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FUNCTIONAL GROUP CONTRIBUTIONS TO THE PARTITIONING OF PHENOLS BETWEEN LIPOSOMES AND WATER

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Summary

The temperature dependency of the partitioning of *p*-alkylphenols and *p*-halophenols has been determined between dimyristoyl phosphatidylcholine liposomes and 0.15 M NaCl. Partition coefficients increased as a function of temperature below the endothermic phase transition temperature (T_c) of the phospholipid but decreased above this temperature. The transfer process was found to be entropy-dominated below and enthalpy-dominated above the T_c, although large negative entropy changes were observed. Regular changes in the thermodynamic functions, partition coefficients and functional group free energies occurred as a function of the alkyl chain length or size of the halogen substituent below but not above the T_c. This has tentatively been attributed to increased phenol-phospholipid interaction at the higher temperatures. The partitioning of *p*-fluorophenol behaved in a manner expected of fluorinated compounds, yielding relatively low partition coefficients, but it produced an additional effect of markedly lowering the T_c of dimyristoyl phosphatidylcholine. Good correlations of the partition coefficients in liposomes with those in bulk organic solvents and with molecular size of the solute have been obtained.

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Abbreviation used: DMPC, dimyristoyl phosphatidylcholine.

Supplementary data to this article are deposited with, and can be obtained from, Elsevier/North-Holland Biomedical Press B.V., BBA Data Deposition, P.O. Box 1345, 100 BN Amsterdam, The Netherlands. Reference should be made to No. BBA/DD/137/78740/598 (1980) 392. The supplementary information includes: linear regression analysis of plots of log K vs. $1/T$ for phenols in dimyristoyl phosphatidylcholine liposomes and correlation of partition coefficients of *p*-alkyl and *p*-halophenols in dimyristoyl phosphatidylcholine liposomes and bulk organic solvents.

Introduction

The ability of a drug to elicit a pharmacological action in the biological system in most cases requires that an interaction take place between the drug and the biological membrane. The response may be as a result of interactions between the drug and the components of the membrane per se, or the membrane may be only a permeating barrier to extracellular agents, and consequently, regulates the rate and extent of passage of materials into and out of the cell. An intense area of investigation for many years has been the search for a suitable model of the biological membrane which lends itself to physical and chemical analysis, which is simplified so that certain parameters may be controlled, and which closely resembles the structure of a biological membrane to the extent of what is presently known about the composition and architecture of this important tissue. Liposomes are now widely studied as model membranes because these units consist of lipid bilayers, either multilayers arranged concentrically or a single bilayer encasing a volume of aqueous medium in its core [1,2]. Thus, studies of the interactions of substances with liposomes may increase our understanding of the interactions of substances with biological membranes, the backbone of which is considered to be a phospholipid bilayer.

The permeability of biological membranes to various molecules with which they make contact is determined by physical factors including the partition coefficient, diffusion coefficient, membrane thickness and interfacial barriers. Except for very small molecules and ions, for which electrical forces may also play a role, and where lipid-independent permeation is observed, permeability is proportional to the product of the membrane partition and diffusion coefficients. The diffusion coefficient is not normally sensitive to small changes in molecular structure of a molecule, since $D \propto M^{-1/3}$ [3]. Where significant changes in permeability are observed when different molecules are compared, for example in a series of functional groups to a parent molecule, the influence is made mainly through changes in the partition coefficient.

The rules of partitioning as given by Meyer [4] and Overton [5], from correlations of nonspecific activity and oil-water partition coefficients, have found general agreement for many drugs. But, such relationships reveal very little concerning the mechanisms of membrane-drug interactions and drug activity. A re-examination of partition coefficients and cell membrane permeabilities by a few groups of workers has very significantly advanced our understanding of the effect of molecular structure on these properties. Collander [6—10] has reported partition coefficients of non-electrolyte organic compounds in oil-water systems as well as bacterial cells. A detailed study of molecular forces governing non-electrolyte permeation through cell membranes has been made by Diamond and Wright [11] from results obtained with the rabbit gall-bladder and this has been succeeded by the comprehensive review of partition coefficients and their uses by Leo et al. [12].

