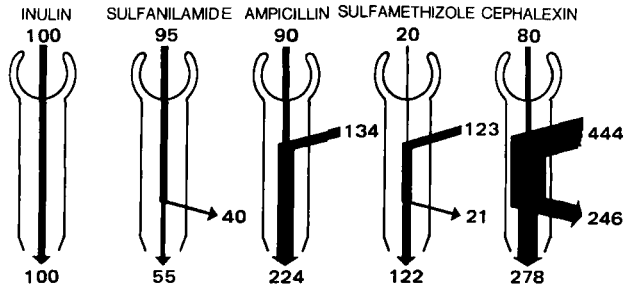

Figure 9—*Schematic representation of renal handling of drugs in the human. The parameters in Table III are used; detailed information can be found in the text.*

typical active reabsorption pattern such as that found for glucose (18). Therefore, the contribution of a nonlinear reabsorption process would not be significant under the conditions used here even if an active-like process were to be included in its reabsorption.

Figure 9 shows a schematic representation of the renal handling of these drugs in humans compared with that of inulin. The numbers given, calculated using a plasma drug concentration of 5 μM and glomerular filtration rate of 120 ml/min, represent the rate (in percent) of the designated transport process (*i.e.*, filtration, secretion, reabsorption, and urinary excretion) normalized for inulin glomerular filtration (600 nmoles/min). Plasma concentration of drugs were corrected by protein-binding percentage. Thus, for example, since sulfanilamide is 5% protein-bound in plasma, its filtration rate is 95% that of inulin (95% of 600 = 570 nmoles/min). Similarly, reabsorption of sulfanilamide occurs at a rate 40% of inulin filtration, *i.e.*, at 40% of 600 = 240 nmoles/min. It is evident that tubular secretion and reabsorption would be the most important process in regulating the urinary excretion of sulfamethizole, cephalexin, and ampicillin. If renal functions, such as renal secretion, were decreased by renal failure, the renal excretion of sulfamethizole, cephalexin, and ampicillin would be reduced greatly resulting in a high concentration in the blood and target organ. The impact of renal failure on the process of filtration, secretion, and reabsorption should be known in order to provide optimal treatment and protection from adverse reactions in patients with this disease state. We have applied the method described in this paper to the analysis of renal handling of drugs in patients with renal failure, the results of which will be discussed in an ensuing manuscript.

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Thermodynamics of Distribution of *p*-Substituted Phenols Between Aqueous Solution and Organic Solvents and Phospholipid Vesicles

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Abstract □ The distribution of *p*-substituted phenols between 0.15 *M* NaCl and a range of organic solvents (including 1-octanol) was examined over a range of temperatures. The thermodynamic parameters of transfer, ΔG, ΔH, and ΔS, were determined and the values examined in the light of Hildebrand and Scott's solubility parameter theory, and the collision complexes between solute and organic solvent. ΔH of transfer was positive for nonpolar solvents and negative for 1-octanol; the transfer processes were entropy and enthalpy dominated, respectively. The distribution of the phenols into phospholipid vesicles was examined below the phase-transition temperature. Although ΔG of transfer for vesicle–water systems was similar to that for octanol–water systems, the full thermodynamic analysis indicated that the two systems were dissimilar. The use of vesicle distribution data in structure–activity studies is discussed.

Keyphrases □ *p*-Substituted phenols—partitioning between water and organic solvents, phospholipid vesicles, thermodynamics □ Phospholipid vesicles—partitioning of *p*-substituted phenols, water and organic solvents, thermodynamics □ Thermodynamics—*p*-substituted phenols, partitioning, water and organic solvents, phospholipid vesicles

The importance of hydrophobicity in drug absorption, drug binding, and drug-receptor site interactions is well known (1, 2). Some measure of the hydrophobicity of a solute is given by the distribution (partition) coefficient between water and a suitable organic solvent (3). Usually the choice of the organic phase has been 1-octanol; however, Rytting *et al.* (4) have argued from a thermodynamic standpoint that a nonpolar inert solvent such as isooctane or cyclohexane would be a more appropriate solvent for distribution studies.

