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The measurement of the rate of transport of solutes in both directions across the aqueous-nonaqueous liquid interface and its significance to membrane permeability

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A technique for measuring the partition coefficients, K_{pc}, and the rate of movement of solutes across an aqueous-nonaqueous interface is described. Using it, measurements of the rate constants for the transport of water and the lower alcohols in both directions across the water-octanol interface were made. The effect of unstirred layers was found to be unimportant in the present case. The partition coefficients of water and the alcohols, calculated as the ratio of the rate constants, showed good agreement with those measured directly. For small molecules, such as the lower alcohols, the permeability constants of natural membranes do not appear to be determined either by K_{pc} or the rate constants, probably due to the presence of pores. Since the resistance offered to water transport across the interface is as high as that of any artificial lipid bilayer for which measurements have been reported, it appears that the main barrier to permeation in these structures is not diffusion within the membrane, but transport across the aqueous-nonequeous interface.

Introduction

When two immiscible liquids are in contact, movement of any solutes which are soluble in both phases will occur across the interface separating them. The rate of solute transfer out of each phase is proportional to its concentration in that phase. Thus for two liquids (labelled 'o' for the organic and 'w' for the water phase) the rates will be $k_{ow}C_o$ from the organic to water phase and $k_{wo}C_w$ in the reverse direction, where C_o and C_w are the respective concentrations and k_{ow} and k_{wo} are the proportionality, or transfer rate constants. Transfer will continue as long as the solute is present and the phases are in contact; however, net transfer between the phases will take place only until the rates of movement in both directions are equal. Such equilibrium will be attained only after the elapse of an infinite time, and if the concentrations at this point are C_o^∞ and C_w^∞, then since $k_{ow}C_o^\infty = k_{wo}C_w^\infty$ we can define a term known as the partition coefficient, K_{pc}, as $K_{pc} = C_o^\infty / C_w^\infty = k_{wo}/k_{ow}$.

The evaluation of K_{pc} for a given solute-solvent system is relatively easy and as a consequence many K_{pc} values appear in the literature (see for example Ref. 1). In contrast, the measurements of individual rate constants require much more sophisticated methods, with the result that relatively few such measurements have been reported. This is true in spite of the obvious importance of the dynamic aspects of interfacial transport, particularly in such fields of study as biological membrane permeability. What is particularly surprising is that so little is known concerning the effect on the aqueous/organic-solvent interfacial transport of substances adsorbed at the interface, and it was with the view of obtaining such knowledge that the present studies were initiated. This report describes the experimental techniques to be employed and illustrates and evaluates them using a relatively simple system involving the movement of water and the lower alcohols between the water and octanol phases. Octanol was chosen as the organic phase since its solvent properties have been found to resemble those of biological systems more closely than other solvents.

Experimental

Three basic experimental systems have been employed in interfacial transport studies: layers, jets and falling drops [2]. Most organic liquids have a density of less than one and can be layered over water. In this configuration the rates of passage of solute from one phase to the othr can be measured simply by quantitative analysis of the solutes in samples taken from either layer as a function of time. The problem with this simple system is that solute concentration gradients, which form on each side of the interface, develop a resistance to solute diffusion with time which can rapidly exceed that of transfer across the interface. Even with stirring there remains a layer of liquid near the interface which is undisturbed. The thickness of this unstirred layer is reduced as the stirring rate increases. At the same time, however, the onset of turbulence with rapid stirring distorts the interface to the point where its area becomes uncertain. This problem is avoided in the rotating diffusion cell [3], in which the organic liquid is confined to a filter paper and is therefore undisturbed by rapid stirring. In this method, rate measurements made at different stirring speeds are extrapolated to infinite speed, at which the resistance of the unstirred layer can be assumed to approach zero.

In the second experimental system, one phase moves through the other in the form of a well-defined jet, the surface area of which can be readily measured. Unstirred layers in such a system will, of course, be negligible. However, only very rapid transport rates can be measured by this method, which is therefore inappropriate for the present study.

In the third system, one phase is stationary while the second passes through it in the form of a drop rising (or falling) under gravity. As the drop passes through the continuous phase, liquid is swept from its leading to its trailing surface, causing rapid internal stirring and reducing the drag which the continuous phase would otherwise produce on the drop. Thus the liquid on both sides of the interface is constantly renewed and unstirred layers have little chance to form [2,4]. At the same time, since the drop is a sphere, its surface area is well defined and can be determined accurately. It is the appealing simplicity of this system which prompted its use in the present study.

