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Evidence that interfacial transport is rate-limiting during passive cell membrane permeation

D.M. Miller

Agricultural Canada Research Centre, London, Ontario (Canada)

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The octanol to water overall transfer rate constants (k'_{ow}), the octanol/water partition coefficients (K_{pc}) and the diffusion coefficients in octanol (D_o) and water (D_w) were measured for 36 compounds. A plot of D_o/k'_{ow} as a function of $D_o \cdot K_{pc}/D_w$ was shown to fit a straight line for compounds having a high K_{pc}, and from the slope of this line, the thickness of the unstirred layer in the water phase was found to be 9.1 μm. Using this value and the data provided by compounds having a low K_{pc}, an estimate of 1.2 μm was obtained for the thickness of the unstirred layer in the octanol phase. This provided estimates for the true interfacial rate constants, k_{ow} (for movement of a compound from octanol into water) and k_{wo} (for movement in the opposite direction), which were corrected for the effects of the unstirred layers. k_{ow} proved to be constant to within one order of magnitude for a series of compounds whose K_{pc} values ranged over three orders of magnitude, while k_{wo} for these same compounds varied directly as K_{pc}. Assuming that octanol has solvent properties similar to those of the lipid bilayer present in natural membranes, the permeability of these membranes to a given compound can be shown to be equal to $k_{wo}/2$. Comparing the permeabilities calculated in this way to measured permeabilities of natural membranes showed them to be of the same order or smaller. These data are consistent with a proposal that the rate-limiting step in passive membrane permeation is not the rate of diffusion within the membrane itself, but rather, transfer of the permeant across the interfaces separating the lipid phase of the cell membrane from the aqueous phases on either side of it.

Introduction

The plasmalemma of most living cells has as its core a bilipid layer. This layer is generally assumed to be the main barrier to movement of compounds in and out of the cell – the evidence for this being that the rate of movement of a specific compound into the cell is proportional to its oil-water partition coefficient (K_{pc}). This is generally true for compounds with a large K_{pc} but for many small compounds, and those with low K_{pc} the relationship does not appear to be clear cut [1].

In an earlier paper [2] it was pointed out that in passing through the plasmalemma, or cell membrane, a compound not only has to traverse the bilayer but must also move across an interface between the water and lipid phases on either side of the bilayer. If k_{wo} is the rate constant for passage of the compound from water into the organic phase (the bilayer) and k_{ow} is the rate constant in the opposite direction, then the overall membrane permeability constant will be $P_m = k_{wo} \cdot P/(k_{ow} + 2P)$, where P, the permeability of the bilayer, $= D/\delta$, D being the diffusion coefficient of the compound in the bilayer and δ the bilayer thickness. From this expression it follows that if the slow step in permeation of the membrane is the movement through the bilayer, (i.e. if $P \ll k_{ow}$) the rate constant for permeation will be $\approx K_{pc} \cdot P$, since the partition coefficient, $K_{pc} = k_{wo}/k_{ow}$. On the other hand, if $P \gg k_{ow}$ and interfacial transport is rate-limiting, the rate constant will approximate $k_{wo}/2$.

Diffusion coefficients usually vary as the cube root of the molecular weight of the compound and therefore P is unlikely to provide the wide range of values necessary to account for the range of permeation rates usually found in living cells, which may be as high as four orders of magnitude. Partition coefficients, on the other hand, and presumably the interfacial transport constants of which they are composed, vary over many orders of magnitude and therefore could account for the range of permeation rates. Partition coefficients are

Correspondence: D.M. Miller, Agricultural Canada Research Centre, 1400 Western Road, London, Ontario, Canada, N6G 2V4.

readily measured and a large number are listed in the literature, allowing comparisons between them and cellular transports to be made [1]. The interfacial transport constants are much more difficult to determine, however, and relatively few have been reported, so that one cannot, at the moment, correlate their values with those of membrane transport. In the present work, k_{ow} and K_{pc} were measured for 36 compounds of widely differing chemical structure, together with their diffusion coefficients, in an octanol-water system and k_{wo} for each calculated.