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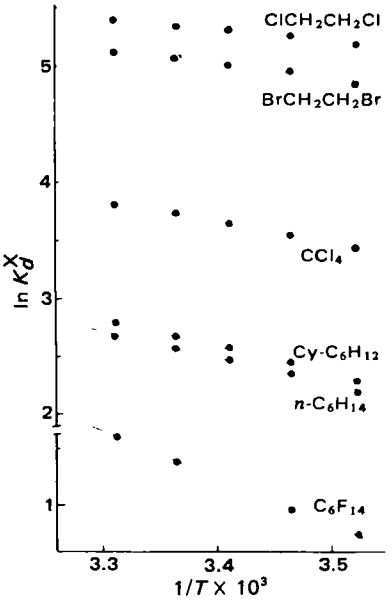

Figure 1—*van't Hoff plots for* p-*ethylphenol distributed between 0.15 M aqueous sodium chloride solution and various nonpolar organic solvents.*

In spite of the wide usage of 1-octanol–water distribution coefficients in structure–activity studies, relatively little work has been done to establish the actual basis of the relationship between distribution with octanol and biological activity (5). Martin (6) has emphasized that various absorption phenomena involving the passage of drugs across biological membranes are not well modeled by 1-octanol–water distribution coefficients. Other groups have suggested that the phospholipid vesicle (liposome) can be used as a model system to study solute distribution into membranes (7–9). Phospholipid vesicles consist of lipid bilayers in the form of multilayers arranged concentrically, or as simple bilayers encapsulating a volume of aqueous medium in the core. The vesicle can exist in liquid-crystalline or solid forms depending on the choice of the constituent phosphatidylcholine(s). Bindsley and Wright (10) have concluded that permeation rates of solutes across the toad bladder were more closely related to vesicle–water distribution data when the vesicles were below their phase-transition temperature (T_c).

The present investigation was designed to evaluate various organic solvents (including 1-octanol) as models for the distribution of solutes into membranes. The distribution of various *p*-substituted phenols between water and different organic phases as well as phospholipid vesicles were studied. The solvents were selected to give a wide range of solvent properties (solubility parameters). Data were obtained at different temperatures, allowing the calculation of the relevant thermodynamic parameters, enthalpy and entropy, which provide insight into the mechanism of solute transfer.

THEORETICAL

Organic Solvent–Water Distribution—The distribution ratio of solute between two immiscible solvents is given by:

$$D = \frac{C_{A,org}}{C_{A,aq}} \qquad \text{(Eq. 1)}$$

where $C_{A,org}$ and $C_{A,aq}$ are the total solute concentration in the organic and aqueous phases respectively. When only monomeric and un-ionized solute exist in both phases, Eq. 1 can be written as:

$$K_d = D = \frac{[\text{Solute}]_{org}}{[\text{Solute}]_{aq}} \qquad \text{(Eq. 2)}$$

The thermodynamic distribution constant K_d^X (mole fraction) (concentration units) can be written as:

$$K_d^X = K_d \left(\frac{V_{org}}{V_{aq}}\right) \qquad \text{(Eq. 3)}$$

where V_{org} and V_{aq} are the molar volumes of the organic solvent and aqueous phase. The distribution constant (K_d^X) is a free-energy (ΔG) related term:

$$\Delta G_{(transfer)} = -RT \ln K_d^X \qquad \text{(Eq. 4)}$$

As such it yields little information about the nature of the transfer process, and a more complete thermodynamic picture can be obtained by studying the change of the distribution coefficient with temperature, to provide enthalpic and entropic contributions to the free energy of transfer (11–15):

$$\ln K_d^X = -\frac{\Delta H}{R}\frac{1}{T} + \frac{\Delta S}{R} \qquad \text{(Eq. 5)}$$

where ΔG, ΔH, and ΔS are the partial molal free energy, enthalpy, and entropy change on transfer of the solute. The parameters ΔH and ΔS can be calculated from the slope and intercept of a plot of $\ln K_d^X$ *versus* $1/T$.

Phospholipid Vesicles: Water Distribution—The molal concentration scale has to be used because of the heterogeneous nature of the system under investigation (7). [A useful appraisal is the choice of concentration scale/standard state in bioenergetics and thermodynamics has been given by Jameson (16).]

The molal concentrations of solutes in the lipid (vesicle) phase (C_0^m) and the aqueous phase (C_w^m) are given by:

$$C_0^m = \frac{(C_T - C_W)w_1}{dMw_2} \qquad \text{(Eq. 6)}$$

$$C_w^m = \frac{C_W}{dM} \qquad \text{(Eq. 7)}$$

Table I—Thermodynamic Values for the Distribution of *p*-Substituted Phenol between 0.15 *M* NaCl and Organic Solvents at 25°