Materials. Octan-1-ol (99%) was obtained from Aldrich and used directly from the bottle after saturation with water; methanol was redistilled reagent-grade; ethanol was absolute from Consolidated Alcohols Ltd. Toronto (pharmaceutical grade); propan-1-ol was obtained from Matheson, Coleman and Bell; butan-1-ol was HPLC-grade supplied by Fisher Scientific Co.; ^{3}HHO was obtained from New England Nuclear with a specific activity of 25 mCi/g.

Apparatus and procedure. Two versions of the apparatus used to measure the rate constants and partition coefficients are shown in Fig. 1. In version A, drops of the organic phase rose through a column of the aqueous phase and were sampled at the top, while in version B the aqueous phase was dropped through a column containing the organic phase and was sampled at the bottom. Before use, each phase was saturated with the other at the temperature used for the measurements.

The measurement procedure for the movement of solute out of an octanol drop in the version shown in Fig. 1A was as follows. Syringe S_1 was filled with the organic phase containing the solute and S_3 was filled with the aqueous phase. Syringe S_2 contained the aqueous phase which had been previously equilibrated with the solute in the organic phase. The reason for this is that drops were formed by injecting 30 μl of the solute-containing organic phase into the capillary from S_1 at the bottom of the apparatus in the position shown (D) and were then forced out of the top of the capillary by rapidly injecting 40–50 μl of fluid from S_2 into the apparatus. Since this fluid had already been equilibrated with the drop, no movement of solute occurred until the fully formed drop was ejected from the capillary. The remainder of the apparatus contained the aqueous phase which the stirring circulated in the direction shown by the arrows. To start a measurement, a sample tube (S) consisting of a piece of melting-point capillary-glass tubing (2–2.5 cm long and about 0.1 cm i.d.) was inserted into the top of a short piece of silicon rubber tubing whose bottom end was connected to the top of the apparatus. After injecting the 30 μl of organic phase into the capillary at the bottom of the apparatus, the meniscus at the top of the aqueous phase (M_1) was positioned by operating S_3 so that, following the injection of the drop using S_2, it would still be a few millimeters below the sample tube. A timer was started at the instant the drop was injected and the stirring rate was adjusted so that the drop rose through the wider section of the column to point L, but was unable to enter the narrower section above it due to the higher velocity there. After the required time had elapsed, stirring was terminated, allowing the drop to continue its rise. At the instant it broke through the upper meniscus (M_1), the timer was stopped and by manipulating S_3 about 10–20 μl of the drop contents were forced into the sample tube, which was immediately separated from the apparatus. Analysis of this liquid provided a measure of the solute concentration in the drop as a function of the time interval. Usually, 15–20 similar measurements, but with varying time intervals, were used to estimate the rate constants, as detailed below. In experi-

