J. Membrane Biol. 17, 101–120 (1974)

Thermodynamic Constants for Nonelectrolyte Partition between Dimyristoyl Lecithin and Water

Yehuda Katz and Jared M. Diamond

Department of Physiology, University of California Medical Center, Los Angeles, California 90024

Received 8 May 1973; revised 28 January 1974

Summary. Partition coefficients (K's) between dimyristoyl lecithin liposomes and water were measured as a function of temperature for 16 nonelectrolytes. From K and its temperature dependence, the partial molar free energy (ΔF), enthalpy (ΔH) and entropy (ΔS) of partition for each solute were extracted. By subtraction of ΔF, ΔH and ΔS of hydration of each solute (where known), ΔF, ΔH and ΔS of solution in lecithin could be calculated for some solutes. Among different solutes ΔS of solution increases approximately linearly with ΔH of solution, as also found in bulk solvents (the so-called Barclay-Butler relations). The Barclay-Butler slope $d\Delta S/d\Delta H$ is approximately twice as steep for lecithin as for bulk nonpolar solvents. This effect is attributed in part to greater immobilization of solutes in bilayers than in bulk solvents.

The previous papers in this series described methods for measuring nonelectrolyte partition coefficients between liposomes and water, and for estimating the amount of nonsolvent water in liposomes (Katz & Diamond, 1974*a*, *b*, referred to as papers I and II, respectively). The present paper reports experimental measurements of partition coefficients between dimyristoyl lecithin liposomes and water, corrected for the effects of trapped water and nonsolvent water in the liposomes. It then extracts the following thermodynamic parameters from these measurements: partial molar free energies, enthalpies, and entropies of partition; partial molar free energies, enthalpies, and entropies of solution in lecithin from the gas phase; and the Barclay-Butler constants for solution in lecithin. Discussion of physical and molecular interpretations except for the significance of the Barclay-Butler constants is reserved to the following paper (Diamond & Katz, 1974, referred to as paper IV).

Methods

Experimental methods were described previously (paper I).

All partition coefficients reported have been corrected for the effect of nonsolvent water, using Eq. (17) of paper I and reading off f values at the appropriate temperature

from the solid lines of Fig. 2, paper II, fitted through the experimental values of f for sucrose. One can easily calculate what the partition coefficient would be if one assumed some different value of f: simply add, to the partition coefficient value we report, the new assumed f value and then subtract the f value given by Fig. 2, paper II.

Results and Discussion

Partition Coefficient Measurements

As illustrated in Figs. 1–4, partition coefficients (K) were measured for 12 solutes over a temperature range of at least 30 to 55 °C. Three solutes were studied at temperatures down to 11 °C. For three additional solutes K's were measured over a range of temperatures too narrow to permit accurate estimation of temperature dependence, hence only the extracted K values at 25 and 40 °C are reported (Table 2).

For analysis of the temperature dependence of K, K values for each solute were plotted against $1/T$, and a straight line was fitted by least-mean-squares through the points (e.g., Figs. 1–4). The thermodynamic rationale behind this analysis is apparent from the following equations (Klotz, 1950;

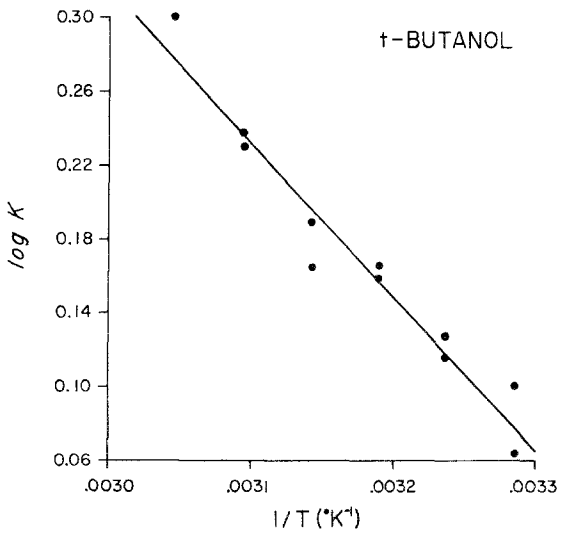


Fig. 1

Figs. 1–4. Experimental measurements of partition coefficients (K's) between dimyristoyl lecithin and water, as a function of temperature. Ordinate, log K; abscissa, $1/T$, in (degrees Kelvin)$^{-1}$. Each point represents one measurement. The straight lines are plotted by least-mean-squares through the points. For the solutes studied both above and below the endothermic phase-transition temperature near 25 °C, separate lines are fitted in each temperature range (Figs. 2–4). The dashed vertical line indicates 25 °C

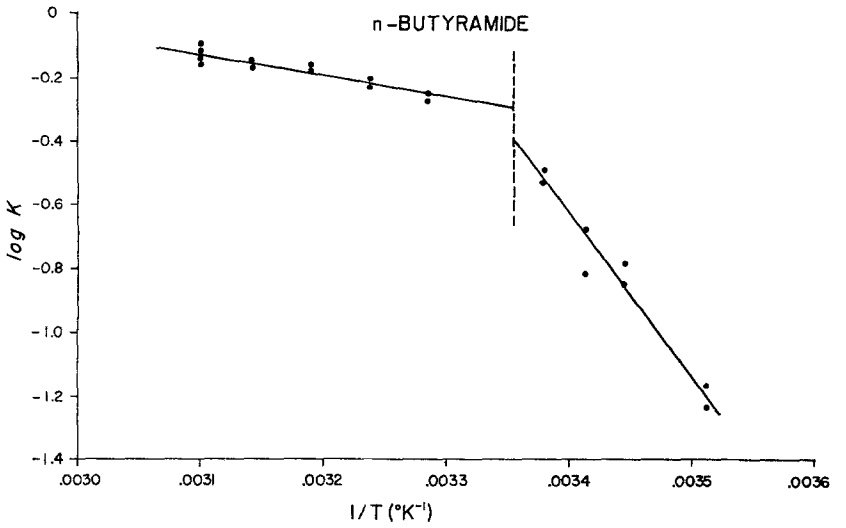


Fig. 2

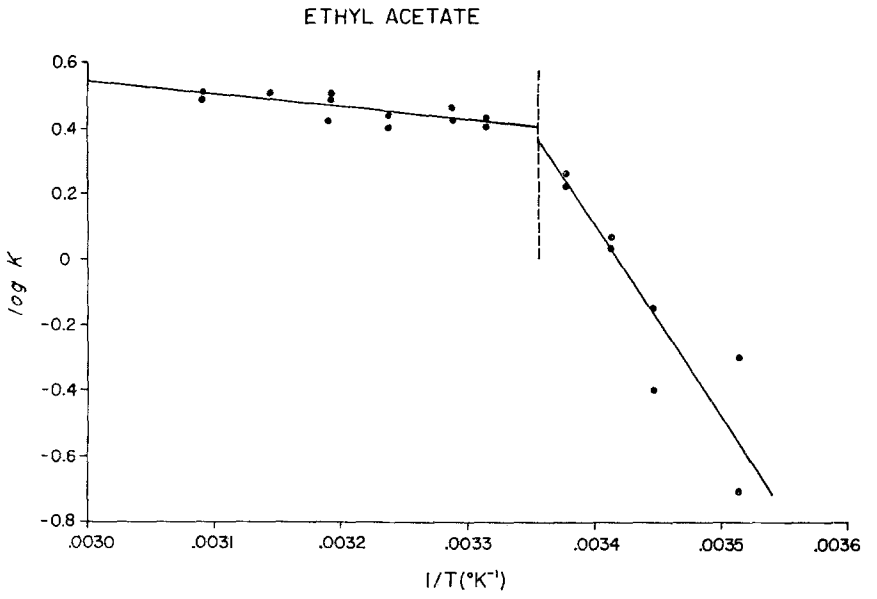


Fig. 3

Guggenheim, 1952):