The technique used to measure k_{ow} is that described in the original report [2] where water and the lower straight chain alcohols were investigated. This technique employs two types of apparatus. In the first (apparatus A), a 30-μl drop of octanol was suspended in a moving stream of an aqueous solution of the compound under investigation for a known time, removed and the amount of compound it contained determined. Repeating this procedure with a number of drops over different time intervals allowed k_{ow} to be determined. During prolonged suspensions, the concentration in the drop reached a steady value, and division of this value by that of the concentration in the aqueous solution, provided an estimate of K_{pc}. The second apparatus (B) was the same as the first with the exception that an aqueous drop was suspended in an octanol solution of the compound. In this case, however, the rate constant determined was k_{wo}.

This work was criticized by Hladky [3] who felt that the resistance to movement imposed by the unstirred layers on either side of the interface would mask that of interfacial transport. Hladky showed that under steady-state conditions, the apparent rate constant, k'_{ow}, would be related to the true rate constant, k_{ow}, by the expression

$$k'_{ow} = \frac{k_{ow}P_oP_w}{P_oP_w + k_{wo}P_o + k_{ow}P_w} \quad (1)$$

where P_o and P_w are the permeabilities of the octanol unstirred layer (o.u.l.) and the water unstirred layer (w.u.l.), respectively. If D_o and D_w are the diffusion coefficients of a compound in octanol and water, and δ_o and δ_w are the thicknesses of the o.u.l. and w.u.l., then $P_o = D_o/\delta_o$ and $P_w = D_w/\delta_w$. Thus, remembering that $K_{pc} = k_{wo}/k_{ow}$, Eqn. 1 may be rearranged to the expression

$$\frac{D_o}{k'_{ow}} = \frac{D_o}{D_w}K_{pc}\delta_w + \frac{D_o}{k_{ow}} + \delta_o \quad (2)$$

If, as Hladky contends, k_{ow} is very large, or $\delta_o \gg D_o/k_{ow}$ for all compounds, Eqn. 2 is reduced to the form

$$\frac{D_o}{k'_{ow}} = \frac{D_o}{D_w}K_{pc}\delta_w + \delta_o \quad (2a)$$

which predicts that a plot of D_o/k'_{ow} as a function of D_oK_{pc}/D_w for a number of compounds should be a straight line with slope δ_w and intercept $= \delta_o$. Hladky showed that this appeared to be true for the data obtained in the original paper [2], since, when plotted in this way they can be fitted to a straight line whose slope provides a value for δ_w of 9 μm and whose intercept $= \delta_o = 6$ μm. A similar plot using the results listed here is made, and it is shown that while data for those compounds with a large K_{pc} can be fited to a straight line, those obtained from compounds having a K_{pc} in the lower range cannot, leading to the conclusion that for these latter compounds, k_{ow} must be of the same order or smaller than P_w and P_o – thus confirming the conclusion reached in the original paper [2].

Experimental

The octanol to water rate constants (k'_{ow}) and the partition coefficients (K_{pc}) were measured at 25°C using the octanol drop apparatus (apparatus A) and the methods previously described [2].

Diffusion coefficients in both octanol (D_o) and water (D_w) were determined at 25°C by the capillary tube method [4,5]. In this method, thin capillary diffusion tubes were filled with a water or octanol solution of the compound under investigation and immersed in a thermostated bath of the same solvent for an appropriate time interval. The diffusion tubes were then removed and placed in centrifuge tubes made from 2 mm i.d. pyrex capillary tubing of the form shown in the inset, Fig. 1. They were then spun in a swinging bucket centrifuge at 3000–4000 $\times g$ for 1 min with the closed end of the capillary resting on the restriction in the centrifuge tube. This procedure removed all solvent from the outside of the capillary tube which was then

