Permeability of Red Cell Membranes to Small Hydrophilic and Lipophilic Solutes

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ABSTRACT The permeability coefficients of a series of amides, ureas, and diols have been measured on red cells of man and dog using the minimum volume method of Sha'afi et al. When the molecules are grouped according to their ether-water partition coefficients, k_{ether}, the behavior of the hydrophilic molecules, with k_{ether} less than water, is different from that of the lipophilic molecules, characterized by k_{ether} greater than water. The rate of permeation of the hydrophilic molecules through an aqueous pathway is determined by the molar volume, a parameter in which the geometrical measure of molecular volume is modified by hydrogen-bonding ability. This indicates the importance of chemical interactions within the aqueous path. The permeation of the lipophilic molecules is determined in the first instance by k_{ether}, taken as a measure of the ease with which the molecule can escape from its aqueous environment. Within the membrane, lipophilic permeability is modified both by steric factors and by the formation of hydrogen bonds with membrane components. These data allow one to infer that lipid-soluble molecules travel through an organized structure within the lipid membrane and come into contact with polar moieties.

Numerous classical studies have been carried out on the permeability of red cells to homologous series of nonelectrolytes, particularly by Jacobs and Höber and Ørskov (see Danielli [1]). These investigators computed permeability coefficients indirectly from the time required for red cells to hemolyze when placed in a solution containing the permeant nonelectrolyte. The red cells swell because the entrance of a permeant solute moving down its concentration gradient causes an imbalance of water activity. Water then moves down its own activity gradient and the process is terminated when

the cells hemolyze. The kinetics clearly depend on other parameters in addition to solute permeability.

More recently Sha'afi et al. (2) have developed a method by which the permeability coefficient may be measured at a time when solvent movement has virtually ceased. When a red cell is placed in a solution containing an isosmolal concentration of nonpermeant solute together with a suitable concentration of the permeant solute, the cells initially shrink as water moves out in response to the applied osmotic pressure gradient. The cell volume then reaches a minimum value when the net volume flow of water *out* equals the net volume flow of solute *in*. Subsequently the direction of net water flux is reversed so that the cells begin to swell and finally return to their initial volume when all the activity gradients have been dissipated. The permeability coefficient may be determined from the minimum cell volume at the precise time when the net volume flow across the membrane is passing through zero.

We have used our method to measure the permeability coefficients of a series of graded amides, of urea and two substituted urea derivatives, and of four diols in which the properties of isomers were of particular interest. The results of the earlier classical studies (1) had already shown that permeability is governed by at least three factors: molecular size, lipid solubility, and the chemical nature of the solute. The results of our measurements have enabled us to assess the importance of each of these three factors separately and to infer some of the important membrane properties that determine permeability.

EXPERIMENTAL METHOD

The permeability coefficient, ω, was determined for membranes of human and dog red cells by the minimum method of which a detailed account has been given by Sha'afi et al. (2). The method depends on measurements of the time course of red cell volume change in the rapid reaction stop-flow apparatus described by Sha'afi et al. (3). Red cells are rapidly mixed with a buffer solution containing a hypoosmolar concentration of salts together with the solute whose permeability is being measured. Under these conditions the cells first shrink to a minimum volume and then swell again to their final volume when both the concentration gradient of the permeant solute and the activity gradient of the water have fallen to zero. ω is determined from the ratio of the minimum volume to the original volume together with the first derivative of the rate of volume change at the minimum volume, $(d^2V/dt^2)_{min}$, according to equations previously given (2). The data obtained by this procedure are taken when net volume flow is zero or close to zero, a necessary condition for accurate measurements of ω as discussed extensively by Sha'afi et al. (2). In a few instances the reflection coefficient, σ, was also measured by the method of Goldstein and Solomon (4).

Measurements were made on several homologous series of compounds: (*a*) all the straight chain amides from formamide to valeramide, (*b*) the branch chain amides,

isobutyramide and isovaleramide, (*c*) urea and two methyl substituted ureas, and (*d*) four short chain diols including three isomers of butanediol. All solutes were reagent grade chemicals, or similar, obtained from Eastman Kodak Co., Rochester, N.Y., Fisher Scientific Co., Boston, Mass., or Aldrich Chemical Co., Cedar Knolls, N.J.

As has been discussed (reference 2; see also the following paper by Savitz and Solomon) the values of ω determined by the minimum method, in which the concentration of the permeant solute is 0.3–0.8 M, agree with ω determinations in tracer experiments, in which the solute concentration is 0.5–5 mM. This suggests that the effect of solute concentration on ω is relatively unimportant. However, the two methods of measurement are quite different, so it is still possible for a concentration effect to be masked by this difference. Tracer experiments in which the solute concentration was varied over wide ranges would be required to evaluate the concentration dependence of ω.

RESULTS AND DISCUSSION

Table I gives the values of all the diffusional permeability coefficients, ω, obtained. In Fig. 1, the permeability coefficients of the straight chain amides for both man and dog are plotted as a function of the number of unscreened CH_2 groups in the solute. The presence of a minimum in the graph indicates that at least two parameters must be involved in amide diffusion through the red cell membrane. One of them decreases in relative importance with the number of CH_2 groups, while the other increases with the same parameter. An examination of the selected physical properties of the diffusing solutes given in Table II shows that in the amide homologous series, the cylindrical radius increases rapidly from formamide to acetamide, and much more slowly from propionamide to valeramide. On the other hand, the ether: water partition coefficient, k_{ether}, taken as an index of lipid solubility, increases rapidly with the number of CH_2 groups.

