Role of Hydrogen-Bonding in Nonelectrolyte Diffusion through Dense Artificial Membranes

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ABSTRACT The diffusion of two series of alcohols and amides through complex cellulose acetate membranes was studied. The thin dense part of these membranes behaves as a nonporous layer of low water content. In this layer, called the skin, the solute diffusion coefficients, ω, depend upon size, steric configuration, and the partition coefficient, K_s, between membrane and bathing solution. From the experimental values of ω and K_s, the over-all friction, f, experienced by the solutes in the membrane was computed. It was found that f depends upon the chemical nature of the solute and is related to hydrogen-bonding ability. In the coarse, porous layer of the cellulose acetate membrane, diffusion occurs mainly through aqueous channels. In this instance also the hydrogen-bonding ability of the solute seems to exercise a smaller but significant influence.

In order to account for the permeability of small hydrophilic solutes through the plasma membrane of *Chara* cells, Collander and Bärlund (1) and Collander (2) postulated that biological membranes may act not only as selective solvents but also as molecular sieves. This latter aspect of membrane properties has been intensively studied with both artificial and natural membranes. These studies have led to the concept of the equivalent pore, which provides a coherent description of the flows of hydrophilic solutes exclusively based upon steric considerations (3). However, the resistance experienced by a solute crossing a membrane may be due to more specific, physical chemical forces. Polarity and hydrogen-bonding ability are important in diffusion, not only in hydrogen-bonding solvents (4, 5) but also in nonpolar solvents (6). Horowitz and Fenichel (7) related the chemical specificity of cellular nonelectrolyte transport to the general diffusional properties of these solutes in hydrogen-bonding solvents. Recently, Stein (8) has made suggestive correla-

tions between solute hydrogen-bonding properties and permeability data obtained with various biological systems. It seems advantageous to approach this problem experimentally using simpler synthetic membranes, with which it is possible to achieve a better control of permeability parameters. Ginzburg and Katchalsky (9) have already shown that pure geometrical considerations are not able to account for the observed diffusion of nonelectrolytes across dialysis tubing. But the large predominance of solute-water interactions in this highly swollen membrane did not allow them to make further analysis and to generalize their conclusions to more dense biological membranes.

In an attempt to obtain more information about the possible influence of physical chemical factors, we have measured the diffusion of two series of alcohols and amides in cellulose acetate membranes. These membranes, first designed by Loeb (10) for desalination by reverse osmosis, are composed of a thin but very dense layer supported by a coarse porous matrix. The water permeability characteristics of this type of membrane have been studied recently by Hays (11). The present study shows the diffusion coefficients of the nonelectrolytes to be related to molecular size and partition coefficient between the membrane and the bathing solution, as measured in separate experiments. It also appears that the chemical nature of the solute is important in controlling diffusion.

MATERIALS AND METHODS

Cellulose Acetate Membrane Preparation

Membranes were prepared from a mixture of cellulose acetate (Eastman 4644, acetyl 39.8%), formamide, and acetone in a proportion by weight of 25:25:50. The viscous solution was cast on glass plates at room temperature, allowed to dry in the open air for 1 min, and then immersed in an ice-water bath for 1 hr. The resulting membranes were finally heated for 5 min in a water bath at 80°C. During the evaporation period, a thin but very dense layer is formed, the "skin" which is supported by the thick coarse matrix formed in the water bath, the "coarse" membrane. In order to study the coarse membrane alone, the skin is removed by sandpapering under water. Skin membranes were prepared by complete evaporation of the mixture in the open air.

L_p and ω Measurements

The hydraulic conductivity measurements were carried out in a Lucite chamber made of two compartments separated by the membrane, supported by a stainless steel screen. The effective area was 7 cm^2. Capillary pipettes were sealed in each compartment for volume measurements. The displacements of the meniscus in the two capillaries, opposite in direction, were checked and found to be equal. Thus any aberrant changes not due to hydrostatic pressure could be discarded. The hydrostatic pressure was provided by a nitrogen tank connected to one capillary through a mercury manometer.