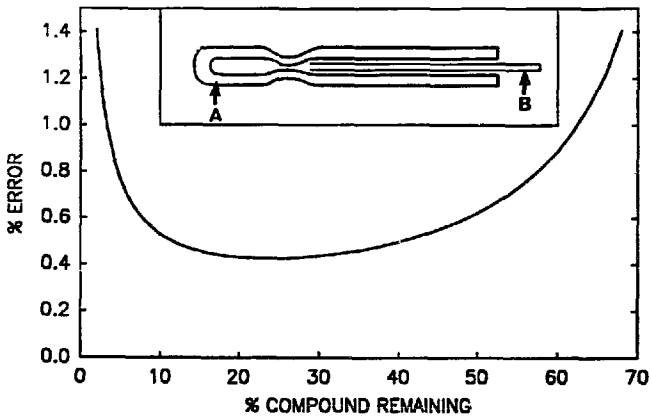

Fig. 1. Graph: plot of the maximum error, E_m, in the determination of the diffusion coefficient as a function of $100C_t/C_o$, the percentage of the compound remaining. The error in the concentrations, ΔC, is taken as 0.1% of C_o. See text for explanation. Inset: A is the centrifuge tube with the diffusion tube, B, inserted in it, in position for transfer of the solution from B to A by centrifugation.

transferred to a second centrifuge tube, this time with its open end inside the latter, where it was again centrifuged, and the solution it contained completely transferred to the centrifuge tube. This solution was mixed by forcing it in and out of a syringe several times after which samples were taken for analysis. The diffusion coefficient was calculated using the equation [5]

$$D = \frac{4L^2}{\pi^2 t} \ln\left(\frac{8}{\pi^2} \cdot \frac{C_o}{C_t}\right) \quad (3)$$

where D is the diffusion coefficient, L is the length of the capillary tube, C_o is the concentration of the original solution, and C_t the average concentration of the contents of the capillary after time t.

The capillary tubes were made from replacement bores for Drummond microdispensers available from Fisher Scientific (whose catalogue number is 21-169A for a 10-μl bore and 21-169C for a 25-μl bore). These tubes are ideal for the present use since they have a highly uniform diameter throughout their length. The 10-μl tubes employed in the aqueous diffusion measurements (which have an internal diameter of 0.7 mm) were cut in half to give a length of 4.5 cm, and the broken end sealed by rotating in a flame. For the octanol diffusion measurements, the 25-μl tubes (whose internal diameter was 1.1 mm) were similarly treated but were cut to about 2.2 cm. Before being filled, the lengths of the tubes were determined to the nearest 0.01 mm using a cathetometer. Since the time and length could be determined with considerable accuracy, the main source of errors probably resided in the determination of the concentrations. To estimate the effect of these errors, the following analysis is useful, If C_o and C_t are the true values of the concentrations, then according to Eqn. 3, for a given time interval the true diffusion coefficient will be $D = A \ln(BC_o/C_t)$, where $A = 4L^2/\pi^2 t$ and $B = 8/\pi^2$. Suppose ΔC is a constant error in the determination of the concentrations, then $D_e = A \ln(B(C_o + \Delta C)/(C_t - \Delta C))$ will be the value for D at maximum error, and the maximum percentage error will be $E_m = 100(D_e - D)/D$. In Fig. 1, E_m is plotted against $100C_t/C_o$ and it can be seen that the lowest error occurs at a time for which 24% of the compound remains. In the present work, the times were selected such that the amount of compound remaining was in the range 18–30%.

Results

Table I lists the results of the various measurements and these are plotted in Fig. 2 according to Eqn. 2a as D_o/k'_{ow} vs. $D_o K_{pc}/D_w$. Note that the numbering of the compounds in the table increases as their value of $D_o K_{pc}/D_w$ increases, i.e. as the distance this ratio is plotted from the ordinate. A second plot of compounds one to 22 is made in Fig. 3.

TABLE I

Partition coefficients, rate constants and diffusion coefficients in water and octanol

The rate constants are in units of μm s^{-1} and the diffusion coefficients are in μm^2 s^{-1}.