$$\ln K = -\Delta F^0_{w\to l}/RT \tag{1}$$

$$= -\Delta H^0_{w\to l}/RT + \Delta S^0_{w\to l}/R \tag{2}$$

where the partition coefficient K on a molal or weight basis is defined by

$$K \equiv C_l/C_w. \tag{3}$$

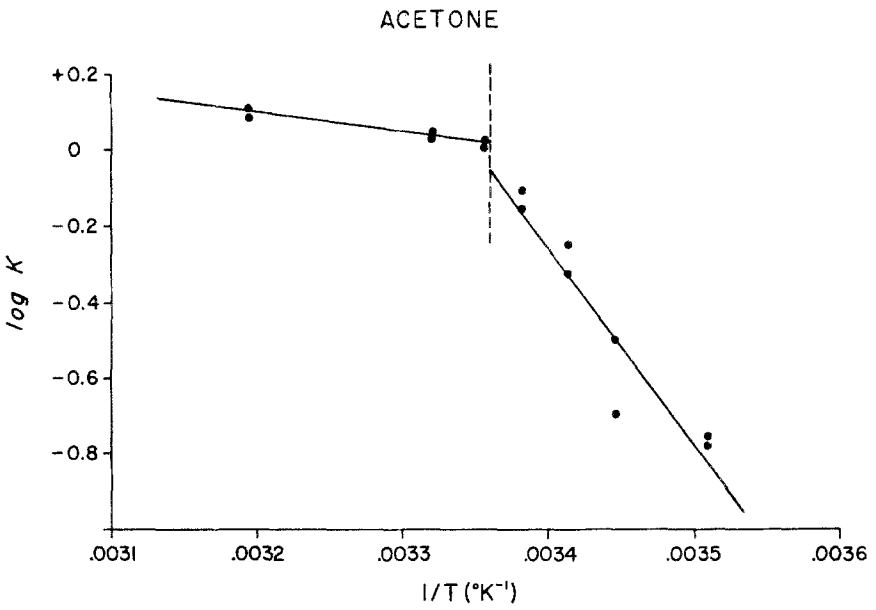


Fig. 4

C represents solute concentrations, and subscripts l and w refer to the lipid phase and aqueous phase, respectively. $\Delta F^0_{w\to l}$, $\Delta H^0_{w\to l}$ and $\Delta S^0_{w\to l}$, the changes in standard partial molar free energy, enthalpy and entropy, respectively, on transferring solute from water to the lipid phase, are given by

$$\Delta F^0_{w\to l} \equiv F^0_l - F^0_w \tag{4}$$

$$\Delta H^0_{w\to l} \equiv H^0_l - H^0_w \tag{5}$$

$$\Delta S^0_{w\to l} \equiv S^0_l - S^0_w \tag{6}$$

where F^0_l, F^0_w, etc., are the standard partial molar state functions of solute in each phase.

Over a range of temperatures sufficiently narrow that $\Delta H^0_{w\to l}$ may be considered approximately independent of temperature, $\Delta S^0_{w\to l}$ will also be independent of temperature, since in general

$$(\partial H/\partial T)_P = T(\partial S/\partial T)_P. \tag{7}$$

Thus, Eq. (2) means that a graph of $\ln K$ (*or* $\log K$) against $(1/T)$ should give a straight line with a slope of $-\Delta H^0_{w\to l}/R$ and intercept on the ordinate of $\Delta S^0_{w\to l}/R$.

The only assumption involved in fitting a straight line through the experimental points is the usual one that over a small temperature range $\Delta H^0_{w\to l}$ may to a first approximation be considered independent of temperature. If $\Delta H^0_{w\to l}$ is not independent of temperature and is represented by a power series in T (Klotz, 1950), then to a next approximation this does not alter the form of the relation between $\ln K$ and $1/T$, which remains linear. What is changed is only the significance of the slope of this line, which is no longer the constant value of $\Delta H^0_{w\to l}$ [from Eq. (2)] but is instead the value of $\Delta H^0_{w\to l}$ at an intermediate temperature. Evaluation of the first two coefficients of the power series from Eq. (1) and the Gibbs-Helmholtz relation combined with experimental values of K and T showed that the range of variation of $\Delta H^0_{w\to l}$ over the experimental temperature range above 25 °C was 15% or less of the mean value.

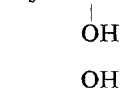
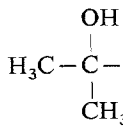
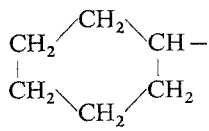
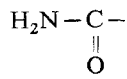
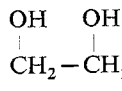
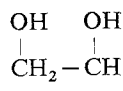
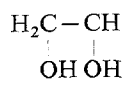
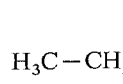
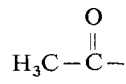
Figs. 2–4 show that, on cooling dimyristoyl lecithin through its endothermic phase-transition temperature, there is a small downwards jump in K, and the slope of $\log K$ *vs.* $1/T$ goes more negative by a factor of 10, for the three solutes studied both above and below 25 °C. The remainder of this paper is concerned solely with the temperature range above 25 °C, in which the great majority of our experimental measurements were made. Evaluation of measurements below 25 °C, and discussion of the remarkable discontinuities at the phase-transition temperature, are reserved for the following paper (paper IV).

For each of the 12 solutes studied over the range 30 to 55 °C, Table 1 gives the intercept a_1 and slope a_2 of the straight line fitted by least-mean-squares to points above 25 °C, the mean square error, and the correlation coefficient. As expected, correlation coefficients are highest (and errors lowest) for the solutes with the highest partition coefficients. Table 2 gives K values for the 12 solutes at three temperatures, calculated from the straight-line parameters extracted in Table 1. Table 2 also gives similarly calculated K values for four additional solutes on which we made six or eight measurements near 40 °C (or 25 °C and 40 °C), sufficient to calculate reliable K values at these temperatures but not to obtain a reliable value of the slope a_2. Subsequent calculations are based on the parameters of Tables 1 and 2. It should be reemphasized that extraction of these parameters assumes nothing except temperature independence of $\Delta H^0_{w\to l}$ over the temperature range studied. The correlation coefficients of Table 1 are a measure of experimental errors rather than a test of some hypothesis. The parameters simply provide a means of expressing average values of measurements made at different temperatures.