Organic Solvent	δ[d]	X[a]	$-\Delta G$[b]	ΔH[b]	ΔS[c]
C_6F_{14}	5.6	CH_3	−3.91	32	94
		C_2H_5	−1.46	35	111
		C_3H_7	1.07	32	111
n-C_6H_{14}	7.4	H	−0.15	21	69
		CH_3	3.18	19	74
		C_2H_5	6.39	19	85
		C_3H_7	10.00	18	95
		Cl	2.96	16	63
Cy-C_6H_{12}	8.2	H	0.03	21	70
		CH_3	3.29	21	80
		C_2H_5	6.67	20	91
		C_3H_7	10.4	19	99
		Cl	3.24	16	66
CCl_4	8.6	H	2.48	15	58
		CH_3	5.94	14	67
		C_2H_5	9.26	15	83
		C_3H_7	12.8	14	90
		Cl	6.03	13	63
$ClCH_2CH_2Cl$	9.8	H	7.39	7	50
		CH_3	10.4	9	64
		C_2H_5	13.3	8	73
		Cl	11.0	4	51
$BrCH_2CH_2Br$	10.4	H	6.42	9	51
		CH_3	9.70	10	66
		C_2H_5	12.6	10	78
		Cl	10.3	6	55
1-$C_8H_{17}OH$[e]	10.3	H	14.07	−7	23
		CH_3	16.54	−7	30
		C_2H_5	18.99	−8	36
		Cl[f]	19.05	−16	10

[a] X is the substituent of *p*-position of phenol. [b] ΔG and ΔH are given in kJ mole^{-1}. [c] ΔS is given in J mole^{-1}°K^{-1}. [d] δ-Solubility parameter in Cal$^{1/2}$cm$^{-3/2}$ from ref. 20. [e] Mean values taken from ref. 13 and F. J. C. Dearden and G. M. Bresnen, *J. Pharm. Pharmac. Suppl.* 107P (1981).

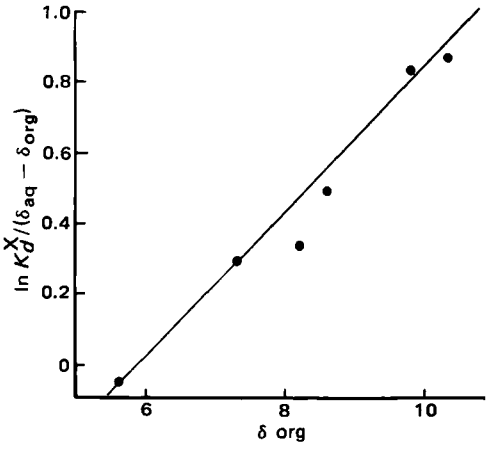

Figure 2—*The analysis of distribution data using solubility parameter theory.*

where C_T is the initial aqueous concentration before equilibrium (mg/ml), C_W is the final aqueous concentration after equilibrium (mg/ml), d is the density of the initial aqueous phase, M is the molecular weight of the solute, w_1 is the weight of aqueous phase in the sample, and w_2 is the weight of phospholipid in the sample. Thus, the molal distribution coefficient K_d^m is given by:

$$K_d^m = \frac{(C_T - C_W)w_1}{C_W w_2} \qquad \text{(Eq. 8)}$$

EXPERIMENTAL

Chemicals—Water was distilled from an all-glass still. The phenolic solutes used were as follows: phenol[1] (99.99%) was used without further purification; *p*-cresol[2], *p*-ethylphenol[2], *p*-chlorophenol[3], *p*-bromophenol[3], *p*-iodophenol[4], and *p*-fluorophenol[4] were used after recrystallization; *p*-propylphenol[4] was used after double distillation; and *p*-chloroanisole[3], resorcinol[3], *m*-methoxyphenol[2], and *m*-ethoxyphenol[2] were used as received.

Aqueous solutions of the phenols (~2 mg/100 ml) were prepared by dissolving samples in 0.15 *M* NaCl solution. The organic solvents used were perfluorohexane[1], *n*-hexane (special for spectroscopy)[3], cyclohexane (analytical grade)[3], 1,2-dichloroethane[4] (>99%) and 1,2-dibromoethane[4] (99%), carbon tetrachloride[5], and 1-octanol[4]. When necessary, they were washed successively with sodium hydroxide solution, distilled water, concentrated sulfuric acid, and finally 5 times with distilled water. L-α-Dimyristoylphosphatidylcholine[6] (DMPC) (98%) was used throughout as the sole lipid in the preparation of phospholipid vesicles.

Thin-layer chromatography of DMPC produced a single spot. Chloroform[1] was reagent grade.

Organic Solvent–Water Distribution—Oil–water distribution coefficients were obtained using the shake flask technique or a rapid mix/filter probe system (14, 17). The aqueous and organic phases were mutually saturated before use. Each experimental arrangement was thermostated at a given temperature (±0.1°), and at equilibrium the concentration of the solute in the aqueous phase was determined from the UV absorbance measured in the linear region of the Beer–Lambert plot.

