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Thermodynamic Analysis of Nonelectrolyte Permeation across the Toad Urinary Bladder

Ernest M. Wright and Niels Bindslev*

Department of Physiology, Center for Health Sciences, University of California, Los Angeles, California 90024

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Summary. Permeability coefficients (P's) and apparent activation energies (E_a's) for nonelectrolyte permeation across the toad urinary bladder have been analyzed in terms of the thermodynamics of partition between membrane lipids and water. Particular attention has been paid to the contributions made by $-CH_2-$ and $-OH$ groups: on the average, the addition of one $-CH_2-$ group to a molecule increases P fourfold, while the addition of one $-OH$ group reduces P 500-fold. Using these changes in P, we have calculated the incremental free energies ($\delta\Delta F$), enthalpies ($\delta\Delta H$), and entropies ($\delta\Delta S$) for partition, hydration, and solution in membrane lipids. The results for toad bladder have been compared and contrasted with those extracted from the literature for red blood cells, lecithin liposomes, and bulk phase lipid solvents. The partition of $-CH_2-$ groups into toad bladder and red cell membranes is dominated by entropy effects, i.e., a decrease in entropy of the aqueous phase that "pushes" the group out of water, and an increase in entropy of the membrane lipid that "pulls" the group into the membrane. This process resembles that in "frozen" liposome membranes. In "melted" liposomes and bulk lipid solvents the free energy of solution in the lipid is controlled by enthalpy of solution. Partition of $-OH$ groups in all systems is governed by hydrogen bonding between the $-OH$ group and water. However, the solution of the $-OH$ group in toad bladder membranes is complex, and processes such as dimer and tetramer formation in the lipid phase may be involved. The results presented in this and the previous paper are discussed in terms of the structure of phospholipid bilayer membranes. Attention is drawn to the possible role of structural defects in the quasi-crystalline structure of the lipid (so-called 2 *gl* kinks) in the permeation of small molecules such as water, urea, methanol and acetamide.

The group contribution concept in permeability studies originates from the early experiments of Overton (1896, 1899, 1902) and Collander and Bärlund (1933), and has been employed in modern permeability studies to rationalize observed permeability patterns (e.g., Stein, 1967; Diamond & Wright, 1969*a*, 1969*b*). Each substituent group in a molecule,

* *Present address:* Institute of Medical Physiology A, University of Copenhagen, 2100 Copenhagen, Denmark.

$-CH_2-$, $-OH$, $-NH_2$, $-COOH$, etc., contributes in an additive fashion to the permeability coefficient (P). In the absence of intramolecular bonding, inductive effects, and chain branching, each methylene group increases P, whereas each polar group reduces P depending on the ability of the group to make H bonds with water. The effect of the $-CH_2-$ group is explained in terms of hydrophobic interactions in the aqueous phase. On the other hand, the effect of the polar groups is explained by the greater amount of energy needed to break the H bonds and tear the solute out of water. These molecular interactions in the aqueous phase account for the frequent observation that the relative ranking of solute P's is largely constant from one membrane to another, and from membranes to such model systems as bulk phase hydrocarbon/water partition coefficients (K's).

To explain the variation in magnitude of the selectivity sequences, i.e., the variation in the P or K ratios for any two solutes from one system to another, which can vary over an order of magnitude or more for any pair of solutes, we have to consider solute/membrane interactions. Little is known about the actual molecular forces involved in solute membrane interactions in biological membranes and differences between one membrane and another are usually ascribed to differences in "hydrophobic character". So in this paper, to gain more information about the molecular interactions between solutes and plasma membranes, we have attempted a thermodynamic analysis of P's and apparent activation energies (E_a's) obtained for the toad urinary bladder (Bindslev & Wright, 1976). We have paid particular attention to the contributions of the $-CH_2-$ and $-OH$ groups, and we have compared and contrasted our results with those that can be extracted from recent studies on red cell membranes (Galey, Owen & Solomon, 1973), liposomes (Diamond & Katz, 1974; Dix, Diamond & Kivelson, 1974; Cohen, 1975*a*, 1975*b*; Katz & Diamond, 1974); and bulk hydrocarbon solvents (e.g., Bindslev & Wright, 1976).

We conclude that in the toad bladder and red cell the interaction of the methylene group with the plasma membranes is mainly entropic in nature, and that this originates in the increase in disorder in the membrane upon creating "holes" or "kinks" in the liquid crystalline membrane matrix to accomodate the larger molecule. We further conclude that this effect is related to the action of lipophilic solutes as local anesthetics. The interactions of the $-OH$ group with toad bladder plasma membranes is complex, and is complicated by such effects as intramolecular H bonding when the solute is transferred from water to the hydrocarbon environment of the membrane interior.

Kinetic Analysis of Permeation

Solubility/diffusion mechanisms appear to be most productive in accounting for permeation of nonelectrolytes across biological membranes. Various expressions have been derived in the literature but here we follow the generalized treatment of Diamond, Szabo and Katz (1974). Their general expression relates the permeability coefficient (P) of the membrane to a nonelectrolyte to the solute's local partition coefficient ($K(x)$) and local diffusion coefficient ($D(x)$) within the membrane, the interfacial resistances (r' and r'') and the membrane thickness (x_0),

$$\text{i.e.} \quad 1/P = r' + \int_{x=0}^{x=x_0} dx/K(x)\,D(x) + r''. \tag{1}$$

The interfacial resistances r' and r'' are defined as $1/k_i'$ and $1/k_i''$ respectively where k_i' and k_i'' are permeability coefficients for the molecule crossing the membrane interfaces ′ and ″ in the water to membrane interior direction. [The interfacial permeability coefficients for the molecule passing from the membrane interior to the external aqueous phases are k_0' and k_0'', respectively, and the solute partition coefficients at $x=x_0(K(x_0))$ and $x=0(K(0))$ are simply given by the ratio of the interfacial permeability coefficients, $K(x_0)=k_i''/k_0''$ and $K(0)=k_i'/k_0'$].

A useful working equation is obtained in the limiting case where the membrane is homogenous (i.e., D and K independent of position in the membrane) and symmetrical (i.e., $k_i'=k_i''=k_i$, $k_0'=k_0''=k_0$, and $k_i/k_0=K$), namely:

$$1/P = 2/k_i + k_0\,x_0/k_i D \tag{2a}$$

or

$$1/P = 2/k_i + x_0/KD \tag{2b}$$

The meaning of these equations is apparent when one considers that the membrane resistance to permeation of the solute ($1/P$) is the sum of three series resistances – the two interfacial resistances ($2/k_i = r' + r''$) and the diffusional resistance of the membrane interior (x_0/KD). In the limit that the interfacial resistances are negligible compared to the diffusional resistance presented by the membrane interior, $x_0/KD \gg 2/k_i$, Eq. 2b can be reduced and rearranged to give a familiar expression for the permeability coefficient, i.e., $P=DK/x_0$. In toad bladder the fact that plots of log $P/1/T$ are straight lines over a large range of temperature (2–32 °C) suggests that only one of the steps in membrane permeation is rate limiting. (If each step contributed significantly to the resistance to permeation,

nonlinear curves would be obtained.) However, there are few clues to which particular step is rate limiting for any given molecule in any given membrane.

