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Interpretation of Nonelectrolyte Partition Coefficients between Dimyristoyl Lecithin and Water

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Summary. Nonelectrolyte partition coefficients (K's) and free energies of solution (ΔF_l's) in dimyristoyl lecithin liposomes and in bulk nonpolar solvents were compared. Individual substituent groups tend to have consistent effects on K, permitting the extraction of incremental free energies ($\delta\Delta F$), enthalpies ($\delta\Delta H$), and entropies ($\delta\Delta S$) of partition and of solution. Values of the selectivity constant s and of $\delta\Delta F_l$ for the $-CH_2-$ and $-OH$ groups in lecithin suggest that partitioned solutes are mainly located in a region slightly less hydrophobic than octanol and similar to $C_5H_{11}OH$ in its solvent properties. Lecithin discriminates against branched solutes more than does a bulk solvent with the same s value. Below the endothermic phase-transition temperature (i.e., when the hydrocarbon tails "freeze"), ΔS and ΔH of partition increase 10-fold, K jumps down slightly, ΔS and ΔH of solution reverse in sign from negative to positive, and the Barclay-Butler constants become more positive. Partition in lecithin and in erythrocytes is similar, except for the absence of surface charge effects in lecithin. Resistance to nonelectrolyte permeation is inhomogeneously distributed through the bilayer, and the region of maximum partition does not provide the rate-limiting barrier. An appendix derives a simple general expression for the nonelectrolyte permeability of a membrane that may be asymmetrical, may have position-dependent partition coefficients and diffusion coefficients, and may have significant interfacial resistances.

This paper, the last in a series of four, analyzes nonelectrolyte average partition coefficients that were measured between water and the sucrose-excluding space of hydrated dimyristoyl lecithin liposomes and water and reported in the preceding paper (Katz & Diamond, 1974*c*, referred to as paper III). As discussed in the Appendix of the present paper, to attain a detailed understanding of permeation in biological membranes and thin lipid membranes will require separating the dependence of permeability coefficients on three groups of factors: those that determine, respectively, equilibrium solute concentrations in the membrane interior, solute mobilities in the membrane interior, and interfacial rate processes. Partition measurements provide information about the first of these three sets of factors.

We consider in turn the following questions: To what extent do the average solvent properties of dispersed lecithin (in the form of bilayers) resemble those of well-studied bulk solvents, such as ether, octanol and benzene? Do bilayers, as a result of their ordered structure, discriminate against the partition of branched solutes more than do bulk solvents? What are the contributions of individual substituent groups, such as $-CH_2-$ and $-OH$, to partition coefficients? What happens to solute partition at the phase transition temperature of lecithin? What can be learned by comparing partition coefficients for dimyristoyl lecithin with partition coefficients in biological membranes, and with permeability coefficients of biological membranes and artificial bilayers?

We emphasize again that what we have measured are *average* partition coefficients for the sucrose-excluding space of liposomes. Within this space and within the bilayer itself, solute concentrations probably vary locally (*see* pp. 142–144 for further discussion). Except for the values in Table 5, values of all parameters calculated for dimyristoyl lecithin at 25 °C in this paper refer to lecithin with the hydrocarbon tails "melted" – i.e., based on values extrapolated from above 25 °C and given in Tables 1–5 of paper III.

The Selectivity Constants

There tend to be systematic relationships among nonelectrolyte partition coefficients measured between different nonpolar solvents and water. Thus, Collander (1947, 1950, 1951) showed that if one studies a group of solutes of comparable acidity (e.g., monocarboxylic acids, or else diamines, or else neutral solutes) and compares their partition coefficients in the systems ether:water, isobutanol:water, oleic alcohol:water, etc., the following expression holds approximately:

$$\log K_{i,y} = s_{x,y} \log K_{i,x} + r_{x,y} \tag{1}$$

or

$$K_{i,y} = c_{x,y} (K_{i,x})^{s_{x,y}}. \tag{2}$$

In these equations $K_{i,x}$ or $K_{i,y}$ is the partition coefficient of solute i between solvent x and water or solvent y and water, respectively, while $s_{x,y}$ and $r_{x,y} (= \log c_{x,y})$ are constants for a particular choice of solvents x and y. The larger the value of s, the greater the selectivity of the system y: water; i.e., the greater are the differences between the partition coefficients of different solutes. Thus, in each solvent studied by Collander, K for propionic acid ($H_3C–CH_2–COOH$) exceeded that for malonic acid ($HOOC–CH_2–COOH$), but the ratio varied systematically with the polarity of the nonpolar

Table 1. Comparison of partition coefficients in different solvent systems

Solvent	Formula	s	r
Water	HOH	0.00	0.00
Isobutanol	C_4H_9OH	0.81	0.42
Isoamyl alcohol	$C_5H_{11}OH$	0.86	0.21
Dimyristoyl lecithin	$C_{36}H_{72}O_8NP$	0.87	−0.13
n-Octanol	$C_8H_{17}OH$	1.00	0.00
Ether	$C_4H_{10}O$	1.07	−0.61
Oleic alcohol	$C_{18}H_{35}OH$	1.46	−1.08
Olive oil		1.57	−1.97
Benzene	C_6H_6	2.17	−1.31

Values of nonpolar-solvent:water partition coefficients for nonelectrolytes were compared for different solvents according to Eq. (1), taking *n*-octanol as the solvent x and the solvent listed in the first column as the solvent y. Values of s and r were calculated from the data and analyses of Collander (1947, 1950, 1951), or, in the case of dimyristoyl lecithin, were calculated by linear regression of K_{lecithin} values (paper III) against K_{octanol} values (Collander, 1951; Leo, Hansch & Church, 1969). K values refer to a temperature of 25 °C for lecithin, 17 to 23 °C for the bulk solvents. Note that, among the bulk solvents, the selectivity constant s increases as the ratio of number of carbons to number of hydrogen-bonding atoms (O and hydroxylic H) increases.

solvent chosen: $K_{\text{propionic acid}}/K_{\text{malonic acid}}$ was 4.7 for the solvent isobutanol = C_4H_9OH (i.e., for the C_4H_9OH: water system), 7.7 for isoamyl alcohol = $C_5H_{11}OH$, 8.5 for *n*-octanol = $C_8H_{17}OH$, 16 for oleic alcohol = $C_{18}H_{35}OH$, 280 for olive oil, and 3,000 for benzene = C_6H_6. Table 1 lists values of s for nine solvents referred to octanol ($C_8H_{17}OH$) as the standard solvent x. It is clear that s, which may be termed the selectivity constant, increases as the solvent becomes less like water and more like a pure hydrocarbon.

What is the significance of the empirical Eq. (1), and of the observed relation between solvent structure and the value of s? Eq. (1) of paper III states that

$$\Delta F_{i,w\to x} = -RT \ln K_{i,x} \tag{3}$$

where $\Delta F_{i,w\to x}$ is the change in standard partial molar free energy, on transferring a given solute i from water to a given solvent x.[1] Furthermore, Eq. (11) of paper III permits the resolution of $\Delta F_{i,w\to x}$ into separate terms depending only on solute-water forces or on solute-solvent forces:

$$\Delta F_{i,x} = \Delta F_{i,w\to x} + \Delta F_{i,w} \tag{4}$$

1 Throughout the present paper we omit the superscript o, used in paper III to distinguish standard partial molar thermodynamic state functions (e.g., $\Delta F^0_{w\to l}$) from the corresponding partial molar functions (e.g., $\Delta F_{w\to l}$). All values of ΔF, ΔH and ΔS in this paper refer to changes between standard states.

where $\Delta F_{i,x} + \Delta F_{i,w}$ represent the free energies for transfer of solute i from the vapor phase to solvent x or water, respectively. Substituting Eq. (3) applied to solvent x and a corresponding equation applied to solvent y into Eq. (1) and adding $\Delta F_{i,w}/RT$ to both sides yields

$$\begin{aligned} \Delta F_{i,y} &= s\Delta F_{i,x} + (1-s)\,\Delta F_{i,w} - 2.303\,rRT \\ &= s\Delta F_{i,x} - 2.303\,r'RT \end{aligned} \tag{5}$$

where $r' \equiv r - (1-s)\,\Delta F_{i,w}/2.303\,RT$.

Eq. (5) states that sets of free energies of solution for different solutes in two different nonpolar solvents may tend to be linearly related.[2] This statement is obviously a gross oversimplification that sweeps all the complexities of nonelectrolyte solubility theory (Hildebrand & Scott, 1964) into the parameters s and r'. Eq. (5) nevertheless often holds to a sufficient degree to be useful (*cf.* Fig. 2), provided that the solutes are a group of simple organic nonelectrolytes of comparable acidity and that the solvents are simple organic alcohols, ethers, acids and hydrocarbons. The reason underlying the usefulness of Eq. (5) is that hydrogen bonds are considerably stronger than the interactions between $-CH_2-$ groups, so that the differences between the hydrogen-bonding abilities of different solvents or solutes largely overshadow effects of other differences of molecular structure in determining nonelectrolyte partition coefficients (Collander, 1949; Diamond & Wright, 1969*a*, *b*). Increasing hydrogen-bonding ability of a solute increases its energy of hydration $\Delta F_{i,w}$ more than $\Delta F_{i,x}$ and lowers K in a given solvent system, while increasing hydrogen-bonding ability of the solvent increases the free energy of solution $\Delta F_{i,x}$ and increases K for a given solute. Thus, the selectivity constant s, which measures the spread of selectivity, increases as the ratio of hydrogen-bonding groups (e.g., $-OH$) to $-CH_2-$ groups decreases in the solvent phase. In addition, the higher this ratio, the greater is the solubility of water itself in the solvent phase, and

2 Even if Eq. (1) holds exactly with s and r constant for a given solvent pair, Eq. (5) will not hold exactly with constant r', because $\Delta F_{i,w}$ and hence r' differ for each solute. If there is no correlation between values of $\Delta F_{i,w}$ and $\Delta F_{i,x}$, this effect will simply increase the scatter in fit of experimental data to Eq. (5) assuming constant coefficients. If there is some correlation between $\Delta F_{i,w}$ and $\Delta F_{i,x}$, then the values of s calculated from Eq. (1) and Eq. (5) will differ. For solvent pairs and solutes such that variation in $s\Delta F_{i,x}$ is much greater than variation in $(1-s)\,\Delta F_{i,w}$ (e.g., because s is near 1), these effects of variable $\Delta F_{i,w}$ will be negligible. This condition is fulfilled for our lecithin-octanol and lecithin-isobutanol comparisons, but not for our lecithin-olive oil and lecithin-ether comparisons, and contributes in the latter two cases to the lower correlation coefficients of Table 3 and differing s values of Tables 2 and 3.