Partial Molar Free Energies of Partition

From Eq. (1) and the K values of Table 2, one can calculate directly the difference in standard chemical potential for a solute between the lipid

Table 1. Linear dependence of log K on $(1/T)$

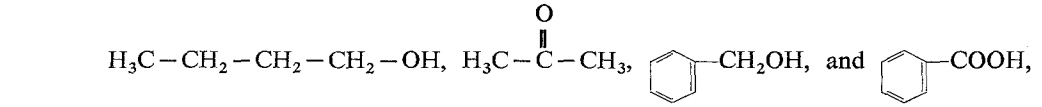
Solute	Formula	a_1	a_2	M.S.E.	Correlation
Methanol	H_3C-OH	3.14	−1,150	0.0062	−0.86
Ethanol	H_3C-CH_2-OH	1.65	− 598	0.0051	−0.94
n-Propanol	$H_3C-CH_2-CH_2-OH$	1.20	− 324	0.0028	−0.94
Isopropanol		2.15	− 686	0.0052	−0.95
t-Butanol		2.81	− 830	0.0046	−0.97
Cyclohexanol		1.87	− 290	0.0047	−0.82
Urea		0.274	− 275	0.0083	−0.63
Ethylene glycol		2.02	− 880	0.017	−0.78
Glycerol		4.10	−1,610	0.030	−0.77
Erythritol		5.67	−2,160	0.046	−0.76
Butyramide		1.79	− 622	0.0037	−0.97
Ethyl acetate		1.70	− 387	0.0074	−0.75

For each solute a straight line, $\log K = a_1 + a_2(1/T)$, was fitted by least-mean-squares to the experimental measurements of log K above 25 °C (as illustrated for four solutes in Figs. 1–3), expressing T in degrees Kelvin. Columns 3, 4, 5 and 6 give the values of a_1, a_2, the mean square error, and the correlation coefficient, respectively, for each line. The mean square error is defined as $\{\sum[(\log K)_{\text{expt}} - (\log K)_{\text{fit}}]^2\}^{1/2}/\text{n}$, where $(\log K)_{\text{expt}}$ is an experimental value of log K, $(\log K)_{\text{fit}}$ is the corresponding fitted value calculated by substituting the experimental value of $(1/T)$ into $\log K = a_1 + a_2(1/T)$, and n is the number of measurements. Since $\Delta F^0_{w\to l}$ is proportional to log K, this mean square error in effect measures the fit of a straight line to $\Delta F^0_{w\to l}$ *vs.* $(1/T)$.

Table 2. Partition coefficients of nonelectrolytes between dimyristoyl lecithin and water

Solute	25 °C	40 °C	55 °C
Methanol	0.206	0.294	0.405
Ethanol	0.441	0.551	0.637
n-Propanol	1.31	1.47	1.64
Isopropanol	0.707	0.912	1.15
t-Butanol	1.05	1.43	1.89
Cyclohexanol	7.94	8.84	9.74
Urea	0.230	0.255	0.279
Ethylene glycol	0.116	0.161	0.216
Glycerol	0.0501	0.0909	0.156
Erythritol	0.0259	0.0577	0.119
Butyramide	0.507	0.638	0.787
Ethyl acetate	2.52	2.91	3.31
n-Butanol	3.16	3.49	—
Acetone	1.05	1.25	—
Benzyl alcohol	—	13.9	—
Benzoic acid	—	8.51	—

The numbers are the partition coefficients, defined as $K \equiv c_{sl}/c_{sw}$ where c_{sl} and c_{sw} are the equilibrium solute concentrations in the lipid phase and water phase, respectively, expressed in grams solute per grams phase. In effect, K is expressed in molal concentration units, since the solutions were dilute. Average K values at each of three temperatures were calculated from the equation $\log K = a_1 + a_2(1/T)$, inserting the values of a_1 and a_2 listed in Table 1 and extracted by least-mean-squares from the measured K values. Structures of *n*-butanol, acetone, benzyl alcohol, and benzoic acid are, respectively,



while structures of the other solutes are given in Table 1.

phase and water, $\Delta F^0_{w\to l} \equiv \Delta F^0_l - \Delta F^0_w$. $\Delta F^0_{w\to l}$ has the physical meaning of the change in free energy on transferring one mole of solute from a hypothetical aqueous solution to a hypothetical solution in lipid, each solution containing solute at a concentration of 1 molal and having the physical properties of an infinitely dilute solution (i.e., obeying Henry's law). The significance of alternative choices of concentration units (e.g., mole fractions instead of molalities) is discussed in the Appendix. Table 3 gives values of $\Delta F^0_{w\to l}$ at 25 and 40 °C for the solutes studied.

Partial Molar Enthalpies of Partition

Eq. (2) implies the following relation between the enthalpy of partition, $\Delta H^0_{w\to l} = H^0_l - H^0_w$, and a_2, the slope calculated by least-mean-squares for

Table 3. Partial molar free energies, enthalpies and entropies of partition

Solute	$\Delta F^0_{w\to l}$ (25 °C) (cal/mole)	$\Delta F^0_{w\to l}$ (40 °C) (cal/mole)	$\Delta H^0_{w\to l}$ (cal/mole)	$\Delta S^0_{w\to l}$ (cal/mole, °K)
Methanol	940	760	4,370	11.5
Ethanol	490	370	2,740	7.55
n-Propanol	− 160	− 240	1,480	5.49
Isopropanol	210	60	3,140	9.82
t-Butanol	− 30	− 220	3,800	12.8
Cyclohexanol	−1,230	−1,360	1,330	8.55
Urea	870	850	1,240	1.24
Ethylene glycol	1,280	1,140	4,030	9.22
Glycerol	1,780	1,490	7,370	18.8
Erythritol	2,160	1.780	9,900	25.9
Butyramide	400	280	2,850	8.19
Ethyl acetate	− 550	− 660	1,770	7.77
n-Butanol	− 680	− 780	—	—
Acetone	− 30	− 140	—	—
Benzyl alcohol	—	−1,640	—	—
Benzoic acid	—	−1,330	—	—

The quantities are the standard partial molar ΔF, ΔH, and ΔS for transfer of the indicated solute from water to dimyristoyl lecithin, both solutions being hypothetical 1-molal solutions obeying Henry's law. $\Delta F^0_{w\to l}$ was calculated as $-RT\ln K$, using the K values of Table 2; $\Delta H^0_{w\to l}$, as $-2.303\ \mathrm{Ra}_2$, using the a_2 values of Table 1; and $\Delta S^0_{w\to l}$, as $(\Delta H^0_{w\to l}-\Delta F^0_{w\to l})/T$.

the graphs of $\log K$ *vs.* $1/T$:

$$\Delta H^0_{w\to l}=-2.303\ \mathrm{Ra}_2. \tag{8}$$

The factor 2.303 enters because of conversion from $\log K$ to $\ln K$. Table 3 gives values of $\Delta H^0_{w\to l}$ calculated from the a_2 values of Table 1 by means of Eq. (8). As discussed previously, $\Delta H^0_{w\to l}$ was assumed independent of T in extracting a_2. $\Delta H^0_{w\to l}$ has the physical meaning of the change in enthalpy when one mole of solute is transferred from water to lipid at infinite dilution. Since $\Delta F^0_{w\to l}$ is obtained directly from K, while $\Delta H^0_{w\to l}$ is obtained from the slope of $\log K$ against $1/T$, $\Delta H^0_{w\to l}$ is more sensitive to experimental errors than is $\Delta F^0_{w\to l}$.