The diffusion coefficients were measured in a two compartment chamber with an effective area for diffusion of 0.785 cm^2. The two compartments, I and II, were filled with the same 10^{-2} M solution of the desired solute in distilled water. At zero time, a known amount of the labeled solute (as a 10^{-2} M solution including labeled and non-labeled species) was introduced in compartment I. After a given diffusion period had elapsed (from 30 to 120 sec depending upon solute and temperature), the solution in compartment II was completely withdrawn and replaced by fresh solution. The time taken by this operation did not exceed 5 sec. Routinely, four to five successive diffusion measurements were thus carried out. Owing to the low membrane permeability, and the low surface to volume ratio of the chamber, the decrease of tracer concentration in I at the end of the five diffusion periods was never greater than 0.5–1 %. Correspondingly, there were no measurable differences in the number of counts obtained in II at the end of each successive period. In some instances the radioactivity was introduced initially into compartment II, rather than into compartment I; the diffusion coefficient was independent of this choice of initial condition. Vigorous stirring was achieved by mechanically driven Teflon propellers.

K_s Measurements

The solute partition coefficients, K_s, between the skin membrane and the solution were measured under the same conditions of concentration and temperature as the diffusion measurements. A known weight, G_m, of wet membrane, reduced to a coarse powder was mixed with a weight, G_s, of solution just sufficient to cover the powder. After equilibrium, K_s was computed as

$$K_s = (A_o - A_f)G_s/G_m A_f \tag{1}$$

in which A_o and A_f are respectively the initial and final equilibrium amounts (in mols) of the solute in the solution. This use of K_s differs slightly from the conventional one since the concentration in the membrane is molal whereas that in the medium is molar.

All the measurements of L_p, ω, and K_s were carried out at room temperature (22°C ± 0.5°C), unless otherwise indicated. THO and ^{14}C-labeled solutes were counted in a liquid scintillation counter (Nuclear Chicago Corp., Des Plaines, Ill., Model 6801). The labeled solutes were obtained from New England Nuclear Corp. (Boston, Mass.) except butyramide and isobutyramide which were obtained from International Chemical and Nuclear Corp. (City of Industry, Cal.). The thickness of wet membranes was measured using a light microscope to determine the distance between two spots marked on opposite surfaces of the membrane. The thickness of the skin was not measured and values were obtained from the electron microscope measurements of Lonsdale (12). The water content, Φ_w, of the membranes was measured by drying to constant weight at 100°C.

THEORY

The hydraulic conductivity, L_p, in the presence of an applied presssure, ΔP, and in the absence of osmotic pressure ($\Delta\pi = 0$) was computed according to

the following equation from Kedem and Katchalsky (13)

$$L_p = (J_v/\Delta P)_{\Delta\pi=0} \tag{2}$$

in which J_v is the water flow in ml cm^{-2} sec^{-1}. The exchange diffusion of isotopically labeled solute in the absence of net volume flow was computed as:

$$\omega = (J_s/RT\Delta C_s)_{J_v=0} \tag{3}$$

in which J_s is the flow of labeled solute in mol cm^{-2} sec^{-1}, and ΔC_s is the isotope concentration difference across the membrane in mol cm^{-3}. The total cellulose acetate membrane (t) is composed of two membranes in series, the skin (s) and the coarse (c); in such a condition, the permeability coefficients are related by (14):

$$1/L_{pt} = 1/L_{ps} + 1/L_{pc} \tag{4}$$

$$1/\omega_t = 1/\omega_s + 1/\omega_c \tag{5}$$

L_{pt}, L_{pc}, ω_t, and ω_c were determined experimentally, and L_{ps} and ω_s were computed by equations 4 and 5.

Each membrane was characterized using the ratio, g, of hydraulic to THO diffusion coefficients, and the equivalent pore radius, r, of the membrane was calculated according to

$$g = L_p/\bar{V}_w\omega_{\text{THO}} = 1 + r^2RT/8\eta D\bar{V}_w \tag{6}$$

in which $\bar{V}_w$, D, and η are the partial molar volume, the self-diffusion coefficient, and the viscosity of water at the temperature of the experiment.