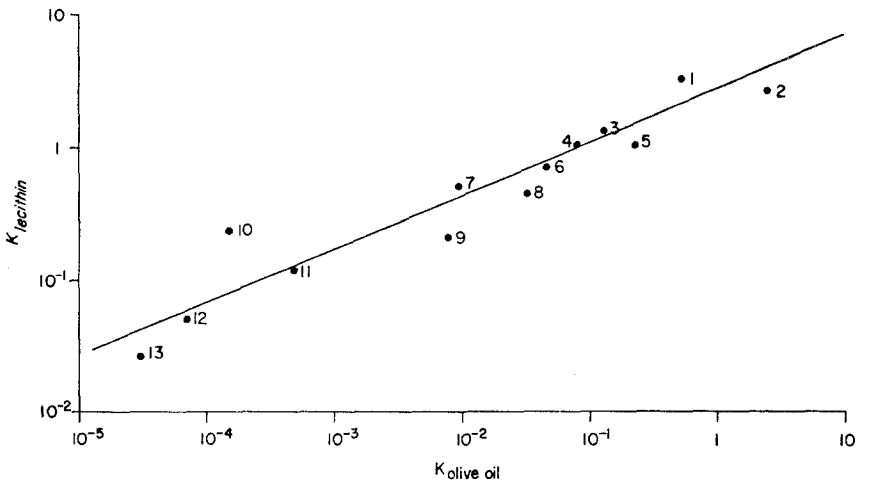


Fig. 1. Lecithin:water partition coefficients for 13 nonelectrolytes (from Table 2 of paper III, at 25 °C), plotted against oliveoil:water partition coefficients of the same solutes (from Collander, 1954) on a double logarithmic scale. Note the approximately linear relation. Solutes are identified by numbers: *1* *n*-butanol, *2* ethyl acetate, *3* *n*-propanol, *4* acetone, *5* *t*-butanol, *6* isopropanol, *7* butyramide, *8* ethanol, *9* methanol, *10* urea, *11* ethylene glycol, *12* glycerol, *13* erythritol

this also reduces the spread of selectivity. These considerations suggest two methods for characterizing the solvent properties of lecithin.

First, K's for lecithin can be correlated with K's for other solvents to determine which solvent yields the value of s nearest 1.0; i.e., is most similar to lecithin. Fig. 1 is a graph of K_{lecithin} plotted against $K_{\text{olive oil}}$, for the 13 solutes for which K values were available in both systems. There is a good correlation ($r = 0.93$), but the slope s is only 0.38, implying that the partitioning of solutes into lecithin is largely into a region less hydrophobic than olive oil. Table 1 lists s values for lecithin and other solvents, referred to octanol as the solvent y. The s value for lecithin is nearest that for isoamyl alcohol ($C_5H_{11}OH$) and below that for more hydrophobic solvents. Table 2 gives the results of correlating K_{lecithin} with K's in olive oil, ether, octanol and isobutanol, these being the solvents in which K's were available for a sufficient number of the solutes studied in lecithin. The conclusion is again that lecithin behaves more similarly to the lower alcohols than to the more hydrophobic solvents, ether and olive oil.

Second, values of $\Delta F_{i,x}$ for lecithin can be correlated with values for other solvents. Fig. 2 depicts $\Delta F_{i,\text{ lecithin}}$ plotted against $\Delta F_{i,\text{ octanol}}$. There is an approximately linear relationship (correlation coefficient of 0.94) with a slope s of 0.73, suggesting that lecithin behaves as somewhat less hydro-

Table 2. Correlation of partition coefficients for lecithin and bulk nonpolar solvents

Solvent	s	r	n	Corr. coef.
Olive oil	0.38	0.37	13	0.95
Ether	0.36	−0.01	13	0.93
n-Octanol	0.87	−0.13	9	0.95
Isobutanol	0.80	−0.35	7	0.95

Partition coefficients for nonelectrolytes in dimyristoyl lecithin at 25 °C (taken from paper III) were compared by logarithmic linear regression [Eq. (1)] to partition coefficients for the four bulk solvents listed in column 1, at 17 to 23 °C (taken from Collander, 1949, 1950, 1951 and 1954). In Eq. (1) the listed bulk solvent was taken as solvent x, while lecithin was taken as solvent y. Columns 2 and 3 give the constants s and r in Eq. (1), column 4 the number of solutes n available for comparison, and column 5 the correlation coefficient.

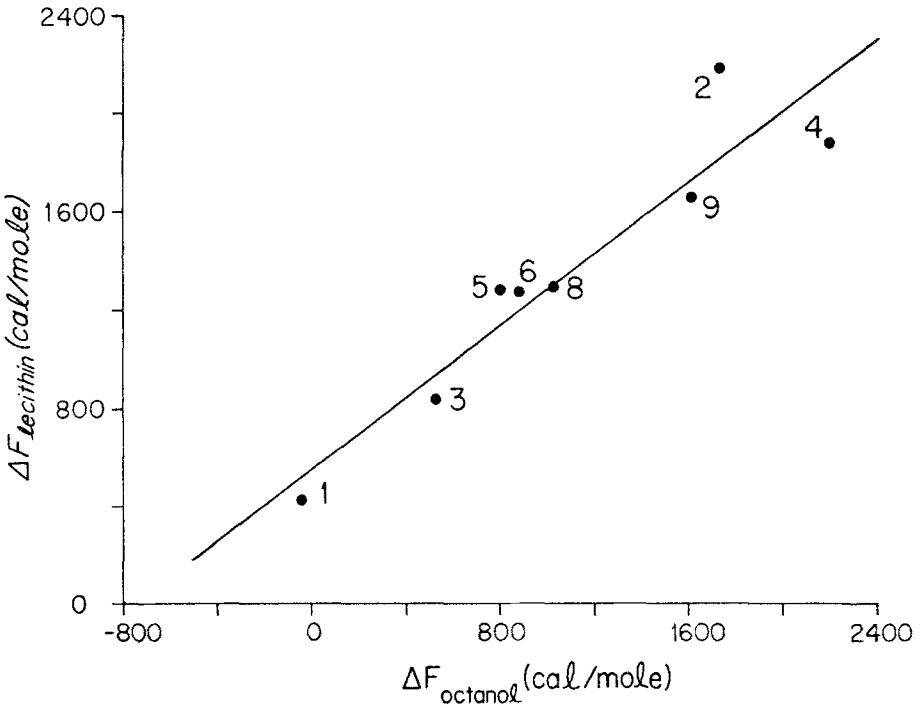


Fig. 2. Free energies of solution in lecithin for eight nonelectrolytes (from Table 5 of paper III), plotted against free energies of solution in octanol for the same solutes (calculated as described in Table 3 of this paper). Numbers to identify solutes have the same meaning as in Fig. 1

phobic than octanol. Table 3 summarizes correlations between values of $\Delta F_{i,x}$ for lecithin and for olive oil, ether, octanol and isobutanol. Lecithin is less hydrophobic than olive oil, ether or octanol ($s < 1$) but is similar to or slightly more hydrophobic than isobutanol ($s = 1.06$).

Table 3. Correlation of $\Delta F_{i,x}$ values for lecithin and bulk nonpolar solvents

Solvent	s	r'	n	Corr. coef.
Olive oil	0.62	−0.54	10	0.34
Ether	0.78	−0.05	10	0.44
n-Octanol	0.73	−0.41	8	0.94
Isobutanol	1.06	−0.38	4	1.00

Free energies of solution ($\Delta F_{i,x}$) for nonelectrolytes in dimyristoyl lecithin at 25 °C (taken from paper III) were compared by linear regression [Eq. (5)] to $\Delta F_{i,x}$ values for the same solutes in the four bulk solvents listed in column 1, at 17 to 23 °C (extracted from partition coefficient values in Collander, 1949, 1950, 1951 and 1954). $\Delta F_{i,x}$ values were calculated by substituting Collander's measured values of partition coefficients into Eq. (3) to obtain $\Delta F_{i,w\to x}$, and substituting these $\Delta F_{i,w\to x}$ values plus $\Delta F_{i,w}$ values from Butler (1937) (recalculated from a mole fraction basis to a molal basis) into Eq. (4) to obtain $\Delta F_{i,x}$. In Eq. (5) the listed solvent was taken as solvent x, while lecithin was taken as solvent y. Columns 2 and 3 give the constants s and r' of Eq. (5), column 4 the number of solutes n available for comparison, and column 5 the correlation coefficient. Standard states are as defined in Tables 3 and 4 and the Appendix of paper III.

Both calculations thus show that the average solvent properties of lecithin are near those for lower alcohols. (The two calculations do not yield the same values of s, partly for the reasons discussed in footnote 2, and partly because, although they start from the same data (measurements of K), the second method can be applied only to those solutes for which ΔF's of hydration are known). We repeat that this conclusion applies to the *average* solvent properties of lecithin, and that the bilayer interior is presumably more hydrophobic than the periphery.

Effect of Chain Branching

Although bulk nonpolar solvents discriminate against branched solutes compared to straight-chain homologues in their partition coefficients, many biological membranes discriminate against branched solutes even more in their permeability properties (Collander, 1954, 1957, 1959; Oura, Suomalainen & Collander, 1959; Diamond & Wright, 1969*b*; Wright & Diamond, 1969; Hingson & Diamond, 1972). This extra discrimination is probably somehow related to the approximately parallel arrangement of hydrocarbon tails in a bilayer interior. Compared to a bulk hydrocarbon, this ordered array might preferentially lower either the diffusion coefficients (Lieb & Stein, 1971) or the partition coefficients (Diamond & Wright, 1969*b*; Hingson & Diamond, 1972) of branched solutes, or both.

The present results provide two opportunities for comparing branched and straight-chain homologues: the highly branched *t*-butanol, $H_3C-\underset{\displaystyle CH_3}{\overset{\displaystyle CH_3}{\overset{|}{\underset{|}{C}}}}-OH$, *vs.* the straight-chain *n*-butanol, $H_3C-CH_2-CH_2-CH_2-OH$; and the somewhat branched isopropanol, $H_3C-\underset{\displaystyle OH}{\overset{}{\overset{|}{CH}}}-CH_3$, *vs.* the straight-chain *n*-propanol, $H_3C-CH_2-CH_2-OH$. Proper choice of a basis for comparison is somewhat tricky. In each pair the branched solute does have a lower value of K_{lecithin}, a more positive $\Delta F_{i,\,\text{water}\to\text{lecithin}}$ and a more positive $\Delta F_{i,\,\text{lecithin}}$ than does the straight-chain solute[3]. However, the same is true in the bulk solvents octanol, ether and olive oil, due to low values of $\Delta F_{i,x}$ for the branched solute[3]. Furthermore, even if no special factor were operating for branched solutes, partition coefficient ratios for any given pair of solutes differ when one compares lecithin with a given bulk solvent, as expressed in different values of the selectivity constant *s*. The question is thus, whether lecithin discriminates against branched solutes *more* than do bulk solvents, and whether this discrimination is *more* than the degree expected just from the different *s* values derived from all solutes together.