No.	Compound	K_{pc}	k'_{ow}	D_w	D_o
1	Tetraethylene glycol	0.0239	26.2	592	107
2	Water	0.0525	44.5	2340 [a]	270 [c]
3	Ethylene glycol	0.0606	40.7	1178	164
4	Acetamide	0.0692	39.2	1320 [b]	164
5	1,3-Butanediol	0.164	29.5	980 [c]	146
6	Methyl carbamate	0.230	51.5	1200	136
7	Propionamide	0.189	31.0	1057	145
8	Methanol	0.225	47.8	1700 [b]	234
9	1,5-Pentanediol	0.372	21.8	865	124
10	Acetic acid	0.481	32.8	1153	181
11	Butyramide	0.573	26.8	1070 [b]	151
12	Ethanol	0.487	42.0	1286	224
13	Urethane	0.716	35.7	1066	212
14	1,6-Hexanediol	0.945	28.3	747	128
15	Pinacol	0.885	15.0	745	151
16	Isopropanol	1.20	33.2	1067	203
17	Isovaleramide	1.54	27.2	855	127
18	*n*-Valeramide	2.35	20.3	954	133
19	Ethyl acetoacetate	1.49	20.7	723	169
20	*n*-Propanol	2.06	27.0	1140	212
21	*t*-Butanol	2.17	22.5	885	157
22	Ethyl acetate	3.59	22.0	1056	166 [c]
23	2-Butanol	3.64	17.4	908	181
24	Butylcarbitol	4.69	12.7	830 [c]	141 [c]
25	Isobutanol	5.96	12.0	970	193
26	*n*-Butanol	7.08	11.2	1027	196 [c]
27	Aniline	8.65	10.8	1039	164 [c]
28	*t*-Amyl alcohol	5.81	10.6	761	182
29	*t*-Amylamine	7.94	9.17	867	175
30	2-Picoline	9.54	7.84	928	203
31	Benzyl alcohol	13.02	7.05	946	196
32	Vanillin	16.44	4.24	726	142
33	Methoxyphenol	21.05	4.30	815	141 [c]
34	Phenylacetic acid	25.45	3.77	933	163
35	*n*-Valeric acid	29.30	3.41	932	172
36	Phenol	30.95	2.87	980	178

[a] Value from Ref. 6.
[b] Values from Ref. 7.
[c] Estimated from other data in this table.

Discussion

Fig. 2 shows that all the data taken together appear to fit Eqn. 2a over a wide range, and provide a value of 9.1 ± 0.2 μm for δ_w. A closer examination shows, however, that the fit to Eqn. 2a does not apply for all the compounds. This is illustrated in Fig. 3 where the data obtained on the 22 compounds having the lowest K_{pc} are replotted. Here it can be seen that there is considerable scattering of the plotted data which we might conclude initially is due to experimental error. This

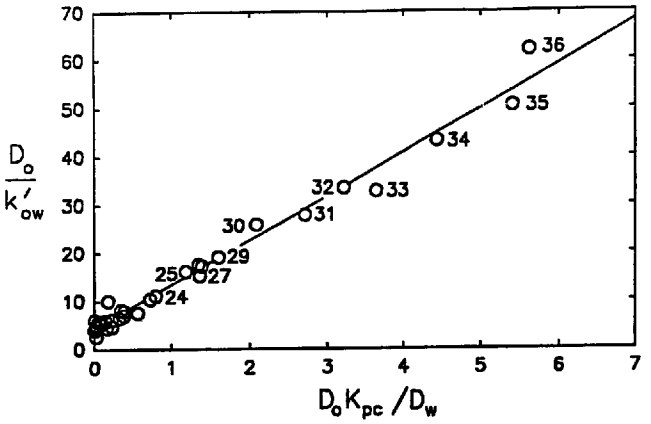

Fig. 2. Plot of the data according to Eqn. 2a. All the data in Table I are plotted in this graph as open circles with the numbers beside them referring to the compounds as numbered in this table. These numbers increase as their distance along the abscissa increases. The solid line is a regression on all the points and has a slope of 9.08 ± 0.21 μm, an intercept of 4.30 ± 0.39 μm, and a correlation coefficient of 0.99.