Partial Molar Entropies of Partition

The familiar relation $\Delta F=\Delta H-T\Delta S$ may be rearranged to:

$$\Delta S^0_{w\to l}=(\Delta H^0_{w\to l}-\Delta F^0_{w\to l})/T. \tag{9}$$

The $\Delta S^0_{w\to l}$ values of Table 3 were calculated by inserting the $\Delta H^0_{w\to l}$ and $\Delta F^0_{w\to l}$ values of Table 3 into Eq. (1). Since $\Delta F^0_{w\to l}$ values were calculated on

the assumption that $\Delta H^0_{w\to l}$ is independent of temperature and since this is equivalent to assuming that $\Delta S^0_{w\to l}$ is independent of temperature [Eq. (7)], the calculated $\Delta S^0_{w\to l}$ values are independent of temperature. This procedure for obtaining $\Delta S^0_{w\to l}$ is equivalent to calculating it as 2.303 Ra_1 from Eq. (3) and the intercepts of graphs such as Figs. 1–4, using the straight-line constant a_1 of Table 1. $\Delta S^0_{w\to l}$ has the physical meaning of the change in entropy when one mole of soltue is transferred between two hypothetical mixtures, in each of which the solute has a molal concentration of 1 but has the same physical properties as in infinitely dilute solution.

Table 3 shows that both the enthalpy and the entropy of partition are positive for all solutes studied, while the free energy of partition in the temperature range studied may be either positive or negative. Since the effects of a positive $\Delta H^0_{w\to l}$ and a positive $\Delta S^0_{w\to l}$ on $\Delta F^0_{w\to l}$ are opposite (*cf.* the relation $\Delta F^0 = \Delta H^0 - T\Delta S^0$), one could speak of partition as being either enthalpy-dominated or entropy-dominated, depending on whether $\Delta F_{w\to l} > 0$ or < 0. Application of Eq. (2) to two different solutes, i and j, shows that their partition coefficients should become equal at an isopartition temperature given by

$$T_{\text{iso}} = (\Delta H^0_{i,\,w\to l} - \Delta H^0_{j,\,w\to l})/(\Delta S^0_{i,\,w\to l} - \Delta S^0_{j,\,w\to l}). \tag{10}$$

That is, the sequence of partition coefficients (hence possibly the sequence of permeability coefficients also) can change with temperature. For the temperature range and solutes we studied, two inversions or crossovers with temperature were observed: between *t*-butanol and *n*-propanol at $T_{\text{iso}} =$ 45 °C (from Table 3 and Eq. (10), $T_{\text{iso}} = (3{,}800 - 1{,}480)/(12.8 - 5.49) =$ 318° K; $K_{n\text{-propanol}} > K_{t\text{-butanol}}$ below this temperature, vice versa above this temperature); and between urea and methanol at $T_{\text{iso}} = 32$ °C.

Free Energies, Enthalpies and Entropies of Solution

Values of the thermodynamic state functions of partition, $F^0_{w\to l}$, $H^0_{w\to l}$ and $S^0_{w\to l}$, depend both upon interactions between solute and water and upon interactions between solute and lipid. In order to obtain quantities that can be discussed solely in terms of solute-lipid interactions, the contributions of solute-water interactions must be removed. This can be accomplished by referring to the following relationships:

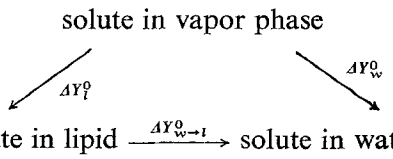


Table 4. Free energies, enthalpies and entropies of hydration at 25 °C

Solute	ΔF_w^0 (cal/mole)	ΔH_w^0 (cal/mole)	ΔS_w^0 (cal/mole, °K)
Methanol	710	−11,240	−40.2
Ethanol	810	−12,880	−46.0
n-Propanol	1,000	−14,420	−51.7
Isopropanol	1,070	−13,450	−48.7
n-Butanol	1,110	−15,940	−57.2
t-Butanol	1,310	−14,440	−52.8
Ethylene glycol	−1,830	—	—
Glycerol	−3,390	−24,730	−72.0
Ethyl acetate	2,730	−11,710	−48.3
Acetone	1,910	−10,090	−40.3

The quantities are the standard partial molar ΔF, ΔH and ΔS for transfer of the indicated solute from an ideal vapor phase at a partial pressure of 1 mm Hg to a hypothetical 1-molal aqueous solution obeying Henry's law. The values were calculated from Tables 1 and 2 of Butler (1937) by multiplying Butler's ΔF's by 1,000 and subtracting 2,380, and by adding 8.0 to his ΔS's (*see* Appendix).

Y stands for any thermodynamic state function whose change can be measured when one mole of solute is transferred between water, lipid and the vapor phase. ΔY_w^0 represents the standard partial molar free energy (enthalpy, or entropy) of solution in water or hydration, while ΔY_l^0 represents correspondingly the standard partial molar free energy (enthalpy, or entropy) of solution in lipid. Thus,

$$\Delta Y_{w\to l}^0 = Y_l^0 - Y_w^0$$

$$\Delta Y_l^0 = Y_l^0 - Y_v^0$$

$$\Delta Y_w^0 = Y_w^0 - Y_v^0$$

where Y_v^0 is the value of the function in the vapor phase. If $\Delta Y_{w\to l}^0$ can be extracted from partition measurements and if ΔY_w^0 is known independently, then ΔY_l^0 may be calculated as

$$\Delta Y_l^0 = \Delta Y_{w\to l}^0 + \Delta Y_w^0. \tag{11}$$

The functions $\Delta Y_{w\to l}^0$ for transfer from water to lipid are the ones given in Table 3 of this paper. The functions ΔY_w^0 for hydration were measured by Butler (1937, Tables 1 and 2) for ten of the solutes we studied, and are given in Table 4. Insertion of the values of Tables 3 and 4 into Eq. (11)