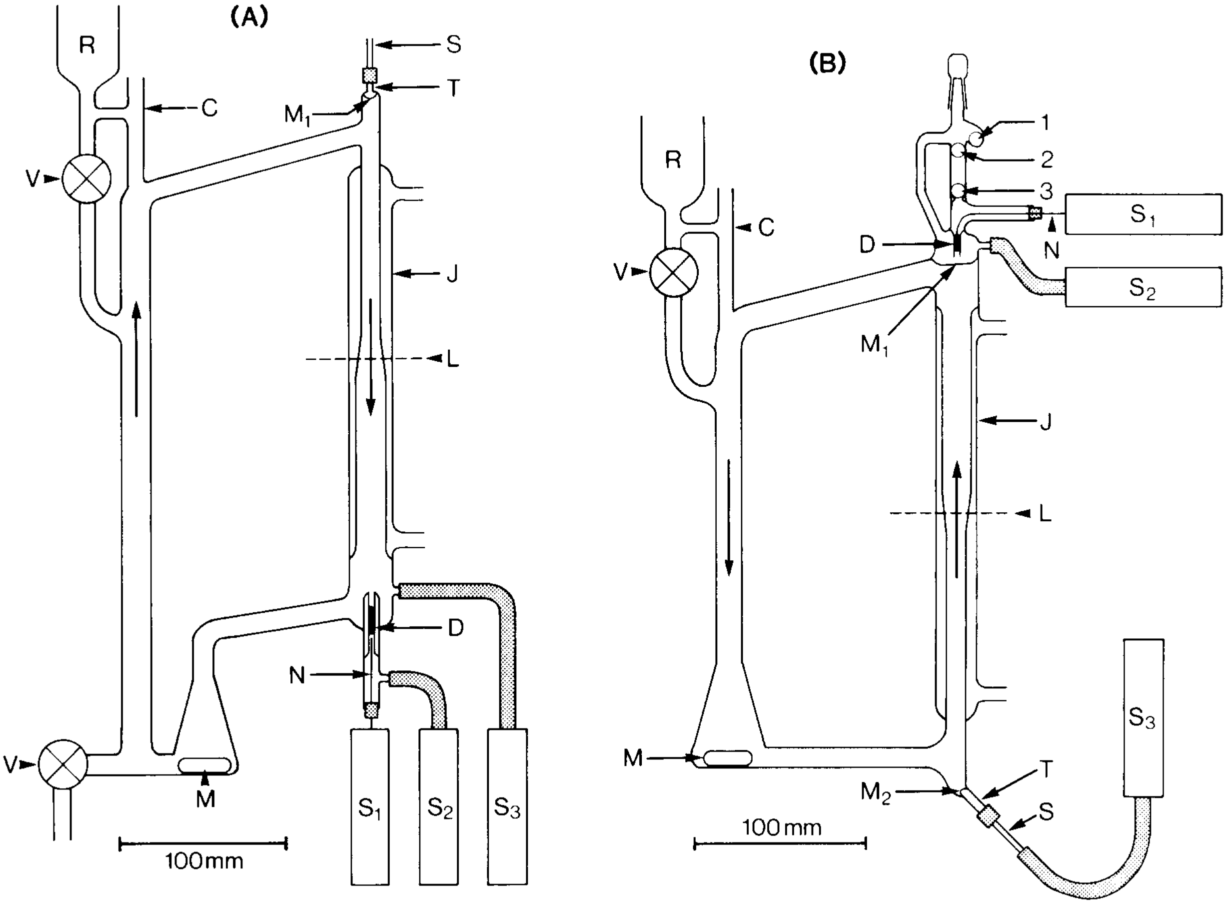

Fig. 1. (A) Rising drop apparatus, for the measurement of the rate of movement of solute between a drop of organic phase rising through a column of aqueous phase. (B) Falling drop apparatus, for the measurement of the rate of movement of solute between a drop of aqueous phase falling through a column of organic phase. C, thermometer port; D, drop-forming medium; J, water jacket; L, level at which drop is suspended by stirring; M, magnetic stirrer; M_i, upper meniscus; M_2, lower meniscus; N, syringe needle; R, reservoir; S, sample collecting tube; S_1, S_2 and S_3, 2 ml micrometer syringes; T, tube to which sample tube is attached; V, glass-Teflon valve; 1, 2 and 3, positions of steel ball.

ments in which solute was moving into the drop, the solute was present in the bulk aqueous phase but absent from the liquids in the syringes. In addition, several measurements were made after time intervals of sufficient length to ensure virtual equilibrium, and the average of these measurements was used to determine K_{pc}.

The rate of movement of solute between an aqueous phase drop and the continuous organic phase was measured using the apparatus shown in Fig. 1B. Since the drop fell through the continuous phase it was introduced at the top and collected at the bottom. In this experiment, syringe S_1 contained the drop-forming solution, while S_2 and S_3 contained air. 30 μl of aqueous solution were deposited in the position shown (D, Fig. 1B) with the sample tube (S) and silicon rubber tubing disconnected from tube T. A steel ball-bearing in the upper part of the apparatus was lifted by a magnet to position 1 (Fig. 1B), and by enlarging the volume of S_2, air was drawn into tube T. A sample tube was then connected to T by means of the silicon rubber tubing and S_3 was connected to its other end. Adjusting S_3 gave a slightly convex meniscus (M_2) at the upper end of T. To start a measurement, the steel ball was moved to position 2, then dropped to position 3, forcing air downward and ejecting the drop which fell into the column of organic liquid. Again, the stirring was adjusted so that the drop was suspended at L for the required time, then allowed to drop further by terminating stirring. A timer which was started when the drop passed through M_1 was stopped when it passed through M_2. As before, 10–20 μl of the drop contents were drawn into the sample tube by manipulating S_3, and analyzed.

When not in use, both versions of the apparatus were filled with normal KOH. This treatment of the glass surface caused it to repel the organic phase so that drops of the latter would not adhere to it and, furthermore, aided sampling by forming more well-defined menisci.