Phospholipid Vesicle–Water Distribution—The vesicles were prepared using a stock solution of DMPC in chloroform containing 20-mg DMPC. This solution was placed in tared 50-ml round-bottom flasks and the chloroform removed rapidly by rotary evaporation at ~40°. The weight of the dried lipid film was measured, and multilamellar vesicles were formed at 40° by transferring 5 ml of highly dilute stock solution of phenol derivative into the flask and swirling the contents. Samples were prepared in duplicate, and the mean size and polydispersity of the vesicles were measured using a laser light scattering method (photon correlation spectroscopy)[7]. Reproducible yields of vesicles could be prepared by controlling strictly the hydration time and method of agitation.

[1] British Drug Houses.
[2] Eastman Kodak Co.
[3] Aldrich Chemical Co.
[4] Bristol Organic Ltd.
[5] Hopkins and Williams Co.
[6] Sigma Chemical Co.
[7] Malvern Instruments.

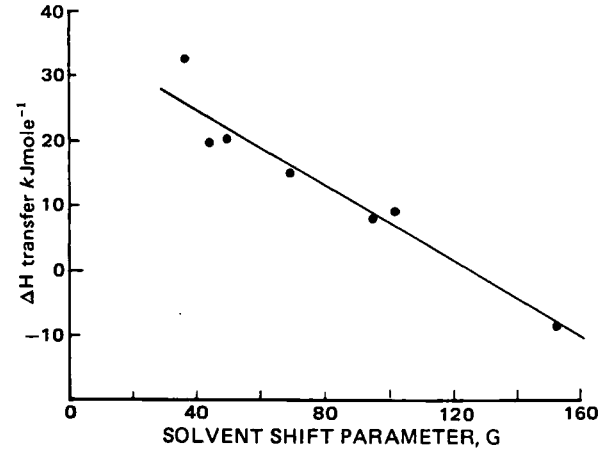

Figure 3—*The relationship between the enthalpy of transfer of* p-*substituted phenols and the solvent shift parameter* P *(Eq. 10).*

An equilibrium time for distribution of 12 hr was established from preliminary studies. The DMPC vesicles were separated from the aqueous phase by centrifugation in a thermostated centrifuge (8). The UV absorbance of the supernatant aqueous phase was measured at the λ_{max} of the phenol. After analysis the solution was returned to the phospholipid vesicle pellet and the system redispersed and re-equilibrated at the next temperature.

The processes of centrifugation, followed by resuspension did not alter significantly the mean size or polydispersity of the vesicles. Distribution studies were conducted over the temperature range 5–22° below the phase transition temperature of the phospholipid (23°) (18).

RESULTS AND DISCUSSION

Organic Solvent–Water Distribution—Preliminary studies indicated that for $C_{A,org}$ of less than 5×10^{-3} *M* the phenols existed as monomers in the organic phase. This is confirmed by literature data (19). Some typical plots of $\ln K_d^X$ *versus* $1/T$ are shown in Fig. 1. Good linearity was obtained in all cases, indicating that ΔH was independent of temperature over the range studied. Thermodynamic values obtained or calculated at 25° are summarized in Table I. The agreement with literature values (11, 13) including those obtained using microcalorimetry (15) is good in most cases, but there is some variation in ΔH (and consequently ΔS values). This is to be expected, since it is well known that the derivation of thermodynamic values from the van't Hoff isochore is dependent on good experimental data.

Examination of Fig. 1 and Table I shows clearly that the thermodynamic values of the transfer of phenols between aqueous environment and organic phases are dependent not only on the nature of the solute but also on the solvent. ΔG of transfer is positive for the lower alkyl phenols in perfluorohexane and for phenol in hexane. An increase in the distribution of the solute into the organic phase (ΔG becoming negative and increasing in magnitude) is accompanied by a decrease in ΔH and

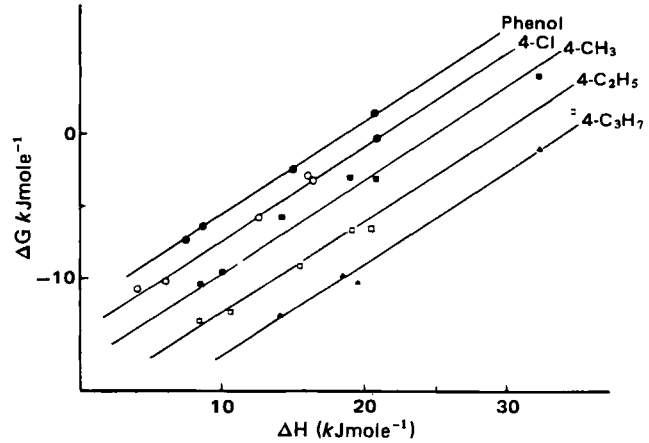

Figure 4—*Evaluation of compensation behavior. Enthalpy–free energy relationship for* p-*substituted phenols partitioned between 0.15* M *aqueous NaCl and nonpolar organic solvents.*