The solute diffusion coefficient through a membrane of a given thickness, Δx, has been related (13) to the solute partition coefficient, K_s, and two frictional coefficients, f_{sm}, solute-membrane, and f_{sw}, solute-water, by

$$\omega = K_s/\Delta x(f_{sm} + f_{sw}) \tag{7}$$

In the present experiments the exchange diffusion of the labeled solute, s^*, with the unlabeled species, s, was measured through a dense membrane, the skin. As pointed out by Kedem and Essig (15) and Curran, Taylor, and Solomon (16), isotopic interactions may not be negligible in this particular condition. Therefore, equation 7 does not describe the phenomenon completely and must be replaced by:

$$\omega_{s^*} = K_{s^*}/\Delta x(f_{s^*m} + f_{s^*w} + 2f_{s^*s}) \tag{8}$$

in which f_{s*s} represents solute-solute interaction.[1] Since it may be reasonably assumed that $K_{s*} = K_s$, $f_{s*m} = f_{sm}$, and $f_{s*w} = f_{sw}$, equations 7 and 8 differ only by the $2f_{s*s}$ term. On the basis of the present experimental data, it was not possible to make a separate analysis for each of these three frictional coefficients. However, the sum of the three frictions may be computed according to:

$$K_s/\omega\Delta x = f_{sm} + f_{sw} + 2f_{s*s} = f \tag{9}$$

For each solute f is related to steric and physical chemical parameters as will be shown below.

RESULTS AND DISCUSSION

Characterization of the Membranes

The main characteristics of the total membrane and its two components are given in Table I. These include thickness, Δx, water content, Φ_w, hydraulic conductivity, L_p, THO diffusion coefficient, ω_{THO}, and the ratio $g = L_p/\bar{V}_w\omega_{\text{THO}}$. L_p measurements were carried out at low pressure (<0.5 atm). In this range, L_p was found to be independent of the pressure as well as of the direction from which the pressure was applied, either towards the skin or towards the coarse side.

TABLE I

CHARACTERIZATION OF THE MEMBRANE

	Total membrane	Coarse membrane	Skin membrane
Thickness, *cm*	$(2\pm0.01)\times10^{-2}$	$(2\pm0.01)\times10^{-2}$	5×10^{-4}
Φ_w *g/g wet membrane*	0.60±0.005	0.60±0.005	0.135±0.005
$L_p\times10^{12}$ $cm^3 dyne^{-1} sec^{-1}$	4.2±0.1	25.5±0.7	5.0±0.8
$\omega_{\text{THO}}\times10^{14}$ $mol\ dyne^{-1} sec^{-1}$	2.55±0.05	2.80±0.05	30±3
$L_p/\bar{V}_w\omega_{\text{THO}}$	9±0.2	50±2	1±0.25

The main difficulty in diffusion measurements is the unstirred layer effect. Preliminary experiments on THO diffusion have shown that ω_{THO} becomes constant independent of the stirring rate above 1000 rpm. All the measurements were carried out at 1500 rpm. Since the membrane resistance is high (ω of the order of magnitude of 10^{-14} or lower), the unstirred layer effect is small and may be neglected without introducing appreciable errors.

[1] According to Kedem and Katchalsky (13) in the steady-state condition one may write:

$$-d\mu^*/dx = f_{s*w}(v_{s*} - v_w) + f_{s*m}v_s + f_{s*s}(v_s^* - v_s)$$

in which v represents velocity. Since $v_w = 0$ and $v_s^* = -v_s$, $-d\mu_s^*/dx = v_s^*(f_{s*m} + f_{s*w} + 2f_{s*s})$, from which one obtains equation 8. For a more complete discussion of this question, see Essig (17).

L_p and ω_{THO} obtained in this membrane are generally smaller than those obtained by Hays (11). These differences are attributable to differences in membrane composition and casting technique. But there is a complete agreement with Hays's observation that resistance to hydraulic flow is determined by the skin and diffusional resistance is mainly due to the thick coarse layer. The g ratio for the skin is practically equal to 1. This indicates that water flows only by diffusion and that this membrane behaves as a dense homogeneous, nonporous layer. For the coarse membrane, $g = 50$. Bulk flow through pores indubitably takes place in this membrane. From equation 6 the equivalent radius of these pores may be computed as 24 A.