The actual magnitude of the apparent activation energies (E_a) for permeation are of little assistance in assigning the rate limiting step because E_a's in model systems for partition and diffusional processes vary widely. In lecithin liposomes activation energies for the partition of nonelectrolytes between water and the membranes range from 1.2 to 27.2 kcal/mole (Dix, Diamond, & Kivelson, 1974; Katz & Diamond, 1974). Furthermore, in polymers the activation energies for diffusion of gases and vapors range up to 40 kcal/mole (*see* Meares, 1965). It is apparent that the relative magnitude of the resistance barriers in biological membranes cannot be obtained from permeability studies alone.

More direct evidence for the distribution of resistance barriers in lipid membrane comes from the study by Griffith, Dehlinger and Van (1974) on ESR spectra of nitroxide spin labels in liposomes and natural membranes. They conclude that the shape of the hydrophobic barrier is trapezoidal with the highest part of the barrier located in the hydrocarbon core of the membrane interior. This, together with the assumption that hydrophilic molecules are partitioned into the polar periphery of membranes, suggests that the interior of the membrane is rate limiting for the hydrophilic solutes. For hydrophobic molecules on the other hand, the rate limiting step is probably at the periphery of the membrane. As the solutes used in this study are relatively hydrophilic (only benzyl alcohol has an olive oil partition coefficient greater than 1), we tentatively conclude that diffusion of these molecules through the interior of toad bladder plasma membranes is the rate limiting step for permeation, i.e., $1/P = x/(K_m D_m)$.

A number of other attempts have been made to assign the rate limiting step in solute permeation across membranes to either the membrane interface or the membrane interior (e.g., Zwolinsky, Eyring, & Reese, 1949; Stein, 1967; Diamond & Wright, 1969*b*; and Galey *et al.*, 1973). The correlation between P's and bulk phase partition coefficients has been used to assign the rate limiting step to one location or the other: the correlation between P and K_{oil} over many orders of magnitude has been used by Diamond and Wright (1969*b*) to propose that the rate limiting step is diffusion through the membrane interior, but the same arguments have been used by Stein (1967) to reason that the interfacial resistances are rate limiting. This arises because the same intermolecular forces in the aqueous phase largely account for the variations in both K's and k_i's with molecular structure, e.g., hydrogen bonding between solutes and

water will account for the correlation between the solute's hydrogen bonding ability and both K's and k_i's ($\Delta K \alpha \Delta k_i$ since variations in k_i are likely to be greater that variations in k_0). In other words the energy required to tear the solute out of the aqueous medium plays a major role in determining the absolute magnitude of both the membrane partition coefficients and the interfacial permeability coefficients.

Both Zwolinsky *et al.* (1949) and Galey *et al.* (1973) have attempted to estimate the magnitude resistance of the steps in membranes. For homologous series of compounds they plot $1/P$ against $1/K$ using oil or ether K's models, and assume that there is little variation in either D's or k_i's from one member of the series to the other. Straight lines were obtained under some conditions for the higher members of the series, and the slopes were used to estimate membrane diffusion coefficients, and the intercepts on the ordinate were used to estimate the interfacial resistances. Although similar curves may be drawn for toad urinary bladder results (straight lines can be obtained that pass through the origin), these are invalid because changes in P caused by the addition of $-CH_2-$ groups to a molecule are at least in part caused by changes in entropy in the aqueous phase, i.e., increases in k_i (*see* pp. 294–301).

Galey *et al.* (1973) also analyzed temperature dependent changes in P by the same procedure making the further assumption that changes in K_m/K_{ether} were independent of temperature. This assumption is invalid because apparent activation energies for the partition of solutes into membranes may exceed 20 kcal/mole (*see* Katz & Diamond, 1974), while the apparent activation energies for partition into bulk lipid solvents are generally less than 5 kcal/mole (*see* Bindslev & Wright, 1976).

Thermodynamic Analysis of Permeation

Considerable insight into permeation processes in the urinary bladder can be gained by the thermodynamic analysis of the permeability coefficients and their apparent activation energies presented in the previous paper. The approach is essentially similar to that developed earlier for the analysis of *Nitella P*'s and bulk phase partition coefficients (Diamond & Wright, 1969*b*). The starting point is the expression that relates lipid/water partition coefficients (K's) to thermodynamic parameters:

$$K = e^{-\Delta F_{wl}/RT} \tag{3a}$$

$$= e^{-(\Delta H_{wl} - T\Delta S_{wl})/RT} \tag{3b}$$

where ΔF_{wl}, ΔH_{wl} and ΔS_{wl} are the standard partial molar free energy, enthalpy and entropy, respectively, on transferring one mole of the molecule from a hypothetical aqueous solution to a hypothetical solution in lipid, each containing the molecule at a concentration of 1 M and obeying Henry's law. $RT = 596$ cal/mole at 22 °C.

Making the assumption that permeation across the toad bladder is limited by diffusion through the interior of the plasma membranes, i.e., $P = K_m D_m / x$ (*see* above), the membrane partition coefficient (K_m) is given by $K_m = Px/D_m$. Furthermore, assuming that variations in P are largely due to variations in K_m's and not D_m's, a reasonable assumption when comparing molecules of similar size and shape, the value of K_m estimated in this way can be inserted into Eq. 3*a*, i.e.,

$$K_m = Px/D_m = e^{-\Delta F_{wl}/RT} \tag{3c}$$

The absence of information about the precise magnitude of both D_m and x does not allow us to estimate the absolute free energy of the transfer of one mole of the molecule from the aqueous phase into the membrane (ΔF_{wl}). However, it is possible to estimate the incremental free energy ($\delta\Delta F_{wl}$) of a functional group, e.g., $-CH_2-$ or $-OH$, which alters the partition equilibrium between the membrane lipids and water, by inserting the ratios of the permeabilities of two molecules differing by one functional group into Eq. 3*c* and solving for $\delta\Delta F_{wl}$. For example, the addition of a $-CH_2-$ to *n*-propanol to give *n*-butanol increases P in the toad bladder from 2240×10^{-7} to $20{,}000 \times 10^{-7}$ cm/sec (Table 1, Bindslev & Wright, 1976). Therefore the incremental free energy for transfer of a $-CH_2-$ group from water into the membrane ($\delta\Delta F_{wl}^{CH_2}$) $= -1280$ cal/mole $[-RT \ln(20{,}000 \times 10^{-7}/2240 \times 10^{-7})]$. Incremental enthalpies of transfer are obtained in a similar fashion using the experimental heats of activation (E_a) and the relation $\Delta H_{wl} = E_a - RT$, and incremental entropies of transfer are calculated from $(\Delta H_{wl} - \Delta F_{wl})/T$.