A second observation about Fig. 1 is that amide permeability in dog red cell membrane is faster than in man, the difference being greater in the case of lipophilic amides. This observation points to significant differences in the properties of the two species, which corroborate previous observations on red cell water permeability by Vieira, Sha'afi and Solomon (5). The data in Fig. 1 appear to be consistent with Collander and Bärlund's hypothesis (6) that biological membranes act not only as a selective solvent but also as a molecular sieve, and to support the principle of a porous model for the red blood cell membrane.

Parameters Governing Red Cell Membrane Permeability

There are three important variables which need to be considered separately in understanding the permeation process for the solutes that we have studied. The first is a parameter describing lipid solubility, the second a parameter

dependent on molecular size, and the third a parameter which is concerned with the chemical nature of the solute. Our studies indicate that three such parameters are sufficient to account for the major permeability properties of all the small nonelectrolytes we have studied. The model is perforce empirical and its specific properties depend upon the exact nature of each of the parameters that have been selected.

The lipid solubility parameter which we have chosen is the ether:water

TABLE I

PERMEABILITY COEFFICIENTS, ω, IN HUMAN AND DOG RED CELLS

Solute	Symbol	Man* $\omega \times 10^{15}$	Dog $\omega \times 10^{15}$
		mol dyne^{-1} sec^{-1}	mol dyne^{-1} sec^{-1}
Water‡	W	136	232
Formamide	F	18±1 (2)	23±5 (4)
Acetamide	A	5.0±0.5 (4)	9±2 (2)
Propionamide	P	4.0±0.5 (3)	7±1 (5)
Butyramide	B	14±1 (3)	21±4 (4)
Isobutyramide	IB	5±1 (3)	8.5±0.5 (2)
Valeramide	V	27±2 (2)	63±8 (4)
Isovaleramide	IV	7.2±0.5 (2)	25±3 (2)
Urea	U	15±1 (6)	
Methyl urea	MU	2.0±0.3 (2)	
1,3-Dimethyl urea	DMU	1.1±0.2 (2)	
1,3-Propanediol	1,3-PD	1.2±0.1 (2)	
1,4-Butanediol	1,4-BD	0.9±0.2 (3)	
1,3-Butanediol	1,3-BD	2.0±0.3 (3)	
2,3-Butanediol	2,3-BD	3.1±0.8 (2)	

* The number of experiments is indicated in parentheses. Errors are standard errors of the mean.
‡ Data for water taken from Vieira, Sha'afi, and Solomon (5).

partition coefficient as determined by Collander and Bärlund (6) and Collander (7, 8). The olive oil:water partition coefficient might have been used, but we have found empirically that the use of k_{ether} gives a better fit to our data. The ratio between these two partition coefficients lies between relatively narrow limits as discussed in detail by Collander and Bärlund (6). The partition coefficients of nonelectrolytes between water and a variety of organic solvents have been studied by Hansch, Quinlan, and Lawrence (9) who found that aqueous solubility was the primary determinant of partition between water and a wide variety of organic solvents including alcohols, ketones, esters, and ethers. Their experiments showed that virtually any

monofunctional organic liquid would serve equally well to represent the lipid phase in partition experiments with water. The values of k_{ether} given in Table II have been generally determined at relatively high solute concentrations of the order of 0.05–5 M in the water phase. In many cases (for example: urea, 5 M; formamide, 5 M; acetamide, 2 M) the concentrations were very much higher than the concentrations at which our measurements were

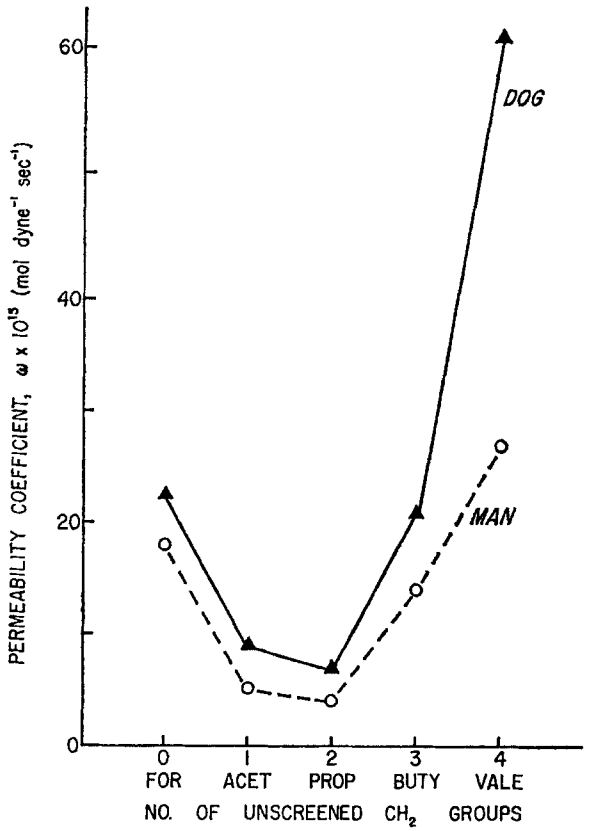

FIGURE 1. Permeability coefficients of a homologous series of straight chain amides in red cell membranes of man and dog.

made, which varied from 0.3 to 0.8 M. No values are known to us for k_{ether} for isobutyramide and valeramide. The values enclosed in square brackets in Table II have been taken arbitrarily as equal to butyramide and isovaleramide, respectively, a process which introduces an error in those instances of the order of 30%. Although the comparisons with k_{ether} have turned out to be very illuminating, it must be kept in mind that more appropriate coefficients than k_{ether} may later become available, as for example, partition coefficients determined in the appropriate concentration range between plasma and a purified phospholipid, or a membrane lipid extract.