Table 5. Free energies, enthalpies and entropies of solution in lecithin at 25 °C

Solute	ΔF_l^0 (cal/mole)	ΔH_l^0 (cal/mole)	ΔS_l^0 (cal/mole, °K)
Methanol	1,650	− 6,870	−28.7
Ethanol	1,300	−10,140	−38.5
n-Propanol	840	−12,940	−46.2
Isopropanol	1,280	−10,310	−38.9
n-Butanol	430	−	−
t-Butanol	1,280	−10,640	−40.0
Ethylene glycol	− 550	−	−
Glycerol	−1,610	−17,360	−53.2
Ethyl acetate	2,180	− 9,940	−40.5
Acetone	1,880	−	−

The quantities are the standard partial molar ΔF, ΔH and ΔS for transfer of the indicated solute from an ideal vapor phase at a partial pressure of 1 mm Hg to a hypothetical 1-molal Henry's-law solution in fully hydrated dimyristoyl lecithin. The values were calculated by adding the ΔY_w^0 values of Table 4 to the $\Delta Y_{w\to l}^0$ values of Table 3 [*cf.* Eq. (11)].

yields the values listed in Table 5 for the free energy, enthalpy and entropy of solution in lipid; i.e., the functions for transfer of solute from the vapor phase into lipid. Insofar as intermolecular forces are negligible in the vapor phase[1], values of ΔF_l^0, ΔH_l^0 and ΔS_l^0 can be interpreted unequivocally in terms of the state of solute in the lipid phase[1,2,3]. These values refer to a lipid phase in equilibrium with water rather than with a vapor phase, but this is also the state that is of biological interest.

1 These assumptions are strictly valid only if solute concentrations are sufficiently low that the solution obeys Henry's law and that imperfections in the vapor phase are negligible. The mole fraction of solute was about 10^{-3} in Butler's (1937) experiments, $<2\times10^{-4}$ in lecithin and $<10^{-5}$ in water in our experiments, so that Henry's law is expected to be valid (Guggenheim, 1952).

2 Butler's (1937) values of ΔY_w^0 refer to water without added salt, whereas our values refer to 0.15 M KCl as the aqueous phase. However, the effect of this KCl concentration on ΔY_w^0 is negligible (Long & McDevit, 1952).

3 Macroscopic thermodynamic state functions may be rigorously defined and experimentally measured for the lecithin phase, even though local physical properties of the phase surely vary with position in the bilayer. This variation may, however, be very important for the physical interpretation of state function values in terms of local bilayer properties. For example, temperature dependence of solute distributional volumes within a lecithin bilayer may contribute a positive term to $\Delta H_{w\to l}^0$. That is, with increasing temperature, increasingly peripheral regions of the bilayer's hydrocarbon tails "melt" and become available to dissolve solutes (*cf.* pp. 137–138 of paper IV, and Dix, Diamond & Kivelson, 1974).

Barclay-Butler Constants for Solution in Lecithin

Barclay and Butler (1938) discovered empirically that if the transfer of different solutes from the vapor phase into the same solvent is studied, there is an approximately linear relation between the ΔH^0 and ΔS^0 of solution. This relation was discussed further by Frank and Evans (1945), who showed that the relation is expected to be valid for dilute solutions obeying Henry's Law. As will be seen, the values of the straight-line constants, which we shall term the Barclay-Butler constants, can be interpreted in terms of the structure of the solvent. Since the solute concentrations in our experiments (mole fractions $< 10^{-5}$ in water, $< 2 \times 10^{-4}$ in lipid) were well within the usual limits for validity of Henry's Law (mole fraction $< 10^{-2}$: Guggenheim, 1952), the Barclay-Butler relation might be expected to apply to ΔH_l^0 and ΔS_l^0 for lecithin. Inspection of the two right-hand columns of Table 5 shows that the entropy of solution in lecithin does in fact go increasingly negative as the enthalpy of solution goes negative. Fig. 5 (experimental points, and

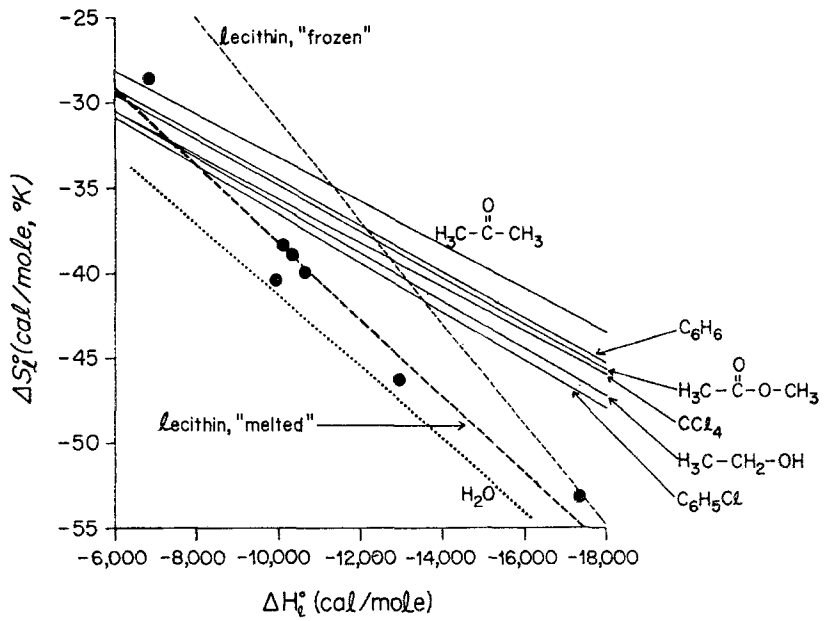


Fig. 5. Barclay-Butler relations for lecithin and bulk solvents compared. The experimental points are values of ΔS_l (the entropy of solution) and ΔH_l (the enthalpy of solution) for lecithin from Table 5, based on temperatures above the phase transition near 25 °C. Each point gives ΔS_l and ΔH_l for one solute. The heavy dashed line (labeled: lecithin, "melted") is the straight line of least-mean-squares fit. The six solid lines plot ΔS_l *vs.* ΔH_l for the six indicated bulk nonpolar solvents, based on the *a* and *b* values of Table 6. The dotted line is the corresponding line for water (ΔS_w *vs.* ΔH_w), also using the *a* and *b* values of Table 6. The light dashed line (labeled: lecithin, "frozen") is the tentative relation for lecithin below the phase-transition temperature, as calculated from Table 5 of paper IV

