffusion coefficient D_{ef} for THO and ^{14}C-glycol in concentrated erythrocyte suspensions at 20 °C[a]

Solute	V_1/V	D_{ef} (10^{-5} cm^2 sec^{-1})
THO	0.0279	0.177 ±0.003 (13)
THO	0.0489	0.218 ±0.004 (20)
^{14}C-glycol	0.0279	0.0404±0.0007 (25)

[a] Mean values ±SEM. Number of experiments in parentheses. THO-values for two different relative extracellular volumes V_1/V.

Table 4. Diffusion coefficients in water for THO and ^{14}C-glycol as solutes at 20 °C[a]

Solute	D_1 (10^{-5} cm^2 sec^{-1})
THO	2.20 ±0.02 (41)
^{14}C-glycol	1.035±0.02 (12)

[a] Number of experiments in parentheses.

Table 5. Diffusion coefficient D_2 for THO and ^{14}C-glycol in concentrated hemoglobin solution at various concentrations c_{Hb} at 20 °C[a]

Solute	c_{Hb} (g/liter)	$D_2(c_{Hb})$ (10^{-5} cm^2 sec^{-1})
THO	304	1.35 ±0.01 (14)
	195	1.58 ±0.03 (4)
	122	1.84 ±0.02 (3)
^{14}C-glycol	304	0.680±0.006 (14)

[a] Mean values ±SD. Number of experiments in parentheses.

ficients for water as solvent are presented in Table 4. The number for THO is the average of all ($n=41$) single values of the test series from Table 2. The accuracy of this value is confirmed by its coincidence with results obtained from the literature, as pointed out above. Diffusion coefficients for ^{14}C-glycol were not available from standard data collections. However, reference data for polar organic solutes with similar molecular weight indicate that the value found is realistic. For subsequent calculations, diffusion coefficients were assumed to be identical for water and isotonic saline as solvents within error limits.

The intracellular fluid of the erythrocyte contains hemoglobin at a concentration of 330 g/liter cells. Diffusion coefficients for the intracellular fluid were measured in reconcentrated hemolysates of lower concentrations (Table 5). Including the mean value for pure water as solvent from Table 4, we obtain four pairs of data for THO as diffusing solute which were well fitted by linear regression and extrapolated over a narrow range to the desired intracellular hemoglobin concentration (Fig. 3). The D_2 value thus obtained is clearly higher than that found by Redwood et al. (1974) by means of the differential method. The integral method may yield erroneous values when diffusion in hemoglobin solutions is measured due to the fact that the 3-liter bath of solvent was free of hemoglobin. A net hemoglobin diffusion flux out of the capillary thus arises, interfering with the diffusion flux of THO by either dilution of hemoglobin in the capillary medium or flux coupling. However, as will be demonstrated in a brief error analysis below, even substantial errors in D_2 contribute very little to the error in the resulting permeability coefficient.

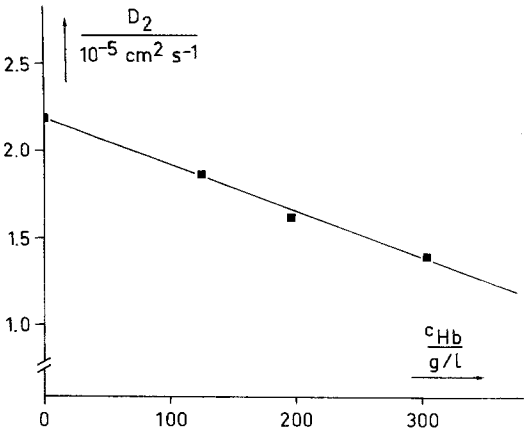

Fig. 3. Diffusion coefficient (D_2) for THO in hemoglobin solutions as a function of hemoglobin concentration. Regression line obtained for the data points according to the equation $D_2 = D_2^0 - a \cdot c_{Hb}$ (cm$^2 \cdot$ sec^{-1}), where $D_2^0 = 2.169 \cdot 10^{-5}$ cm$^2 \cdot$ sec^{-1} and $a = 2.781 \cdot 10^{-8}$ cm$^2 \cdot 1 \cdot$ sec$^{-1} \cdot$ g^{-1}

The diffusion coefficient of ^{14}C-glycol was assumed to depend linearly on the hemoglobin concentration, as found for THO as solute. Diffusion coefficients of ^{14}C-glycol for water and hemoglobin (304 g/liter) solutions as solvents were used to extrapolate to a hemoglobin concentration of 330 g/liter (Table 6). No data to compare with are yet available.

Calculation of P_d Values

Permeability coefficients P_d were calculated from Eqs. (1) to (6) using diffusion coefficients D_1, D_2, and D_{ef} as determined by the integral method, and the corresponding geometrical parameters V_1/V, L, S/A_2 and L_1/L_2 as defined and specified above. The data are compiled in Table 7, which also contains a result from our own differential measurements.