One of us (C. M. G-B.) has made preliminary measurements of partition coefficients of amides between red cell water and bulk water. The ratios are very close to unity for formamide, acetamide, and propionamide. The partition coefficient is greater than unity for butyramide and isobutyramide; furthermore, it is not a function of concentration between 10^{-1} and 10^{-3} M.

The cylindrical radius of the permeating molecule is the measure of molecular size which Soll (10) has shown to be of most importance in the steric

TABLE II

PHYSICAL CHEMICAL PROPERTIES OF SOLUTES*

Solute	Molar volume	Cylindrical radius	Density	k_{ether}
	$cm^3\ mol^{-1}$	A	$g\ cm^{-3}$	
Water	18.00	1.50	1.00	0.003
Formamide	40.00	2.07	1.134	0.0014
Acetamide	59.06	2.38	0.998	0.0025
Propionamide	70.14	2.61	1.042	0.013
Butyramide	84.41	2.68	1.032	0.058
Isobutyramide	86.00	2.97	1.013	[0.058]
Valeramide	98.87	2.75	1.023	[0.17]
Isovaleramide	104.8	3.08	0.965	0.17
Urea	44.98	2.41	1.32	0.00047
Methyl urea	61.52	2.60	1.204	0.0012
1,3-Dimethyl urea	77.15	2.70	1.142	0.0031
1,3-Propanediol	72.28	2.56	1.053	0.012
1,4-Butanediol	88.30	2.61	1.017	0.019
1,3-Butanediol	89.67	2.77	1.005	0.042
2,3-Butanediol	86.00	2.98	0.987	0.029

* The values for k_{ether} have been taken from Collander (7, 8) except for water which was determined by one of us (C. M. G-B) using THO. The molar volume is obtained from the molecular weight and the density of the pure crystal as given in the usual handbooks. The cylindrical radius is the minimum cylindrial radius which will contain the nonhydrated molecule as measured with molecular models.

interactions which govern the values of the reflection coefficient, σ, for human red cells. Gary-Bobo, DiPolo, and Solomon (11) have also found the cylindrical radius to be the parameter of choice in studies of the diffusion of small nonelectrolytes through nonporous cellulose acetate membranes. In the present study, we have examined a number of other geometrical parameters and have found that the cylindrical radius gives the best fit in those cases in which purely geometrical factors are dominant.

The molar volume is another parameter which includes geometrical fac-

tors, being equal to the molecular weight divided by the density of the pure compound. The molecular weight may be construed as a measure of molecular size based on a spherical model. Division by the density modifies the strictly geometrical interpretation by introduction of the hydrogen-bonding ability because, as Pimentel and McClellan (12) have pointed out, hydrogen bonds generally increase the density and lower the molar volume. For example, the 1.32 density of urea may be taken as an index of the hydrogen-bonding ability of this solute. The correlation of hydrogen-bonding ability with density is illustrated particularly effectively by the butanediol series in which the ability to hydrogen bond with other molecules decreases as the hydroxyl groups move closer together and become able to form intramolecular hydrogen bonds. As Table II shows the density of the isomers increases with increasing separation of the hydroxyl groups. Other convincing examples are given by Pimentel and McClellan (12).

We interpret the molar volume as a mixed parameter, a geometrical construct modified by chemical properties, primarily hydrogen-bonding ability. Though the compound nature of this parameter makes it difficult to assign exact weights to the relative contributions of geometry and chemical reactivity, it has the important advantage of being a combined measure that can be specified exactly by quantitative measurements, and one that is widely available in physical chemical tables.

Some discussion of the effect of hydrogen bonding on diffusion coefficients is pertinent to an understanding of membrane permeability. Horowitz and Fenichel (13) have shown that hydrogen bonding hinders nonelectrolyte diffusion in formamide-swollen dextran gels. These authors found that the greater the number and the strength of solute-solvent hydrogen bonds, the slower the diffusion in a hydrogen-bonding solute such as formamide. On the other hand when similar studies were made with water-swollen dextran gels, the reverse phenomenon was observed, and an increase in hydrogen bonding led to an increase in diffusion. Thus, for the same molecular weight, ureas diffused faster than amides and amides faster than alcohols, forming three quite distinct series when plotted against inverse molecular weight. Horowitz and Fenichel assumed that, in water, a second effect was superimposed on the original hydrogen-bonding effect, through a solute-induced modification of the water structure which favored solute diffusion. This process is peculiar to water. A similar explanation has been given by Gary-Bobo and Weber (14) to account for their observation that amides, as a class, diffuse faster than alcohols in bulk water. Horowitz and Fenichel also point out that "the use of the molar volume will bring the series together, so that differences between compounds of the same molar volume can be seen in most cases only by careful examination of the data."

Relation of ω to Partition Coefficient and Cylindrical Radius

In order to have an overview of two of the factors affecting permeation, ln (ω/k_{ether}) has been plotted as a function of the cylindrical radius in Fig. 2. Katchalsky and Curran (15) define ω as follows:

$$\omega = (J_s/\Delta\pi)_{J_v=0} = K_s/\Delta x\ (f_{sw} + f_{sm}) \quad (1)$$

in which J represents either solute flow, J_s, or volume flow, J_v. $\Delta\pi$ is the osmotic pressure difference, K_s is the partition coefficient of the solute be-