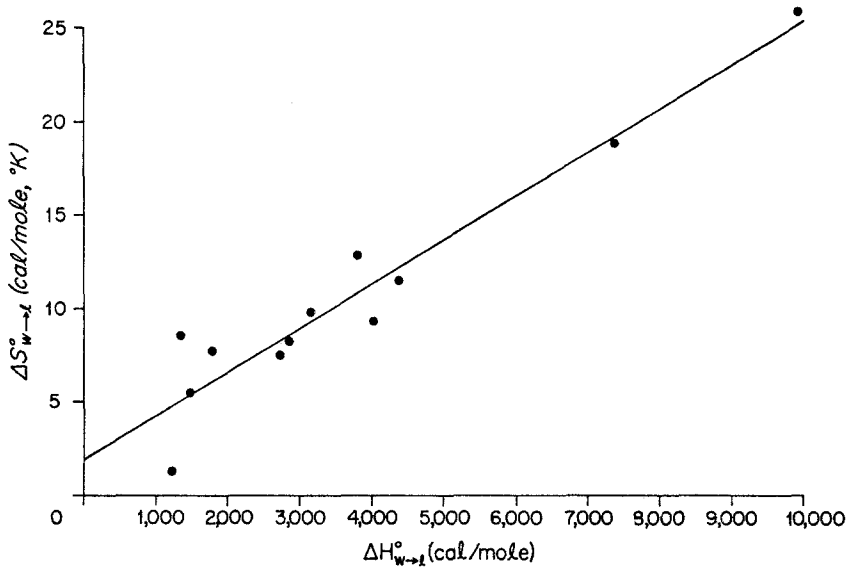


Fig. 6. The entropy of partition between dimyristoyl lecithin and water ($\Delta S^0_{w\to l}$) above 25 °C, plotted against the enthalpy of partition ($\Delta H^0_{w\to l}$). Each point represents one solute. $\Delta S^0_{w\to l}$ and $\Delta H^0_{w\to l}$ values are from Table 3. The straight line gives the least-mean-squares fit

heavy dashed curve captioned: lecithin, "melted") confirms the existence of an approximately linear Barclay-Butler relation for lecithin. The least-mean-squares linear fit is

$$\Delta S^0_l = -15.6 + 0.00226\,\Delta H^0_l \tag{12}$$

with a mean square error of 0.58 and correlation coefficient of 0.975.

Just as one can write a relation

$$\Delta S^0_l = a_l + b_l \Delta H^0_l \tag{13}$$

for the lipid phase, one can write a similar relation for the aqueous phase with different constants:

$$\Delta S^0_w = a_w + b_w \Delta H^0_w. \tag{14}$$

Inserting Eqs. (13) and (14) into Eq. (11) applied to ΔS^0 and to ΔH^0 yields

$$\Delta S^0_{w\to l} = a_l - a_w + \Delta H^0_l (b_l - b_w) + b_w \Delta H^0_{w\to l}. \tag{15}$$

From Eq. (15) it is apparent that a linear relation between $\Delta S^0_{w\to l}$ and $\Delta H^0_{w\to l}$ is not expected, unless either $b_w \Delta H^0_{w\to l} \gg \Delta H^0_l (b_1 - b_w)$ (e.g., because $b_l \doteq b_w$) or else there is a linear correlation between ΔH^0_l and ΔH^0_w (and hence between ΔH^0_l and $\Delta H^0_{w\to l}$). Fig. 6 shows that there is in fact a good

correlation between $\Delta S^0_{w\to l}$ and $\Delta H^0_{w\to l}$:

$$\Delta S^0_{w\to l} = 1.9 + 0.00235\,\Delta H^0_{w\to l} \tag{16}$$

with a mean square error of 0.52 and correlation coefficient of 0.956. The values of the constants b_1 (for lecithin) and b_w (for water) (Table 6) differ by less than 4%, so that in Eq. (15) the term $b_w \Delta H^0_{w\to l}$ is considerably larger than the term $\Delta H^0_l (b_l - b_w)$ for all solutes. Fig. 6 is based on almost twice as many solutes as Fig. 5. (The reason is that construction of Fig. 5 requires knowledge of ΔH^0_w and ΔS^0_w, which Butler determined for only seven of the solutes we studied.) Thus, the approximate linearity of Fig. 6 simply confirms the conclusion that followed more directly from Fig. 5 but was based there on fewer experimental points – viz., that the value of the Barclay-Butler slope b for lecithin is close to that for water.

Significance of the Barclay-Butler Relation

We consider here the possible significance of the Barclay-Butler relation for lecithin, while further discussion is reserved to the following paper (paper IV).

A physical interpretation of the Barclay-Butler relation for simple nonpolar solvents such as benzene may be given pictorially as follows (Barclay & Butler, 1938; Frank, 1945; Frank & Evans, 1945). When a molecule is taken from the liquid phase into the gas phase, work must be done against the intermolecular forces that hold it to its neighbors, so that the enthalpy of vaporization ($-\Delta H_l$) is positive and the enthalpy of solution (ΔH_l) is negative. The stronger the intermolecular forces, the more negative is ΔH_l; i.e., the deeper is the energy well into which the molecule condenses. The intermolecular forces in the liquid phase also restrict the molecule's rotational and translational freedom and its available free volume, so that the entropy of vaporization ($-\Delta S_l$) is positive and the entropy of solution (ΔS_l) is negative. The stronger the intermolecular forces, the more negative is ΔS_l; i.e., the greater is the loss of freedom when the molecule condenses. These considerations apply either to a pure liquid or to a solution.

Frank and Evans (1945, Table 3) have compiled values of ΔH and ΔS of vaporization for solutes in six nonpolar solvents. From these values converted to the units and standard states discussed in the Appendix, we have calculated the Barclay-Butler constants a and b for these six solvents as we have done in Eqs. (12) and (13) for lecithin. The six solvents vary over a range of only about 10% in their a and b values. The Barclay-Butler slope

Table 6. Barclay-Butler constants of solvents

Solvent	a (cal/mole, °K)	b $(°K)^{-1}$
Acetone	−20.6	0.00127
Carbon tetrachloride	−22.8	0.00129
Benzene	−21.3	0.00133
Methyl acetate	−21.4	0.00135
Ethanol	−22.0	0.00140
Chlorobenzene	−22.4	0.00142
Water	−20.1	0.00212
Dimyristoyl lecithin, >25 °C	−15.6	0.00226
(Dimyristoyl lecithin, <25 °C	− 1.3	0.00296)

Entropies and enthalpies of solution for various solutes in a given solvent were fitted to the equation $\Delta S_l^0 = a + b\,\Delta H_l^0$, and values of the constants a and b for each solvent were calculated by least-mean-squares. Standard states were taken as 1 mm Hg partial pressure of solute in the gas phase, a hypothetical Henry's-law 1-molal solution in the liquid. Values of ΔS_l^0 and ΔH_l^0 for the six solvents listed first were calculated from Frank and Evans (1945, Table 3) (all values refer to 25 °C except those for ethanol, which refer to 30 °C); values for water (at 25 °C), from Butler (1937, Tables 1 and 2), using the seven solutes for which Table 5 lists ΔH_l^0 and ΔS_l^0; values for lecithin above 25 °C, from Table 5; and values for lecithin below 25 °C, from paper IV (Table 5). The extracted a and b values for lecithin below 25 °C are put in parentheses to emphasize that they are tentative, based on limited data.