Analytical procedures. Two methods were used to analyze the samples: gas chromatography for the alcohols, and scintillation counting for the ^{3}HHO. For GC, 0.5–2 μl samples of the liquid in the sample tube were withdrawn and injected into a Hewlett Packard 5880A series chromatograph with flame ionization detector and a Chrompack (Middelburg, The Netherlands) CP^{TM} Wax-57-CB fused silica capillary column. The procedure used to measure tritiated water movement comprised weighing scintillation vials containing sample tubes, removing the tubes to collect the sample, returning them to the vial and reweighing to give the sample weight by difference. Scintillation fluid was then added to the vials and the radioactive contents were assayed.

Calculations

If n is the number of moles of solute in an octanol drop of area A, the flux of solute into the drop will be

$$dn/dt = A(k_{wo}C_w - k_{ow}C_o)$$

Due to the large volume of the water phase relative to that of the drop, C_w is a constant and only C_o is a variable. After an infinite time the system reaches equilibrium and net transport ceases. At this point, $dn/dt = 0$ and C_o has the value C_o^∞. Furthermore, since influx must equal efflux, $k_{wo}C_w = k_{ow}C_o^\infty$ and therefore we can rewrite the original equation as $dn/dt = Ak_{ow}(C_o^\infty - C_o)$ or, for a drop of volume V, letting $C_o = n/V$,

$$dC_o/dt = (A/V)k_{ow}(C_o^\infty - C_o)$$

Integrating this expression between the limits $C_o = C_o^0$ at $t = 0$ and C_o at time $= t$, gives

$$\ln(C_o^\infty - C_o) = \ln(C_o^\infty - C_o^0) - Ak_{ow}t/V \qquad (1)$$

Measuring C_o as a function of t and plotting the results according to Eqn. 1 should provide a straight line whose slope is $-Ak_{ow}/V$, or for a drop whose volume is 0.030 cm^3, k_{ow} = the slope multiplied by -642.5 μm. The advantage of this procedure for measuring k_{ow} is that the initial concentration in the drop, C_o^0, need not be known. Thus if there is a constant error in initiating the time measurement, in which case the assumed value of C_o^0 is not the true concentration of the drop-forming solution, this will not affect the measurement of k_{ow}, and one possible source of error is eliminated.

Rate measurements may be made in two ways: either the solute is initially present in the drop, but not in the continuous phase, and its loss from the drop is measured; or it is present initially only in the continuous phase, in which case uptake by the drop is determined. When the rate of loss is measured, $C_o^\infty = 0$ and Eqn. 1 can be rearranged to

$$\ln C_o = \ln C_o^0 - Ak_{ow}t/V \qquad (2)$$

For uptake measurements, expression 1 is used but C_o^∞ must be determined. In practice, a sufficient number of measurements at different times were made to estimate $t_{1/2}$, the half-time of the uptake. Measurements of C_o were then made after a time interval of ten half-lives, at which point the concentration should be greater than 99.9% of the true infinite time value. This quantity (taken as C_o^∞) can be used to determine K_{pc}, which is equal to C_o^∞ divided by C_w, the concentration of solute in the water phase.

It should be noted that whether solute movement, as measured by the apparatus in Fig. 1A, is into or out of the octanol drop, only k_{ow} is determined according to Eqns. 1 and 2. The rate constant k_{wo} must be measured using a water drop in the apparatus depicted in Fig. 1B, and is calculated from equations derived in a similar manner to 1 and 2. These have the form

$$\ln(C_w^\infty - C_w) = \ln(C_w^\infty - C_w^0) - Ak_{wo}t/V \qquad (3)$$

and

$$\ln(C_w) = \ln(C_w^0) - Ak_{wo}t/V \qquad (4)$$

where C_w^∞ and C_w^0 are the infinite and zero-time concentrations of solute in the water drop. Since 30 μl drops were used in all the measurements reported here, the rate constants were found by multiplying the slopes of the lines, plotted according to Eqns. 1–4, by $-V/A = -642.5$ μm. C_w^∞ was determined in the same way as C_o^∞ and was used to estimate the partition coefficient $K_{pc} = C_o/C_w^\infty$.