The analysis can be taken one step further to yield incremental quantities for the solution of the functional groups in the membrane lipid ($\delta\Delta F_l$, $\delta\Delta H_l$ and $\delta\Delta S_l$). This is accomplished by splitting the transfer of the molecules from water to lipid into two steps, i.e., vaporization of the molecule from the aqueous solution at infinite dilution into a vacuum, followed by the solution of the molecule in the lipid phase. Then we can write

$$\delta\Delta F_l = \delta\Delta F_{wl} + \delta\Delta F_w, \tag{4a}$$

$$\delta\Delta H_l = \delta\Delta H_{wl} + \delta\Delta H_w, \tag{4b}$$

Table 1. Incremental thermodynamic functions for the methylene group, $-CH_2-$

Process	$\delta\Delta F$ cal/mole	$\delta\Delta H$ cal/mole	$\delta\Delta S$ cal/mole, K
Transfer from water to lipid (wl)	−1280	2100	11.5
Solute hydration (w)	110	−1520	−5.5
Solution in lipid (l)	−1170	580	6.0

The incremental thermodynamic functions for the $-CH_2-$ group are calculated from the butanol-propanol pair and are given here as an example of how the functions were obtained. $\delta\Delta F_{wl}^{CH_2}$ for the transfer from water to membrane was estimated from the relation $\delta\Delta F_{wl}^{CH_2} = -RT\ln(K_{butanol}/K_{propanol})$ assuming that $P_{butanol}/P_{propanol} = K_{butanol}/K_{propanol}$. Although the diffusion coefficient for butanol is expected to be slightly less than that for propanol, the difference is in the wrong direction to account for changes in P; i.e., in this particular case the incremental quantities may be underestimated, but by not more than 30%. Note that this procedure cannot be used for the smaller solutes since the change in P on addition of a $-CH_2-$ group (a decrease) is probably dominated by a reduced diffusion coefficient. $\delta\Delta H_{wl}^{CH_2}$ is obtained from the difference in the apparent activation energies ($\Delta H = E_a - RT$), assuming that the contribution of the diffusion coefficients is identical for the two solutes. The entropy term is then estimated from the relation $(\Delta H - \Delta F)/T$. The hydration energies were taken from Butler (1937). The incremental changes in free energy, enthalpy and entropy for solution in the membrane were obtained from the sum of the incremental changes on going from water to membrane and those for hydration, e.g., $\delta\Delta F_m^{CH_2} = \delta\Delta F_{wl}^{CH_2} + \delta\Delta F_w^{CH_2}$. For further details *see* the text.

and

$$\delta\Delta S_l = \delta\Delta S_{wl} + \delta\Delta S_w, \tag{4c}$$

where $\delta\Delta F_l$, $\delta\Delta H_l$ and $\delta\Delta S_l$ are the incremental standard partial molar free energy, enthalpy and entropy of solution in the membrane lipid, respectively, and $\delta\Delta F_w$, $\delta\Delta H_w$, and $\delta\Delta S_w$ are the corresponding quantities for hydration. Assuming that intermolecular forces in the vapor phase are negligible, $\delta\Delta F_w$, $\delta\Delta H_w$, and $\delta\Delta S_w$ can be interpreted in terms of the state of the molecule in the water phase, while $\delta\Delta F_l$, $\delta\Delta H_l$, and $\delta\Delta S_l$ can be interpreted in terms of the state of the molecule in the lipid membrane phase. The thermodynamic parameters for solution in the toad bladder membranes are then obtained from equations using the thermodynamic parameters for the transfer of the molecule from the aqueous phase into the membrane ($\delta\Delta F_{wl}$, $\delta\Delta H_{wl}$, and $\delta\Delta S_{wl}$) estimated from the data presented in the previous paper and the parameters obtained from the literature for the solution of nonelectrolytes in water. Again the results are expressed in terms of incremental quantities ($\delta\Delta F_l$, $\delta\Delta H_l$, and $\delta\Delta S_l$) for functional groups such as $-CH_2-$ and $-OH$. For more detailed discussions of the procedures involved *see* Diamond and Wright (1969*b*), Diamond and Katz (1974), and Katz and Diamond (1974).

An example of the procedure involved in calculating $\delta\Delta F_l$, $\delta\Delta H_l$, and $\delta\Delta S_l$ is given in Table 1 for the addition of a $-CH_2-$ group to *n*-propanol to give *n*-butanol: $\delta\Delta F_{wl}^{CH_2}$ (-1280 cal/mole) is first calculated from the ratio of the *P*'s as outlined above; $\delta\Delta F_w^{CH_2}$ (110 cal/mole) is obtained directly from the difference between the hydration energies for butanol and propanol given by Butler (1937); and, finally, $\delta\Delta F_l^{CH_2}$ (-1170 cal/mole) is obtained from the relation $\delta\Delta F_l^{CH_2} = \delta\Delta F_{wl}^{CH_2} + \delta\Delta F_w^{CH_2}$ (Eq. 4*a*). The incremental enthalpies were obtained in the same manner from the apparent activation energies for permeation presented in Table 4 of the previous paper and from the enthalpies of hydration reported for these solutes by Butler (1937). The incremental entropies were calculated from the free energies and enthalpies using relations of the type $\delta\Delta S^{CH_2} = (\delta\Delta H^{CH_2} - \delta\Delta F^{CH_2})/T$. The results show that the increase in permeation caused by the addition of the $-CH_2-$ group ($\delta\Delta F_{wl}^{CH_2} = -1280$ cal/mole) is due to an increase in the free energy of hydration ($\delta\Delta F_w^{CH_2} = +110$ cal/mole) and a decrease in the free energy of solution in the bladder membranes ($\delta\Delta F_l^{CH_2} = -1170$ cal/mole). In both the aqueous phase and the membrane the changes in free energy are controlled by entropy changes, i.e., $T\delta\Delta S > \delta\Delta H$.[1]

The average incremental quantities for solution of $-CH_2-$ and $-OH$ groups in the bladder membranes are summarized in Tables 2 and 3, respectively. Also included for comparison are the tentative values estimated for the solution of the $-CH_2-$ and $-OH$ groups in olive oil (*from* Table 5, Bindslev & Wright, 1976), benzene (Butler, 1937; Butler & Harrower, 1937; Tanford, 1973), dimyristoyl lecithin liposomes (Diamond & Katz, 1974; Katz & Diamond, 1974), egg lecithin liposomes with and without 48% cholesterol (Cohen, 1975*a*, 1975*b*), and red blood cell membranes (Galey *et al.*, 1973). At the outset it should be made clear that any differences between the membranes, and between membranes and model systems, *originate with interactions between solutes and membrane lipids*

1 This analysis has been carried out assuming that the epithelium of toad bladder can be treated as a single membrane. Of course it is appreciated that toad bladder is a heterogenous epithelium with granular rich cells (83%), mitochondria rich cells (11%) and goblet cells (6%), and that molecules must cross two plasma membranes (the apical and baso-lateral membranes) to traverse each cell type. Assignment of the derived average membrane properties to the plasma membranes of the epithelial cells will depend on which membrane limits permeation across the bladder. If the apical membranes of the granular rich cells represent the rate limiting barrier, the conclusions drawn refer to this membrane. On the other hand, if the apical and baso-lateral membranes contribute equally to the resistance the conclusions refer to the average properties of the two plasma membranes (it can be shown in this case that the overall *P*'s and E_a's are linear functions of the *P*'s and E_a's for each separate membrane).