Investigations of the partitioning of solutes into liposomes may provide some insight into the role of the particular chemical structure of a molecule in determining the degree of interaction with membrane components. Some liposome partition coefficients have been reported [13—15], but the only extensive temperature-dependent study has been carried out by Katz and Diamond [16].

The influence of certain functional groups on the partition coefficient of a solute may be conveniently compared from the energetics of the process. Thus, these authors have reported results of the thermodynamics of transfer of water-soluble alcohols and amides [17], their solutes having partition coefficients of less than 10. In this report, partition coefficients of *p*-alkylphenols and *p*-halophenols in dimyristoyl phosphatidylcholine liposomes are examined over a temperature range which includes the phase transition temperature of this phospholipid. The partition coefficients in nearly all cases are 80 or greater.

Experimental procedure

Materials

L-α-Dimyristoyl phosphatidylcholine (DMPC) (Sigma Chemical Co., 98%) was used throughout as the sole lipid in the preparation of liposomes. Thin-layer chromatography of DMPC produced a single spot. Chloroform was BDH reagent grade. The phenolic solutes used were as follows: *p*-methylphenol, *p*-chlorophenol (more than 99.5%), *p*-bromophenol (99—100%) obtained from BDH; *p*-ethylphenol (99%), *p*-(*n*-butyl)phenol (98%) from Eastman Kodak, *p*-(*n*-propyl)phenol, *p*-iodophenol, *p*-fluorophenol (all 99%, Aldrich). Sodium chloride (BDH, analar) and double-distilled water were used to prepare the aqueous phases.

Methods

Stock solutions of *p*-alkylphenols or *p*-halophenols in 0.15 M NaCl were prepared at 0.1 mg/ml. These phenols rendered a pH of 5.0—5.5 of their aqueous solutions. 5 ml of a 10 mg/ml stock solution of DMPC in chloroform were delivered to tared 50-ml round-bottom flasks, then the chloroform was rapidly removed by rotary evaporation at approx. 40°C. Evaporation was continued for 1 h followed by overnight drying in a vacuum oven at 35°C. This resulted in smooth uniform dry lipid films being formed on the inside walls of the flasks. The weight of DMPC was then determined. Multilamellar liposomes were formed by transferring 5 ml stock solution of phenol derivative, warmed to approx. 40°C, to the appropriate flask containing the dried lipid film along with 4 or 5 small glass beads, then swirling with the aid of a vortex mixer until all of the lipid was dispersed. Subsequently, the flasks were placed in a water-bath (±0.1°C) to equilibrate. Preliminary runs indicated that equilibrium distribution of solute required approx. 40 h in some cases, so this period of equilibration was maintained throughout. Samples were prepared in duplicate for each phenol-liposome system.

Separation of the DMPC and aqueous phases was accomplished by centrifugation. The liposome preparation was transferred to a tared 10 ml centrifuge tube then placed in a fixed-angle rotor precooled or prewarmed to the desired temperature (average transfer time 1.33 min). These were centrifuged in an MSE High Speed 25 centrifuge using an 8 × 50-ml fixed-angle aluminium rotor with 10-ml adaptors at 25 000 rev./min for 1 h or 20 000 rev./min for 1.5 h which resulted in clear supernatants in all cases. The centrifuge was thermostatically controlled to maintain temperature within ±0.5°C. After completion

of the centrifugation cycle, samples of supernatant were carefully removed with a Pasteur pipette and transferred directly to silica cells. Absorbances were measured using a Beckman Model 25 spectrophotometer at λ_{max} for each phenol. Following spectrophotometric analysis of the supernatant, the solution was returned to the same centrifuge tube and the liposomal pellet was redispersed. This was accomplished by repeatedly withdrawing and expelling the dispersion using the same Pasteur pipette used to remove the sample of supernatant originally. Losses were minimized by rinsing the silica cell with reconstituted dispersion. Finally, the actual losses in weight of dispersion were measured. The contents of the centrifuge tube were vigorously shaken for 20 s employing a vortex mixer, then the tube was returned to the water bath which had been reset to the next temperaure. The temperatures at which studies could be made were restricted to the temperature range of the centrifuge. Thus, measurements were conducted over the range 5—31°C.