Fig. 1 shows that K_{lecithin} values for the two straight-chain solutes lie slightly above, and values for the two branched solutes lie slightly below, the least-mean-square line fitted through all solutes. Fig. 2 shows that $\Delta F_{\text{lecithin}}$ values deviate in the positive direction for the branched solutes, and in the negative direction for the straight-chain solutes, referred to the least-mean-square line for all solutes. This conclusion holds whether the reference solvent is taken as octanol, ether or olive oil (no *K* or $\Delta F_{i,x}$ values are available for comparison in isobutanol). The magnitude of these deviations gives the *extra* discrimination in lecithin compared to a bulk solvent with the same value of the selectivity constant *s*. Averaging the results of comparisons based on each of the three available reference solvents, one finds that the ratio $K_{\text{straight}}/K_{\text{branched}}$ is increased in lecithin by a factor of 1.54 for the *n*- and *t*-butanol pair, and by 1.19 for the *n*- and isopropanol pair. The difference $[\Delta F_{i,x\,(\text{straight})} - \Delta F_{i,x\,(\text{branched})}]$ is increased in lecithin by -262 calories/mole (implying an additional factor of 1.56 in the *K* ratio) for the butanols, and by -26 calories/mole ($\sim$factor of 1.04

3 A more positive $\Delta F_{i,w\to x}$ corresponds to a lower *K*, and to either a more positive $\Delta F_{i,x}$ (weaker solute-membrane forces), more negative $\Delta F_{i,w}$ (stronger solute-water forces), or both.

in the K ratio) for the propanol pair. (The two comparisons need not yield identical results, because fewer solutes are available for constructing $\Delta F_{i,x}$ curves similar to Fig. 2 than K curves similar to Fig. 1).

Thus, lecithin appears to exhibit an extra discrimination against branched solutes qualitatively different from that seen in bulk solvents (i.e., beyond that predicted from the s value); and this discrimination is more marked against the doubly branched $H_3C-\overset{\overset{CH_3}{|}}{C}-OH$ than against the singly branched $H_3C-\overset{\overset{OH}{|}}{CH}-CH_3$. Further evidence comes from the finding of Lange, Gary-Bobo and Solomon (1974), that the ratio of K for valeramide to K for its branched analogue isovaleramide in hydrated egg yolk lecithin lamellae is close to or slightly higher than the ratio in several mixtures of pure hydrocarbons. If the s value for egg yolk lecithin is similar to that for dimyristoyl lecithin (i.e., less than one-half of that for a pure hydrocarbon), then the results of Lange *et al.* mean that egg yolk lecithin also exhibits an extra discrimination against branched solutes. This discrimination against the partition of branched solutes in an ordered bilayer is in the correct direction to explain the lowered permeabilities of branched solutes in biological membranes. However, the possibility of an additional contribution to lowered permeabilities from lowered diffusion coefficients is not thereby precluded, and is in fact indicated by the results of Lange *et al.* (1974).

Contributions of Individual Substituent Groups

As summarized in Overton's empirical rules (Overton, 1896, 1899, 1902), particular substituent groups tend to have qualitatively consistent effects on permeability coefficients in different biological membranes and on partition coefficients in different nonpolar-solvent:water systems. $-CH_2-$, $-Cl$ and $-NO_2$ groups usually increase permeability or partition coefficients, while other groups reduce them in the approximate sequence (of increasing effect in reducing permeability or K's) $-C\equiv N$, $-O-$, $-\overset{\overset{O}{\|}}{C}-OR$ $<-C=O<-OH$, $-COOH<-NH_2<-\overset{\overset{O}{\|}}{C}-NH_2$, $2-OH$. Examination of the lecithin:water K's in Table 2 of paper III shows that $-CH_2-$ groups increase K, while, if one compares solutes with the same number of carbon

atoms, $-C=O < -OH < -\overset{O}{\overset{\|}{C}}-OR < -\overset{O}{\overset{\|}{C}}-NH_2$, $2-OH$ decrease K's in that sequence of increasing effect. Thus, Overton's rules apply to lecithin. The molecular interpretation of these rules in terms of strength and number of solute-solvent hydrogen bonds and entropy effects in the aqueous phase has been discussed in detail by Collander (1949) and Diamond and Wright (1969*a*, *b*).

The regularities expressed in Overton's rules are quantitative as well as qualitative. Thus, at 25 °C values of K_{lecithin} for the primary alcohols are (from Table 2 of paper III): CH_3-OH, 0.206; CH_3-CH_2-OH, 0.441; $CH_3-CH_2-CH_2-OH$, 1.31; $CH_3-CH_2-CH_2-CH_2-OH$, 3.16. The addition of each successive $-CH_2-$ group increases K by about 2.5 times ($0.441/0.206 = 2.1$, $1.31/0.441 = 3.0$, $3.16/1.31 = 2.4$). The value of this multiplicative factor is characteristic of a given group in a given membrane or solvent. For instance, introduction of one $-OH-$ group into a solute reduces the solute's $K_{\text{isobutanol}}$ by about 5 times, its K_{octanol} by about 9 times, its $K_{\text{olive oil}}$ by about 107 times, its permeability coefficient in the alga *Nitella mucronata* by about 450 times, and its K_{benzene} by about 1,300 times.

From such ratios Diamond and Wright (1969*b*) calculated incremental free energies of solution ($\delta\Delta F$) of various substituent groups in bulk solvents and in cell membranes. The partition measurements of paper III make it possible to estimate some $\delta\Delta F$'s for lecithin as well as to extend the treatment to incremental enthalpies ($\delta\Delta H$) and entropies ($\delta\Delta S$).

1. $-CH_2-$. The relatively constant successive *ratios* in K's as one ascends the primary alcohol series, cited in the previous paragraph, imply relatively constant *differences* in values of $\Delta F_{w\to l}$[4]. Thus at 25 °C, $\Delta F_{w\to l}$

4 The basis for this relation is as follows: Empirically, introduction of a given substituent group G into molecule i increases the partition coefficient of that molecule K_i by a factor j_G, where j_G is to a coarse first approximation independent of K_i or the nature of i (*see* Diamond & Wright, 1969*b*, pp. 639–640, for qualifications to this statement). That is, $K_{i+G} \sim j_G K_i$, where K_{i+G} is the partition coefficient of the G-substituted derivative. Since $\Delta F_{w\to l} = -RT \ln K$ [Eq. (3)], one can write $\Delta F^i_{w\to l} = -RT \ln K_i$, $\Delta F^{i+G}_{w\to l} = -RT \ln K_{i+G} = -RT \ln j_G K_i = -RT \ln j_G - RT \ln K_i = \Delta F^i_{w\to l} - RT \ln j_G$,

$$\Delta F^{i+G}_{w\to l} - \Delta F^i_{w\to l} = -RT \ln j_G. \tag{6}$$

This constant difference $-RT \ln j_G$ may be conveniently referred to as $\delta\Delta F^G_{w\to l}$, the amount by which the group G increases molar free energies for transfer from water to lipid. Similarly, one expects group increments $\delta\Delta F_w$ and $\delta\Delta F_l$ for free energies of solution in water or lipid, such that $\delta\Delta F_{w\to l} = \delta\Delta F_l - \delta\Delta F_w$ [in analogy to Eq. (4)]. Corresponding group increments also apply to enthalpy and entropy terms, as well as to free energy terms.

is 940 cal/mole for CH_3-OH, 490 for CH_3-CH_2-OH, -160 for $CH_3-CH_2-CH_2-OH$, and -680 for $CH_3-CH_2-CH_2-CH_2-OH$. The successive increments are $490-940=-450$, $-160-490=-650$, and $-680-(-160)=-520$ cal/mole. These increments may be termed $\delta\Delta F_{w\to l}^{CH_2}$, the incremental change in standard partial molar free energy when a $-CH_2-$ group is transferred from water to lecithin. These values of $\Delta F_{w\to l}^{CH_2}$ are entered into column 3 of Table 4, along with the corresponding values of $\delta\Delta H_{w\to l}^{CH_2}$ and $\delta\Delta S_{w\to l}^{CH_2}$ in columns 4 and 5 in the first two cases (we did not measure $\Delta H_{w\to l}$ or $\Delta S_{w\to l}$ for *n*-butanol).

Butler (1937) measured ΔF, ΔH and ΔS of hydration for the same alcohols, yielding by differences the estimates of $\delta\Delta F_w^{CH_2}$, $\delta\Delta H_w^{CH_2}$ and $\delta\Delta S_w^{CH_2}$ entered into columns 6–8 of Table 4. For example, values of ΔH_w for CH_3-OH, CH_3-CH_2-OH, $CH_3-CH_2-CH_2-OH$, and $CH_3-CH_2-CH_2-CH_2-OH$ are $-11{,}240$, $-12{,}880$, $-14{,}420$ and $-15{,}940$ cal/mole, respectively, yielding the estimates of $\delta\Delta H_w^{CH_2}$ of $-12{,}880-(-11{,}240)=-1{,}640$, $-14{,}420-(-12{,}880)=-1{,}540$, and $-15{,}940-(-14{,}420)=-1{,}520$ cal/mole in column 7.

Finally, using Eq. (4), one may add these values of $\delta\Delta F_{w\to l}^{CH_2}$ and $\delta\Delta F_w^{CH_2}$ to obtain the values of $\delta\Delta F_l^{CH_2}$ in column 9, the incremental free energy of solution of $-CH_2-$ in lecithin. Columns 10 and 11 give corresponding values of $\delta\Delta H_l^{CH_2}$ and $\delta\Delta S_l^{CH_2}$.

These numbers in Table 4 yield the following conclusions:

Differences between pairs of the four primary alcohols studied yield two or three independent estimates of each quantity $\delta\Delta Y_{w\to l}^{CH_2}$, $\delta\Delta Y_w^{CH_2}$ and $\delta\Delta Y_l^{CH_2}$ (where Y means any of the three state functions F, H and S). Different estimates of the same quantity agree fairly well, considering that they were obtained as differences between large numbers.

A $-CH_2-$ group promotes solution of a solute in lecithin from the vapor phase (i.e., $\delta\Delta F_l^{CH_2}<0$), because of an enthalpy effect ($\delta\Delta H_l^{CH_2}<0$) and despite an opposing decrease in entropy ($\delta\Delta S_l^{CH_2}<0$). The enthalpy term arises simply from van der Waals' forces between $-CH_2-$ and lecithin. The decrease in entropy arises from the loss in freedom of motion caused by these intermolecular forces, as implicit in the Barclay-Butler equation relating entropies of solution to enthalpies of solution (paper III, Eqs. (13) and (14)].

A $-CH_2-$ group actually retards solution of a solute in water from the vapor phase ($\delta\Delta F_w^{CH_2}>0$), due to an entropy effect ($\delta\Delta S_w^{CH_2}<0$) and despite a favorable enthalpy change ($\delta\Delta H_w^{CH_2}<0$). That is, the decrease in entropy is larger in relation to the enthalpy change for solution in water than in lecithin and more than counterbalances the effect of van der Waals' forces.