In measuring the tritiated water transfer, the exchange of tritium atoms between water and octanol, $\text{OcOH} + {}^3\text{HHO} \rightleftharpoons \text{OcO}^3\text{H} + \text{H}_2\text{O}$ (where Oc represents the octyl radical), must be considered. This reaction has been shown [5] to occur with a half-time of 10^{-2}–10^{-3} s and may therefore be considered to be instantaneous for the present purposes. Thus, when tritiated water enters an octanol drop, some of the tritium will exchange with octanol, reducing the radioactivity of the water which has entered the drop. The net movement of ^{3}HHO will continue into the drop until its activity there equals that in the water phase. This will require that more ^{3}HHO enter the drop than is required to raise the activity of the water in the drop alone, since that of the octanol must also rise to its equilibrium value. Since there is 1 mol of exchangeable hydrogen atom per mol of octanol and 2 mol of exchangeable hydrogen atoms per mol of water, the radioactive dpm of 9 g water should be equal to that of 130.23 g octanol at equilibrium. Defining the activities as a_w = dpm/g water in the drop and a_o = dpm/g octanol in the drop, then $a_o/a_w = 0.06911$ at all times. The total dpm in the drop will be the sum of that due to the water and octanol in the drop, or $a_d w_d = a_w w_w + a_o w_o$, where a_d is the total activity of the drop, and the weight of the drop, w_d, is the weight of the water, w_w, plus that of the octanol in the drop, w_o. Thus $a_d/a_w = 0.9309(w_w/w_d) + 0.06911$. Since the octanol phase was saturated with water throughout the measurements, w_w and w_d are constant and their ratio $w_w/w_d = S$, the solubility of water in the octanol phase in g/g. After the two phases have been in contact for an infinite length of time, the activity of the water in the drop, a_w^∞, will equal that of the bulk water phase, and a_d will have reached its equilibrium value, a_d^∞. Letting the ratio at this point be $R^\infty = a_d^\infty/a_w^\infty$ (i.e., the dpm/g of the octanol phase divided by the dpm/g of the water phase at infinite time),

$$S = (R^\infty - 0.06911)/0.9309$$

To derive an expression for ^{3}HHO movement equivalent to Eqn. 1, let n_t be the total number of moles of ^{3}H in the drop (as both ^{3}HHO and octan-1-[^{3}H]ol) while C_w and C_o are the concentrations of ^{3}HHO alone in the bulk and drop, respectively. If n_w = moles of ^{3}HHO in the drop, $C_o = n_w/V$ and the ratio of this to n_t will be

$$\frac{n_w}{n_t} = \frac{\text{dpm}_w}{\text{dpm}_d} = \frac{a_w w_w}{a_d w_d} = S\frac{a_w}{a_d}$$

Due to the rapidity of exchange of ^{3}H between water and octan-1-ol, the ratio of the activity of the water in the drop to that of the drop as a whole will be constant and therefore equal to its value at infinite time. Thus $a_d/a_w = R^\infty$ and $n_w = (S/R^\infty)n_t$. Next, letting $C_t = n_t/V$ be the total concentration of ^{3}H in the drop, and C_b that in the bulk water, (which is constant because of the large volume),

$$\frac{dC_t}{dt} = \frac{A}{V}\left(k_{wo}C_b - \left(\frac{S}{R^\infty}\right)k_{ow}C_t\right)$$

At infinite time $dC_t/dt = 0$ and C_t reaches the value C_t^∞. Thus

$$\frac{dC_t}{dt} = \frac{AS}{VR^\infty}k_{ow}(C_t^\infty - C_t)$$

The activity of the drop will be proportional to C_t, or $C_t = pa_d$, where p is a constant. In practice R is taken as the ratio of the cpm per g of drop phase divided by the cpm per g of the water phase and as this equals a_d/a_b, substituting pa_bR for C_t and pa_bR^∞ for C_t^∞ and integrating, we obtain

$$\ln(R^\infty - R) = \ln(R^\infty - R^0) - (AS/VR^\infty)k_{ow}t$$

In this case, k_{ow} is the slope of the line multiplied by the factor $(-VR^\infty/AS)$. This is true also for the loss of ^{3}HHO from an octan-1-ol drop (equivalent to Eqn. 2) but not for the measurements with a water drop where the factor is, as before, $(-V/A)$, so that Eqns. 3 and 4 still apply here.

TABLE II

TRANSFER RATE CONSTANTS AND PARTITION COEFFICIENT FOR *n*-BUTANOL BETWEEN WATER AND OCTANOL AS A FUNCTION OF TEMPERATURE

Both k_{ow} and K_{pc} were measured as uptake of butanol into an octanol drop from a water solution of 10 mM. The k_{wo} values (in brackets) were calculated as $k_{wo} = k_{ow}K_{pc}$.