Variation of V_1/V strongly affects the measured bulk diffusion coefficient, but will certainly not change P_d, the membrane permeability. Therefore,

Table 6. Diffusion coefficient D_2 for THO and ^{14}C-glycol in hemoglobin solution at a hemoglobin concentration of 330 g/liter, at 20 °C, as established by three approaches

Method	D (10^{-5} cm^2 sec^{-1})	
	THO	^{14}C-glycol
Differential, this work	1.004 ± 0.026 [a]	
Differential, Redwood et al. (1974)	1.06 ± 0.05 (SEM)	
Integral, this work [b]	1.25 ± 0.01	0.650 [c] ± 0.006

[a] Obtained by extrapolation from measurements of D_2 at lower hemoglobin concentrations. Standard error of the estimate obtained from the regression function.
[b] Errors taken as those given in Table 5 for $c_{Hb} = 304$ g/liter.
[c] Obtained by linear extrapolation of the data from Tables 4 and 5.

Table 7. Permeability coefficients P_d at 20 °C [a]

Solute	V_1/V	D_1	D_2	D_{ef}	P_d (10^{-3} cm sec^{-1})
		10^{-5} cm^2 sec^{-1}			
THO	0.0279	2.20	1.25	0.177	3.72 ± 0.14
	0.0489			0.218	3.54 ± 0.14
^{14}C-glycol	0.0279	1.035	0.650	0.0404	0.326 ± 0.034

[a] Errors as given in the text (V_1/V) and in Tables 4 to 6 (diffusion coefficients). The error in P_d (SEM) was calculated as described in the text.

one should obtain the same permeability coefficient for each pair of data (V_1/V; D_{ef}) provided the other geometrical parameters are invariant. Table 7 shows a remarkable agreement of P_d values for two different relative extracellular volumes and corresponding D_{ef}'s, measured for THO as test solute.

Error Analysis

The statistical errors in the measured values of D_1, D_2, D_{ef}, and V_1/V (*see* Tables 3 to 5) propagate to a statistical error in P_d according to the Gaussian law of error propagation. Calculated errors in P_d are shown in Table 7. They amount to less than 4 % of the mean for THO, and about 10 % of the mean for ^{14}C-glycol as solute.

P_d is affected by different errors to a varying extent. It is strongly affected by the errors in D_{ef} and V_1/V, much less by error in D_1, and even less by the error in D_2.

As stated above, the D_2 values obtained by the integral method may well be too high by about 25 % (Table 6). It can be shown, however, that this possible systematic error enlarges P_d by no more than 2.7 % for THO, and 0.5 % for ^{14}C-glycol which is clearly smaller than the errors in P_d from Table 7. Therefore, a rough estimate of D_2, assuming values in the range of, say, 0.40 to 0.60 times that of D_1 (*see also* Redwood et al., 1974), will generally be sufficient.

It may be shown in a similar way, that uncorrect assumptions about the intracellular hemoglobin concentration of a blood sample under investigation − 330 g/liter instead of an unrecognized correct value of, say, 250 g/liter − will affect P_d by 2 % at most, mainly depending on the measured value of D_{ef}.

The values of the geometrical parameters L, S/A_2, and L_1/L_2 are estimates based on plausibility assumptions. Further justification of these values emerges from the following comparison with data available in the literature.

Comparative Evaluation and Conclusions

Diffusional permeability coefficients for the solutes used in our study, as obtained by several authors and different methods, have been compiled in Table 8. Permeability coefficients for water given in the literature vary by a factor of about 2 (Table 8 and

Table 8. Comparison of permeability coefficients P_d as obtained by different methods [a]

Solute	This work	Rapid flow method	NMR method
THO	3.72 ± 0.14 (SEM) [b] (20 °C)	3.31 ± 0.37 (SEM) [c] (22 °C)	4.27 ± 0.35 (SD) [d] (20 °C)
		5.30 ± 1.27 (SD) [e] (23 °C)	2.48 ± 0.07 (SEM) [f] (23 °C)
^{14}C-glycol	0.326 ± 0.034 (SEM) (20 °C)	0.147 ± 0.071 (SD) [g] (21 °C)	

[a] Values of P_d in units of 10^{-3} cm sec^{-1} for slightly different temperatures as indicated in the Table.
[b] Value obtained for $V_1/V = 0.0279$.
[c] Vieira, Sha'afi and Solomon (1970).
[d] Conlon and Outhred (1978).
[e] Paganelli and Solomon (1957).
[f] Pirkle, Ashley and Goldstein (1979).
[g] Savitz and Solomon (1971).

Conlon & Outhred, 1978, Table III). The values obtained for THO by our procedure fit well into that range. On the other hand, the only value for ^{14}C-glycol previously reported (Savitz & Solomon, 1971) is about half that obtained by our method. Such differences are most likely mainly caused by differences in the techniques of transport measurements and in the assumptions underlying the evaluation.

The method presented here proved to be simple and reliable and therefore may be well-suited for serial measurements dealing with the molecular basis of the diffusional permeability of highly permeable nonelectrolytes. Studies on this subject have hitherto been hampered considerably by the lack of such simple methods. For this reason not much is known about transport of water through the human erythrocyte membrane except that two parallel pathways contribute, one of which is related to proteins while the other seems to involve the membrane lipid phase (*see* Deuticke, 1977 for a review, and Kutchai, Cooper & Forster, 1980, for recent studies).