Table 2. Incremental thermodynamic functions for the $-CH_2-$ group

	Transfer from water to lipid $\delta\Delta F_{wl}^{CH_2}$ cal/mole	$\delta\Delta H_{wl}^{CH_2}$ cal/mole	$\delta\Delta S_{wl}^{CH_2}$ cal/mole, °K
toad bladder	−780	2,460	11.0
red cell	−630	9,500	34.0
liposomes	−550	−1,450	−3.0
benzene	−780	540	4.3
olive oil	−650	2,020	9.0
	Hydration $\delta\Delta F_{w}^{CH_2}$	$\delta\Delta H_{w}^{CH_2}$	$\delta\Delta S_{w}^{CH_2}$
	130	−1,570	−5.7
	Solution in lipid $\delta\Delta F_{l}^{CH_2}$	$\delta\Delta H_{l}^{CH_2}$	$\delta\Delta S_{l}^{CH_2}$
toad bladder	−650	890	5.2
red cell	−490	7,930	28.3
liposomes	−410	−3,040	−8.8
benzene	−650	−1,030	−1.4
olive oil	−520	450	3.3

The incremental thermodynamic functions were estimated as described in the text and in Table 1. In the case of toad bladder, these were calculated using the data in Tables 1 and 4 of the previous paper, but were restricted to those compounds with three or more carbon atoms. The red cell values are calculated from the permeability coefficients and temperature coefficients for amide permeation (Tables 1 and 3, Galey *et al.*, 1973), where again the calculations were restricted to the larger molecules. The functions for liposomes refer to the nonelectrolyte partition coefficients (methanol, *n*-propanol and *n*-butanol) between dimyristoyl lecithin liposomes, and were taken directly from Table 4 of the paper by Diamond and Katz (1974). Note these values for the liposomes refer to temperatures *above* the phase transition temperature. The values for benzene and olive oil are for bulk phase partition coefficients between the hydrocarbon and water. The functions for benzene were obtained from Butler and Harrower (1937) from the solutes methanol, ethanol, *n*-propanol, and *n*-butanol. The preliminary values for olive oil are from Table 5 of the previous paper. The hydration functions are from the data given by Butler (1937) for methanol, ethanol, *n*-propanol and *n*-butanol.

since the intermolecular forces in the aqueous phase are identical for all these diverse systems.

In performing the analysis we have attempted to minimize errors by considering only a limited group of molecules for all transfer processes. Ideally, we would have prefered to consider only the same pairs of solutes for all permeation, partition and hydration steps, in order to eliminate such variables as intramolecular H bonding and inductive effects, and to draw more accurate conclusions about the differences between membranes and between membranes and model systems. In the various membrane systems we have only considered molecules with more than two carbon

Table 3. Incremental thermodynamic functions for the $-$OH group[a]

	Transfer from water to lipid $\delta\Delta F_{wl}^{OH}$ cal/mole	$\delta\Delta H_{wl}^{OH}$ cal/mole	$\delta\Delta S_{wl}^{OH}$ cal/mole, °K
toad bladder	3,780	−8,650	−42.3
liposome K's[b]	970	2,950	6.7
liposome P's			
a. cholesterol free	940	3,600	9.0
b. 48% cholesterol	1,290	3,600	7.0
benzene	4,500	4,100	− 4.8
olive oil	2,520	−1,600	−14.0
	Hydration $\delta\Delta F_{w}^{OH}$	$\delta\Delta H_{w}^{OH}$	$\delta\Delta S_{w}^{OH}$
	−7,000	−8,260	− 4.3
	Solution in lipid $\delta\Delta F_{l}^{OH}$	$\delta\Delta H_{l}^{OH}$	$\delta\Delta S_{l}^{OH}$
toad bladder	−3,230	−16,900	−46.6
liposome K's[b]	−1,230	−2,210	− 3.5
liposome P's			
a. cholesterol free	−6,060	−4,660	4.73
b. 48% cholesterol	−5,710	−4,660	3.53
benzene	−2,280	−4,370	− 9.1
olive oil	−4,480	−9,860	−18.3

[a] Incremental functions were calculated in the same fashion as for the methylene group (*see* text, Tables 1 and 2). In toad bladder these were estimated from the P's and E's for the pairs of solutes 1,3-propanediol *vs.* *n*-propanol and 1,4-butanediol *vs.* *n*-butanol (Tables 1 and 4, Bindslev & Wright, 1976). The liposome K's refer to partition of solutes between dimyristoyl lecithin liposomes and water, and the values quoted are those taken from Table 4 of the paper by Diamond and Katz (1974). The liposome P's refer to permeability studies on egg lecithin liposomes containing 48% cholesterol or no cholesterol (Cohen, 1975*a*, 1975*b*). The numbers were estimated from the average changes in P and E_a upon introduction of one $-$OH group into a molecule (two H bonds). The olive oil and benzene functions are for the bulk phase partition of solutes between the hydrocarbon and water. Those for benzene are deduced from the experimental data presented by Butler (1937), Butler and Harrower (1937) and Tanford (1973) for the solution of ethane and ethanol in benzene and in water. The numbers agree well with the rough estimates of Laiken and Némethy (1970). The olive oil estimates come from Table 5 of our preceding paper. The hydration values come from Butler (1937) for the pairs of solutes methanol *vs.* methane and ethanol *vs.* ethane. Finally, it has to be recorded that our functions strictly apply to the substitution of an H atom with an $-$OH group, and as Davis (1973) pointed out, the incremental quantities for the methylene group are not identical to a terminal methyl group. This leads to an overestimate of the free energy of transfer by about 1 kcal/mole depending on the solvent. We have not attempted to make this correction in view of the absence of information about the enthalpy and entropy terms.