Calculation of partition coefficients

Aqueous supernatant concentrations were obtained from previously prepared calibration curves in mg/ml of phenol derivative. Molal concentrations in the lipid phase (C_0^m) and the aqueous phase (C_w^m) are given by Eqns. 1 and 2, respectively:

$$C_0^m = \frac{(C_T - C_w)\, w_1}{dM_r w_2} \tag{1}$$

$$C_w^m = \frac{C_w}{dM_r} \tag{2}$$

where C_T = initial aqueous concentration before equilibration (mg/ml), C_w = final aqueous concentration after equilibration (mg/ml), d = density of the initial aqueous phase, M_r = molecular weight of phenol derivative, w_1 = weight of aqueous phase in the sample and w_2 = weight of DMPC in the sample. Thus, the molal partition coefficient, K, is given by:

$$K = \frac{(C_T - C_w)\, w_1}{C_w w_2} \tag{3}$$

Concentrations were expressed in molal units for the reasons described previously by others [16]. The assumption is made that the concentration of phenol in the aqueous phase entrapped in the liposomes is equal to the concentration of the supernatant.

Monolayer studies

Monolayers of DMPC were spread from 30 μl of a 0.4 mg/ml solution in double-distilled hexane/ethanol (9 : 1) onto the surface of a substrate of 0.15 M NaCl containing 2.5, 10.0 or 100 μg/ml phenol derivative. After evaporation of the spreading solvent and film equilibration, films were compressed at a rate of 2.06 cm/min. A modified version of the surface balance described by Doroszkowski and Monk [18] was employed. Surface tensions were measured by the Wilhelmy plate method and continuously recorded on a Bryans (29000 A4) recorder connected to a Cahn electrobalance. Surface

pressures were calculated from the difference between surface tensions in the absence and the presence of film at various areas of the trough and the corresponding areas occupied per molecule at the surface were calculated knowing the amount of DMPC on the surface. Experiments were performed in triplicate and the results averaged. After a compression cycle was completed, the film was removed by suction, the surface tension of the subphase was checked and a fresh film was spread. At the concentrations employed, the *p*-alkylphenols did not lower the surface tension of the aqueous phase. These measurements were carried out at 22 ± 1°C.

Results

Temperature dependence of K

The temperature dependence of the K of *p*-alkylphenols between DMPC liposomes and 0.15 M NaCl is shown in Fig. 1. These results yield double linear relationships for each phenol. Pure DMPC is reported to undergo an endothermic phase transition (T_c) near 23°C [19]. Below this temperature, the DMPC molecules exist in a crystalline or 'frozen' state whereas above this temperature they exist in a liquid crystalline or 'melted' state. This type of phase behavior profoundly affects the partitioning of solutes between water and the lipid as seen in Fig. 1 and also as reported by Katz and Diamond [17]. Below the T_c of DMPC, K increases gradually with temperature to approx. 18°C then K increases rapidly to a maximum value at 22°C in the case of *p*-(*n*-propyl)phenol and *p*-(*n*-butyl)phenol but not until 25°C in the case of *p*-methylphenol and *p*-ethylphenol. An intermediate value of K was obtained at 22°C for the latter two solutes. Above the T_c, K decreases with temperature at a rate which increases with the alkyl chain length.

The presence of solutes in the bilayers may alter the characteristic T_c of the particular phospholipid depending on the nature of the solute molecules.