error is unlikely to be random, however, since in the previous work [2,8] it was shown that rate measurements are usually good to better than ±10% for a given compound, whereas the difference between the rates predicted by Eqn. 2a and those measured for various compounds is much greater. Systematic errors, such as those produced by the presence of impurities, are unlikely to account for rates which are too high. On the other hand, the low value for k'_{ow} for compounds such as pinacol (number 15) might result from the presence of surface active impurities. These, as was pointed out in the previous publication [8], can inhibit stirring within the drop with a consequent enlargement of unstirred layers present at the interface and a reduction in the overall rate of transfer across the interface. To test for the presence of such impurities, the measurements on pinacol, the compound having the widest positive deviation from the straight line in Fig. 3, were repeated on a recrystallized sample. This produced little change in the value of k'_{ow} and a second quantity of this compound was ordered from the manufacturer. Again the measurements showed no significant difference from the original value, forcing the conclusion that the scatter among points in Fig. 3 does not arise from any known experimental error. An alternate explanation for these discrepancies is that the assumption on which the plot in Fig. 2 is based, i.e. that k_{ow} is very large relative to the other constants in Eqn. 2, is not valid for these less lipid soluble compounds and we shall now examine this possibility.

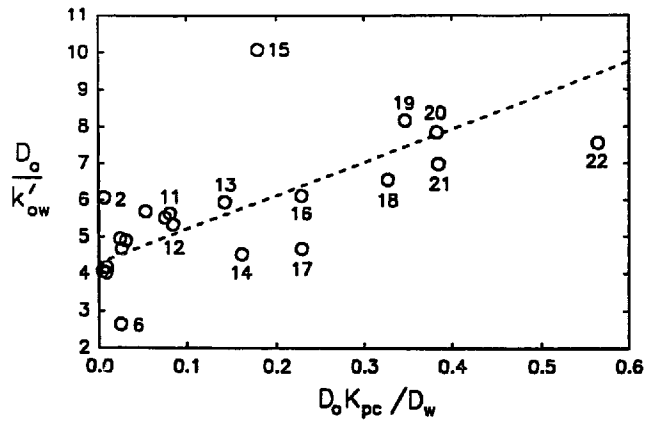

Fig. 3. The data for compounds numbered 1 to 22 in Table I are plotted here according to Eqn. 2a. The dotted line is the same as the solid line in Fig. 2, which is a regression on all the data listed in Table I.

Eqn. 2 can be rearranged to the form

$$\frac{1}{P_o} + \frac{1}{k_{ow}} = \frac{1}{k'_{ow}} - \frac{K_{pc} \cdot \delta_w}{D_w} = R$$

Using this equation, a lower limit for P_o for each compound can be estimated by assuming that k_{ow} is infinitely large, and from this, a upper limit for δ_o can be obtained. This calculation was made for each of the compounds listed in Table I, with $\delta_w = 9.1$ μm, and listed in column 2 of Table II. At the same time the ratio of the residue, R, to $1/k'_{ow}$, was calculated as a percentage for each compound and listed in column 3 of Table II as the 'confidence ratio'. The significance of this ratio is that since the maximum value of $1/P_o$ (and therefore δ_o) is calculated as the difference between the measured quantities $1/k'_{ow}$ and $K_{pc} \cdot \delta_w / D_w$ (see Eqn. 2), the errors in this calculation will increase as the difference decreases relative to the larger of the two quantities (usually $1/k'_{ow}$). Thus, this ratio provides an indication of the confidence which one might have in the result since the errors will be lower the larger this relative difference.

δ_o ranges from 2.4 μm for methyl carbamate and ethyl acetate, and 2.6 μm for isovaleramide, to 8.4 μm for pinacol. However, as these are upper limits, the lowest, 2.4 μm, must be accepted as the *true* (or overall) upper limit. Substituting this value together with other known quantities into expression 2 rearranged to the form

$$k_{ow} = \frac{1}{(1/k'_{ow}) - (\delta_w K_{pc}/D_w) - (\delta_o/D_o)}$$

allows us to calculate k_{ow}, the true rate constant. Since 2.4 μm is the largest δ_o can be, this calculation results in a *maximum* value for k_{ow}. On the other hand, substituting the lowest possible value for δ_o (which, of course, is zero) into this equation provides a *minimum* estimate of k_{ow}. These calculation have been made and the minimum and maximum estimates for k_{ow} listed in Table II. A similar listing for k_{wo} (found by multiplying k_{ow} by K_{pc}) is also included in Table II. The confidence ratios