[b] It should be mentioned that the values for liposome K's were derived from the partition coefficients of glycerol and *n*-propanol, and these values are likely to be underestimates owing to intramolecular hydrogen bonding.

atoms to avoid complications posed by the anomalous high permeation of small molecules (*see* Bindslev & Wright, 1976) where variations in size of the molecule may contribute to both the partition and diffusion steps. In the case of methylene groups the data in the literature is more than adequate for our approach, but with polar groups the data is far from complete and so our discussion of the $-OH$ group is on less secure ground. However, following Butler's (1937) principle of additive contributions of the various constituent groups in a molecule, it is apparent from the literature that the addition of $-OH$ and $-CH_2$ groups to alkyl chains between three and seven carbons long produce roughly constant effects on P, K, $\delta\Delta F_{wl}$, $\delta\Delta H_{wl}$, $\delta\Delta S_{wl}$, $\delta\Delta F_w$, $\delta\Delta H_w$ and $\delta\Delta S_w$, at least in the absence of chain branching and intramolecular H bonding.

The Methylene Group

In membranes and model systems the addition of $-CH_2$ groups increase P and/or K, i.e., $\delta\Delta F_{wl}^{CH_2} < 0$. This increase in P and K is due in part to the positive free energy of hydration ($\delta\Delta F_w^{CH_2} > 0$) and in part to the negative free energy of solution in the lipid ($\delta\Delta F_l^{CH_2} < 0$).

The change in free energy of hydration by introducing a $-CH_2-$ group in the aqueous phase, $\delta\Delta F_w^{CH_2}$, is dominated by a decrease in entropy, $|T\delta\Delta S_w^{CH_2}(-1690 \text{ cal/mole})| > |\delta\Delta H^{CH_2}(-1570 \text{ cal/mole})|$. The entropy decrease is due to the stabilizing action of the hydrocarbon residue on the local order of water molecules, i.e., hydrocarbons promote "iceberg" formation in water. The negative hydration enthalpy also reflects the stronger water/water H-bonding in the vicinity of the residue. A possible contribution to $\delta\Delta H_w^{CH_2}$ from van der Waal forces between $-CH_2-$ and water is likely to be small as the mutual perturbation induced in the two groups, $-CH_2-$ and $-OH$, is out of phase. Thus, the methylene group is not immobilized but rather squeezed out of the water (*see* Diamond & Wright, 1969*a*, 1969*b*).

The positive enthalpy of solution in the toad bladder membrane ($\delta\Delta H_l^{CH_2} = +890$ cal/mole) probably reflects the energy required to insert the larger molecule between the hydrocarbon tails of the lipids, i.e., the energy required to overcome the attractive forces between the membrane lipid chains, and to overcome the repulsion forces encountered during the increase in packing of the membrane lipid to accommodate the larger solute, outweighs any increase in the attractive forces (dispersion forces) between the $-CH_2-$ group and the membrane lipids. The large increase

in the entropy of solution in the membrane ($T\delta\Delta S_l^{CH_2} = 1540$ cal/mole) presumably arises from the disruption of the ordered crystalline array of the membrane lipids outweighing any immobilization of the solute in the membrane. This can be formulated as $|\delta\Delta H_l(\text{lipid/lipid})| > |\delta\Delta H_l(\text{solute/lipid})|$ and $|\delta\Delta S_l(\text{lipid/lipid})| > |\delta\Delta S_l(\text{solute/lipid})|$.[2]

The value obtained for $\delta\Delta F_l^{CH_2}$ in the case of the toad bladder (-650 cal/mole) is very similar to that obtained for other membranes (red blood cells, -490; Nitella, -450; liposomes, -410) and bulk lipid solvents (benzene, -650; olive oil, -520; ether, -510; isobutanol, -370; octanol, -502; cyclohexane, -615; and carbon tetrachloride, -630) (*see* Table 2; Diamond & Wright, 1969*b*; Diamond & Katz, 1974). However, the agreement between the biological membranes and the model systems is mainly coincidental as $\delta\Delta H_l^{CH_2}$ and $\delta\Delta S_l^{CH_2}$ are both positive for the toad bladder and red blood cell but are negative for most other systems (Table 2; Diamond & Katz, 1974).

The reason for the discrepancy between the biological membranes and the model systems probably lies in the fact that the lipids are in a highly ordered configuration in both the bladder and red cell compared with the more random configurations in both the liposomes above their phase transition temperature and the bulk lipid solvents. In the case of the more fluid lipids a negative $\delta\Delta H_l^{CH_2}$ is accounted for by the increase in van der Waal's forces (dispersive forces) between the hydrocarbon and the lipids, whereas in biological membranes a positive $\delta\Delta H_l^{CH_2}$ is a consequence of the energy needed to insert the large molecule in between the lipids of the quasi crystalline structure of the membrane. Positive values of the entropy of solution ($\delta\Delta S_l^{CH_2} > 0$) in both the bladder and red cell suggest that the introduction of the larger molecule causes a disruption of membrane structure, i.e., the membrane becomes more fluid. On the other hand the negative values of the entropy of solution ($\delta\Delta S_l^{CH_2} < 0$) in the bulk lipids and the liposomes above the phase transition temperature indicate that the dominant effect here is the relative immobilization of the solute in the lipid compared with the vapor state.

Evidence to substantiate this picture of the solubilization of the larger hydrocarbon molecules in biological membranes comes from experiments on the partition of solutes into "frozen" liposome membranes, where it was observed that the enthalpy and entropy of solution of molecules in

2 Similar reasoning accounts for the high value of the Barclay-Butler slope in toad urinary bladder, i.e., $b^{CH_2} = d\delta\Delta S_l^{CH_2}/d\delta\Delta H_l^{CH_2} = 5.8 \times 10^{-3}$, $^0K^{-1}$ (*see* Diamond & Katz, 1974; Katz & Diamond, 1974). The value of δb^{CH_2} is much lower in the case of lecithin liposomes (2.9×10^{-3}, $^0K^{-1}$) and bulk solvents (e.g., benzene, 1.4×10^{-3}, $^0K^{-1}$).

the membranes were both *positive*, i.e., the enthalpy and entropy of solution *reverse* in sign on lowering the temperature of the liposomes down through their phase transition point (Diamond & Katz, 1974; Dix, Diamond, & Kivelson, 1974). In other words the partition of solutes into liposome membranes in the "frozen" state closely resembles that for the toad bladder and red blood cell.