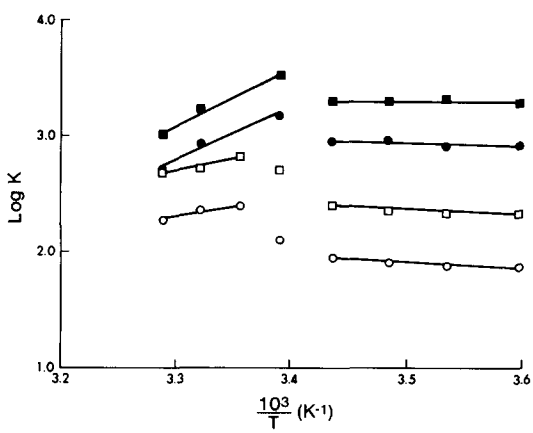

Fig. 1. Temperature dependence of the partition coefficient of *p*-alkylphenols between dimyristoyl phosphatidylcholine liposomes and 0.15 M NaCl. ○, *p*-methylphenol; □, *p*-ethylphenol; ●, *p*-(*n*-propyl)phenol; ■, *p*-(*n*-butyl)phenol. Equilibrium concentrations of *p*-alkylphenol were determined in supernatant phases by ultraviolet analysis, concentrations in the lipid phase were determined by difference and expressed in molality.

Certain water-soluble solutes which have been partitioned in DMPC liposomes resulted in an apparent T_c of 25°C [17]. The apparent T_c of DMPC in the presence of the *p*-alkylphenols is not sharp but extends over a 4—6°C range above 18°C depending on the alkyl chain length (Fig. 1). When the *p*-halophenols are incorporated in liposomes, T_c is near 15°C with the exception of *p*-fluorophenol (Fig. 2). The *p*-fluoro substituent of phenol apparently disrupts the order of the bilayer to an extent which lowers T_c to approx. 10°C. A shifting of T_c of the phospholipid to lower temperatures in the presence of small molecular lipid-soluble compounds [20] and charged drugs [21] has previously been reported. The type of effect on K upon cooling through T_c is related to the position of localization of the solute along the thickness of the bilayer [20]. Thus, K of butyramide, ethyl acetate and acetone decreased by 5—9% in DMPC liposomes [17], K of the spin-labelled solute, di-*t*-butyl nitroxide, in dipalmitoyl phosphatidylcholine liposomes decreased by 55% [22] and our studies indicate that a 40—63% decrease in K occurs with the *p*-alkylphenols upon cooling through T_c whereas almost no change in K is found in systems containing *p*-halophenols (except for *p*-fluorophenol).

Generally, K of a *p*-halophenol in DMPC liposomes increases as a function of the size of the halogen substituent. A detailed analysis of halogen substitution and its influence on the permeation of nonelectrolytes through cell membranes by Diamond and Wright [11] reveals (i) that halogens are simply equivalent to a hydrocarbon residue of the same size in its direct effect on permeability or K, (ii) that halogens, with the exception of fluorine, form weak hydrogen bonds with water, and (iii) that halogens have an inductive effect on other substituents (eg., hydroxyl group) to cause decreased hydrogen-bonding strength of polar substituents with water. The inductive effect of the halogen is in the order F > Cl > Br > I [23] and corresponding values of K should decrease in the same order. The K values of *p*-methylphenol and *p*-chlorophenol are comparable in DMPC liposomes below T_c (where hydrocarbon chain interac-