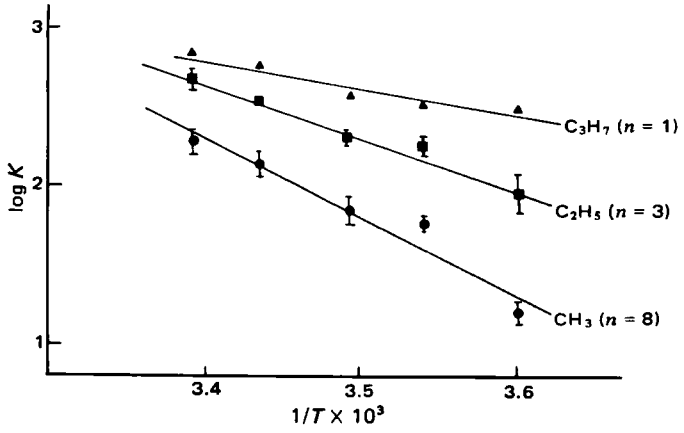

Figure 5—*The distribution of* p-*alkylphenols into DMPC vesicles—van't Hoff plots.*

a corresponding decrease in ΔS. 1-Octanol with a high extractive capacity provides large negative ΔH values and relatively small ΔS values. Such thermodynamic values reflect the important differences between the nature of the distribution process for different solvents. For inert hydrocarbon solvents, the distribution process reflects the loss of hydrogen bonding interactions between the hydroxyl group of the phenols and the aqueous phase that is compensated (in some cases) by an increase in ΔS. Any interaction between the phenolic hydroxyl group and the solvent [as well as the increased water content of the solvent and hydration of the monomeric phenol in the organic phase (21, 22)] would also be expected to lead to a decrease in the change in ΔH and a corresponding decrease in the change in ΔS. For 1-octanol, strong hydrogen bonding between the phenolic and alcohol hydroxyl groups will lead to a large and favorable ΔH (15). Consequently, control of the transfer passes from entropy domination to enthalpy domination.

When the concentration of a solute is sufficiently low in both phases, the distribution coefficient of solute distributed between water and a nonpolar organic solvent can be defined according to the theory of regular solutions (20):

$$\ln K_d^X = \frac{V_A}{RT}(\delta_{aq} - \delta_{org})(\delta_{aq} + \delta_{org} - 2\delta_A) \qquad \text{(Eq. 9)}$$

where V_A and δ_A are the molar volume and solubility parameter of solute A; δ_{aq} and δ_{org} are the solubility parameters of the aqueous solution and the organic solvent, respectively. Thus, a plot of $\ln K_d^X/(\delta_{aq} - \delta_{org})$ against δ_{org} should yield a straight line with a theoretical slope of V_A/RT. As shown in Fig. 2, using *p*-ethylphenol as an example, a linear relationship is obtained. The slope of the line is 0.206, in good agreement with the theoretical value of 0.203 calculated using $V_A = 120$ cm^3. A value of $\delta_{aq} = 16.5$ was employed rather than the usual value of 24. Davis *et al.* (23) and others (24) have shown previously that the lower value for δ_{aq} is valid for use in distribution studies involving organic solvents and aqueous phases.

Solvent effects can be considered also in terms of collision complexes between the phenolic solutes and the solvent. The aromatic carbon-hydrogen (A—H) stretching frequency in the IR is sensitive to hydrogen bond formation. Solvent shift theories and experimental studies on solvent effects have been reviewed (25). The frequency shift ΔV can be represented as the difference between the stretching frequency for the monomeric A—H in the vapor phase (V_0) and the lowered stretching frequency for A—H . . . B in the solvent in question (V_s). Badger and Bauer in 1937 (26) proposed that a linear relationship existed between the enthalpy of hydrogen bond formation and the frequency shift of the A—H stretching vibration.

In Fig. 3, the enthalpy of transfer has been plotted against the solvent shift parameter *P* (27) defined as:

$$\frac{V_0 - V_s}{V_0} = a\,P \qquad \text{(Eq. 10)}$$

The *P* values have been taken from literature sources (25, 27) on phenol in different solvents or calculated from values presented by Nakanishi *et al.* (28) for methanol and butanol systems[8]. The correlation between ΔH and *P* is good even though the *P* values refer to the anhydrous condition, and the phenols may be hydrated in the organic phase chosen for the distribution system.