TABLE II

Estimated values for δ_o and the rate constants

The values in column 2 are estimated maximum values for δ_o in μm. The vlaues of the rate constants are reported as $\mu m\ s^{-1}$. The confidence ratios are an indication of the reliability of δ_o and the lower values of the rate constants. (See text for explanation). Calculations on the data for compounds 33–35 are not complete due to their low confidence ratios.

Compound	δ_o	Confidence ratio	Minimum–maximum		$\delta_o = 1.2\ \mu m$	
			k_{ow}	k_{wo}	k_{ow}	k_{wo}
1 Tetraethylene glycol	4.2	99	27– 65	0.6– 1.6	38	1
2 Water	6.0	99	45– 75	2 – 4	56	3
3 Ethylene glycol	4.0	98	42–110	3 – 6	60	4
4 Acetamide	4.1	98	40– 96	3 – 7	56	4
5 1,3-Butanediol	4.7	96	31– 63	5 – 10	41	7
6 Methyl carbamate	2.4	91	56–∞	13 –∞	110	26
7 Propionamide	4.4	95	33– 71	6 – 13	45	9
8 Methanol	4.6	94	51–110	11 – 24	69	15
9 1,5-Pentanediol	5.2	92	24– 44	9 – 17	31	12
10 Acetic acid	4.8	88	38– 74	18 – 36	50	24
11 Butyramide	4.9	87	31– 60	18 – 35	41	23
12 Ethanol	4.6	86	49–100	24 – 51	67	33
13 Urethane	4.6	78	46– 95	33 – 68	62	44
14 1,6-Hexanediol	5.0	67	42–200	40 – 190	70	66
15 Pinacol	8.4	84	18– 25	16 – 22	21	19
16 Isopropanol	4.0	66	50–120	60 – 150	72	86
17 Isovaleramide	2.6	55	49–680	76 –1000	92	140
18 *n*-Valeramide	3.6	55	37–110	88 – 270	56	130
19 Ethyl acetoacetate	5.0	61	34– 65	50 – 97	45	66
20 *n*-Propanol	4.4	56	49–110	100 – 220	67	140
21 *t*-Butanol	3.5	50	45–150	98 – 320	69	150
22 Ethyl acetate	2.4	32	68–∞	250 –∞	140	490
23 2-Butanol	3.8	37	48–130	170 – 470	69	250
24 Butylcarbitol	3.9	35	37– 97	170 – 460	53	250
25 Isobutanol	5.3	33	36– 66	220 – 390	47	280
26 *n*-Butanol	5.2	30	38– 70	270 – 490	49	350
27 Aniline	2.8	18	58–400	510 –3500	100	880
28 *t*-Amyl alcohol	4.5	26	40– 86	230 – 500	55	320
29 *t*-Amylamine	4.5	24	39– 83	310 – 660	53	420
30 2-Picoline	6.9	27	29– 45	280 – 430	36	340
31 Benzyl alcohol	3.2	12	61–250	800 –3200	98	1300
32 Vanillin	4.2	13	34– 78	550 –1300	47	770
33 Methoxyphenol	−0.3	−1				
34 Phenylacetic acid	2.8	6				
35 *n*-Valeric acid	1.2	2				
36 Phenol	10.9	18	16– 21	510 – 650	18	570

in Table II apply to the minimum values of the rate constants, but not to the much less reliable maximum values.

It is unlikely that δ_o would be as large as 2.4 μm since this would make k_{ow} for methyl carbamate infinitely large, while that for all other compounds near it in Table II are less than 100 $\mu m\ s^{-1}$. On the other hand it is also unlikely to be zero, leaving the best estimate as an average of these two extremes – 1.2 μm. For the purpose of further discussion this value was adopted in calculating the estimates for the rate constants listed in the last two columns of Table II.