Even less is known about the pathways of transfer of other small hydrophilic or larger lipophilic nonelectrolytes, such as glycol, amides or aliphatic alcohols. Patterns of inhibition or enhancement by alcohols and phloretin also suggest the involvement of two pathways (Deuticke, 1977) but the problem needs more experimental work.

In addition, the integral technique of diffusion measurements presented here may also be of potential value for permeability studies on other single cells, cell organelles or membrane vesicle suspensions as long as they can be tightly packed and modelled, for evaluation, as a two-compartment system.

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References

Barton, T.C., Brown, D.A.J. 1964. Water permeability to the fetal erythrocyte. *J. Gen. Physiol.* **47**:839

Chien, S., Dellenback, R.J., Gregersen, S.U., Gregersen, M.I. 1965. Plasma trapping in hematocrit determination. Differences among animal species. *Proc. Soc. Exp. Biol. Med.* **119**:115

Conlon, T., Outhred, R. 1972. Water diffusion permeability of erythrocytes using an NMR technique. *Biochim. Biophys. Acta* **288**:354–361

Conlon, T., Outhred, R. 1978. The temperature dependence of erythrocyte water diffusion permeability. *Biochim. Biophys. Acta* **511**:408–418

Deuticke, B. 1977. Properties and structural basis of simple diffusion pathways in the erythrocyte membrane. *Rev. Physiol. Biochem. Pharmacol.* **78**:1–97

Garrick, R.A., Redwood, W.R. 1977. Membrane permeability of isolated lung cells to nonelectrolytes. *Am. J. Physiol.* **233**:104–109

Garrick, R.A., Patel, B.C., Chinard, F.R. 1980. Permeability of dog erythrocytes to lipophilic molecules: Solubility and volume effects. *Am. J. Physiol.* **238**:107–113

Kutchai, H., Cooper, R.A., Forster, R.E. 1980. Erythrocyte water permeability. The effects of anesthetic alcohols and alterations in the level of membrane cholesterol. *Biochim. Biophys. Acta* **600**:542–552

Mills, R. 1973. Self diffusion in normal and heavy water in the range 1–45°. *J. Phys. Chem.* **77**:685–688

Osberghaus, U. 1980. Messung schneller Transportvorgänge an Erythrozytenmembranen. Doktordissertation. Rheinisch-Westfälische Technische Hochschule, Aachen

Paganelli, C.V., Solomon, A.K. 1957. The rate of exchange of tritiated water across the human red cell membrane. *J. Gen. Physiol.* **41**:259–277

Pirkle, J.L., Ashley, D.L., Goldstein, J.H. 1979. Pulse nuclear magnetic resonance measurements of water exchange across the erythrocyte membrane employing a low Mn concentration. *Biophys. J.* **25**:389–406

Redwood, W.R., Rall, E., Perl, W. 1974. Red cell membrane permeability deduced from bulk diffusion coefficients. *J. Gen. Physiol.* **64**:706–729

Rich, G.T., Sha'aif, R.I., Barton, T.C., Solomon, A.K. 1967. Permeability studies on red cell membranes of dog, cat and beef. *J. Gen. Physiol.* **50**:2391–2405

Saraf, D.N., Witherspoon, P.A., Cohen, L.H. 1963. Diffusion coefficients of hydrocarbons in water: Method for measuring. *Science* **142**:955–956

Savitz, D., Solomon, A.K. 1971. Tracer determinations of human red cell membrane permeability to small nonelectrolytes. *J. Gen. Physiol.* **58**:259–266

Sha'afi, R.I., Rich, G.T., Mikulecky, D.C., Solomon, A.K. 1970. Determination of urea permeability in red cells by minimum method. *J. Gen. Physiol.* **55**:427–450

Sha'afi, R.I., Gary-Bobo, C.M., Solomon, A.K. 1971. Permeability of red cell membranes to small hydrophilic and lipophilic solutes. *J. Gen. Physiol.* **58**:238–258

Vieira, F.L., Sha'afi, R.I., Solomon, A.K. 1970. The state of water in human and dog red cell membranes. *J. Gen. Physiol.* **55**:451–466

Wang, J.H. 1951. Self-diffusion and structure of liquid water with deuterium as tracer. *J. Am. Chem. Soc.* **73**:510–513

Wang, J.H. 1952. Tracer-diffusion of tracer amount of sodium ion in aqueous potassium chloride solutions. *J. Am. Chem. Soc.* **74**:1182–1186

Wang, J.H., Robinson, C.V., Edelman, I.S. 1953. Self-diffusion and structure of liquid water with ^2H, ^3H and ^{18}O as tracers. *J. Am. Chem. Soc.* **75**:466–470

Witherspoon, P.A., Saraf, D.N. 1965. Diffusion of methane, ethane, propane, and *n*-butane in water from 25 to 43 °C. *J. Phys. Chem.* **69**:3752–3755

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