TABLE II

PERMEABILITY COEFFICIENTS OF TOTAL, COARSE, AND SKIN MEMBRANE

Solute	Code	Permeability coefficient		
		Total $\omega_t \times 10^{14}$	Coarse membrane $\omega_c \times 10^{14}$	Skin $\omega_s \times 10^{14}$
			mol dyne^{-1} *sec*$^{-1}$	
Methanol	1	1.208	1.255	32.5
Ethanol	2	0.533	0.583	6.3
sec-Propanol	5	0.167	0.230	0.6
ter-Butanol	6	0.038	0.102	0.06
n-Propanol	3	0.422	0.472	3.98
n-Butanol	4	0.366	0.375	15.1
Ethylene glycol	12	0.157	0.246	0.43
Glycerol	13	0.033	0.097	0.05
Formamide	7	0.748	0.804	10.7
Acetamide	8	0.293	0.364	1.5
Propionamide	9	0.141	0.220	0.39
Isobutyramide	11	0.051	0.118	0.089
Butyramide	10	0.128	0.198	0.36
Malonamide	14	0.070	0.141	0.139
Water	15 (see Table I)			

Solute Diffusion

The diffusion coefficients of the two series of alcohols and amides are listed in Table II. Each experimental measurement of a diffusion coefficient for both the total and the coarse membrane was determined to an accuracy of ± 0.2 %. The data listed represent the average of at least 10 measurements. The error of the skin permeability coefficient, calculated from equation 5, varies from about 10 % for the fastest solute (methanol) to less than 1 % for the slowest (glycerol). Since we are primarily concerned with the behavior of solutes in

the dense homogeneous skin, the following discussion will be mainly devoted to the diffusion coefficients obtained in this membrane.

To facilitate comparison, a plot of these coefficients, ω_s, on a logarithmic scale, as a function of the number, n, of carbon atoms is given in Fig. 1. Two series may be distinguished among the alcohols: the normal series, methanol (1), ethanol (2), n-propanol (3), and n-butanol (4); and the branched series, including methanol, ethanol, sec-propanol (5), and ter-butanol (6). In the