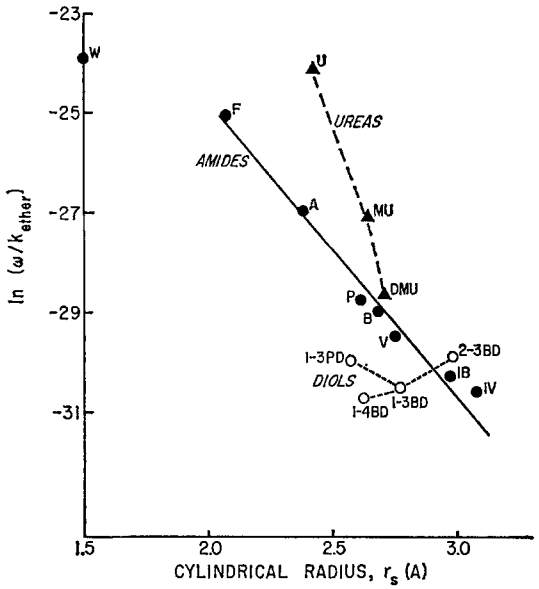

FIGURE 2. Relation among permeability coefficient, partition coefficient, and cylindrical radius for a series of amides, diols, and ureas in human red cells. Table I gives the code for the solutes.

tween membrane and external solution, and Δx is the path length through the membrane. Solute-water and solute-membrane frictions are denoted by f_{sw} and f_{sm}. Assuming k_{ether} to serve as a qualitative indicator of K_s, the ratio ω/k_{ether} should be inversely proportional to the sum of the frictional coefficients, Δx being assumed constant. By plotting ln (ω/k_{ether}) as a function of the cylindrical radius, as has been done in Fig. 2, it is possible to evaluate the importance of the purely steric factors.

Fig. 2 shows that steric hindrance has a consistent effect on the entire series including both hydrophilic and lipophilic molecules. It is clear that the cylindrical radius is an important parameter to be considered in understanding the permeation process. Fig. 2 also shows that chemical factors are

of great importance since the urea family falls on an entirely different curve than the amides. The cylindrical radius has little effect on the permeability of the diols, whose behavior is entirely different from either of the other two series. Water also occupies a unique place apparently unrelated to any of the other solutes. The lack of uniformity among the solutes in Fig. 2 indicates quite clearly that no unitary hypothesis will serve to account for the behavior of all the solutes that have been studied. We may conclude rather that chemical properties play a role beyond that reflected in the partition coefficient, and that geometrical factors are important in most but not all instances.

We have therefore formed a composite model by which the data shown in Fig. 2 may be interpreted in a coherent fashion. Drawing on inferences from the data in Fig. 1, and previous observations reviewed by Solomon (16), we have assumed that the membrane has one pathway for hydrophilic molecules, and a second pathway for lipophilic molecules. Neither pathway is exclusive and for molecules such as propionamide which lies at the minimum in Fig. 1, both pathways are open. Steric factors are important to permeation by either route but are not sufficient in themselves to account for all the observations. Chemical interactions between the solute and the membrane also play a significant role as do those between solute and solvent.

Permeation through Aqueous Pathways

We have defined hydrophilic solutes to include all those studied for which k_{ether} is ≤ 0.003, the partition coefficient for water. The hydrophilic class includes all three ureas, the two shortest chain amides, and, of course, water. The data in Fig. 2 show that a simple geometric approach will not be satisfactory even for these six molecules. Since they are all hydrophilic there is no reason to expect k_{ether} to be an important criterion.

The relevant distribution parameter for these solutes would be their partition coefficient between the water in the membrane and the water outside. These coefficients are unknown, and implicitly taken equal to unity. However, the partition ratio for urea (17) between red cell water (including the membrane) and external solution water is greater than unity, having a value of 1.06 at 0.3 M, probably as a result of the "salting-in" effect, which is particularly important for polar molecules such as urea.

In view of Horowitz and Fenichel's finding (13) that the molar volume is a parameter that brings together the diffusion coefficients in water of a series of ureas, amides, and alcohols, we have plotted ln ω as a function of the molar volume for the hydrophilic solutes we have studied. This treatment provides a very good empirical fit for all six molecules, as illustrated in Fig. 3. The implication is that hydrogen bonding with both membrane

and solvent cannot be neglected in understanding the mechanism of transport of hydrophilic molecules through aqueous channels. The available data for the dog red cell membrane include only two hydrophilic solutes and water. Though the data are few, the results are consistent with those in man.

As DiPolo, Sha'afi, and Solomon (18) have pointed out, the effect of hydrogen bond formation is apparently much more important with respect to ω than to σ in porous cellulose acetate membranes. Thus it is not surprising that allowance for hydrogen bonding must be made in considering the behavior of ω in red cells whereas hydrogen bonding has hitherto appeared to be unimportant as a determinant of σ for the human red blood cell.

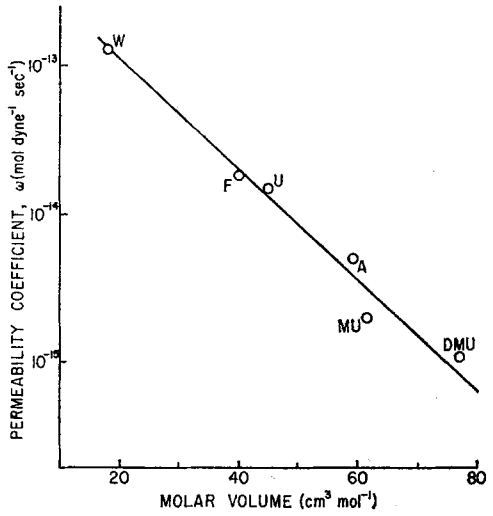

FIGURE 3. Permeability coefficient for hydrophilic solutes in human red cells as a function of molar volume.