[8] It should be noted that the *P* values were derived for anhydrous conditions, and the hydration of phenol in the organic phase may well alter the absolute values.

Table II—Thermodynamic Values for the Distribution of *p*-Substituted Phenols between 0.15 *M* NaCl and DMPC Vesicles at 22°

X[a]	−ΔG[b]	ΔH[b]	ΔS[c]
4-CH_3	13.3	119	448
4-C_2H_5	14.8	81	324
4-C_3H_7	15.5	31	157
4-F	12.4	37	168
4-Cl	14.6	19	112
4-Br	15.7	17	111
4-Cl anisole	17.9	102	405
3-OH	14.1	67	273
3-OCH_3	13.5	130	485
3-OC_2H_5	13.0	102	390

[a] Substituted phenol. [b] ΔG and ΔH are given in *k*J mole^{-1}. [c] ΔS is given in J mole^{-1}°K^{-1}.

Although there is no satisfactory treatment to account for solvent shifts of stretching frequencies, there is general agreement that the bulk properties of the solvent such as dielectric constant are important only for nonpolar solvents (25). Complexes between phenols and nonpolar solvents have been referred to as collision complexes to emphasize that although the interaction is very weak, it is sufficient to influence the IR spectrum (25).

Procedures for converting thermodynamic data obtained in polar solvents to those expected in an inert solvent have been proposed (25). One method is based on the assumption that the enthalpy of transfer can be written as (25):

$$-\Delta H\ (\text{nonpolar media}) = -\Delta H\ (\text{polar media}) + A \qquad \text{(Eq. 11)}$$

where *A* is a constant for the solvent pair. A free-energy related equation of similar type was proposed by Leo *et al.* (3) for the interconversion of distribution coefficient data obtained with nonpolar solvents and those obtained with octanol:

$$\log K_{d,\text{solvent}} + I_h = \log K_{d,\text{octanol}} + b \qquad \text{(Eq. 12)}$$

where I_h were additive increments to hydrogen bonding.

The use of enthalpy–entropy compensation plots has been exploited when the thermodynamic data have been evaluated. [Proportionality between ΔH and ΔS is believed to imply a single unique mechanism for a series of solutes or solvents (29).] However, when ΔH and ΔS are both derived from the van't Hoff relationship, good correlations can be obtained that arise from statistical artifacts. Krug *et al.* (30, 31) have discussed this in detail, and more recently Kinkel *et al.* (14) have shown that a so-called excellent correlation between ΔH and ΔS for the transfer of various phenols between water and 1-octanol (13) was spurious. Enthalpy–entropy compensation can be tested by plotting ΔH *versus* ΔG at the harmonic mean temperature of the experiments (31). Figure 4 shows the ΔH *versus* ΔG relationship for the various solvent systems examined. A relationship of the form:

$$\Delta G^X = 0.7\,\Delta H^X - B \qquad \text{(Eq. 13)}$$

can be obtained, where *B* is a constant that is related to the size of the phenolic solute. Similar compensation relationships have been reported by others (14, 32). (No linear relationships were observed when ΔG was plotted *versus* ΔH for a set of solutes and a given solvent.)

The linear relationship defined by Eq. 13 suggests that the various solutes are distributed between water and the various inert solvents by the same mechanism; *i.e.*, that the aqueous phase provides the major driving force for the transfer process: the hydrophobic effect. The ΔG and ΔH values for the 1-octanol system have not been shown. The values given in Table I deviate from the linear relationship. The ΔH values corresponding to measured ΔG values are much larger than would be predicted from Eq. 13.

For example:

Phenol	0.4 kJ mole^{-1}
Cresol	0.8 kJ mole^{-1}
p-Ethylphenol	1.0 kJ mole^{-1}
p-Propylphenol	1.5 kJ mole^{-1}
p-Chlorophenol	1.0 kJ mole^{-1}

These data indicate that the transfer of phenolic solutes between water and 1-octanol follows a different mechanism than that for the transfer