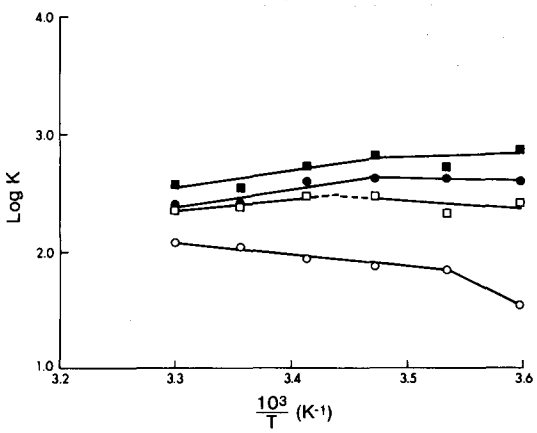

Fig. 2. Temperature dependence of the partition coefficient of *p*-halophenols between dimyristoyl phosphatidylcholine liposomes and 0.15 M NaCl. ○, *p*-fluorophenol; □, *p*-chlorophenol; ●, *p*-bromophenol; ■, *p*-iodophenol. Equilibrium concentrations of *p*-halophenol were determined in supernatant phases by ultraviolet analysis, concentrations in the lipid phase were determined by difference and expressed in molality.

tion is minimal) suggesting that points ii and iii above are probably insignificant. K of the p-halophenols is found to be in reverse order to that expected if the halogen group exerted an inductive effect with respect to the phenolic OH group. Furthermore, fluorine substitution in a solute reduces intermolecular forces between the solute and hydrocarbon tails of phospholipids in membranes based upon observations that intermolecular forces between fluorocarbons and hydrocarbons are much weaker than those between hydrocarbons [11]. This may account for our observations that p-fluoro substitution of phenol yields markedly lower values of K than the other p-halophenols but does not explain the observed lowering of T_c by p-fluorophenol. Linear regression analysis of the K data yielded the slopes and intercepts below and above the apparent T_c in Figs. 1 and 2.

Thermodynamic analysis of partitioning

The thermodynamics of partitioning of a solute between an aqueous phase (w) and a lipid phase (l) are described by:

$$\Delta G_{w\to l} = -RT \cdot \ln K \tag{4}$$

$$\Delta H_{w\to l} = T\Delta S_{w\to l} - RT \cdot \ln K \tag{5}$$

$$\Delta S_{w\to l} = \frac{\Delta H_{w\to l} - \Delta G_{w\to l}}{T} \tag{6}$$

where $\Delta G_{w\to l}$ is the partial molar free energy of transfer of a mole of solute from an aqueous phase to a lipid phase, $\Delta H_{w\to l}$ is the corresponding partial molar enthalpy and $\Delta S_{w\to l}$ is the corresponding partial molar entropy. Partition coefficients derived from the linear regression equation at each temperature were used to obtain the calculated thermodynamic functions for the p-alkylphenols and p-halophenols presented in Table I. These are shown at only one temperature below and above the T_c of DMPC. The assumption is made by fitting lines through the experimental points that, over a small temperaure range, $\Delta H_{w\to l}$ is independent of temperature [17]. The results are consistent for both groups of phenols in that the energetics of partitioning become more favorable as the series is ascended. It is interesting to note that the free energy change for partitioning of a particular phenol is approximately constant in each series of phenols, irrespective of whether transfer has taken place above or below the T_c of the phospholipid. However, below T_c the partitioning is an entropy-dominated process ($\Delta S_{w\to l}$ positive and greater than $\Delta H_{w\to l}$) whereas above T_c it is an enthalpy-dominated process ($\Delta H_{w\to l}$ negative and greater than $\Delta S_{w\to l}$) (the data included for p-fluorophenol at 15°C are actually above the apparent T_c of DMPC in this system (Fig. 2) and cannot properly be used for comparison). The removal of a hydrocarbon from water generally results in a large gain in entropy due to loss of water structure imposed by hydrocarbons and hydrocarbon moieties of molecules [11,24]. On the other hand, a greater restriction of movement of solute molecules in an ordered environment favors a loss of entropy. The observed positive entropies below the T_c of the liposome would indicate that the former influence is stronger whereas the converse is true above T_c. Likewise, the change in enthalpy as a result of partitioning is the net effect of broken hydrogen bonds between phenol and water and forces of

TABLE I

PARTIAL MOLAR FREE ENERGIES ENTHALPIES AND ENTROPIES OF PARTITION OF *p*-ALKYLPHENOLS INTO DIMYRISTOYL PHOSPHATIDYLCHOLINE LIPOSOMES

$\Delta H_{w\to l}$ was obtained from the slopes of the curves in Figs. 1 and 2 and calculations were based on Eqns. 4, 5 and 6, respectively. The stated temperatures are those immediately below and above the observed T_c of the phospholipid in each case.