Permeation by Lipophilic Amides

Five of the amides that have been studied may be classified as lipophilic: propionamide and the two isomers each of butyramide and valeramide. The ether:water partition coefficient for the least lipid-soluble of these amides is three times greater than that of water, and the coefficient for the most soluble is almost two orders of magnitude greater than for water. The cylindrical radii of all these molecules lie between 2.6 and 3.1 A so that permeation through the aqueous pathway may not be excluded, but a reasonable picture emerges if we consider all five as a lipophilic class whose route of entrance is primarily by dissolution in the membrane. In Fig. 4, ω is plotted as a function of k_{ether} and it is seen that for the straight chain amides, ω increases directly with the partition coefficient, in entire agreement with

Overton's rule. Comparison of the results for dog and man indicates that the species differences are quite important, as was suggested by the data in Fig. 1. Since the fractional difference in ω increases as k_{ether} increases, it is likely that important species differences lie in the lipid moiety of the membrane.

The most striking feature of Fig. 4 is the difference between the pairs of isomers. The quantitative values of the difference might be altered if the

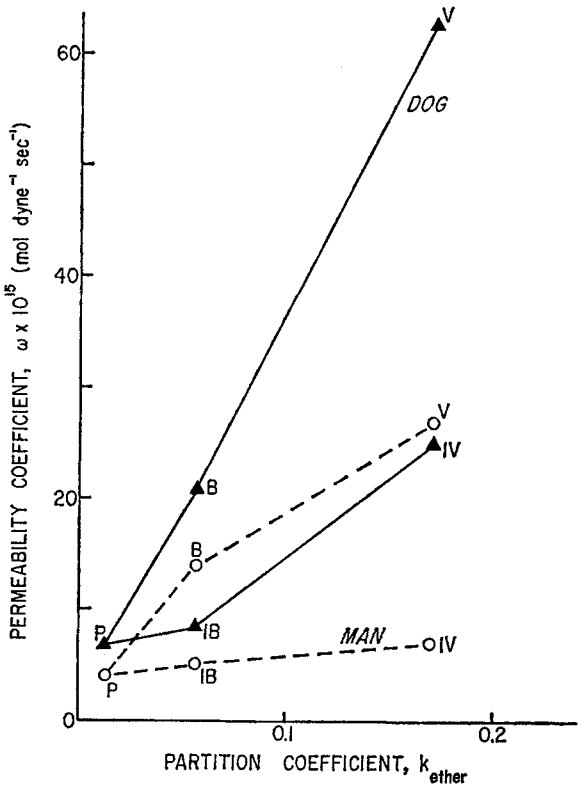

FIGURE 4. Relation between permeability coefficient and partition coefficient for lipophilic amides in red cell membranes of man and dog.

partition coefficient of the other member of each pair were known but, notwithstanding this possibility, it is clear that branched chain compounds behave consistently differently from straight chain ones. Introduction of the branch in the chain increases both the cylindrical radius and the molar volume as can be seen in Table II.

When ω/k_{ether} is plotted as a function of molar volume, the differences, both between species and between isomers, remain as impressive as in Fig. 4. This is not surprising since the molar volume is a composite of the spherical volume and the hydrogen-bonding ability. The isomers have similar hydro-

gen-bonding abilities since it is the methyl group furthest from the amide group whose position is shifted. Furthermore the spherical volume is a geometrical parameter which minimizes the differences between isomers, whereas the cylindrical radius provides a much more representative view of the molecule. We have therefore plotted ln (ω/k_{ether}) against the cylindrical radius in Fig. 5. It may be seen that the entire series may be fitted reasonably well by a single straight line for man and another for dog. Thus steric hindrance appears to be the most important factor in differentiating the branched

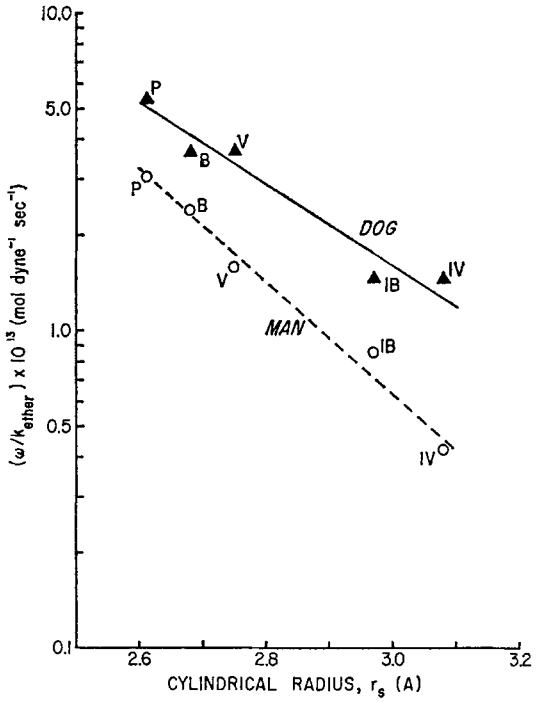

FIGURE 5. Permeability of red cells to lipophilic amides as a function of the cylindrical radius of the solute in red cells of man and dog.

chain from the straight chain compound. Permeation through the human red cell membrane is entirely comparable with permeation through a nonporous cellulose acetate membrane in which Gary-Bobo, DiPolo, and Solomon (11) have previously observed a logarithmic relationship between $\omega/k_{membrane}$ and the cylindrical radius. In water, Gary-Bobo and Weber (14) have found very little difference between the diffusion coefficients of butyramide and isobutyramide which are 1.07 and 1.02 $\times$ 10^{-5} cm^2 sec^{-1} at 24°C. This behavior may be contrasted with that in the human red cell membrane in which ω is 14 $\times$ 10^{-15} mol dyne^{-1} sec^{-1} for butyramide, almost three times greater than ω for the branched chain isobutyramide, for which

ω is 5×10^{-15} mol dyne^{-1} sec^{-1}. It appears therefore that the lipids in the red cell membrane are very much less fluid than water and must be held together in an organized structure.