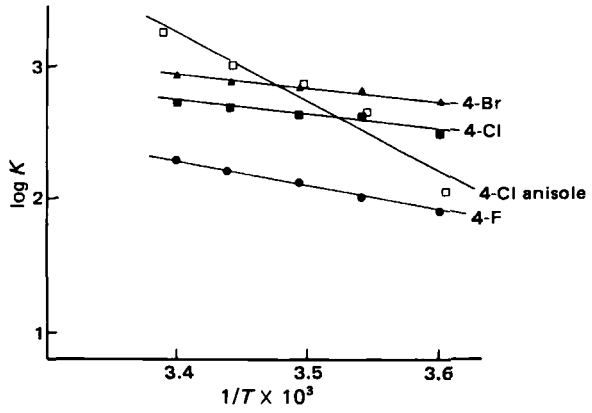

Figure 6—*The distribution of* p-*halophenols into DMPC vesicles—van't Hoff plots.*

of the same solutes between water and nonpolar solvents and, thus, ΔH–ΔS compensation is not obtained. A similar finding was reported recently by Kinkel *et al.* (14). These authors pointed out that mutually saturated 1-octanol contains a considerable quantity of dissolved water (27% on a mole fraction basis) and that water-centered aggregates (4:1 alcohol–water) have been described (5). In addition, linear aliphatic alcohols can exist in polymeric forms (33). Changes in temperature will affect these associations as well as the association between phenolic solutes and 1-octanol itself. Because of these factors, it was suggested (14) that the van't Hoff method should not be used to obtain thermodynamic quantities from the 1-octanol–water system, and microcalorimetry was proposed as an alternative. This is an attractive possibility provided the methodology can be refined to give reliable results. An alternative suggestion would be to avoid the use of 1-octanol when studying the thermodynamics of the distribution process.

Phospholipid Vesicle–Water Distribution—The temperature dependence of the molal distribution coefficient (K_d^m) for *p*-substituted phenols distributed between DMPC vesicles (below their phase transition temperatures) and 0.15 *M* NaCl is shown in Figs. 5 and 6.

The derived thermodynamic quantities are given in Table II. All values refer to initial phenol concentrations of 2×10^{-4} *M* and 20 mg of DMPC. The ΔH is large and positive for all solutes studied. The negative ΔG is the result of a large compensating increase in entropy: the process is entropy dominated. The magnitude of the absolute values is dependent on the nature of the experimental system used, and different values can result from using different phenol concentrations, phospholipid concentration, and experimental protocol. Thus, the data should be regarded as giving a qualitative rather than a precise quantitative picture (34).

In both absolute and relative terms the log K_d^m values for the alkyl and halophenols are more closely related to the distribution coefficients for the 1-octanol–water system rather than those for the so-called inert solvents such as cyclohexane. However, for the vesicles, ΔH is positive throughout which is similar to the situation for the nonpolar solvents and in direct contrast to the negative ΔH values found for the 1-octanol–water system.

Previously, Rogers and Davis (8) have reported that the thermodynamic parameters for the transfer of phenols between aqueous environment and DMPC vesicles change in sign and magnitude at the phase transition temperature. Above T_c, ΔH and ΔS are both negative. Similar changes in thermodynamic parameters at temperatures in the region of T_c have been reported by others (7, 9, 35). Diamond and Katz (7) interpreted observed enthalpy changes by proposing that in the crystalline state below T_c the hydrocarbon tails of DMPC pack closely and uniformly along their length. Thus, insertion of a solute into the membrane requires the breaking of strong intermolecular forces between the hydrocarbon tails, and entropy changes can be attributed to the disruption of the orderly crystalline array by the inserted solute. Thus, large and compensating changes in ΔH and ΔS attributable to the change in liposome structure can mask the smaller changes in ΔH and ΔS due to the actual transfer of the solute. In this respect, Lumry (36) has warned recently that ΔH and ΔS quantities each contain two parts: one (motive) that contributes to the free energy (of transfer) and one that does not (compensation) but that reflects enthalpy and entropy fluctuations. In water and most biological systems these compensation effects can dominate the motive (work-doing) contribution. Consequently, discrepancies can arise in attempts to formulate mechanistic hypotheses using ΔH and ΔS information.

The phospholipid vesicles are isotropic and will have markedly different polarity regions; consequently, the different phenolic solutes are likely to distribute into different regions. This will affect both the distribution coefficient and the thermodynamics of transfer. The high log K_d^m values obtained for the phenols with phospholipid vesicle systems indicate that the phenols could be hydrogen bonded to the phosphate group of DMPC. Comparison of the data obtained for chloroanisole with those for chlorophenol (Fig. 6) indicates that ΔH for the anisole, where there can be no hydrogen bonding, is much larger. At lower temperatures the anisole has a lower K_d^m value than the phenol, the K_d^m value being much more temperature dependent than for the phenol. The hypothesis that the phenolic hydroxyl group can (hydrogen) bond to the DMPC phosphate group was further strengthened by studies on the resorcinol monoethers. Again the hydroxy compound has a lower ΔH value than the corresponding methoxy or ethoxy compound (Table II). This suggests that the resorcinol is able to interact favorably with the phospholipid head group through both of its phenolic hydroxyl groups and that the orientation of these compounds in the lipid bilayer is quite different from that of the alkyl phenols. The ΔH values for the resorcinols were all positive (67–140 kJ mole^{-1}), whereas Beezer *et al.* (12) have shown for the 1-octanol–water system that ΔH is negative and in the range of −15 to −7 kJ mole^{-1}.