T (°C)	k_{ow}	K_{pc}	k_{wo}
15	8.8 ± 0.5	6.07	(53.4)
20	10.6 ± 0.5	6.01	(63.7)
25	10.0 ± 0.4	6.46	(64.6)
30	14.0 ± 0.5	7.02	(98.3)
35	15.4 ± 0.5	7.67	(118)

Results

Table I lists the rate constants and partition coefficients of all compounds at 25°C. Similar determinations made at various temperatures are reported for butanol in Table II and water in Table III. Arrhenius plots of these data give activation energies for the transport of butanol which are 4.9 ± 1.0 kcal · mol^{-1} in the direction octanol to water and 2.2 ± 0.4 kcal · mol^{-1} in the opposite direction. The corresponding values for water are 6.9 ± 0.4 and 6.6 ± 0.7 kcal · mol^{-1}. The density of water-saturated octanol was found to be 0.833 g/cm^3 at 25°C.

According to equations in the previous section, letting $C_t = C_t^\infty$ (and $dC_t/dt = 0$) and substituting pa_w^∞ for C_b, and pa_d^∞ for C_t^∞, it can be shown that $k_{wo}/k_{ow} = S$. Thus the solubility of water in the octanol phase can be obtained in three ways:

TABLE I

TRANSFER RATE CONSTANTS AND OCTANOL/WATER PARTITION COEFFICIENTS FOR ^{3}HHO AND THE LOWER ALCOHOLS

Temperature was 25°C. Rate constants are in units of μm · s^{-1} ± S.D.

Compound	k_{ow} (octanol drop)		k_{wo} (water drop)		Partition coefficient		
	loss	uptake	loss	uptake	octanol drop	water drop	k_{wo}/k_{ow}
^{3}HHO	44.4 ± 3.4	44.6 ± 4.5	2.3 ± 0.2	2.3 ± 0.2	0.0524	0.0535	0.0517
Methanol	50.5 ± 3.7	45.0 ± 3.0	11.5 ± 1.0	10.1 ± 0.9	0.245	0.203	0.227
Ethanol	43.9 ± 1.5	40.1 ± 1.7	20.1 ± 1.3	20.9 ± 2.8	0.493	0.481	0.488
n-Propanol	25.6 ± 0.5	28.3 ± 2.3	56.4 ± 2.7	52.3 ± 2.7	2.21	1.94	2.02
n-Butanol	11.5 ± 0.3	10.8 ± 0.2	65 ± 17	73 ± 20	7.12	7.03	6.2

TABLE III

TRANSFER RATE CONSTANTS AND THE SOLUBILITY IN OCTANOL FOR WATER AS A FUNCTION OF TEMPERATURE

All constants were measured as uptake into a drop with the exception of those marked by an asterisk, which were measured as loss. Units are $\mu m \cdot s^{-1}$ for the rate constants and g water per g water-saturated-octanol for the water solubility.

T (°C)	Octanol drop		Water drop		k_{wo}/k_{ow}
	k_{ow}	solubility	k_{wo}	solubility	
15	29.2 ± 2.9	0.0452	1.44 ± 0.15	0.0504	0.0493
20	38.5 ± 3.1	0.0483	2.04 ± 0.12	0.0528	0.0530
25	44.6 ± 4.9	0.0524	2.28 ± 0.20	0.0535	0.0511
25	44.4 ± 3.4 *		2.31 ± 0.19 *		0.0520
30	51.5 ± 4.2	0.0509	2.74 ± 0.35	0.0594	0.0532
35	68.7 ± 4.0	0.0544	3.53 ± 0.21	0.0560	0.0514
40	77.3 ± 6.1	0.0500	3.58 ± 0.20	0.0557	0.0463

by the direct measurement of equilibrium concentration in an octanol drop; in the same way in a water drop; and from the ratio of the rate constants. These results may be compared directly in Tables I and III. Averaging these results at 25°C (Table III) the solubility of water is found to be 0.052 ± 0.002 g per g octanol phase.