Perhaps one of the most striking features of these results is that the range of values for k_{ow} is so narrow (one order of magnitude) in spite of a wide range for the values of K_{pc} (greater than three orders of magnitude). On the other hand, k_{wo} varies in proportion to K_{pc}. These facts are illustrated in Fig. 4 where the two rate constants are plotted against the partition coefficients and fitted with regression lines. The latter has a slope of virtually zero for the k_{ow} data and, since $k_{wo} = K_{pc} \cdot k_{ow}$, a slope of one is obtained for the k_{wo} data. It was shown previously [2] that if movement across the interface is the rate-limiting step in membrane transport, the membrane permeability constants, P_m, will equal $k_{wo}/2$. It is a well established observation that the permeability constants for most cells vary directly as K_{pc} and therefore the assumption that interfacial transport is rate-limiting is consistent with this observation, at least in a qualitative sense, since, as we

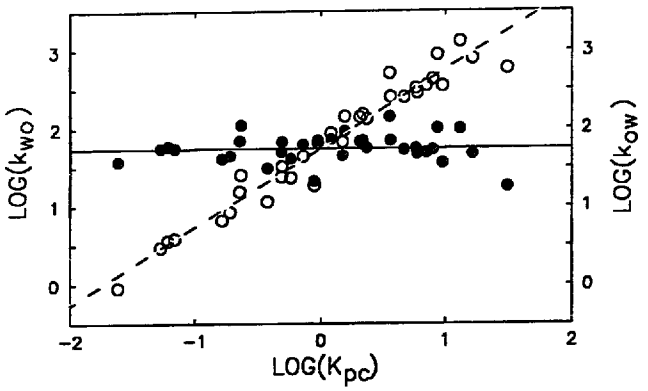

Fig. 4. Plot of the rate constants calculated with the assumption that $\delta_o = 1.2$ μm (Table II) vs. the partition coefficients as listed in Table I. Solid circles: k_{ow} data; open circles: k_{wo} data. The solid line is a regression on the k_{ow} data and has a slope of 0.001 ± 0.04 μm s^{-1} and an intercept with $\log(K_{pc}) = 0$ at 1.74 ± 0.03 μm s^{-1}. The broken line is a regression on the k_{wo} data and has a slope of 1.00 ± 0.04 μm s^{-1}, an intercept with $\log(K_{pc}) = 0$ at 1.74 ± 0.03 μm s^{-1} and a correlation coefficient of 0.97.

have just seen, a calculated P_m would vary as k_{wo} which in turn varies as K_{pc}.

The scattering of the data points around the regression lines in Fig. 4 is no doubt due in part to experimental error. However, a major part of the scattering must also be due to physical causes arising from the variety of chemical structures of the compounds tested. This is perhaps best illustrated by the compounds pinacol ($HOC(CH_3)_2C(CH_3)_2OH$) and methyl carbamate ($H_2NCO_2CH_3$), which, although they exhibit similar water-octanol transfer rate constants ($k_{wo} = 19$ and 26 μm s^{-1}, respectively) have widely differing k_{ow} values (21 and 110 μm s^{-1}, respectively). This may possibly be due to the methyl groups shielding each hydroxyl group on the pinacol molecule, thus slowing the hydration of the hydroxyl groups during entry into the water phase, and, at the same time, ease its entry into the organic phase. Such speculation is premature, however, in the absence of measurements on a greater variety of compounds.

Brodin and Agren [9] measured the rate of mass transfer across a cyclohexane/water interface using a similar moving drop technique to that used here. Although they did not make allowances for unstirred layers in their calculations, assuming them to be negligible, it is interesting to note that their values for k'_{ow} were similar to those reported here, ranging from 10 to 100 μm s^{-1} in spite of a wide range in partition coefficients (0.4 to 160000). The similarity in absolute values of the rate constants to those reported here is also significant in view of the much more lipophilic organic phase they employed, since it seems to indicate that the nature of the organic phase is not important in determining the rate of movement of a compound into the water phase (k_{ow}).