Solute	$\Delta G_{w\to l}$ (kJ/mol)	$\Delta H_{w\to l}$ (kJ/mol)	$\Delta S_{w\to l}$ ($J \cdot mol^{-1} \cdot K^{-1}$)
18°C *p*-methylphenol	−10.88	8.8	67.6
p-ethylphenol	−13.39	8.6	75.7
p-(*n*-propyl)phenol	−16.44	5.6	75.5
p-(*n*-butyl)phenol	−18.41	0.8	65.9
28°C *p*-methylphenol	−13.51	−31.5	−59.8
p-ethylphenol	−15.86	−38.9	−76.6
p-(*n*-propyl)phenol	−16.65	−83.4	−221.8
p-(*n*-butyl)phenol	−18.41	−96.3	−258.6
15°C *p*-fluorophenol	−10.44	19.6 *	104.4 *
p-chlorophenol	−13.48	10.3	82.4
p-bromophenol	−14.43	2.7	59.5
p-iodophenol	−15.32	−8.0	25.5
25°C *p*-fluorophenol	−11.48	19.6	104.4
p-chlorophenol	−13.67	−16.8	−10.4
p-bromophenol	−13.99	−30.7	−56.2
p-iodophenol	−14.92	−32.5	−59.1

* These values actually correspond to dimyristoyl phosphatidylcholine in a 'melted' state at 15°C in the presence of *p*-fluorophenol as shown in Fig.2.

attraction which develop in the bilayers between solute and DMPC molecules. The results in Table I indicate that $\Delta H_{w\to l}$ is dependent on the functional group both above and below the T_c, suggesting that attractive forces in the bilayers are very significant. These may arise through van der Waals forces between hydrocarbon chains and hydrogen bonding of the phenolic OH with the phospholipid.

Monolayer studies

These measurements were undertaken in an attempt to evaluate the nature of the interactions occurring with the phenols and DMPC molecules. Further work is underway to obtain more detailed results with additional phenolic derivatives. No attempt was made to purify or dry the DMPC. The surface pressure-area curve obtained for a DMPC monolayer on 0.15 M NaCl has the same shape as previously reported for this phospholipid [25] but is somewhat more expanded (Fig. 3, curve 1). This may be due to DMPC existing initially in a hydrated rather than a dry state [25]. When spread on substrates containing *p*-alkylphenols at 2.5 or 10.0 μg/ml, no significant change in the pressure-area curve was observed (not shown) indicating no penetration or interaction of the dissolved solute with the monolayer. However, at a substrate concentration of 100 μg/ml (the same concentration used in the liposome studies), marked condensation of the film occurred which appears to be a function of the alkyl chain length. This would suggest penetration of the film by the *p*-alkylphenols

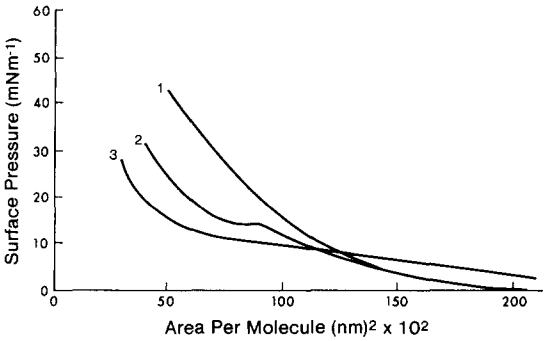

Fig. 3. Surface pressure versus area per molecule of dimyristoyl phosphatidylcholine films spread on 0.15 M NaCl containing 0.1 mg/ml *p*-alkylphenol. Curve 1, no *p*-alkylphenol; curve 2, *p*-methylphenol; curve 3, *p*-ethylphenol. Surface tensions were obtained by the Wilhelmy plate method during compression of films at 2.06 cm/min using a surface balance at 22 ± 1°C as modified by Doroszkowski and Monk [18].