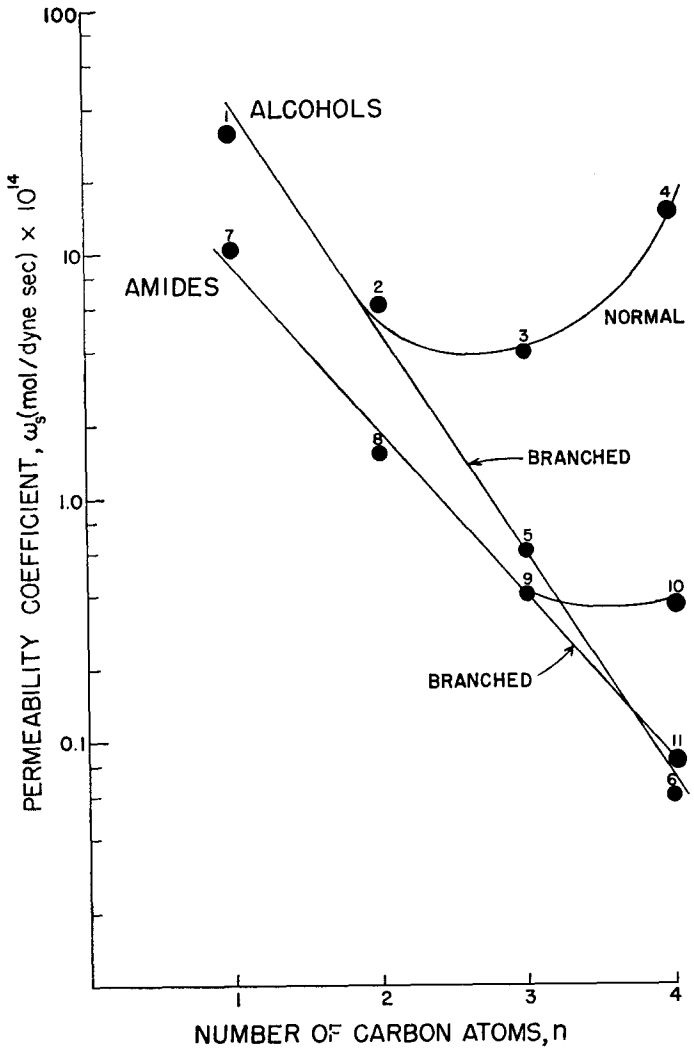

FIGURE 1. Nonelectrolyte permeability coefficients, through the skin membrane, as a function of the number, n, of carbon atoms. The numbers refer to the solute code numbers listed in Table II. The errors are proportional to the size of the circles in this and subsequent figures.

normal series, the curve passes through a minimum at n-propanol. In the branched series, log ω_s decreases linearly with n. The same general pattern is obtained for the amides: formamide (7), acetamide (8), propionamide (9), and isobutyramide (11) fall on the same line, and butyramide (10) shows a deviation from the linearity. This particular feature of nonelectrolyte diffusion is related to the partition coefficient, K_s, between membrane and bathing

TABLE III

THE RADIUS, PARTITION COEFFICIENT, AND HYDROGEN-BONDING ABILITY OF THE SOLUTES STUDIED

	Radius* r	Partition coefficient‡ Membrane/Solution K_s	Partition coefficient‡ Castor oil/Water K_o	Hydrogen-bonding ability N_H
	A			
Methanol	2.03	0.21	0.06	2
Ethanol	2.32	0.25	0.15	2
n-Propanol	2.49	0.63	0.57	2
sec-Propanol	2.73	0.34	0.30	2
n-Butanol	2.60	4.12	2.20	2
ter-Butanol	3.17	0.55	0.48	2
Ethylene glycol	2.45	0.20	—	4
Glycerol	2.77	0.20	—	6
Formamide	2.07	0.30	—	3
Acetamide	2.38	0.32	—	3
Propionamide	2.61	0.35	—	3
Isobutyramide	2.97	0.45	—	3
Butyramide	2.68	0.50	—	3
Malonamide	2.59	0.28	—	6
Water (THO)	1.65	0.135	—	4

* Radius of the narrowest cylinder fitting the molecular model.

‡ K_s was obtained within ±5%; K_o was obtained from Lindenberg (18).

solution, and the molecular dimensions, which are not explicitly related to the parameter, n.

K_s was measured under the same conditions of concentration and temperature as the diffusion measurements. The values are given in the third column of Table III and large differences can be seen between isomers. K_s increases much more rapidly in the normal series than in the branched one. The 10-fold increase in K_s between n-butanol and ter-butanol may account, at least partially, for the diffusional differences between these two solutes. It is interesting to note that all the solute K_s's are greater than the membrane water content (0.135). K_s's are much higher than the corresponding olive oil/water parti-

tion coefficient, but they are quite comparable to the partition coefficient between castor oil, a more polar oil containing hydroxyl groups, and water (Table III, fourth column). These facts indicate that the membrane material including water, behaves in this respect as a homogeneous amphophilic phase.

There is some difficulty in choosing the appropriate size parameter. The Stokes-Einstein hydrodynamic radii, as well as the partial molar volume in solution reflect solute-solvent interactions which may not be extrapolated to the particular case of the membrane. It seems preferable to use the pure geometrical measurements taken on molecular models [Stuart and Briegleb (E. Leybold's Nachfolger, Cologne, Germany)] although there is some arbitrariness in the choice of the suitable model dimension. The parameter which fits our diffusion data best is the radius, r, of the narrowest cylinder sufficient to contain the molecular model. This radius was first proposed by Soll (19) to fit solute reflection coefficients in porous membranes. He suggested that the dynamics of quasi-laminar flow oriented the solute lengthwise in its passage through the pores. Obviously such an assumption does not hold in the case of diffusion through a nonporous membrane. Nonetheless, this radius provides by far the best fit for our diffusion measurements. Diffusion in dense membranes depends critically upon steric configuration. The radius classically obtained by averaging the three mutually perpendicular dimensions of the model, including the shortest and the longest, considers molecules as spheres; this procedure minimizes the steric differences between linear and branched chains. On the contrary these differences are fully taken into account by the cylindrical model.