Another approach to the free energy of transfer of a methylene group between water and membranes that is illuminating is to split the partition term into two other components – the adsorption of the solute at the membrane interface and the subsequent transfer of the solute from the interface into the interior of the membrane. The free energy of the partition process is now given by

$$\delta \Delta F_{wl}^{CH_2} = \delta \Delta F_{wi}^{CH_2} + \delta \Delta F_{il}^{CH_2}$$

where $\delta \Delta F_{wi}^{CH_2}$ and $\delta \Delta F_{il}^{CH_2}$ are the free energies of the adsorption step from the water to the interface, and the transfer from the interface into the interior of the lipid respectively. The incremental free energy for the adsorption of a methylene group at the decane/water interface is -825 cal/mole (Haydon & Taylor, 1960; Wang, Rich, Galey & Solomon, 1972) and at the dodecane/0.1 M NaCl interface is -415 cal/mole (Gillap, Weiner, & Gibaldi, 1968). These free energy changes of adsorption are comparable to the free energy of transfer from the water into the membrane (Table 1 and 2), and it can be concluded that the major effect of the methylene group is on the adsorption step. This conclusion is supported by observations which show that the free energy of adsorption is entropic rather than enthalpic (Gillap *et al.*, 1968), i.e., the positive enthalpy term is more than offset by the large increase in entropy. These results indicate that there is little difference in the environment of the $-CH_2-$ group whether it is absorbed on the surface of the membrane or distributed in the hydrocarbon interior of the membrane.

The Hydroxyl Group

The addition of a $-OH$ group to a molecule reduces the permeability and partition coefficients, i.e., $\delta \Delta F_{wl}^{OH} > 0$. In the absence of intramolecular H bonding P's decrease by as much as 500-fold in toad urinary bladder (Table 3) to as little as twofold in rabbit gall bladder (Smulders & Wright, 1971). Likewise for partition coefficients, the factor ranges from 2000 for benzene to 5 for isobutanol (from Table 3, and Diamond & Wright,

1969*b*, Table 1). In the extreme case where solutes are partitioned between water and an inert hydrocarbon it can be estimated from the paper by Laiken and Némethy (1970) that an $-$OH group reduces partition coefficients by 5000-fold. Thus, on the basis of the free energies of transfer alone, it can be concluded that the membranes of the toad urinary bladder are much more hydrophobic than olive oil and the liposome membranes, but less hydrophobic than benzene (*see* Table 3).

On examining the enthalpy and entropy of the transfer process in toad bladder we find that both terms are large and negative with the entropy term dominating, i.e., $|T\delta\Delta S_{wl}^{OH}|$ ($-12{,}480$ cal/mole) $>|\delta\Delta H_{wl}^{OH}|$ (-8700 cal/mole). This is in direct contrast to the liposomes and benzene where the enthalpy term is positive, i.e., heat is required to transfer the $-$OH group from water into the lipid (2950 to 4080 cal/mole), and the entropy term ranges from -4.8 cal/mole, degree for benzene to 9.03 cal/mole, degree for the liposomes. [These latter values are similar to those group quantities estimated by Laiken & Némethy (1970) for the transfer of the $-$OH group from water into inert hydrocarbons; $\delta\Delta F_{wl}^{OH}$ 5100 to 4700 cal/mole, $\delta\Delta H_{wl}^{OH}$ 700 cal/mole, and $\delta\Delta S_{wl}^{OH}$ 7.0 to 8.5 cal/mole, degree.] So we conclude that the processes underlying the free energy of transfer of the $-$OH group into toad bladder membranes are quite distinct from those involved in the transfer process into liposomes or bulk hydrocarbons. The group transfer quantities for olive oil are intermediate between those of the bladder and those of the liposomes and benzene.

As with the methylene group, additional insight can be obtained by separating the transfer process into two steps—the hydration step and solution in the membrane lipid. First let us consider the hydration energies which were obtained from Butler (1937). The free energy of hydration (-7000 cal/mole) is controlled by the enthalpy of solution (-8500 cal/mole), which reflects the formation of the two H bonds between the $-$OH group and water. The entropy of hydration (-4.3 cal/mole, degree) is also interpreted in terms of the increased H bonding.

Turning to the transfer of the $-$OH group from the vapor state into the membranes and lipids, we find that the free energy of transfer in all cases is negative, but less negative than the free energy of hydration, i.e., $\delta\Delta F_w^{OH} < \delta\Delta F_l^{OH} < 0$. This is quite consistent with the established view that the reduction in P's and K's by polar groups is controlled by the increase in bonding between the solute and water.

The molecular events underlying the free energy of solution in the membrane are more obscure than those underlying the free energy of

hydration. In toad bladder the enthalpy of solution is $-16{,}900$ cal/mole (about twice as high as the value for hydration), and the entropy term is almost as large ($T\Delta S_l^{OH} = -13{,}750$ cal/mole). In the model systems, however, the enthalpy of solution in lipid is generally less than the corresponding hydration term, $\delta\Delta H_l^{OH} < \delta\Delta H_w^{OH}$. These results imply that intermolecular bonding between the $-$OH group and toad bladder membranes is much greater than both H bond formation in the aqueous phase and bonding between the group and the model lipid systems.

There are two explanations for the apparent excessive negative enthalpy and entropy of solution in toad bladder membranes. (1) *The assumption underlying the extraction of K's from P's may be invalid.* We assume that variations in P among different molecules mainly reflect variations in K's rather than variations in D's, i.e., we assume $P_1/P_2 \sim K_1/K_2$, where 1 and 2 are different molecules, and calculate $\delta\Delta F$'s from the P ratios. Although this may be quite valid for $\delta\Delta F$'s it may not be correct for $\delta\Delta H$'s and $\delta\Delta S$'s, because variations in P with T for any given molecule may be due to variations in D rather than K. Thus $\delta\Delta H$'s of permeation may reflect $\delta\Delta H$'s of diffusion through the membrane interior rather than $\delta\Delta H$'s of partition. The limited information in the literature suggests that ΔH's for diffusion range from 7 kcal/mole for the diffusion of water in hydrocarbons (Schatzberg, 1965) to a maximum of 40 kcal/mole for diffusion of gases and vapors in hydrophobic polymers (Meares, 1965). [The activation energy for diffusion of valinomycin across lecithin bilayers has been estimated by Benz, Stark, Janko & Lauger (1973) to be 14 kcal/mole.] However, irrespective of the absolute values of the activation energies for diffusion in membranes, we expect that they may increase slightly with molecular size up to a maximal value and to be independent of the hydrogen bonding ability of the solute. Strong evidence in favor of the validity of our analysis comes from the similarities between the incremental thermodynamic quantities for lecithin liposomes obtained from P measurements and from K measurements (Table 3).