Table 4. Incremental thermodynamic

Group	Comparison	$\delta\Delta F_{w\to l}$
$-CH_2-$	CH_3CH_2OH *vs.* CH_3OH	−450
	$CH_3CH_2CH_2OH$ *vs.* CH_3CH_2OH	−650
	$CH_3CH_2CH_2CH_2OH$ *vs.* $CH_3CH_2CH_2OH$	−520
$-OH$	$HOCH_2CH_2OH$ *vs.* CH_3CH_2OH	790
	1/2[$HOCH_2CH(OH)CH_2OH$ *vs.* $CH_3CH_2CH_2OH$]	970
	1/3[$HOCH_2CH(OH)CH(OH)CH_2OH$ *vs.* $CH_3CH_2CH_2CH_2OH$]	950
$=O$ *vs.* $-OH$	$H_3C-\overset{\overset{O}{\|}}{C}-CH_3$ *vs.* $H_3C-\overset{\overset{OH}{\|}}{CH}-CH_3$	−240
$-O-\overset{\overset{O}{\|}}{C}=O$ *vs.* $-OH$	$H_3C-\overset{\overset{O}{\|}}{C}-O-CH_2-CH_3$ *vs.* $H_3C-CH_2-CH_2-CH_2-OH$	130
$-\overset{\overset{OH}{\|}}{\underset{\|}{C}}-$	$H_3C-\overset{\overset{OH}{\|}}{CH}-CH_3$ *vs.* $H_3C-CH_2-CH_2-OH$	370
$-\overset{\overset{CH_3}{\|}}{\underset{\|}{C}}-$	$H_3C-\overset{\overset{CH_3}{\|}}{\underset{\|}{C}}-OH$ *vs.* $H_3C-CH_2-CH_2-CH_2-OH$	650

To estimate the incremental thermodynamic functions listed in columns 3–11 for the group named in the first column, thermodynamic functions for the second-named solute in the second column were subtracted from the corresponding functions for the first-named solute. Values of $\Delta F_{w\to l}$, $\Delta H_{w\to l}$ and $\Delta S_{w\to l}$ were taken from Table 3 of paper III. Values of ΔF_w, ΔH_w and ΔS_w were taken from Tables 1 and 2 of Butler (1937). All values are at 25 °C. In the first six rows the pair of solutes listed differ in

These large entropy effects by which hydrocarbons in aqueous solution locally order the surrounding water have been frequently discussed by physical chemists, using pictorial terms such as "icebergs" (Frank & Evans, 1945; Franks, 1965).

The average value of $\delta\Delta F_l^{CH_2}$ for lecithin (−410 cal/mole) is similar to that for isobutanol (−370), and slightly below that for ether (−510), olive oil (−500), octanol (−502), cyclohexane (−615), carbon tetrachloride (−630) or benzene (−650). [The first three of these $\delta\Delta F_l^{CH_2}$ values for bulk solvents were calculated by Diamond and Wright (1969*b*) from data of Collander (1949, 1950, 1954), while the last four values have been calculated by us from data of Collander (1951) and of Butler and Harrower (1937)].

functions for lecithin

$\delta\Delta H_{w\to l}$	$\delta\Delta S_{w\to l}$	$\delta\Delta F_w$	$\delta\Delta H_w$	$\delta\Delta S_w$	$\delta\Delta F_l$	$\delta\Delta H_l$	$\delta\Delta S_l$
−1,630	−3.95	100	−1,640	−5.8	−350	−3,270	−9.8
−1,260	−2.06	190	−1,540	−5.7	−460	−2,800	−7.7
—	—	110	−1,520	−5.5	−410	—	—
1,290	1.67	−2,640	—	—	−1,850	—	—
2,950	6.66	−2,200	−5,160	−10.2	−1,230	−2,210	−3.5
—	—	—	—	—	—	—	—
—	—	840	3,360	8.4	600	—	—
—	—	1,620	4,230	8.9	1,750	—	—
1,660	4.33	70	970	3.0	440	2,630	7.3
—	—	200	1,500	4.4	850	—	—

the presence and absence of a single group, and columns 3–11 give the incremental quantities for that group: $\delta\Delta F_{w\to l}^{CH_2}$, $\delta\Delta H_w^{OH}$, etc. In rows 7 and 8 the solutes differ in the substitution of one group by another, so that columns 3–11 give the difference in group incremental quantities: $\delta\Delta S_l^{=O} - \delta\Delta S_l^{OH}$, etc. In rows 9 and 10, columns 3–11 give incremental quantities for a secondary and tertiary branch point, respectively. Units are cal/mole for $\delta\Delta F$'s and $\delta\Delta H$'s, cal/mole, °K for $\delta\Delta S$'s.

The similarity of the $\delta\Delta F_l^{CH_2}$ value for lecithin to the values for bulk solvents may be a coincidence: $\delta\Delta H_l^{CH_2}$ and $\delta\Delta S_l^{CH_2}$ values for lecithin greatly exceed those for bulk solvents but affect $\delta\Delta F_l^{CH_2}$ in opposite directions. As regards $\delta\Delta H_l^{CH_2}$, we calculate a value of −1000 cal/mole for benzene from data of Butler and Harrower (1937), while Krishnan and Friedman (1971) obtained values all falling between −860 and −1,200 cal/mole for 11 bulk solvents such as small alcohols, amides, methyl cyanide and nitromethane. The value of $\delta\Delta H_l^{CH_2}$ for lecithin, based on three of the same solutes tested on the solvents studied by Butler and Harrower and by Krishnan and Friedman, is −2,800 or −3,270 cal/mole, three times larger than the bulk-solvent values. This may be because solvent molecules are

less mobile and more closely-packed in a lecithin bilayer than in bulk solvents, so that solute-solvent forces are stronger. $\delta\Delta S_l^{CH_2}$ in lecithin, -7.7 or -9.8 cal/mole, °K, is far greater (more negative) than the value for benzene, -1.2 cal/mole, °K [calculated from data of Butler and Harrower (1937)]. The explanation is probably the same as the one suggested for the steep Barclay-Butler slope of lecithin (paper III, p. 116), namely, immobilization of solute molecules on dissolving in a lecithin bilayer. Just as one can analyze the Barclay-Butler slope $b = d\Delta S_l/d\Delta H_l$ for the thermodynamic state functions of whole molecules, so one can also consider an "incremental slope", $\delta b = d\delta\Delta S_l/d\delta\Delta H_l$, based on incremental state functions for individual substituent groups. The incremental slope δb for the $-CH_2-$ group is much steeper in lecithin (0.0029 $°K^{-1}$) than in benzene (0.0012 $°K^{-1}$), just as is true of the whole-molecule slope b, and presumably for the same reason. The δb^{CH_2} values for lecithin and benzene are close to the b values for these solvents (Table 6 of paper III).

2. $-OH-$. In three cases one can compare thermodynamic constants for pairs of solutes differing in the presence or absence of one or more hydroxyl groups. These cases are of interest in illustrating the multiplicative effect on K of adding successive groups to a molecule, a consequence of the relation $\ln K = -\Delta F_{w\to l}/RT$ and of linear addition of $\delta\Delta F_{w\to l}$ increments to $\Delta F_{w\to l}$. That is, similar estimates for the effect of $-OH-$ are obtained by comparing K's of CH_3-CH_2-OH ($K=0.441$: Table 2 of paper III) and $HO-CH_2-CH_2-OH$ ($K=0.116$), which differ in one $-OH$ ($0.441/0.116=3.8$); $HO-CH_2-CH_2-CH_3$ ($K=1.31$) and $HO-CH_2-CH(OH)-CH_2-OH$ ($K=0.0501$), which differ in two $-OH$ ($\sqrt{1.31/0.0501}=5.1$): and $HO-CH_2-CH_2-CH_2-CH_3$ ($K=3.16$) and $HO-CH_2-CH(OH)-CH(OH)-CH_2-OH$ ($K=0.0259$), which differ in three $-OH$ ($\sqrt[3]{3.16/0.0259}=5.0$). Unfortunately, these comparisons involve the three solutes with the lowest values of K_{lecithin} measured (hence subject to the largest experimental uncertainties). Furthermore, the added $-OH$ is in each case immediately adjacent to another $-OH$, permitting formation of an intramolecular hydrogen bond and reducing the opportunity for solute-solvent hydrogen bonding. As a result of this intramolecular H-bonding, the estimates of $\delta\Delta F_w^{OH}$ for these comparisons ($-2{,}640$ and $-2{,}200$ cal/mole, Table 4, column 6) are far below the value of $-7{,}000$ obtained for solutes in which there is no opportunity for intramolecular H-bonding (Diamond & Wright, 1969*b*, Table 1).

We may still conclude qualitatively from Table 4 that $-OH$ reduces lecithin:water partition coefficients ($\delta\Delta F_{w\to l}^{OH} > 0$), primarily because more

energy is required to break hydrogen bonds between $-OH$ and water than is gained back from the weaker forces between $-OH$ and lecithin ($\delta\Delta H^{OH}_{w\to l} > 0$, $|\delta\Delta H^{OH}_{w}| > |\delta\Delta H^{OH}_{l}|$). For these same three pairs of solutes from which we estimate $\delta\Delta F^{OH}_{w\to l}$ as 790 to 970 cal/mole for lecithin, we calculate $\delta\Delta F^{OH}_{w\to l}$ as 2,200 to 2,350 cal/mole for ether, and 1,930 to 3,830 cal/mole for olive oil, based on Collander's (1949, 1954) measurements of K's for these solvents. The lower values of $\delta\Delta F^{OH}_{w\to l}$ for lecithin are due to larger negative values of $\delta\Delta F^{OH}_{l}$; i.e., a less hydrophobic environment in lecithin than in ether or olive oil, offering more opportunities for H-bonding. The corresponding value of $\delta\Delta F^{OH}_{w\to l}$ for these solutes in isobutanol is approximately 1,000 to 1,080 cal/mole, close to the lecithin value.

From comparison of $HO-CH_2-CH_2-OH$ with $HO-CH_2-CH(OH)-CH_2-OH$, and of the latter with $HO-CH_2-CH(OH)-CH(OH)-CH_2-OH$, one may estimate $\delta\Delta F_{w\to l}$ as 440 cal/mole for the $-CHOH-$ group in lecithin. This estimate agrees well with the sum of the average values of $\delta\Delta F_{w\to l}$ for $-CH_2-$ and $-OH$ ($-540+900=360$, from Table 4).

3. $-\overset{\overset{O}{\|}}{C}-$. There is no instance in which we determined K_{lecithin} for pairs of solutes differing by the presence or absence of a ketone group. However, comparison of acetone and isopropyl alcohol permits one to compare the effect of $=O$ with the effect of $-OH$ (Table 4). $\delta\Delta F_l^{=O}$ is more positive than $\delta\Delta F_l^{-OH}$ by 600 cal/mole, close to the value of $\delta\Delta F_l^{=O} - \delta\Delta F_l^{-OH}$ based on the same two solutes in olive oil (530 cal/mole) and ether (860 cal/mole). However, $\delta\Delta F_w^{=O} - \delta\Delta F_w^{OH}$ is larger, 840 cal/mole. That is, $-OH$ is attracted both to water and to lecithin more than is $-C=O$, but the water-*vs.*-lecithin difference is larger in the case of $-OH$, since it can form more hydrogen bonds than can $=O$ and since water is a better hydrogen bonding solvent than is lecithin. Thus, the ketone has a higher partition coefficient than the alcohol.