With the use of the experimental values of K_s and equation 9, the over-all friction experienced by the solutes in the skin membrane, denoted by f, was computed and plotted in Fig. 2 on a log scale as a function of the cylindrical radius. Two conclusions may be drawn from this graph. First, there is no deviation in either series from the logarithmic relationship between f and radius. The differences in diffusion between isomers exhibited in Fig. 1 appear to be completely ascribable to differences in K_s and in steric configuration. Second, two distinct lines are obtained for the two chemical series, the amides, as a group, experiencing greater friction in the membrane than the alcohols.

This chemical specificity exhibited by the nonelectrolyte diffusion through the dense cellulose acetate membrane, provides strong support for the hypothesis of Horowitz and Fenichel (7) and Stein (8), which relates permeability to solute hydrogen-bonding ability. The present experimental data offer no possibility for a separate analysis of each of the three components that comprise f, as already stated. The solute-solute friction, the solute-water friction, and the solute-membrane friction all reflect the physical realities of diffusion in the dense membrane, a quite different process from that in free solution or in the less dense membrane. It may be reasonably assumed that the several

frictional components depend, in various degrees, upon the hydrogen-bonding ability of both solute and membrane components. In Fig. 2 account has already been taken of K_s and the cylindrical radius, yet amides diffuse more slowly than the alcohols presumably because of the greater hydrogen-bonding ability of the amides. Complete information about the number, the strength, and the geometrical arrangements of hydrogen bonds for each solute, and the corresponding properties of the membrane components are not yet available.

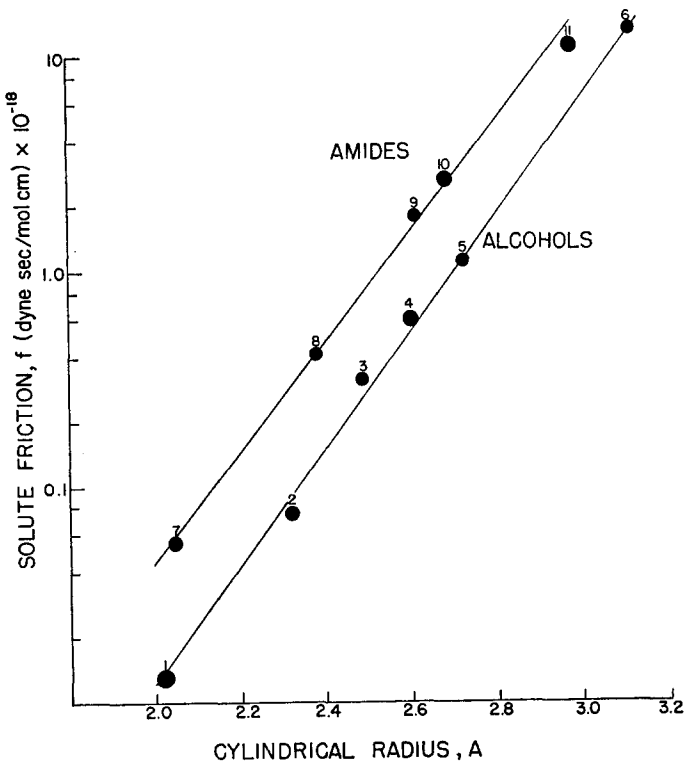

FIGURE 2. Solute friction, f, in the skin membrane as a function of the cylindrical radius of the solute. The numbers refer to the solute code numbers listed in Table II.

Though only empirical correlations can be made, these are nevertheless very suggestive. As a first approximation, one may count the number, N_H, of possible hydrogen bonds for each solute. This number for water, four, is well-established. For the alcohol group, $N_H = 2$ (Franks and Ives [20]). For the amide group the most likely value is three (21, 22). Thus, assuming simple additivity in the case of polyfunctional molecules, a number, N_H, may be assigned to each solute we have studied, as given in the last column of Table III. These values of N_H take no account of the strength and the steric disposition of the hydrogen bonds and are only to be considered as a rough index of

hydrogen-bonding ability. Since Fig. 2 shows that friction increases with N_H, the ratio f/N_H was plotted as a logarithmic function of radius in Fig. 3. It may be seen that all the solutes tested including methanol ($N_H = 2$), water ($N_H = 4$), and malonamide and glycerol ($N_H = 6$), fall close to a single