(2) *The group transfer parameters could be seriously influenced by self association and/or intramolecular hydrogen bonding.* It is well known that molecules with polar groups associate by way of hydrogen bonds when they are dissolved in hydrocarbon solvents, and this shows up as an increase in lipid/water partition coefficients as the concentration of the polar molecule is increased (*see* for example Aveyard & Mitchell, 1969). In toad bladder such association of alcohol molecules in the membrane could explain the large negative enthalpy and entropy of solution. In other words, dimer formation in the membrane lipids would lead to

overestimates of alcohol P's and "errors" in derived thermodynamic parameters. For example, the large enthalpy of association, -5195 cal/mole of hydrogen bonds formed (Fletcher & Heller, 1967), could account for much of the enthalpy of solution of the $-OH$ group in the membrane lipids. Evidence against this hypothesis is that P's in the toad bladder are relatively independent of solute concentration over a wide range in concentration (from 30 μM to as high as 100 mM). However, in dilute solutions of nonaqueous solvents *intramolecular* hydrogen bonding is known to accur with diols such as 1,3-propanediol and 1,4-butanediol (Pimentel & McClellan, 1960, p. 175). During permeation of these molecules across lipid membranes, intramolecular H bonding is expected to occur; we predict that this would enhance the permeability of the diols relative to their monohydroxy alcohols and lead to a larger than expected enthalpy of solution in the membrane. (This hypothesis might be tested by recording the IR spectra of alcohols in isolated purified membranes.) Likewise, the associated decrease in molecular volume with intramolecular H bonding would be expected to increase the diffusion coefficient of the molecules in the membrane.

Splitting $\delta \Delta F_{wl}^{OH}$ into adsorption ($\delta \Delta F_{wi}^{OH}$) and transfer ($\delta \Delta F_{il}^{OH}$) steps is less informative than was the case with $\delta \Delta F_{wl}^{CH_2}$. All that is known about the incremental quantities for adsorption at water/lipid interface is that $\delta \Delta F_{wi}^{OH}$ and $\delta \Delta F_{wi}^{CONH_2}$ are -800 and -400 cal/mole for the water decane interface respectively, and that the free energy of adsorption appears to be entropic (Haydon & Taylor, 1960; and Wang *et al.*, 1972). This suggests that the adsorption step at the water/decane interface becomes energetically unfavorable with increasing ability of the substituent group to make H bonds (three bonds for $-CONH_2$ *vs.* two bonds for $-OH$). Clearly it would be desirable to have more information about the adsorption of a wide range of solutes to membrane interfaces.

Model for Permeation

A mosaic of lipid and protein is the starting point for current models of plasma membranes. The lipids, which form the backbone of the membrane, are arranged in a bimolecular layer 50 Å thick with their ionic head groups in the aqueous phase on each side of the membrane, and their hydrocarbon tails directed towards the center of the membrane (Fig. 1). Globular proteins float in this lipid sea with their highly polar groups exposed on one or both surfaces of the membrane, and their hydrophobic

regions embedded in the hydrocarbon core of the lipid bilayer (Singer & Nicholson, 1972). The lipids in plasma membranes are glycerides, and sterols. The glycerides are mainly phospholipids of long chain fatty acids (16–24 carbon atoms long), and cholesterol is the most abundant sterol. In eukaryotic cells the membranes are in an anisotropic, or liquid crystalline state, owing to the presence of unsaturated fatty acids (50% of the fatty acids are unsaturated) and the high cholesterol content (up to 50 moles% of the total membrane lipids). Parenthetically this is probably the explanation of the lack of real "phase transitions" in the plots of log P *vs.* $1/T$ (*see* Fig. 4, Bindslev & Wright, 1976).

It is against this background of membrane structure and composition that we describe nonelectrolyte permeation. Historically, it is the correlation between permeability coefficients and oil partition coefficients that lead to current concepts about the role of lipids in membrane structure and function. This has been dramatically reinforced in recent studies which show that lipid bilayers mimic many of the properties of biological membranes. Differences in detail, however, may be due to such differences in lipid composition and configuration and the presence of proteins in biological membranes. In this discussion it is assumed that the passive permeation of simple nonelectrolytes reflects the properties of the lipids, but it should be borne in mind that the permeation of these molecules may in part reflect the properties of the hydrophobic regions of the globular proteins that span biological membranes. We consider, irrespective of the actual pathway, that permeation occurs by a solubility/diffusion mechanism, and we shall deal with each in turn.

Partition into Membrane

The partition of molecules from the aqueous phase into the membrane occurs in two steps: the adsorption of the molecule at the membrane/solution interface and the subsequent transfer of the molecule into the membrane. Thermodynamic considerations (pp. 301 and 304) suggest that the adsorption step is energetically favorable for the nonpolar regions of the molecule, but is less favorable for the highly polar regions. The transfer of the molecule from the interface into the membrane involves breaking the bonds which hold the polar groups on the molecule to the interface and the insertion of the molecule into a "hole" in the membrane lattice. The amount of energy required for this step depends on (1) the number and strength of the bonds to be broken at the interface – the more polar

Fig. 1. Space filling models showing the arrangement of phospholipid molecules in biological membranes. The phospholipid molecules are arranged as a bilayer with their polar head groups associated with the aqueous solutions on each side of the membrane, and with their hydrocarbon tails directed towards the center of membrane. In closely packed membranes there is little free volume within the membrane. Model *b* shows the creation of free volumes in a membrane by conformational changes in the hydrocarbon tails. These defects, so-called 2 *gl*

the molecule the higher the energy required [this accounts for the increase in E_a with the number of H bonds each solute can form with water that is observed in some systems, such as the permeation and partition of molecules in lecithin liposomes (Cohen, 1975*a*, 1975*b*; Katz & Diamond, 1974)]; (2) the size of the molecule—the larger the molecule the more energy is required (e.g., Bindslev & Wright, 1976, Fig. 6); and (3) the detailed architecture of the membrane—such as the density of packing of the lipids, the amount of free volume, and the presence or absence of structural defects (kinks) between the hydrocarbon tails (*see* below). Molecules smaller than the size of the kinks in the membrane may "dissolve" more readily in the membrane than large molecules. The solution of large molecules in the membrane requires energy to break the attractive forces (van der Waal's forces) between the hydrocarbon tails, and to distort the packing of neighboring chains. The disruption of the orderly crystalline array in the membrane in turn explains the increase in the entropy that accompanies the addition of $-CH_2$ groups to a molecule (*see* pp. 299–300). Thermodynamic evidence also strongly suggests that the energetics of partition of small molecules into toad bladder membranes resemble the partition of larger molecules; i.e., the changes in free energy, enthalpy and entropy accompanying the addition of a second $-OH$ group to ethanol to form ethylene glycol are virtually identical to those observed on the addition of a $-OH$ group to larger molecules (*see also* Bindslev & Wright, 1976). (However, with the addition of $-CH_2$ groups to small molecules the reduction in P is probably due to changes in diffusion within the membrane rather than changes in partition into the membrane; *see* below.) Overall, the partition of solutes into the plasma membrane closely resembles the partition of solutes into "frozen" liposomes (*see* Diamond & Katz, 1974; and Dix *et al.*, 1974), where the enthalpy of partition is high (up to 27 kcal/mole) and the entropy of solution in the membrane is positive (up to 90 cal/mole, °K).