4. $-\overset{\overset{O}{\|}}{C}-O-R$. As in the preceding case of $=O$, the ester group can only be compared to $-OH$ (Table 4, ethyl acetate *vs.* *n*-butanol) since we studied no pair of solutes differing by the presence or absence of an ester link. $\delta\Delta F_l^{-O-C=O} - \delta\Delta F_l^{OH}$ is 1,750 cal/mole for lecithin, similar to the value for the same two solutes in isobutanol (1,730 cal/mole) but higher than the value in ether (1,560 cal/mole) or olive oil (700 cal/mole). Since $\delta\Delta F_w^{-O-C=O} - \delta\Delta F_w^{OH}$ is 1,620 cal/mole (because $-OH$ forms stronger hydrogen bonds),

the ester has a slightly lower partition coefficient than the alcohol ($\delta\Delta F_{w\to l}^{-O-C=O} - \delta\Delta F_{w\to l}^{OH} > 0$) in lecithin and isobutanol, while the reverse is true in olive oil and ether.

5. *Branching.* Introduction of a branch point (last two rows of Table 4) reduces solubility both in water and in lecithin ($\delta\Delta F_w > 0$, $\delta\Delta F_l > 0$) by an enthalpic effect ($\delta\Delta H_w > 0$, $\delta\Delta H_l > 0$). These effects are twice as large for a tertiary branch (-|-) as for a secondary branch (-'-). Since the effects are larger in the lecithin phase ($\delta\Delta F_l > \delta\Delta F_w$), the branched solute has the lower partition coefficient. The differences arise ultimately from the fact that a branched molecule has some of its atoms buried in the center, has less surface area than a straight-chain homologue, and therefore has weaker van der Waals' attraction to the solvent, since van der Waals' forces are very short-range ($\propto r^{-6}$).

Conclusion. The effects of substituent groups on K_{lecithin} are qualitatively similar to those summarized by Overton's rules for permeation and partition in other nonpolar-solvent:water systems. The effects are mostly enthalpic effects explicable in terms of hydrogen bonds, with the notable exception of the disproportionately large increase in entropy when a $-CH_2-$ group leaves water. Quantitative consideration of the effects of $-CH_2-$, $-OH$, $-\overset{\overset{O}{\|}}{C}-O-R$ and indicates that lecithin as a solvent is more similar to isobutanol than to more hydrophobic solvents like ether and olive oil.

Changes in Lecithin at the Transition Temperature

When dimyristoyl lecithin is heated, an endothermic phase transition occurs near 25 °C, at which temperature the hydrocarbon tails pass from a crystalline state to a liquid crystalline state (Chapman, Williams & Ladbrooke, 1967). We measured K's of three solutes below as well as above this temperature. As depicted in Figs. 2–4 of paper III and summarized in Table 5 of the present paper, these measurements revealed a gross discontinuity in the temperature coefficient of K, as well as a slight change in the value of K.

K jumps down on cooling through the transition temperature, by 5 to 19% of the value of K above the transition. That is, some solute is "frozen out" when the hydrocarbon tails pass into the crystalline state. This conclusion agrees with evidence (Dix, Diamond & Kivelson, 1974) from electron spin resonance techniques, that K for the solute di-*t*-butyl nitroxide in

Table 5. Changes in partition at the transition temperature of lecithin

	Temp.	Butyramide	Ethyl acetate	Acetone
K	>25 °C	0.507	2.52	1.05
	<25 °C	0.409	2.39	0.953
$\Delta H_{w\to l}$ (cal/mole)	>25 °C	2,850	1,770	—
	<25 °C	23,500	27,200	23,600
$\Delta S_{w\to l}$ (cal/mole, °K)	>25 °C	8.19	7.77	—
	<25 °C	77.1	93.0	79.1
ΔH_l (cal/mole)	>25 °C	—	−9,940	—
	<25 °C	—	15,500	13,500
ΔS_l (cal/mole, °K)	>25 °C	—	−40.5	—
	<25 °C	—	44.7	38.8

From K measurements (Figs. 2–4 of paper III) above and below the endothermic transition temperature of dimyristoyl lecithin near 25 °C, K extrapolated to 25 °C from above (symbol >) or below (symbol <) the transition was calculated as in Table 2 of paper III, and $\Delta H_{w\to l}$, $\Delta S_{w\to l}$, ΔH_l and ΔS_l above (>) and below (<) the transition were calculated as in Tables 3 and 5 of paper III.

dipalmitoyl lecithin liposomes jumps down on cooling through the transition temperature at 41 °C, and that the extent of this "freezing out" is modest.

The enthalpy and entropy of partition, $\Delta H_{w\to l}$ and $\Delta S_{w\to l}$, increase enormously on cooling through the transition, by approximately a factor of 10. The same conclusion follows from the results which Dix *et al.* (1974) obtained by electron spin resonance spectroscopy. The enthalpy and entropy of solution in lecithin, ΔH_l and ΔS_l, reverse in sign from negative to positive[5]. We interpret the enthalpy changes in terms of the fact that in the crystalline state the hydrocarbon tails of lecithin pack more closely, more uniformly along their length, and with fewer "kinks". Thus, insertion of a solute into the membrane requires breaking much stronger intermolecular forces between the hydrocarbon tails than is required in the liquid crystalline state. It is possible to further interpret the positive enthalpy of partition as an increase in solute distributional volume within the bilayer with temperature. Estimates of translational diffusion coefficients in lecithin for a hydrophobic solute suggest that at low temperatures the solute is concentrated

5 Our measurements of K_{acetone} above 25 °C were too few to permit accurate estimates of $\Delta H_{w\to l}$, $\Delta S_{w\to l}$, ΔH_l and ΔS_l. However, the change in slope of log K_{acetone} *vs.* $(1/T)$ is sufficiently obvious (paper III, Fig. 4) to justify the conclusion that the changes produced by freezing on these thermodynamic constants for acetone are qualitatively similar to the changes for butyramide and ethyl acetate.

in the center of the bilayer, the most fluid region; and that at higher temperatures the periphery of the bilayer's hydrocarbon region becomes increasingly fluid and available to solute (Dix *et al.*, 1974). Thus, the fraction of bilayer volume occupied by solute may increase with increasing temperature as "melting" spreads peripherally, perhaps abruptly at the transition temperature, but also more gradually over a wide temperature range.

The entropy changes at the transition temperature may be attributed to disruption of the orderly crystalline array by the inserted solute. Since the ΔS and ΔH changes act in opposite directions on ΔF, the change in the partition coefficient, or "freezing out", is much smaller than the change in temperature dependence of K.

The Barclay-Butler constants of "frozen" lecithin may be estimated tentatively from the values of ΔS_l and ΔH_l for acetone and ethyl acetate below 25 °C (Table 6 and Fig. 5 of paper III). The Barclay-Butler slope $b = 0.00296\ (°K)^{-1}$, and the Barclay-Butler intercept $a = -1.3$ cal/mole, °K, are more positive than in any other solvent available for comparison. Conversion from molal to mole fraction units (Appendix of paper III) would leave b unchanged but would make the a value of frozen lecithin even more aberrantly positive. Both shifts are consistent with the positive entropy changes expected for insertion of a solute molecule into the ordered matrix of hydrocarbon tails.

Comparison with Partition Coefficients in Biological Membranes

The few other measurements of K's in artificial phospholipid bilayers (cited on p. 83 of Katz and Diamond, 1974*a*) have mostly utilized solutes much more hydrophobic than our test solutes. The estimate most nearly comparable to ours is for the solute *n*-valeramide, which Lange *et al.* (1974) found to have $K = 1.64$ (uncorrected for nonsolvent water) between egg yolk lecithin and water at 20 °C. Since our value for *n*-butyramide in dimyristoyl lecithin at 25 °C is 0.51, and since each $-CH_2-$ group multiplies our K's by about 2.5, the predicted value for *n*-valeramide in dimyristoyl lecithin is 1.28, fairly close to the egg yolk lecithin value.

For biological membranes the sole comparable measurements are by Seeman, Roth and co-workers (Metcalfe, Seeman & Burgen, 1968; Kwant & Seeman, 1969; Seeman, 1969; Seeman, Roth & Schneider, 1971; Machleidt, Roth & Seeman, 1972; Roth & Seeman, 1972; Roth, Seeman, Åkerman & Chau-Wong, 1972). In an extensive series of studies on the mechanism of action of anesthetics, these workers measured K's of about 17 anesthetics in erythrocyte ghost membranes as a function of concentration, and also

measured K's of several of the same solutes in brain synaptosome membranes and in sarcoplasmic reticulum membranes. The principal findings concerning K's were as follows:

1. *Numerical Values.* The sole solute measured both by us and by Seeman, Roth and co-workers is benzyl alcohol. Its K in erythrocytes at 23 °C is 4.0 (Roth & Seeman, 1972; slightly lower values were obtained earlier by Metcalfe *et al.*, 1968, and by Seeman, 1969). By extrapolation from studies of temperature dependence by Seeman (1969), the value at 400 °C would be about 5, compared to 13.9 in lecithin at 40 °C (paper III, Table 2). Extrapolating from measurements by Seeman *et al.* (1971, Table 2) on pentanol and higher alcohols, one estimates 1.05 for *n*-butanol in erythrocytes at 25 °C, compared to 3.16 in lecithin at the same temperature. K's had the same values in synaptosomes as in erythrocytes for the five solutes compared, and were up to twofold higher in sarcoplasmic reticulum for three solutes compared (Roth & Seeman, 1972, Fig. 6). Thus, the lecithin K's are within a factor of 3 of the erythrocyte values in the two available cases.

2. *Selectivity Constant s.* Using octanol as the reference solvent, s is about 1.0 for erythrocytes (Roth & Seeman, 1972, Fig. 5), compared to 0.87 for lecithin (this paper, Tables 1 and 2). Thus, erythrocyte behaves as slightly more hydrophobic than lecithin. It may be significant, however, that many of the solutes used by Roth and Seeman were more hydrophobic than any of the solutes we used (*see* p. 143 for further discussion).

3. $\delta\Delta F_l^{CH_2}$. From comparison of erythrocyte K's for butyric acid and valeric acid (Roth & Seeman, 1972, Table 1) and for five alcohols of different chain lengths (Seeman *et al.*, 1971, Table 2), one estimates an average value of -681 cal/mole for $\delta\Delta F_{w\to l}^{CH_2}$ in the erythrocyte, using Eq. (6). Taking 160 cal/mole as $\delta\Delta F_w^{CH_2}$ (Diamond & Wright, 1969*b*, Table 1), one obtains -521 cal/mole as $\delta\Delta F_l^{CH_2}$ for erythrocyte, compared to an average value of -410 cal/mole for lecithin (this paper, Table 4). The comparison again suggests that erythrocyte behaves as slightly more hydrophobic than lecithin.