$b \equiv d\Delta S_l / d\Delta H_l$ is almost twice as large for lecithin as it is for these bulk nonpolar solvents. The value of the Barclay-Butler intercept a depends on the choice of concentration units, but the slope b does not. Use of mole fraction units instead of molal units would displace the "melted lecithin" curve of Fig. 5 towards the curves for bulk organic solvents (*see* Appendix for discussion of units).

As first recognized by Barclay and Butler (1938) and discussed in detail by Frank and Evans (1945), values of ΔS_w in water are considerably more negative than predicted by inserting ΔH_w into the Barclay-Butler equation for nonpolar solvents. In Table 6 we have determined the Barclay-Butler constants for water, using Butler's (1937) values of ΔS_w and ΔH_w for the same seven solutes which we used to extract the constants for lecithin. The Barclay-Butler slope b for water is similar to that for lecithin and higher than that for bulk nonpolar solvents. The strongly negative values of ΔS_w in water have been interpreted to mean that solutes with a weaker hydrogen-bonding ability than that of water permit adjacent water molecules to form hydrogen-bonded clusters, which are more stable adjacent to the solute than they would be if adjacent to other water molecules (Frank & Evans, 1945; Franks, 1965). Thus, the solute becomes surrounded by a molecular-

sized "iceberg" of ordered water, which is responsible for the extra decrease in entropy on adding the solute to water, hence the high b value.

The high b value of lecithin probably has a different explanation, namely, that a molecule suffers a much greater loss of mobility when it dissolves in a lecithin bilayer than in a bulk solvent. As documented by the spin-label experiments of Hubbell and McConnell (1971; *see also* McConnell & McFarland, 1970), the flexibility of the hydrocarbon tails in a phospholipid bilayer is low near the bilayer periphery and increases towards the terminal methyl groups. The behavior of spin-labeled solutes (Hubbell & McConnell, 1971, Fig. 5*b*) similarly indicates reduced solute mobility towards the bilayer periphery. This immobilization of solute molecules should cause an extra decrease in entropy when solutes dissolve in a bilayer compared to solution in a bulk solvent, and should therefore contribute to the high b value of lecithin[4]. Analysis of incremental state functions for solution of $-CH_2-$ groups in lecithin supports this picture (paper IV).

Appendix

Concentration Units and Standard States

Depending on whether one expresses concentrations as molalities, molarities, or mole fractions, different numerical values will be obtained for $\Delta F^0_{w\to l}$ and $\Delta S^0_{w\to l}$, and the hypothetical solution that serves as the standard state will differ. Most thermodynamic discussions of mixtures [including the papers by Barclay and Butler (1938) and by Frank and Evans (1945), from which we extract values on pp. 110–115 and in paper IV] find it advantageous to express concentrations as mole fractions (*cf.* Kauzmann, 1959). However, we use molal concentration units for several reasons:

1. The extensive K determinations by Collander (1947, 1949, 1950, 1951, 1954) for model solvents, with which we shall compare lecithin, are all expressed in these units. This is a minor consideration, since Collander's K's could of course be re-expressed in other units.

---

4 Two further factors may contribute. First, the value of b for a given solvent may depend somewhat on the choice of solutes used to calculate it, as suggested for water by Fig. 1 of Butler (1937) or Fig. 3 of Frank and Evans (1945) and for nonpolar solvents by the anomalous behavior of the solute SO_2 (Frank & Evans, 1945, Fig. 2). We used the same set of solutes to extract b for lecithin as for water, while the solutes available for calculating b for the six nonpolar solvents were themselves relatively nonpolar. Second, most of the partitioned solutes in lecithin may be located in a relatively hydrated portion of the lecithin molecule, as suggested by several other lines of evidence (paper IV). This effect could yield a value of b_{lecithin} intermediate between the b values of benzene and of water, but does not explain why b_{lecithin} equals or exceeds b_{water}.

2. K determinations for biological membranes of unknown structure and complex composition will necessarily be expressed in molal units, since the mole fraction scale cannot be used without knowledge of the "molecular weight" of the membrane. K's for other membranes and solvents being compared with biological membranes would have to be converted to the same units for this purpose.

3. Simpler relations between solvent structure and values of incremental state functions of solution or partition emerge with molal units than with mole fraction units (paper IV; Diamond & Wright, 1969, Table 1). For example, consider the incremental free energy of partition of the hydroxyl group, $\delta\Delta F^{OH}_{w\to l}$, from water to each of the following solvents, arranged in sequence of increasing hydrophobicity: C_4H_9OH, $C_5H_{11}OH$, $C_8H_{17}OH$, $C_{18}H_{35}OH$, and C_6H_6. The respective $\delta\Delta F^{OH}_{w\to l}$ values expressed in molal units form a monotonic sequence: 984, 1,179, 1,279, 1,604, and 4,280 cal/mole (calculated from Diamond & Wright, 1969, and from paper IV, Table 6). The respective $\delta\Delta F^{OH}_{w\to l}$ values expressed in mole fraction units do not form a sequence: 138, 213, 99, −12, and 3,404 cal/mole. Thus, as discussed in paper IV, measurements of incremental state functions for a membrane of unknown structure can be used to match the membrane's solvent properties to those of model solvents or membranes if one uses the molal scale, but not if one uses the mole fraction scale.

The $\Delta F^0_{w\to l}$ values in molal units, used throughout papers III and IV, can be converted to mole fraction units as follows. The conversion factor from molal concentration C (moles of solute per 1,000 g of solvent) to mole fractions X (moles of solute, divided by moles solute plus moles solvent) becomes, for dilute solutions,

$$C_{\text{solute}} = 1{,}000\, X_{\text{solute}}/M_{\text{solvent}} \tag{A.1}$$

where M is the molecular weight of the solvent. Combination of Eqs. (1), (3) and (A.1) yields

$$\Delta F^0_{w\to l} = -RT\ln(X_l/X_w) - RT\ln(M_w/M_l) \tag{A.2}$$

where X_1, M_1, X_w and M_w are the mole fraction of solute and the molecular weight of solvent in the lipid phase and aqueous phase, respectively, and $\Delta F^0_{w\to l}$ is still on a molal basis. Since the term $-RT\ln(M_w/M_l)$ is independent of the solute, it can be absorbed into a new definition of the difference in standard chemical potential:

$$\Delta F^0_{w\to l}(\text{m.f.}) = \Delta F^0_{w\to l}(\text{molal}) + RT\ln(M_w/M_l) \tag{A.3}$$

where $\Delta F^0_{w\to l}$ (molal) is the difference in standard chemical potential on a molal basis and $\Delta F^0_{w\to l}$ (m.f.) is the difference on a mole fraction basis. $\Delta F^0_{w\to l}$ (m.f.) has the physical meaning of the difference in chemical potential between two hypothetical mixtures (a solute-water mixture and a solute-lipid mixture), in each of which the solute has a mole fraction of 1 (i.e., a hypothetical solution of pure solute) but has the same physical properties as in infinitely dilute solution or as in a real solution obeying Henry's law (Klotz, 1950, chapter 19).