The observation that the lines for the two species in Fig. 5 are not far from parallel is consistent with the view that the degree of organization in the membranes, as reflected in the steric hindrance, is about the same. The higher permeability coefficients in the dog probably reflect differences in the detailed lipid composition of the membrane leading to increased solubility of the amide series in the dog red cell membrane. Striking species differences in lipid content have been reported (19) which support this view. For example, phosphatidylcholine makes up to 30–35% of the total phospholipid in human red cell membranes as compared to 47% in the dog; in the case of sphingomyelin the figures are: 25–32% man; 11% dog.

Permeation by Diols

Two variables have been changed systematically in our study of the diol group. 1,3-butanediol differs from 1,3-propanediol by the addition of a methyl group. The positions of the two hydroxyl groups in the three butanediols have been permuted to give further insight into the effect of hydrogen bonding on membrane permeation. It is instructive first to compare the behavior of 1,3-propanediol with propionamide. These molecules have very similar physical properties, with molecular weights of 73.09:76.10, cylindrical radii of 2.61:2.56 A, and k_{ether} of 0.013:0.012. The major difference arises from the difference in the hydrogen-bonding ability of the solute as a result of replacing a single amide group with two hydroxyls.

Hydrogen-bonding ability is somewhat greater for amides than for alcohols as illustrated by differences in N_H, the number of possible hydrogen bonds that may be formed for the solute. Franks and Ives (20) give this number as 2 for the alcohol group, whereas the most likely value for the amide group is 3 (12, 21). Gary-Bobo, DiPolo, and Solomon (11) have shown that a series of amides experiences greater friction than an analogous series of alcohols when diffusing across a nonporous cellulose acetate membrane and that the ratio of the frictions is about 3:2. Assuming simple additivity of hydrogen-bonding ability as a first approximation, it is apparent that the hydrogen-bonding ability of the diols should be greater than that of amides. The density of 1,3-propanediol is also slightly greater than that of propionamide, though differences in density of solutes of different reactivity are less significant than differences between solutes with similar reactive groups. Since the hydrogen-bonding ability of 1,3-propanediol is greater than that of propionamide, the sharp decrease in ω from 4.0×10^{-15} mol dyne^{-1} sec^{-1} for propionamide to 1.2×10^{-15} mol dyne^{-1} sec^{-1} for 1,3-propanediol may

be attributed to an increase in solute-membrane friction due to the increased hydrogen-bonding ability of the diol.

The effect of the addition of a methyl group as one goes from 1,3-propanediol to 1,3-butanediol is to increase both k_{ether} and the cylindrical radius. As k_{ether} increases, ω/k_{ether} decreases from 1.0×10^{-13} mol dyne^{-1} sec^{-1} for 1,3-propanediol to 0.48×10^{-13} mol dyne^{-1} sec^{-1} for 1,3-butanediol. This effect is in the same direction, and of about the same magnitude, as the steric hindrance effect on amide isomers as can be seen in Fig. 2. Thus, when hydrogen bonding is kept constant by keeping the separation between the two hydroxyls the same, increases in cylindrical radius produce consistent effects in diols and amides alike.

However, the situation is very different when the separation of the two hydroxyls in the butanediol isomers is altered systematically. Under these conditions, the permeability of the three butanediols, expressed as $\ln \omega/k_{ether}$ in Fig. 2, increases with cylindrical radius, rather than decreasing as is the case for the amide and urea series. More importantly the hydroxyl groups are brought closer together as the hydroxyls are moved from the 1,4-position to the 1,3- and finally reach the 2,3-isomer which is characterized by the largest cylindrical radius. As the hydroxyl groups are brought into increasingly close apposition intramolecular hydrogen bonding increases at the expense of the ability to form hydrogen bonds with external acceptors. Since the strength of the solute-membrane hydrogen bonds has decreased, the solute diffuses through the membrane more readily and $\ln \omega/k_{ether}$ increases despite the concomitant increase in cylindrical radius. This indicates clearly that hydrogen bonding is a more important determinant of membrane permeability than is cylindrical radius.

Davson and Danielli have reviewed (1) a number of earlier observations indicating that apposition of hydroxyl groups increases solute permeability. Using the hemolysis method Jacobs (1) found that 1,2-propanediol permeated the red cells of ox and rabbit more rapidly than 1,3-propanediol. Diamond and Wright (22) have also pointed out that the importance of intramolecular hydrogen bonds increases as the hydroxyl groups move more closely together and have shown that this process is reflected in a resultant decrease of solute reflection coefficients in the gallbladder.

Comparison of ω with Permeability Coefficients Determined by the Hemolysis Method Höber and Ørskov (23) measured the time of hemolysis of human red cells for a number of solutes including four whose permeability coefficients are given in Table I. Jacobs (24) has given an equation by which permeability coefficients may be derived from hemolysis time. Use of this equation gives a value of ω of 5×10^{-16} mol dyne^{-1} sec^{-1} for acetamide, which is one order of magnitude less than our value in Table I. According to Jacobs' equation,

ω is inversely proportional to the hemolysis time, t_h, and is related to it by a series of factors which are constant for any given species. In Table III we have taken the permeability of acetamide as a standard and computed relative permeability ratios both from the data in Table I and the inverse hemolysis times taken from Höber and Ørskov. The agreement in the relative data in these two series of quite different kinds of experiments is surprisingly good. However, since the permeability coefficients computed from hemolysis times differ by an order of magnitude from those obtained using rapid reaction techniques, it is not possible to combine data obtained by both methods into a single ratio, as was done, for example, by Stein (25) in a discussion of the equivalent pore radius in human red cells.