Discussion

Effect of unstirred layers

The validity of the results depends on the magnitude of the effects of the unstirred layers. According to Dainty [6], if δ_o and δ_w are the thicknesses of these layers in octanol and water, and D_o and D_w are the diffusion coefficients in the same media, the apparent rate constants k'_{ow} and k'_{wo} will be given by the expressions

$$\frac{1}{k'_{ow}} = \frac{1}{k_{ow}} + \frac{\delta_o}{D_o} + \frac{\delta_w}{D_w} \quad \text{and} \quad \frac{1}{k'_{wo}} = \frac{1}{k_{wo}} + \frac{\delta_o}{D_o} + \frac{\delta_w}{D_w}$$

TABLE IV

COMPARISON OF THE MEASURED AND CALCULATED VALUES OF MEMBRANE PERMEABILITY

The partition coefficients, K_{pc}, are averages from the present work. The calculated permeability constants $= k_{wo}/2$, while the measured constants are for plant and animal cells. All rate constants are in $\mu m \cdot s^{-1}$.

Compound	K_{pc}	Calculated	Measured values (Ref.)		
			8	9	10
Water	0.052	1.2	91.5	–	25
Methanol	0.225	5.4	1.14	3.3	5.7
Ethanol	0.487	10	0.88	2.4	5.5
n-Propanol	2.06	27	0.64	2.6	7.2
n-Butanol	7.08	35	0.41	2.5	–

Since the resistance to diffusion can be taken as the reciprocal of the rate constant, then if the resistances to passage of the solute across the interface in the two directions are $R_{ow} = 1/k_{ow}$ and $R_{wo} = 1/k_{wo}$, and $R_o = \delta_o/D_o$ and $R_w = \delta_w/D_w$ are the resistances of the unstirred layers, the apparent resistances will be $R'_{ow} = R_{ow} + R_u$ and $R'_{wo} = R_{wo} + R_u$, where $R_u = R_o + R_w$. (δ_o is assumed to be the same in the drop as in the continuous medium. The same assumption is made about δ_w.) The apparent partition coefficient as derived from the rate constants will therefore be

$$K'_{pc} = \frac{k'_{wo}}{k'_{ow}} = \frac{R'_{ow}}{R'_{wo}} = \frac{R_{ow} + R_u}{R_{wo} + R_u} = \frac{K_{pc} + r}{1 + r}$$

whereas the true constant $K_{pc} = R_{ow}/R_{wo}$. Here r is the ratio of the unstirred layer resistance to that of the interface ($r = R_u/R_{wo}$). This ratio may also be written as $r = R_u k_{wo}$, which tells us that when δ and hence R_u are small, $K'_{pc} \approx K_{pc}$, but as the layer thickness, or the constant k_{wo}, increases, K'_{pc} approaches one. This effect is apparent in Table I where, for compounds having lower values of k_{wo}, there is good agreement between the values for K_{pc} measured directly as a ratio of concentrations in the two phases and those measured indirectly as the ratio k_{wo}/k_{ow}. For butanol, which has the highest value of k_{wo} reported here, however, the latter value appears to be lower. Thus we may conclude that for 30 μl drops of these two liquids, internal stirring is sufficient to eliminate unstirred layer effects, provided the interfacial transfer rate constants do not exceed 60 $\mu m \cdot s^{-1}$.

The importance of stirring within the drop can be further illustrated by comparing the rates measured here against those expected in the absence of stirring. The latter may be obtained from the equation given by Jost [7] which provides the average concentration of a diffusing solute, $\bar{C}$, in a sphere of radius r_o, as a function of time, t. If C^0 is the initial and C^∞ the final concentration, then for t which is sufficiently large:

$$\frac{\bar{C}-C^\infty}{C^0-C^\infty} \approx \frac{6}{\pi^2}\exp\left[-\frac{\pi^2 D}{r_o^2}t\right]$$

Letting $\bar{C}=0.5C^\infty$, $C^0=0$ (or conversely $\bar{C}=0.5C^0$ and $C^\infty=0$) and $D=10^{-5}$ cm^2 s^{-1}, the half-time for diffusion in a 30 μl sphere (i.e., in a drop with no stirring) should be 73.6 s. In contrast, half-times less than 5 s were found for the movement by butanol out of a 30 μl water drop, showing that stirring is indeed active in the drops used here.