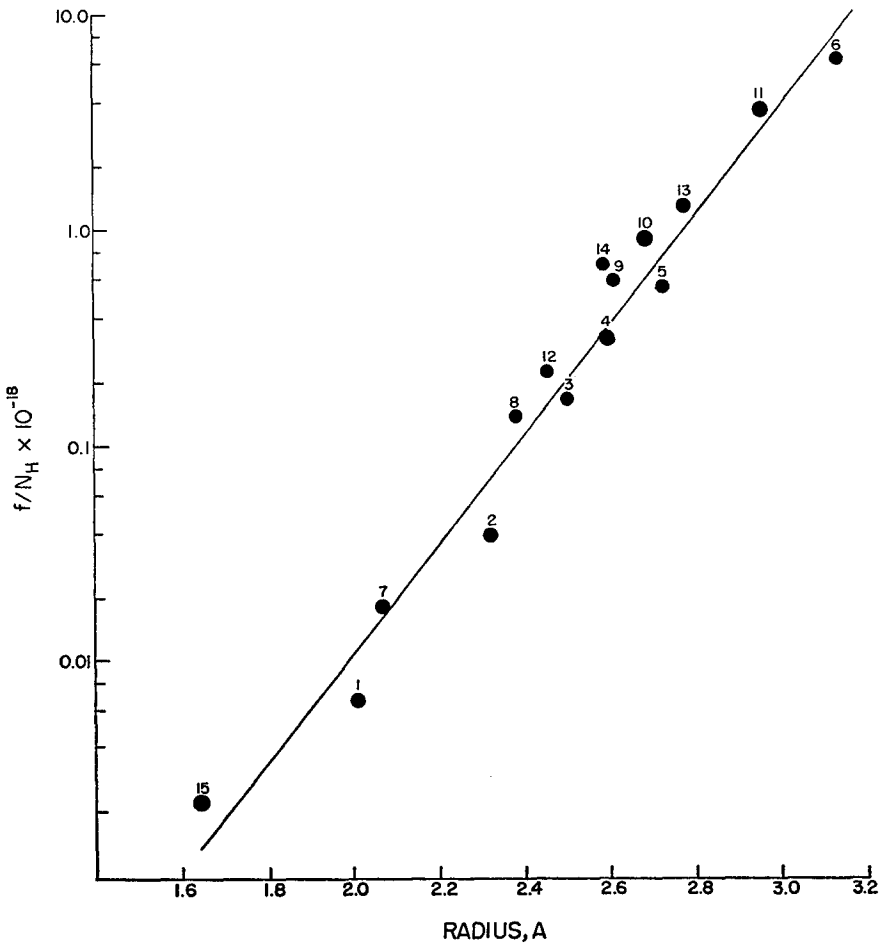

FIGURE 3. Solute friction/hydrogen-bonding ratios as a function of cylindrical molecular radius.

straight line. This correspondence fits very well with the hypothesis that hydrogen-bonding ability is an important factor in diffusion through the skin membrane. However, all the points for the alcohols fall below the line, whereas the rest of the solutes lie above it. This would make it seem that the empirical ratio, f/N_H, may not be the quantitatively appropriate index for the effect of hydrogen-bonding on diffusion, though it properly calls attention to the importance of the hydrogen bonds.

A series of experiments was carried out in order to study the temperature coefficient of diffusion across the total membrane for propionamide and sec-propanol, both with practically the same molecular radius. Diffusion coefficients were measured at 2°, 22°, and 37°C and plotted logarithmically against the temperature. A linear relationship was found and from the slope of the curve an apparent activation energy was computed. This was found equal to 11.4 ± 0.9(SD) kcal/mol for propionamide and 7.8 ± 0.8(SD) kcal/mol for