4. $\Delta H_{w\to l}$, *and Temperature Dependence of Partition.* Temperature dependence of partition was studied for two solutes in erythrocytes. For benzyl alcohol ($K=4$ at 23 °C), K increased with temperature, and $\delta\Delta H_{w\to l}$ was positive (Seeman, 1969). This was also true for all solutes studied by us in lecithin. For chlorpromazine ($K=1{,}600$ at 23 °C), K decreased with temperature and $\delta\Delta H_{w\to l}$ was negative (Kwant & Seeman, 1969). The explanation follows from Table 4: $-CH_2-$ is unusual among substituent groups in that it increases K's and has a negative value of $\delta\Delta H_{w\to l}$. Thus, when one

studies solutes with increasingly large hydrocarbon moieties, K should become increasingly large, $\Delta H_{w\to l}$ should shift from positive towards negative, and K should decrease rather than increase with temperature. This trend is obvious in lecithin as one proceeds from methanol to ethanol to *n*-propanol to *n*-butanol (K values in Table 2 of paper III, $\Delta H_{w\to l}$ values in Table 3 of paper III). By extrapolation, an alcohol with as large a hydrocarbon moiety as that of chlorpromazine ($C_{17}H_{19}ClN_2S$), sufficient to yield a K value over 1,000, would also have yielded a negative $\Delta H_{w\to l}$ in lecithin.

5. *Partition of Charged Solutes and Effects of Surface Charge.* The solutes we studied in lecithin were all neutral except for benzoic acid, on which we made few measurements. Roth and Seeman (1972) measured K's of neutral, positively charged, and negatively charged anesthetics in erythrocytes and noticed striking differences; K's for neutral and negatively charged solutes were independent of concentration, while K's for positively charged solutes decreased with concentration (Roth & Seeman, 1972, Fig. 3). Increasing ionic strength increased K's of negatively charged solutes, decreased K's of positively charged solutes, and did not affect K's of neutral solutes (Roth & Seeman, 1972, Fig. 4). K's of neutral solutes in erythrocytes varied linearly as the solutes' K's in octanol; K's of negatively charged solutes varied linearly as K_{octanol} with the same slope but with a lower intercept; and K's of positively charged solutes did not correlate with K_{octanol}.

Every one of these findings agrees well with the interpretation that they are due to a negative surface charge on the erythrocyte. As demonstrated theoretically and experimentally by McLaughlin, Szabo, Eisenman and Ciani (1970), the presence of a surface charge, by skewing ion concentrations in the aqueous phase within a few Debye lengths of the membrane, increases membrane concentrations of counter-ions and decreases concentrations of co-ions. Increasing ionic strength screens the surface charges and reduces these effects. Certain counter-ions reduce these effects further by binding to surface charges. Membrane concentrations of such counter-ions may be approximately independent of their aqueous concentrations, since aqueous concentrations *per se* and binding act in opposite directions. Figs. 2–5 of McLaughlin *et al.* (1970) and Figs. 28–31, 34, and 35 of Szabo, Eisenman, Laprade, Ciani and Krasne (1973) demonstrate effects equivalent to the above findings by Roth and Seeman (1972), but using phospholipid bilayers and positively or negatively charged carriers of ions. The negative surface charge responsible for these effects in erythrocytes could be sialic acid, negatively charged phospholipids, or both. Since dimyristoyl lecithin

is neutral, effects such as those described above for partition of anesthetics in erythrocytes should not exist in dimyristoyl lecithin. These results raise the possibility that surface charge effects may be important in understanding the biological mechanism of action of some anesthetics.

Comparison with Permeability Coefficients in Biological Membranes

1. *Selectivity Constant s.* Collander (1954) analyzed the empirical dependence of measured permeability coefficients in three species of alga on molecular weight and $K_{\text{olive oil}}$ and calculated s [Eq. (1)] for the algae with respect to olive oil or to each other. In effect, he assumed that the dependence of P on molecular weight entered through diffusion coefficients rather than through partition coefficients. Smulders and Wright (1971) performed a similar analysis for P values in rabbit gallbladder. Recalculating these results so that the reference solvent (solvent x) in Eq. (1) becomes octanol, one obtains $s = 2.07$ for the alga *Nitella mucronata*, 1.78 for *Nitellopsis obtusulus*, 1.47 for *Chara ceratophylla* and 0.29 for gallbladder, compared to 0.87 for lecithin. The rate-controlling barrier for permeation in gallbladder thus behaves as if it is more hydrophilic than the environment of the average

Table 6. Comparison of the effect of −OH on partition coefficients or permeability coefficients

Solvent or membrane	Method	−OH Factor
Rabbit gallbladder	*P*	2
Rat jejunum	*P*	3.4
Dimyristoyl lecithin	*K*	4.6
C_4H_9OH	*K*	5.2
$C_5H_{11}OH$	*K*	7.2
Egg lecithin + cholesterol + PA	*P*	7.6
$C_8H_{17}OH$	*K*	8.5
$C_{18}H_{35}OH$	*K*	14.7
Chara ceratophylla	*P*	23
Olive oil	*K*	107
Nitella mucronata	*P*	450
Benzene	*K*	1,300

"−OH factor" means the average factor by which introduction of one −OH group reduces either partition coefficients or permeability coefficients (K or P, respectively, in "method" column) in the indicated system. As discussed in connection with Eq. (6), this factor equals $\exp(-\Delta F^{OH}_{w\to l}/RT)$. The value for dimyristoyl lecithin is from the present study; gallbladder, from Smulders and Wright (1971); rat jejunum, from Schiff, Small and Dietschy (1972); egg lecithin + cholesterol + PA (= phosphatidic acid), estimated from Fig. 2 of Cohen and Bangham (1972); and all other values, from Diamond and Wright (1969*b*), calculated from data of Collander (1947, 1950, 1951, 1954).

nonelectrolyte molecule in lecithin, while the barrier in the algae is more hydrophobic.

2. *Effect of* $-OH$. As discussed on p. 134, the $-$OH group tends to reduce partition coefficients or permeability coefficients in a given system by a constant factor, which can be translated into values $\delta\Delta F_{w\to l}^{OH}$ or $\delta\Delta F_l^{OH}$. The more hydrocarbon-like and the more devoid of hydrogen-bonding groups is the membrane or solvent phase, the higher is this factor. Table 6 lists this factor for partition coefficients in lecithin and in bulk solvents, and for permeability coefficients in several biological membranes and in an artificial phospholipid membrane. The comparison can be considered only approximate, since the opportunities for intramolecular hydrogen bonding varied among the solutes examined in different systems. It appears, however, that the effect of $-$OH varies greatly among different systems; that biological membranes, considered apart from model systems, span nearly the full range of variation of both; that the value based on partition in lecithin (4.6) is similar to the very tentative value based on permeation in lecithin (7.6); and that the lecithin values lie towards the lower end of the spectrum.

Where are Most of the Partitioned Solutes Located?

Several findings suggest how hydrophobic is the average environment of the partitioned molecules in lecithin. First, the selectivity constant s for lecithin (Tables 1–3) is similar to that for C_4H_9OH or $C_5H_{11}OH$, and lower than that for higher alcohols, hexane or benzene. Second, $\delta\Delta F_l^{CH_2}$ for lecithin is slightly higher than that for C_4H_9OH and lower than that for octanol, olive oil, ether or benzene. Third, $\delta\Delta F_l^{OH}$ for lecithin is similar to that for C_4H_9OH, and lower than that for higher alcohols or benzene. Finally, the relative effects of an ester group and $-$OH on partition in lecithin are similar to those in C_4H_9OH rather than those in olive oil and ether, but this conclusion is based on only one case and must be considered tentative.

The obvious conclusions from these facts are that most of the solute molecules which we measure as partitioned (i.e., as present in the sucrose-excluding space of liposomes) are not simply sitting in water; and that they are also not sitting in a region like a pure hydrocarbon. If our measured K' values (i.e., uncorrected for nonsolvent water: *see* Eq. (1) of Katz and Diamond, 1974*b*) had been mostly less than the f value based on sucrose, these conclusions might have been suspected of being distorted by solutes

having access to the sucrose-excluding water. However, K' is higher than f for 11 of our 16 solutes, more than $2f$ for 9 solutes, and more than $3f$ for 8 solutes. Our estimates of s, $\delta\Delta F_l^{CH_2}$, and the Barclay-Butler constants are little changed if only the solutes with the higher K' or K values are analyzed. Thus, these conclusions are unlikely to be affected by uncertainties about the nonsolvent water correction.

If one thinks of the interior of a bilayer as a hydrophobic region similar to a pure hydrocarbon and expects partitioned solute molecules mostly to be located in this region, our results would be difficult to understand. However, this expectation would be unjustified. The most biologically significant fact about phospholipids, and the one that causes them to form a bilayer in water, is that they combine regions of very different polarity. According to a solute's chemical properties (especially its relative number of $-CH_2-$ groups and hydrogen-bonding groups), a solute will be partitioned between different regions of the bilayer just as between bulk phases of different hydrogen-bonding abilities. Two examples of this effect of membrane heterogeneity may be cited. Levine, Birdsall, Lee and Metcalfe (1972) used ^{13}C nuclear magnetic resonance to study the effect of stearic acid analogues, spin-labeled at different positions with nitroxide groups, on spin-lattice relaxation times of various carbon nuclei in dipalmitoyl lecithin. The results showed that the carboxyl end of these spin-labeled solutes was predominantly located near the membrane surface, while the terminal carbons of the solute were located near the membrane interior. The same conclusion follows from the paramagnetic resonance spectra of the spin-labeled solutes themselves (Hubbell & McConnell, 1971). While these studies involved a single long solute molecule with hydrophilic and hydrophobic ends, the same considerations should apply to small solutes of different polarities. The more hydrophilic solutes should be located predominantly nearer the polar head groups, while the more hydrophobic solutes should be predominantly in the membrane interior (Fig. 3). The solutes that we used were all relatively hydrophilic, none having an octanol: water partition coefficient higher than 13. Probably most molecules of these solutes were located towards the membrane surface rather than interior, and this is the region whose solvent properties resemble those of isoamyl alcohol. Our measurements do not distinguish molecules adsorbed on the polar head groups from molecules near the membrane glyceryl carbons or first carbons of the membrane hydrocarbon tails. However, since our average effective solvent zone resembles isoamyl alcohol rather than water, many of the partitioned solute molecules are probably near the glyceryl or outer carbons and not just adsorbed on polar head groups.

To summarize, partition coefficients are probably a function of position in the bilayer, and the position where a solute's local partition coefficient has the same value as the solute's average partition coefficient for the whole membrane probably varies with the solute used.