The anesthetic action of the aliphatic alcohols and related compounds is closely associated with the positive entropy of solution of methylene

kinks, are formed in a straight hydrocarbon chain by two rotations ($\pm 120°$) about two C–C bonds separated by one chain link. Kinks are favored thermodynamically, and it is estimated that up to 50% of the available methylene groups are in this conformation and that about half of the small mobile pockets of free volume are occupied by water molecules. Kinks are more readily formed at the surface of the membrane where they are able to migrate into the membrane interior. Fairly large free volumes will be formed in the membrane by suitable three dimensional arrangements of the $2gl$ kinks. See Träuble (1971) for a more complete review of kinks and their possible role in membrane permeation

groups in membranes ($\delta \Delta S_l^{CH_2} > 0$). The potency of alcohols as local anesthetics increases with chain length, and when the concentration of the anesthetic reaches the critical level for conduction block there is membrane expansion, "melting" and disordering of lipids, a reduction in membrane viscosity, and an increase in anesthetic mobility within the membrane (Seeman, 1972). Furthermore, thermodynamic analysis of the action of local anesthetics on liposome membranes (Johnson & Bangham, 1969) shows that the free energy change associated with potassium permeation is dominated by the entropy term (+2 cal/mole/degree). In other words the normal permeation processes are perturbed when enough of the anesthetic is partitioned into the membrane to "melt" the lipid.

The effects of the local anesthetics are parallel to the action of antidiuretic hormone (ADH) in toad bladder (Pietras & Wright, 1974, 1975). ADH increases the permeability of the bladder to nonelectrolytes. This is associated with: (1) no change in the apparent activation energies of permeation (Hays & Leaf, 1962; Hays, Franki & Soberman, 1971), i.e., the free energy change associated with the increase in permeability is controlled by the increase in entropy of the system; (2) a reduction in the discrimination between branched and straight chain isomers (Pietras & Wright, 1974, 1975); (3) an increase in the mobility of the lectin binding sites on isolated epithelial cells (Pietras, Naujokaitis & Szego, 1975); and (4) a decrease in the fluorescence polarization of perylene in toad bladder membranes (Nikesch, W., Pietras, R. J. & Wright, E. M. *unpublished observations;* and Masters, Fanestil & Yguerabide, 1976).

Diffusion in Membranes

As first pointed out by Lieb and Stein (1969, 1971), diffusion across biological membranes resembles diffusion in nonporous hydrophobic polymers (*see* Meares, 1965). In both systems: (1) *Diffusion is a steep function of the size of the diffusing molecules.* In toad bladder $D_m \alpha MV^{-2.7}$ (*see* Bindslev & Wright, 1976) and so halving the size of a molecule increases P about 6.5-fold if the two solutes have identical partition coefficients. Thus, it is unnecessary to invoke the presence of pores in toad bladder to explain the high permeation of small molecules including water and methanol. (2) *Diffusion is a function of molecular shape.* Branched molecules have lower D's than their straight chain isomers (*cf.* Bindslev and Wright, 1976). (3) *Temperature coefficients are higher in polymers than in simple solvents.* For example in natural rubber the activation

energies of diffusion of butane and octadecane are about 12 kcal/mole (Meares, 1965, Chapter 12). In liposomes it has been estimated that the valinomycin translocation rate constant has an apparent activation energy of 14 kcal/mole (Benz *et al.*, 1973). Diffusion is controlled by the motions of the polymer molecules and, apart from very small molecules, the activation energy of diffusion is independent of the size of the diffusing molecule, i.e., the kinetic motions involved in diffusion are those which determine the viscosity of the polymer (*see* below). In bulk hydrocarbons it is estimated that the maximal activation energy for diffusion is not greater than 6–8 kcal/mole (Glasstone, Laidler & Erying, 1941), and this is comparable to that deduced from the motions of fluorescent probes in the center of cell membranes (Rudy & Gitler, 1972). Very small molecules on the other hand, i.e., small relative to the monomer units of the polymers, can jump quite readily, and diffusion is simply a function of the thermal energy of the diffusing molecule and the "free volume" of the polymer (*see* below). (4) *Absolute values of D are a function of membrane packing.* "Fillers" reduce diffusion coefficients in both membranes and polymers, e.g., cholesterol increases the microviscosity of membrane lipids in the liquid-crystalline state and reduces permeation. And (5) *Diffusion coefficients are increased by the presence of "plasticizers" and "local anaesthetics"* (*see* p. 308 and Seeman, 1972).

A most attractive molecular theory of diffusion across lipid membranes is that proposed by Träuble (1971). The starting point of this theory is that hydrocarbons contain mobile structural defects – the so-called $2\,gl$ kink – which arise as a result of thermal rotation of the hydrocarbon chains $\pm 120°$ about C–C bonds (Fig. 1). The free volume formed by these defects in the lipid is sufficient to accept small molecules, and so molecules in the aqueous phase may partition into the kinks at the interface and diffuse across the membrane interior within the mobile kink. The actual number of kinks within the membrane are a function of the degree of unsaturation and packing of the hydrocarbons, and this can account for variations in P with changes in composition and configuration of the membrane lipids.

Träuble has estimated the "diffusion coefficient" for the kinks to be about 1×10^{-5} cm^2/sec which is of the same order of magnitude as the diffusion of small molecules in water. Furthermore, using estimates of the kink concentration in a bilayer and the partition coefficient of water into hydrocarbons, he calculates a value for water permeability which is in the range measured experimentally. Additional attractive features of this theory of diffusion in lipid membranes are that the mass selectivity of

diffusion of solutes in the hydrocarbon could be accounted for by kinks with different free volumes and diffusion coefficients, and that fluxes of small nonelectrolytes are expected to show saturation behavior. This in fact could be the explanation for the saturation of acetamide fluxes, and the competition between urea and acetamide fluxes in toad bladder (Levine, Franki & Hays, 1973).

Here the partition and diffusion of solutes in membranes have been treated independently, but it is obvious that in practice it is difficult to separate the two processes. Changes in membrane structure and composition will effect both, e.g., incorporation of cholesterol into membranes will reduce the partition of solutes into the membrane, and will reduce the mobility of the solute within the membrane by increasing the packing of the bilayer. Much is already known about the partitioning of solutes into membranes (*see* Diamond & Katz, 1974; and Seeman, 1972), but little is known about the actual diffusion of solutes across membranes. NMR, fluorescence and spin label techniques have been used to obtain measures of translational and lateral diffusion coefficients, but it has not been possible to obtain direct estimates of the diffusion coefficients perpendicular to the bilayer surface. Nevertheless, it seems likely that in membranes such as those of toad urinary bladder that the permeation of simple nonelectrolytes can be explained in terms of the structure and composition of the membrane lipid. The discrepancy between the behavior of the toad bladder membranes and bulk lipid solvents appears to simply reflect the highly ordered configuration of the lipid molecules in the membranes, rather than the presence of special aqueous channels or carriers.

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