The ability of a solute-like phenol to undergo specific interactions with the bilayer membrane calls into question the use of inert hydrocarbons as model solvents for use in correlating structure with biological activity, even though inert hydrocarbons do find a role as reference states (4) and data obtained using such solvents can be converted to values more relevant to polar environments (37). A reasonable correlation can be obtained between liposome–water partition coefficients and octanol–water partition coefficients (7, 8). However, the derivation of the more complete thermodynamic picture in terms of ΔH and ΔS values indicates that the two systems are very different.

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Influence of Premicellar and Micellar Association on the Reactivity of Methylprednisolone 21-Hemiesters in Aqueous Solution

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Abstract □ Self-association of drug molecules at formulation concentrations can have a major impact on formulation properties. In this study a homologous series of methylprednisolone 21-hemiesters were found to undergo self-association in aqueous solution. The effect of aggregate formation on the solution degradation of these compounds was examined. To determine the nature and extent of association of these steroidal esters, partition coefficients between butyronitrile and aqueous buffer (pH 8.5) were measured as a function of ester concentration. The partitioning data were found to be consistent with dimer formation at low concentration followed by true micelle formation at higher concentration. Chain length increases favored micelle formation, but appeared to have little effect on dimerization. The first-order rate constants for ester hydrolysis and 21 → 17 acyl migration in aqueous buffer (pH 8.5) were also found to be dependent on ester concentration. The kinetic data are consistent with a model which assumes stabilization by both dimer and micelle formation, the limiting factor at high concentration being the reactivity of the ester in the micelles. The degree of stabilization due to self-association was found to increase with chain length.

Keyphrases □ Methylprednisolone—synthesis of 21-hemiester homologues, influence of premicellar and micellar association on reactivity □ Steroids—methylprednisolone, synthesis of 21-hemiester homologues, influence of premicellar and micellar association on reactivity □ Association, micellar—influence on the reactivity of methylprednisolone 21-hemiesters □ Association, premicellar—influence on the reactivity of methylprednisolone 21-hemiesters

Self-association of hydrophobic drug molecules in aqueous solution can have a profound effect on formulation properties due to the reduced effective concentration of drug at high total concentration. Specifically, molecular aggregation may result in higher drug solubility, increased or decreased solution stability, or transient masking of local biological effects.

While there is voluminous literature on the effect of micelle-forming additives on the chemical reactivity of various substrates, very few cases have been reported in which a labile substrate itself forms molecular aggregates resulting in self-stabilization. The few studies which do exist suggest that reactivity can be significantly altered either favorably or unfavorably by substrate self-aggregation into micelles (1, 2). Premicellar aggregation has also been found to dramatically alter reactivity (3–5).

Steroidal molecules, particularly bile salts, are known to undergo self-association in aqueous solution to form aggregates varying in size from dimers to much larger oligomers (6–8). Self-association has also been observed for the corticosteroid methylprednisolone 21-phosphate (9). In this case a marked acceleration in reactivity in more concentrated solutions was attributed to micelle formation.

A recent study showed that methylprednisolone 21-succinate[1] decomposes initially in aqueous solution *via* two parallel pathways (10). In addition to the well-known ester hydrolysis reaction, acyl migration from the 21- to the 17-OH occurs at a rate comparable to hydrolysis (Scheme I).

Since it was suspected that methylprednisolone 21-succinate may self-associate at formulation concentrations, a study was initiated to determine: (*a*) the nature and extent of self-association, (*b*) the effect of aggregation on the solution kinetics, and (*c*) the effect of molecular modification (increasing the hydrophobicity through increases in hemiester chain length) on both the aggregation and kinetics. To determine unambiguously the nature and extent of self-association, a partitioning method was developed enabling calculation of the monomer concentration as a function of total concentration. The initial rates of ester hydrolysis and acyl migration as a function of concentration were then combined with the partitioning data to elucidate the relative reactivities of monomeric and aggregated species.

EXPERIMENTAL

Materials—All reagents and chemicals were either analytical reagent grade or known to be of high purity. Methylprednisolone 21-hemisucci-

[1] SOLU-MEDROL (Upjohn brand of methylprednisolone sodium succinate).

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