Permeation Barriers in Lecithin

Eq. (A.22) of the Appendix derives an expression for the permeability coefficient P_j of the solute species j in terms of the interfacial resistances r_j' and r_j'', the solute partition coefficient $K_j(x)$, the solute diffusion coefficient $D_j(x)$, and the membrane thickness x_0, taking the plane of the membrane as perpendicular to the x-axis:

$$P_j = \left[r_j' + \int_0^{x_0} \frac{dx}{K_j(x) D_j(x)} + r_j'' \right]^{-1}. \tag{A.22}$$

As discussed in the preceding section, K's are a function of position in the membrane. In addition, D's are likely to be a function of position (Fig. 4): the mobility of bilayer components, as well as of portions of long-chain solutes dissolved in a bilayer, decreases progressively below free-solution

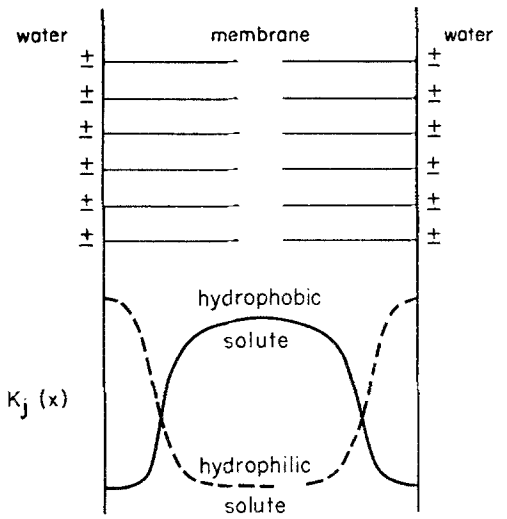


Fig. 3. *Below*: expected form of dependence of nonelectrolyte partition coefficients ($K_j(x)$) on position within a phospholipid bilayer. *Above*: sketch of a bilayer, with polar head groups at the periphery and hydrocarbon tails towards the interior. $K_j(x)$ increases towards the interior for hydrophobic solutes but increases towards the periphery for hydrophilic solutes

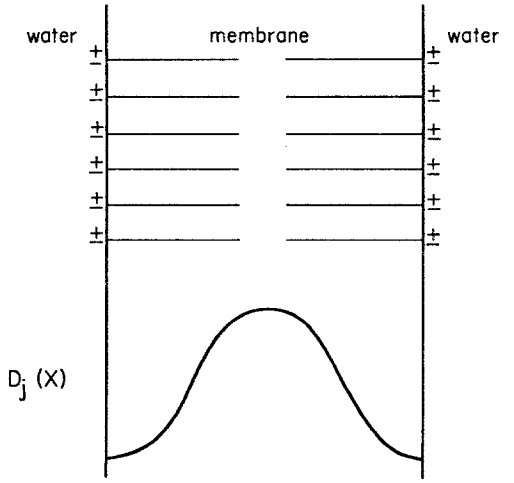


Fig. 4. Expected form of dependence of nonelectrolyte diffusion coefficients ($D_j(x)$) on position within a phospholipid bilayer. $D_j(x)$ increases towards the bilayer interior

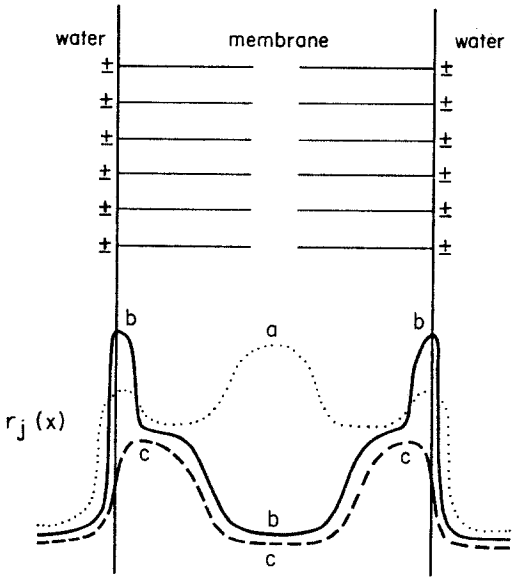


Fig. 5. Examples of expected resistance profiles for nonelectrolyte permeation in bilayers. In the membrane interior the resistance per unit path length, $r_j(x)$, is given by $1/K_j(x)\,D_j(x)$. Interfacial resistances are depicted as significant for solutes *a* and *b* but negligible for solute *c*. Profiles *b* or *c* might apply to a hydrophobic solute, profile *a* to a hydrophilic solute

values from the interior towards the surface of a bilayer (McConnell & McFarland, 1970; Hubbell & McConnell, 1971). *D*'s may also be anisotropic in a bilayer: e.g., higher in a direction parallel to the plane of the membrane

than in a perpendicular direction, the latter being the direction relevant to transmembrane permeation. There is no information as to whether the form of the D profile differs among different solutes, as true of the K profile.

If interfacial resistances were negligible and if K and D were position-independent, Eq. (A.22) would simplify to

$$P = KD/x_0. \tag{A.24}$$

Even though the assumption of position independence is unreasonable, Eq. (A.24) might still provide a fair estimate of P if the rate-limiting barrier to permeation were distributed over a significant fraction of the membrane thickness and if one used average K and D values for this region. For example, Finkelstein and Cass (1968) obtained a good prediction for the measured permeability of an egg lecithin bilayer to water, by substituting into Eq. (A.24) the bilayer thickness together with K and D of water in the bulk hydrocarbon hexadecane. Unless this agreement is coincidental, it suggests that the rate-limiting barrier for water permeation is provided by the bilayer interior, and that solubility and diffusion of water in the bilayer interior and in a bulk hydrocarbon are not too different. We can similarly combine our estimates of K's with other estimates of P's and D's, in order to assess whether the bilayer region where most of our partitioned solutes are located provides the rate-limiting barrier to permeation.

Properly, this calculation requires estimates of P's and D's in dimyristoyl lecithin for the same solutes as used for the K measurements, and the D estimate should be in the direction perpendicular to the membrane plane and should refer to the region where most of the partitioned solute is located. Although such estimates are unavailable, P's for five of the solutes we studied have been measured in other lecithin bilayers. Vreeman (1966) measured P values of 4.2×10^{-6} cm/sec for urea, 4.6×10^{-6} cm/sec for glycerol, and 0.75×10^{-6} cm/sec for erythritol, in thin lipid membranes of egg yolk phospholipids, largely lecithin, at 20 °C. Lippe (1969) obtained $P_{\text{urea}} = 3.65 \times 10^{-6}$ cm/sec in thin lipid membranes of egg lecithin or α-dioleoyl lecithin. Gallucci, Micelli and Lippe (1971) obtained P values of 3.7×10^{-6} cm/sec for urea, 18×10^{-6} cm/sec for ethylene glycol, and 5.7×10^{-6} cm/sec for glycerol in thin lipid membranes of egg lecithin or α-dioleoyl lecithin at 28 °C. From Fig. 2 of Cohen and Bangham (1972) one can estimate that the relative permeabilities of urea, ethylene glycol, glycerol and butyramide in liposomes of lecithin, cholesterol and phosphatidic acid (molar ratios 48:48:4) at 10 °C are in the ratios 1.0:12.2:0.47:18.6. If one converts the relative P's of Cohen and Bangham to absolute P's on the

Table 7. Estimation of rate barriers to permeation in lecithin

Solute	K	KD/x_0	P	$(x_0/KD)/(1/P)$
Butyramide	0.507	1.69	73×10^{-6}	1/23,000
Urea	0.230	0.77	3.9×10^{-6}	1/197,000
Ethylene glycol	0.116	0.39	33×10^{-6}	1/12,000
Glycerol	0.0501	0.17	4.0×10^{-6}	1/42,000
Erythritol	0.0259	0.09	0.75×10^{-6}	1/12,000

Partition coefficients (K) are experimental values for dimyristoyl lecithin (paper III). Permeability coefficients (P, in cm/sec) are experimental values for various lecithins as discussed in the text. Taking an approximate diffusion coefficient (D) of 10^{-6} cm^2/sec based on several solute-lecithin systems, and taking the bilayer thickness x_0 as 30 Å, the column KD/x_0 (in cm/sec) gives the expected permeability coefficient if these K and D values held throughout the bilayer and if interfacial resistances were negligible. The last column represents the fraction of the actual resistance to permeation which a uniform bilayer with these K and D values would possess. The conclusion to be drawn from the very low fractions in the last column, and from the inequality $KD/x_0 \gg P$, is that the rate-limiting barrier to permeation is not the zone where most partitioned solute molecules are located.

assumption that their P_{urea} equals the average of the three values obtained by Vreeman (1966), Lippe (1969), and Gallucci *et al.* (1971), and then averages the P values of all four sets of authors, one obtains the P estimates given in Table 7.

Three estimates of D are available for related systems. By electron spin resonance (e.s.r.) studies of the relatively hydrophobic solute di-*t*-butyl nitroxide in dipalmitoyl lecithin liposomes, Dix *et al.* (1974) estimated $D \sim 10^{-6}$ to 10^{-5} cm^2/sec at 33 to 63 °C as some kind of weighted mean value over all directions. Estimates for diffusion parallel to the plane of the bilayer in hydrated egg yolk lecithin are $\sim 4 \times 10^{-6}$ cm^2/sec for benzene at 22 °C (Rigaud, Gary-Bobo & Lange, 1972) and $\sim 6.5 \times 10^{-7}$ cm^2/sec for valeramide at 20 °C (Lange *et al.*, 1974).

Table 7 combines an approximated D value of 10^{-6} cm^2/sec with a bilayer thickness of 30 Å and measured K values of dimyristoyl lecithin at 25 °C, to calculate x_0/KD. From Eq. (A.22), this factor has the significance of the resistance that the bilayer would offer to permeation, if the region to which our measured K's apply were the rate-limiting barrier (and if the D estimates applied to the same region and that region occupied most of the bilayer thickness). Since $1/P$ has the significance of a resistance, the ratio $(x_0/KD)/(1/P) = Px_0/KD$, where P is the measured permeability coefficient, represents the fraction of the actual bilayer resistance residing in the zone with most of the partitioned solute molecules. Table 7 shows that this ratio is less than 1/12,000 for all five solutes.

The conclusion is that the zone of maximum partition accounts for only a small fraction of the resistance to permeation. This conclusion is entirely reasonable and likely to be generally valid, since Eq. (A.22) indicates that the resistance of a section dx of membrane is proportional to $dx/K(x)D(x)$ and therefore that the zone of *minimum* partition accounts disproportionately for the resistance. If these five relatively hydrophilic solutes are partitioned mainly into the periphery of the bilayer as discussed above, the rate-limiting barrier is probably either the hydrophobic center of the bilayer, where $K(x)$ is very low for these solutes, or else the membrane/solution interfaces.