Measurements of interfacial transport rates have also been made using the rotating diffusion cell [10,11]. In this procedure, the organic phase, which is contained within a Millipore filter disk whose pores it completely fills, separates two compartments containing the aqueous phases. Intense stirring of the aqueous phases is possible since the surfaces of the interface are not disturbed by the stirring and their areas therefore remain constant. Thus by measuring the rate of transport of substances between the two aqueous compartments as a function of the degree of stirring, and extrapolating to an infinite stirring rate, the effect of unstirred layers in the aqueous phases can be eliminated. At the same time, the unstirred layer in the organic phase is defined by the thickness of the filter disk which is usually 150 μm. This imposes a permeability constant, P_o, of approximately 7 μm s^{-1} for compounds with a diffusion coefficient of 1000 μm^2 s^{-1}, as found for most compounds in water. If octanol were the organic phase, however, P_o would be only about 1 μm s^{-1}. Thus meaningful measurements with this system (which measures k_{wo}) can only be made on compounds having low partition coefficients and thus low values for k_{wo}. Nevertheless, despite this and other criticisms levelled at this method, the results reported for it are similar to those reported here in spite of a wide variety of solutes and solvents used. For example, Guy, Aquino and Honda [10] found values for k_{ow} between 18 and 30 μm s^{-1} for salicylic acid and methyl nicotinate moving through a layer of C_9 of C_{16} hydrocarbons while Fleming, Guy and Hadgraft [11] found k_{ow} to be 10 μm s^{-1} for the movement of methyl nicotinate from isopropyl myristate into water. These results further confirm the conclusion that k_{ow} is constant to within an order of magnitude regardless of the chemical structure of the compound and of the nature of the organic phase.

Stein [1] culled from the literature a number of what he considered to be reliable values of 'basal permeabilities' of human red blood cells to nonelectrolyte molecules. The term 'basal' suggests that the membrane permeabilities, P_m, are unaffected by specific transport systems, or that they are purely passive in nature. In Table III, a number of these values are compared to those derived from the present data using the relationship $P_m(\text{calculated}) = k_{wo}/2$ [2]. This comparison shows that for the smaller molecules, water and methanol, the measured values are larger than those calculated. This may reflect the presence of small pores in the natural membrane through which these molecules can diffuse. For the remaining molecules, however, the rates appear to be similar, in spite of the fact that the solvent properties of octanol may not correspond closely to those of the lipid bilayer in the membranes. Had we chosen a more lipophilic solvent, for example, K_{pc} and consequently the calculated P_m values, would have been greater for lipophilic compounds, but less for more lipophobic compounds.

TABLE III

Comparison of measured to calculated permeability coefficients

The values for the measured permeabilities, P_m, were taken from Stein's Table 2.1 [1]. MW is the molecular weight of the compound. Calculated permeability coefficients are reported as $k_{wo}/2$. The values for k_{wo} used are listed in Table II with $\delta_o = 1.2\ \mu m\ s^{-1}$.

Compound	MW	P_m	$k_{wo}/2$
Water	18	12	1.5
Methanol	32	37	7.8
Ethanol	46	21	16
n-Propanol	60	65	69
Ethylene glycol	62	0.29	1.8

But even if the resistance to movement of molecules which is imposed by the lipid bilayer/water interface were no greater than that of octanol/water, it would still be high enough to account for the permeability of natural membranes. By contrast, there is no *direct* experimental evidence that the diffusion coefficients of compounds in the transverse direction within the bilayer are 3–4 orders of magnitude lower than they are in water, which they would have to be, to be rate-limiting during permeation. Thus we may conclude that the lipid bilayer in the plasmalemma may act as a barrier to the movement of molecules not be restricting their diffusion as Stein suggests [1], but by requiring them to negotiate a transfer from water to a lipid phase and back again. This, in turn, necessitates the rupturing of bonds between the permeant and water molecules, replacing them with bonds to molecules in the lipid phase, then reversing this process, with all the consequent enthalpic and entropic restrictions, and it may well be these restrictions which inhibits the free movement of molecules through the cell membrane.

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