TABLE III

COMPARISON OF ω WITH HEMOLYSIS DATA

Solute	Hemolysis time	Relative inverse hemolysis time	Relative ω
	sec		
Acetamide	0.9	1.0	1.0
Propionamide	1.2	0.7	0.8
Urea	0.3	3.0	3.0
Methyl urea	2.3	0.4	0.4

Membrane Properties Inferred from Permeability Measurements

The classical studies of Collander and Bärlund (6) and Collander (26) have provided strong evidence that small hydrophilic molecules cross the membrane through a pathway different from that followed by lipophilic molecules. For *Chara*, Collander and Bärlund give a figure of approximately 4 A as the radius of the equivalent pore. These authors' evidence for the existence of an aqueous path has been generally accepted, although Danielli (1) does not share this view. Recently, Lieb and Stein (27) have suggested that cell membranes should be treated as homogeneous membranes in which the permeability coefficient may be computed from an equation in which the only variables are molecular weight and the oil-water partition coefficient. In the case of bovine red cells they have fitted permeability data obtained from hemolysis measurements to the equation

$$P = P_o \beta^n M^{-p} \qquad (2)$$

in which P is the permeability coefficient and P_o, n, and p are adjustable constants, β the oil-water partition coefficient, and M the molecular weight, relative to methanol. Lieb and Stein obtained values of 1.4 for n and 6.0 for p. The least squares fit to our data on human red cells in Table I gives the

following values: P_o, 0.4; n, 1.0; p, 6.0, when M is used to represent molecular weight. The correlation is very poor, as shown not only by the correlation coefficient of 0.64 but also by the very great scatter when the values predicted according to equation 2 are compared with the experimentally determined ones. There appears to be no simple physical exaplanation as to why the molecular weight should enter the equation to the inverse sixth power. In view of the absence of a convincing physical basis for the equation and the poor correlation of the equation with our data, we conclude that the Lieb and Stein treatment does not provide convincing evidence that the human red cell membrane behaves as a homogeneous structure.

On the contrary, the present results are consistent with the characterization of the red cell membrane in terms of equivalent pores. The primary support for this conclusion is given by the clear differences in the factors governing the diffusion of hydrophilic and lipophilic molecules.

The equivalent pore radius was initially based on analysis of water diffusion and filtration data and of nonelectrolyte reflection coefficients in which the only parameter considered was steric hindrance. The present study of nonelectrolyte diffusion clearly focuses attention on the importance of the physical chemical factors governing membrane permeability. The study of the temperature dependence of water diffusion and filtration, both in red blood cells (5) and artificial membrane models (28), has already shown that chemical factors play an important role in addition to geometrical factors. Hence, the measurement of an equivalent pore radius must be affected by the chemical nature of the membrane fabric. This fact, together with the recent evidence of the duplex nature of the red blood cell membrane (29), makes it impossible to interpret hydrophilic diffusion solely in terms of a quantitative estimate of a geometrical equivalent pore radius.

Lipophilic solutes permeate by dissolution in the membrane. The partition coefficient, which may be interpreted primarily as a measure of the ease with which the lipophilic solute can escape from its aqueous environment, is of overwhelming importance. Once the solute has entered the membrane, steric hindrance plays a role, as illustrated by the comparison between the two pairs of lipophilic amide isomers. It is clear from these data that there is an organized structure within the membrane of a higher order than that characteristic of the liquid state and comparable to the relatively rigid matrix of the cellulose acetate membrane. There are significant differences in lipophilic amide diffusion in dog and man. This species specificity indicates that in biological membranes the lipid route cannot be considered simply as consisting of undifferentiated hydrocarbon layers. The study of the diols shows the importance of hydrogen bonding. From this we infer that lipid-soluble solutes traverse a path that comes into contact with polar moieties within the membrane, such as are provided by phosphatides or proteins.

An entirely different situation prevails in the case of hydrophilic solutes. For molecules with k_{ether} smaller than that for water, the partition coefficient does not play any part. This is not surprising, for if these molecules cross an aqueous path, the only relevant ratio would be the partition ratio between the water in the pathway and the water outside. Although this ratio is not known, there is reason to think that it plays a significant part, inasmuch as the solubility of nonelectrolytes in water in close contact with macromolecular surfaces may be very different from their solubility in the bulk phase. The predominant diffusion factor is steric hindrance. The extreme sensitivity to molecular size and shape reflects not only the geometry of the aqueous pore but also molecular solubility in the water contained in the pore. These geometrical constraints are further modulated by chemical factors as indicated by the role of hydrogen bonding.

Further evidence for the reality of aqueous paths and the effect of hydrogen bonding is afforded by the results of Holz and Finkelstein (30) on nonelectrolyte diffusion through aqueous pores induced by nystatin in thin lipid membranes. These authors have measured diffusion coefficients through the membrane of: H_2O, urea, thiourea, ethylene glycol, and glycerol. We have plotted the natural log of their diffusion coefficients against molar volume and have found a reasonable linear fit, much closer than the fit observed when the same data are plotted against the cylindrical radius.

An increasing amount of evidence thus appears to support the porous model of biological membranes. The concept of the equivalent pore, which initially was purely geometrical, has been expanded by detailed consideration of further physical chemical interactions, which make it a functional entity better able to perform its multiple and complex physiological task.