Significance of the measurements to membrane transport

There are three steps involved in the passive transport of a solute (X) through the lipid bilayers of the plasmalemma:

$$X_o \underset{k_o}{\overset{k_i}{\rightleftharpoons}} M_o \underset{D}{\overset{D}{\rightleftharpoons}} M_i \underset{k_i'}{\overset{k_o'}{\rightleftharpoons}} X_i$$

Starting at a concentration X_o outside the cell, the solute moves across the outer membrane interface, at a rate determined by the constant k_i, to establish a concentration M_o just inside the membrane. Diffusion through the membrane next takes place at a velocity dependent on the diffusion coefficient, D, as a result of which it arrives at the opposite interface, where its concentration is M_i. Finally, movement occurs through the inner membrane interface to the cell interior (rate constant k_o'), where its concentration is X_i. The rate of net inward transport across the outer membrane surface will be the rate into the membrane, Ak_iX_o, minus that out, Ak_oM_o. Similarly, for the inner surface the rate will be $A(k_o'M_i - k_i'X_i)$. Diffusion is usually assumed to obey Fick's law from which the inward rate of diffusion can be shown to approximate $(AD/\delta)(M_o - M_i)$, where δ is the membrane thickness. Under steady-state conditions, all these rates must be equal so that if n is the number of moles of solute in the cell

$$\frac{dn}{dt} = \frac{A(D/\delta)(k_o'k_iX_o - k_ok_i'X_i)}{k_ok_o' + (D/\delta)(k_o + k_o')}$$

At infinite time, $dn/dt = 0$ and since this type of transport is passive, X_o will equal X_i so that $k_o'k_i$ must equal k_ok_i' and the rate expression becomes

$$\frac{dn}{dt} = \frac{A(D/\delta)k_o'k_i(X_o - X_i)}{k_ok_o' + (D/\delta)(k_o + k_o')}$$

Finally, if $k_o' = k_o$, as would be the case for a symmetrical bilayer

$$\frac{dn}{dt} = \frac{Ak_i(D/\delta)}{k_o + 2(D/\delta)}(X_o - X_i) = AP(X_o - X_i)$$

with $P = k_i(D/\delta)/(k_o + 2(D/\delta))$ being the permeability constant. If the slow step is assumed to be diffusion within the membrane, i.e. if $k_o > D/\delta$, then $P \approx K_{pc}D/\delta$, where $K_{pc} = k_i/k_o$ is the partition coefficient of the solute between the water and the membrane lipids. This assumption is usually made, since it is found in general that for a series of compounds, P is proportional to K_{pc}. In some cases, notably with the series of compounds examined here, this simple relation does not hold and it is worth considering the alternative assumption, that transport across the interface is the slow process, or that $D/\delta \gg k_o$, making $P \approx k_i/2$. Assuming that the solvent properties of octanol closely approximate those of the membrane lipids, k_o and k_i may be replaced by k_{ow} and k_{wo}, allowing an estimate of the permeability constants for water and the lower alcohols to be made for this case. These are listed in Table IV, together with their partition coefficients, and are compared with three sets of experimental data [8–10]. Here it can be seen that while K_{pc} and the calculated values of the permeability constant increase with molecular weight, the measured permeabilities appear to be roughly constant (except for water, of course, which is anomalously high). This, coupled with the fact that the calculated values of P (again excluding water) tend to be higher than the measured values, appears to argue against interfacial transport as the slow step in passive membrane transport.

The high value for the permeability of natural membranes to water probably reflects the presence of pores [11], which might also account for the greater-than-expected permeance of smaller molecules such as methanol [9]. Lipid bilayers, on the other hand, are about an order of magnitude less permeable to water than natural membranes, having values for P which range from 1 to ≈ 50 $\mu m \cdot s^{-1}$ [11]. Since the calculated value for P found here is at the lower end of this range, interfacial transport must be the rate-limiting process for water movement in artificial bilayers, since if diffusion were slower the measured P would have to have an even lower value than the calculated value in Table IV. In short, the resistance at the aqueous-nonaqueous interface appears to account fully for the total resistance to water movement through artificial bilayers. It would be interesting to know if the same were true for the lower alcohols. Unfortunately, such a comparison cannot be made at the present time, since the values of the permeability constants of these compounds in artificial membranes have not been reported.

The activation energy for water transport across the water-octanol interface is also at the lower end of the range found for lipid bilayers at or above their transition temperatures (≈ 6–15 $kcal \cdot mol^{-1}$ [11,12]). Here again, interfacial transport could account for this value, but in this case the comparison is less convincing.

The higher alcohols are well-known surface-active agents and as a consequence a high degree of orientation of octanol molecules at the water interface is to be expected. The effect on transport rates produced by such orientation and packing, not only of solvent but also of other molecules (particularly lipids), is of considerable interest, especially in relation to natural membrane permeability. Such a study, using the methods described here, is presently under way.

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