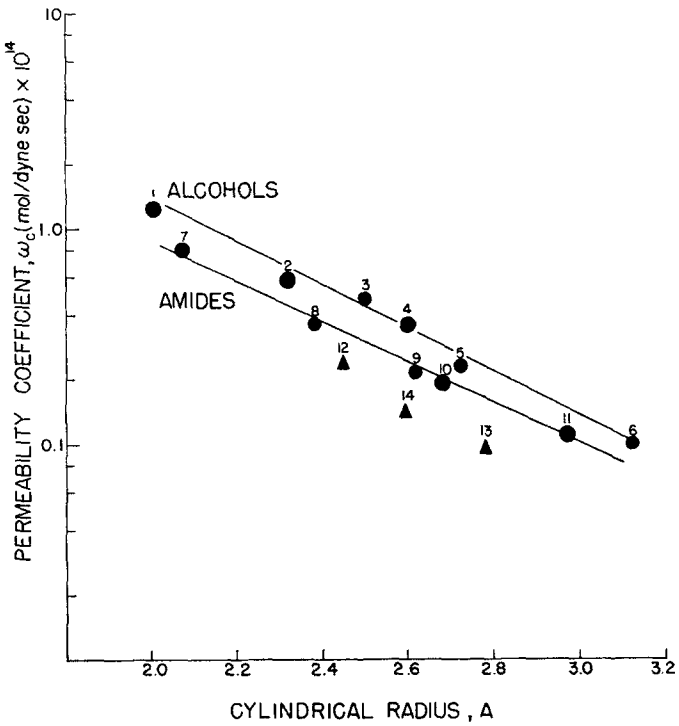

FIGURE 4. Nonelectrolyte permeability coefficients in the coarse membrane as a function of cylindrical molecular radius. Triangles denote the three polyfunctional solutes, ethylene glycol, glycerol, and malonamide.

sec-propanol. The fact that the 1.46 ratio of these activation energies is equal to the 3/2 N_H ratio of these two molecules provides additional support for the role of hydrogen-bonding in diffusion.

It is also interesting to consider solute diffusion in the coarse membrane. Since solute distribution ratios were not measured in this membrane, a simple plot of ω_c as a function of radius is given in Fig. 4, and it can be seen that there is no apparent difference between isomers. This indicates that K_s is essentially independent of differences between isomers and it is very likely that diffusion occurs mainly through aqueous channels. However, the effect of the hydro-

gen-bonding ability on diffusoin is still apparent in the coarse membrane, although considerably smaller than in the dense skin as can be seen by a comparison of Figs. 2 and 4. It is possible to account for this phenomenon by assuming that the solute-membrane friction is due not only to steric hinbrance in the tortuous channels, but also reflects interactions through hydrogen bonds with corresponding sites on the membrane matrix. At first sight it would appear that the collision probability of a 2.5 A radius molecule with the walls of a 24 A radius equivalent pore would be small. Howveer, a simple geometrical calculation indicates that 36 % of the area of a 48 A diameter pore is within 5 A of the pore wall, so that at any one time 36% of the solute molecules should be in contact with the pore wall. Alternatively one might consider that water in the pores is an ordered state different from that in bulk water, and a diffusional mechanism comparable to the mechanism analyzed by Horowitz and Fenichel (7) in a water protein–ordered lattice would apply. In this case, the activation energy for water diffusion would be different from that in the bulk phase, and it might be expected that the diffusion of a given solute would be affected markedly by the presence of another solute having a pronounced effect on the water structure, such as urea. However, Solomon (3) has summarized the evidence that the properties of water in small equivalent pores in cellulose membranes are not different from those in bulk solution, and the present results do not seem to cast doubt on this conclusion.

The present data obtained on both dense homogeneous membranes and on porous membranes seem to demonstrate that in addition to steric factors, the chemical nature of the solute as indicated by its hydrogen-bonding ability is important in controlling diffusion. This implies that the membrane matrix not only has an effect upon the partition coefficient of the solute, but also exercises an important influence upon the diffusion process. It seems reasonable to expect that the hydrogen-bonding ability will affect diffusion differently in polar than in nonpolar membranes, even though it may be assumed that the diffusion occurs in an aqueous phase included in the membrane matrix. The recognition of this fact may lead to a better understanding of the nature of the diffusion barriers offered by biological membranes.

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