APPENDIX

Frictional Interpretation of Phenomenological Coefficients

DiPolo, Sha'afi, and Solomon (18) have pointed out that the partition coefficient and hydrogen bonding exercise opposing actions on ω and σ as measured in a porous cellulose acetate membrane. Phenomenologically ω and σ are entirely independent parameters. However, according to the frictional interpretation of phenomenological coefficients (see reference 15) ω and σ can be related to one another by the equation

$$(1 - \sigma - \omega\bar{V}_s/L_p)/\omega = f_{sw}\,\Phi_w/\Delta x \qquad (3)$$

in which $\bar{V}_s$ is partial molar volume of the solute and L_p, the hydraulic conductivity. Kedem and Katchalsky (see reference 15) have pointed out that in a very loose membrane f_{sw} approaches the value for free solution f°_{sw} which is related to the diffusion coefficient according to $D = RT/f^\circ_{sw}$. However the relationship between f_{sw} and D both in the red cell and in porous cellophane membranes is complex and very difficult to understand. This means that the friction between solute and water in

aqueous pathways is very sensitive to the specific chemical and physical properties of membrane and solute, as has been discussed extensively in the main body of this paper.

In the present study, values of σ have been determined for four of the solutes whose ω's are given in Table I. Table IV contains a comparison of the values computed for $f_{sw}\,\Phi_w/x$ from these data with the diffusion coefficients in water for these four solutes

TABLE IV

RELATION BETWEEN DIFFUSION COEFFICIENT AND f_{sw}

	$D_w \times 10^5$	$f_{sw}\,\Delta x/\Phi_w \times 10^{17}$
	cm² sec⁻¹	*dyne sec mol⁻¹*
Urea	1.382	1.5
Methyl urea	1.168	8.7
Acetamide	1.252	7.3
Propionamide	1.093	3.5

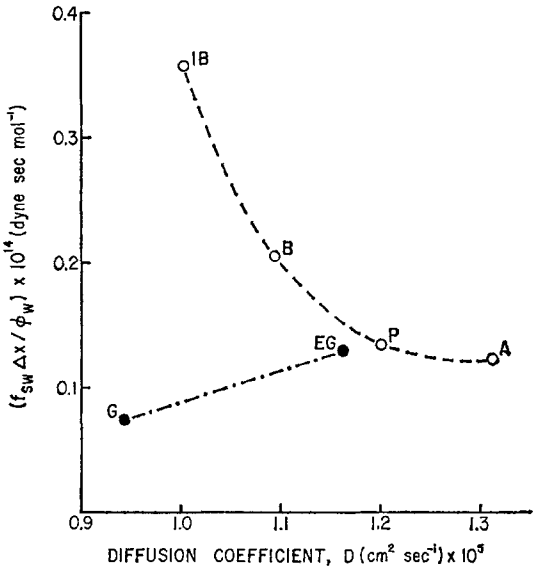

FIGURE 6. Relation between solute-water friction in porous membranes and solute diffusion coefficients in water. *G* stands for glycerol and *EG* for ethylene glycol.

at 25°C. Since L_p and σ are known functions of osmolality, and the parameters were not measured at identical osmolalities, the comparison is qualitative. On the basis of the reasonable assumption that Δx and Φ_w remain essentially constant during all the measurements of σ, ω, and L_p, f_{sw} should be inversely proportional to the diffusion coefficient, except as modified by molecular size. Ginzburg and Katchalsky (31) have measured f_{sw} for a series of solutes that penetrate porous artificial membranes through aqueous channels and have shown that steric factors play an important and consistent

role in this frictional term. This is not the case for the four molecules in Table IV. The acetamide-propionamide pair are particularly puzzling since propionamide is larger than acetamide and yet the frictional term is smaller by a factor of two. This is not to be attributed to that moiety of propionamide that dissolves in the membrane fabric since the introduction of the term, $\omega\bar{V}_s/L_p$, should take account of that contribution.

The three solutes, acetamide, methyl urea, and urea, are all in the hydrophilic class and might therefore be expected to behave consistently. Even this expectation is not satisfied since urea, whose cylindrical radius is approximately equal to that of acetamide, is characterized by a very much smaller value of $f_{sw}\Delta x/\Phi_w$, corresponding to the very large difference in ω in Table I.

We have also examined the relationship of σ to ω using the data from measurements on a porous cellulose acetate membrane given by DiPolo, Sha'afi, and Solomon (18). Their data were obtained on a thin porous membrane in which Δx and Φ_w could each be measured. $f_{sw}\Delta x/\Phi_w$ is plotted in Fig. 6 as a function of D_w for some members of the amide series and for glycerol and ethylene glycol for which k_{ether} is 0.00066 and 0.0053, respectively. The frictional term for the amide series decreases with decrease in molecular size, as expected on steric grounds. The frictional term for ethylene glycol is greater than that for glycerol, a larger molecule. The two series fall on different parts of the graph. This diversity of behavior in an artificial membrane only underlines the fact that the diffusion coefficient in water does not bear a predictive relationship to f_{sw}, the solute-water friction in the membrane. In other words, even in an artificial membrane, it is not possible to obtain reliable estimates of ω from measurements of σ.

The situation is even more complicated in red cells because evidence has been presented by Sha'afi et al. (2) and by Rich et al. (29) indicating that the human red cell membrane acts as a series membrane so that equation 3 would not necessarily be expected to apply to this membrane. The point to be stressed is that the frictional treatment does not have any predictive value in the case of the human red cell membrane. The clear implication of this observation is that grave reservations must be maintained about the results of studies in which σ is used as a measure of membrane permeability as has been done, for example, by Diamond and Wright (22) and Wright and Prather (32).

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