In general, the resistance profile in the membrane interior (Fig. 5) may be obtained by multiplying K profiles such as Fig. 3 times D profiles such as Fig. 4. Fig. 5 indicates that resistance to permeation will usually be inhomogeneously distributed through the membrane. Direct experimental demonstrations of nonhorizontal resistance profiles ("energy barriers") within bilayers have been made for ion-solubilizing carriers by Stark, Ketterer, Benz and Läuger (1971), Ciani, Eisenman, Laprade and Szabo (1973), and Hall, Mead and Szabo (1973). Several types of profiles may be distinguished: (1) For hydrophobic solutes the resistance is likely to be maximal near the periphery, where both K and D are lowest (curve c or else b, Fig. 5). An example is the hydrophobic solute di-*t*-butyl nitroxide as shown by Dix *et al.* (1974). (2) For hydrophilic solutes the form of the resistance profile depends on whether the central trough in the K profile (Fig. 3) is relatively deeper than the relative height of the central maximum in the D profile (Fig. 4). The above-cited calculation for water by Finkelstein and Cass (1968) suggests that the barrier to water is in the bilayer center (curve a, Fig. 5). This could also be the meaning of the calculations of Table 7 for the five hydrophilic solutes analyzed there. (3) For some solutes the membrane/solution interfaces may be rate-limiting (e.g., curve b, Fig. 5). Foster and McLaughlin (1974) have shown this to be the case for the solute 5,6-dichloro-2-trifluoro-methylbenzimidazole.

Appendix

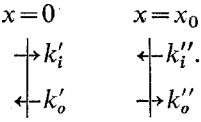
Theory of Nonelectrolyte Permeation in a Generalized Membrane

Jared M. Diamond, Gabor Szabo and Yehuda Katz

In this appendix we derive a general expression by which the permeability coefficient of a membrane to a nonelectrolyte solute is related to the solute's local partition coefficient and local diffusion coefficient within the membrane, the interfacial rate constants, and the membrane thickness. Previous

treatments of nonelectrolyte permeation that derived an explicit expression for the permeability coefficient in terms of these variables have made one or more of the following restrictive assumptions: that partition coefficients (or standard chemical potentials) are uniform throughout the membrane; that diffusion coefficients are uniform throughout the membrane; that the membrane is symmetrical; that interfacial resistances are negligible, i.e., that there is no immediate change in solute chemical potentials on crossing either of the water/membrane interfaces; or that some other specific resistance profile or form of energy barrier to solute permeation applies through the membrane. Ciani, Eisenman, Laprade and Szabo (1973) have treated a similar problem for carrier-mediated ion permeation, except that they assumed a symmetrical membrane.

Consider a membrane of thickness x_0 with planar surfaces oriented perpendicular to the x-axis, at $x=0$ and $x=x_0$. The bathing solutions adjacent to the surfaces $x=0$ and $x=x_0$ are referred to as $'$ and $''$, respectively. Effects of unstirred layers in the adjacent bathing solutions are not considered. The membrane is not necessarily symmetrical, the resistances that the interfaces offer to solute permeation are not necessarily negligible, and the membrane is not necessarily homogeneous along the x-axis. However, the membrane is either homogeneous along a path parallel to the plane of the membrane, or else is a mosaic of two regions, some sharing a certain set of permeability characteristics and some with zero permeability. Chemical potentials are written as μ, standard chemical potentials as μ^0, concentrations as c, mobilities as u, diffusion coefficients as D, velocities as v, fluxes as J, permeability coefficients as P, partition coefficients as K, the gas constant as R, and absolute temperature as T. The rate constants for solute crossing the $x=0$ interface in the water-to-membrane direction and vice versa are written as k_i' and k_o', while the corresponding rate constants at the $x=x_0$ interface are k_i'' and k_o''. μ, μ_0, c, u, D, v and K may be functions of position in the membrane, i.e., functions of x. The Nernst-Planck equation is assumed to hold for diffusion in the membrane interior. The standard chemical potential of solute in water is taken as zero. Only the case of a single solute will be considered. μ, μ_0, C, u, D, v, J, P, K, k_i', k_o', k_i'' and k_o'' will in general have different values for different solutes.



Diffusion. First, consider diffusion within the membrane interior. At any position within the membrane at a distance x from the $x=0$ interface,

one can write:

$$\mu(x)=\mu^0(x)+RT\ln c(x) \tag{A.1}$$

$$v(x)=-u(x)\frac{d\mu(x)}{dx} \tag{A.2}$$

$$J(x)=c(x)v(x) \tag{A.3}$$

$$D(x)=u(x)RT. \tag{A.4}$$

Substitution of (A.1), (A.2) and (A.4) into (A.3) gives the Nernst-Planck equation:

$$J(x)=-D(x)c(x)\frac{d(\mu^0(x)/RT)}{dx}-D(x)\frac{dc(x)}{dx}. \tag{A.5}$$

The local partition coefficient $K(x)$ is defined by

$$-RT\ln K(x)\equiv\mu^0(x). \tag{A.6}$$

Substituting (A.6) into (A.5) gives

$$J(x)=D(x)c(x)\frac{d(\ln K(x))}{dx}-D(x)\frac{dc(x)}{dx}. \tag{A.7}$$

Adding and subtracting $c(x)\dfrac{dD(x)}{dx}$ on the right-hand side of (A.7):

$$\begin{aligned}J(x)=&\frac{D(x)c(x)}{K(x)D(x)}D(x)\frac{dK(x)}{dx}+\frac{D(x)c(x)}{K(x)D(x)}K(x)\frac{dD(x)}{dx}\\&-c(x)\frac{dD(x)}{dx}-D(x)\frac{dc(x)}{dx}\end{aligned} \tag{A.8}$$

$$=D(x)c(x)\frac{d\ln(K(x)D(x))}{dx}-\frac{d(c(x)D(x))}{dx}. \tag{A.9}$$

Multiplying both sides of (A.9) by $(-1/K(x)D(x))$:

$$\frac{-J(x)}{K(x)D(x)}=\frac{d(c(x)/K(x))}{dx}. \tag{A.10}$$

In the steady state J is independent of x, and (A.10) may be integrated between $x=0$ and $x=x_0$ to yield

$$-J=\left[\frac{c(x_0)}{K(x_0)}-\frac{c(0)}{K(0)}\right]\Big/\int_{x=0}^{x=x_0}\frac{dx}{K(x)D(x)} \tag{A.11}$$

or

$$-J=\frac{c(x_0)}{aK(x_0)}-\frac{c(0)}{aK(0)} \tag{A.12}$$

where

$$a\equiv\int_{x=0}^{x=x_0}\frac{dx}{K(x)D(x)}. \tag{A.13}$$

Boundary Conditions. In the steady state, when J is constant through the system,

$$-J=k_i''c''-k_o''c(x_0)=\frac{c(x_0)}{aK(x_0)}-\frac{c(0)}{aK(0)}=k_o'c(0)-k_i'c'. \tag{A.14}$$

In this equation c' and c'' are the solute concentrations in the bathing solutions ′ and ″, respectively, while $c(0)$ and $c(x_0)$ are the solute concentrations in the membrane immediately adjacent to the $x=0$ and $x=x_0$ interfaces, respectively.

Solving (A.14) for $c(0)$ in terms of $c(x_0)$ and c':

$$c(0)=\left[\frac{c(x_0)}{aK(x_0)}+k_i'c'\right]\Big/\left[\frac{ak_i'+1}{aK(0)}\right]. \tag{A.15}$$

Substituting (A.15) into (A.14) to solve for $c(x_0)$ in terms of c' and c'':

$$c(x_0)=[c''K(x_0)(ak_i'+1)+k_i'c'/k_o'']/(ak_i'+1+k_i'/k_i''). \tag{A.16}$$

Substituting (A.16) into (A.14) to solve for J in terms of c' and c'':

$$-J=(c''-c')/(a+1/k_i'+1/k_i''). \tag{A.17}$$

In proceeding from (A.14) to (A.15), (A.16) and (A.17), the algebra was simplified by recognizing that the local partition coefficient within the membrane adjacent to the $x=0$ or $x=x_0$ interface, $K(0)$ or $K(x_0)$, respectively, is simply the ratio of the opposite interfacial rate constants:

$$k_i'/k_o'=K(0), \quad k_i''/k_o''=K(x_0). \tag{A.18}$$

Permeability Coefficient. The permeability coefficient P is defined by

$$P\equiv -J/(c''-c'). \tag{A.19}$$

Comparison of (A.13), (A.17) and (A.19) yields

$$(1/P)=(1/k')+\int_{x=0}^{x=x_0}\frac{dx}{K(x)D(x)}+(1/k''). \tag{A.20}$$

It is convenient to define interfacial resistances r' and r'', as

$$r'\equiv 1/k_i', \quad r''\equiv 1/k_i'', \tag{A.21}$$

thereby transforming (A.20) into

$$(1/P)=r'+\int_{x=0}^{x=x_0}\frac{dx}{K(x)D(x)}+r''. \tag{A.22}$$

The intuitive meaning of (A.22) is as follows. The reciprocal of the permeability coefficient has the significance of a resistance. This resistance consists of three resistances in series: the interfacial resistances r' and r'',

and the diffusional resistance of the membrane interior $\int_{x=0}^{x=x_0} \frac{dx}{K(x)D(x)}$. The latter term consists of the sum of resistance elements $dx/K(x)D(x)$, each arising from a planar section of membrane of thickness dx and at a distance x from the $x=0$ interface. For hydrocarbon-like solutes in a phospholipid bilayer or biological membrane, $K(x/2)$ is likely to be much greater than values of $K(x)$ near $x=0$ or $x=x_0$, $D(x/2)$ is likely to be much greater than values of $D(x)$ near $x=0$ or $x=x_0$, and the peripheral resistance elements will greatly outweigh the central elements in determining the interior diffusional resistance. For hydrophilic solutes in a phospholipid bilayer or biological membrane, $K(x/2)$ is likely to be much lower than values of $K(x)$ near $x=0$ or $x=x_0$, and the central resistance elements may dominate the interior diffusional resistance.

Three limiting cases of (A.22) deserve mention. First, if the interfacial rate constants k_i' and k_i'' are high and if $K(x)$, $D(x)$, or both are low, then the diffusional resistance of the membrane interior becomes rate-limiting. Second, if the membrane is homogeneous ($K(x)$ and $D(x)$ constant) and symmetrical ($k_i' = k_i'' = k_i$, $k_o' = k_o'' = k^0$, $k_i/k^0 = K$) but the interfacial resistances are not negligible, (A.22) becomes

$$1/P = (2/k_i) + k_o x_0/k_i D. \tag{A.23}$$

Finally, if the membrane is homogeneous and the interfacial resistances are negligible, (A.22) reduces to

$$P = KD/x_0, \tag{A.24}$$

the familiar equation for the permeability coefficient of a homogeneous membrane with negligible interfacial resistances.

Note Added in Proof: Relevant to our discussion (pp. 142–152) of solute position in bilayers, as a function of solute polarity and local polarity in the membrane, is an important recent paper on the polarity gradient in bilayers by O. H. Griffith, P. J. Dehlinger and S. P. Van (*J. Membrane Biol.* **15**:159, 1974).

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