The value of $\Delta H^0_{w\to l}$ is independent of whether concentrations are expressed on a molar, molal or mole fraction basis. This can be seen by inserting $\Delta F^0_{w\to l}$ expressed on each basis into the Gibbs-Helmholtz relation, $\partial(\Delta F^0_{w\to l}/T)\,\partial T = -\Delta H^0_{w\to l}/T^2$.

Since the numerical values of $\Delta F^0_{w\to l}$ depend on the units of concentration chosen but the values of $\Delta H^0_{w\to l}$ do not, the values of $\Delta S^0_{w\to l}(=[\Delta H^0_{w\to l} - \Delta F^0_{w\to l}]/T)$ must also depend on the units of concentration.

The confusingly different standard states and units used by Butler (1937), Barclay and Butler (1938), Frank (1954) and Frank and Evans (1945), and by us may be reconciled as follows. Frank, Frank and Evans, and we express ΔH in cal/mole, ΔS in cal/mole, °K, while Barclay and Butler, and Butler express ΔH and $T\Delta S$ in kcal/mole, and Butler expresses ΔS in cal/mole, °K. Butler, Barclay and Butler, and we take as the standard state for solute in the gas phase a solute partial pressure of 1 mm Hg at 25 °C, while Frank and Evans, and Frank, use a partial pressure of 1 atmosphere. To convert ΔS^0 of vaporization as tabulated by Frank or Frank and Evans to the standard state used by the other authors, add 13.2 cal/mole, °K. Butler, Barclay and Butler, and Frank and Evans use the mole fraction concentration scale and take as the standard state for solute in the liquid phase a hypothetical solution with a solute mole fraction of 1 but with the same physical properties as in an infinitely dilute solution (as discussed on p. 107). We take a hypothetical 1-molal solution with these same physical properties for the solute standard state in the liquid phase. To convert a ΔF^0 of solution (e.g., ΔF^0_w or ΔF^0_l) from the mole-fraction standard state to the molal standard state, subtract $RT\ln(1{,}000/M_{\text{solvent}})$ where M_{solvent} is the molecular weight of the solvent. For example, we subtracted $RT\ln 1{,}000/18 = 2{,}380$ cal/mole at 25 °C to convert the mole-fraction ΔF^0_w values listed in Butler's Tables 1 and 2 (after conversion from kcal/mole to cal/mole) to the molal values in our Table 4. To convert a ΔS^0 of solution (e.g., ΔS^0_w or ΔS^0_l) from the mole-fraction standard state to the molal standard state, add $R\ln(1{,}000/M_{\text{solvent}})$. For example, we added $R\ln(1{,}000/18) = 8.0$ cal/mole, °K to Butler's values of ΔS^0_w to convert them

to the molal values in our Table 4. One must also beware of whether the state function tabulated is one of solution ($\Delta Y_w^0 = Y_w^0 - Y_v^0$, and $\Delta Y_l^0 = Y_l^0 - Y_v^0$) or one of vaporization ($Y_v^0 - Y_w^0 = -\Delta Y_w^0$, $Y_v^0 - Y_l^0 = -\Delta Y_l^0$). The quantities in our Tables 4 and 5 and in Butler's Tables 1 and 2 are of solution. Frank's Table 1, and Tables 3 and 4 of Frank and Evans, give ΔH and ΔS of vaporization. Tables 1, 2 and 4 of Barclay and Butler give ΔF of solution along with ΔH and ΔS of vaporization. In Table 2 of Butler (1937, p. 231), "ΔF" ($= \Delta F_w^0$) for ethylene glycol should be 0.55, not 5.50.

References

Barclay, I. M., Butler, J. A. V. 1938. The entropy of solution. *Trans. Faraday Soc.* **34**:1445

Butler, J. A. V. 1937. The energy and entropy of hydration of organic compounds. *Trans. Faraday Soc.* **33**:229

Collander, R. 1947. On "lipoid solubility". *Acta Physiol. Scand.* **13**:363

Collander, R. 1949. Die Verteilung organischer Verbindungen zwischen Äther und Wasser. *Acta Chem. Scand.* **3**:717

Collander, R. 1950. The distribution of organic compounds between isobutanol and water. *Acta Chem. Scand.* **4**:1085

Collander, R. 1951. The partition of organic compounds between higher alcohols and water. *Acta Chem. Scand.* **5**:774

Collander, R. 1954. The permeability of *Nitella* cells to non-electrolytes. *Physiol. Pl.* **7**:420

Diamond, J. M., Katz, Y. 1974. Interpretation of nonelectrolyte partition coefficients between dimyristoyl lecithin and water. *J. Membrane Biol.* **17**:121

Diamond, J. M., Wright, E. M. 1969. Biological membranes: The physical basis of ion and non-electrolyte selectivity. *Annu. Rev. Physiol.* **31**:581

Dix, J. A., Diamond, J. M., Kivelson, D. 1974. Translational diffusion coefficient and partition coefficient of a spin-labeled solute in lecithin bilayer membranes. *Proc. Nat. Acad. Sci.* **71**:474

Frank, H. S. 1945. Free volume and entropy in condensed systems. II. Entropy of vaporization in liquids and the pictorial theory of the liquid state. *J. Chem. Phys.* **13**:493

Frank, H. S., Evans, M. W. 1945. Free volume and entropy in condensed systems. III. Entropy in binary liquid mixtures; partial molal entropy in dilute solutions; structure and thermodynamics in aqueous electrolytes. *J. Chem. Phys.* **13**:507

Franks, F. 1965. Hydrophobic hydration and the effect of hydrogen bonding solutes on the structure of water. *Ann. N. Y. Acad. Sci.* **125**:277

Guggenheim, E. A. 1952. Mixtures. Clarendon Press, Oxford, England

Hubbell, W. J., McConnell, H. M. 1971. Molecular motion in spin-labeled phospholipids and membranes. *J. Amer. Chem. Soc.* **93**:314

Katz, Y., Diamond, J. M. 1974*a*. A method for measuring nonelectrolyte partition coefficients between liposomes and water. *J. Membrane Biol.* **17**:69

Katz, Y., Diamond, J. M. 1974*b*. Nonsolvent water in liposomes. *J. Membrane Biol.* **17**:87

Kauzmann, W. 1959. Some factors in the interpretation of protein denaturation. *Advanc. Protein Chem.* **14**:1

Klotz, I. M. 1950. Chemical Thermodynamics. Prentice-Hall, Englewood Cliffs, N.J.

Long, F. A., McDevit, W. F. 1952. Activity coefficients of nonelectrolyte solutes in aqueous salt solutions. *Chem. Rev.* **51**:119

McConnell, H. M., McFarland, B. G. 1970. The flexibility gradient in biological membranes. *Quart. Rev. Biophys.* 3:91