resulting in a re-orientation of the phospholipid molecules towards a more vertical conformation at the air/water surface. Van der Waals forces of attraction operating between hydrocarbon chains of solute and DMPC could account for such a change, if it is assumed that such forces of attraction would increase as a function of alkyl chain length of the solute. Compressing the monolayer to the collapse point did not expel the phenols from the monolayer suggesting that the solute-lipid interactions are substantial. This is in contrast to results with *p*-chlorophenol and phosphatidylethanolamine monolayers obtained by Proudfoot and Davdani [26]. This phenol expanded monolayers but was subsequently ejected from the monolayers after compression. Also, Kaye and Proudfoot [27] observed an expansion of phosphatidylethanolamine monolayers in the presence of phenol, *o*-cresol, *p*-cresol and 2,6-xylenol at a subphase concentration of $4.2 \cdot 10^{-3}$ M (approximately four times the concentration used in our studies), but is should be pointed out that a monolayer of phosphatidylethanolamine at ambient temperature is well below its endothermic phase transition temperature [25].

Functional group contributions

Partition coefficient data are commonly employed to determine the substituent or hydrophobic bonding constant, π, of functional groups [12,28]. These have mainly been calculated based on solute distribution between an *n*-octanol/water system, although others have determined the π substituent value using an ether/water system [29] or even a biological phase/water system [30]. Table II gives π_{DMPC} values, calculated using the K values of phenols between DMPC and aqueous phase from:

$$\pi_{DMPC} = \log K_{RX} - \log K_{RH} \quad (7)$$

where K_{RX} and K_{RH} are the partition coefficients of the substituted phenol and phenol, respectively. Plots of log K_{RX} vs. n, where n is the number of methylene groups in the alkyl chain of the *p*-alkylphenols, were constructed at each temperature and extrapolated to $n = 0$ to obtain log K_{RH} (correlation coefficient = 0.99). Linear regression analysis of the temperature dependency

TABLE II

π_{DMPC} AND $\Delta G^{GFE}_{w\to l}$ VALUES FOR VARIOUS FUNCTIONAL GROUPS PARA-SUBSTITUTED TO PHENOL

π_{DMPC} is given by log K_{RX} — log K_{RH} corresponding to the partition coefficient of the substituted phenol (RX) and phenol (RH). $\Delta G^{GFE}_{w\to l}$ is the Gibbs free energy of transfer per functional group from aqueous phase to phospholipid phase (Eqn. 8).

Group	π_{DMPC}	$\Delta G^{GFE}_{w\to l}$(kJ/mol)	Group	π_{DMPC}	$\Delta G^{GFE}_{w\to l}$(kJ/mol)
18°C methyl	0.45	2.50	28°C methyl	0.21	1.19
ethyl	0.91	5.05	ethyl	0.61	3.51
propyl	1.48	8.12	propyl	0.75	4.33
butyl	1.80	10.04	butyl	1.06	6.09
15°C fluoro	0.42	2.31	25°C fluoro	−0.16	−0.92
chloro	0.97	5.35	chloro	0.22	1.27
bromo	1.14	6.30	bromo	0.28	1.59
iodo	1.30	7.19	iodo	0.44	2.51

of log K_{RH} produced the log K_{RH} values for calculation of π_{DMPC} at each temperature in Table II (correlation coefficient was 0.91 below and 0.79 above T_c). Group free energies of transfer from aqueous to lipid phase ($\Delta G^{GFE}_{w\to l}$) were determined from:

$$\Delta G^{GFE}_{w\to l} = 2.303 \cdot RT \cdot \pi_{DMPC} \quad (8)$$

and also appear in Table II. Both of these functions increase in each series as expected for substitution on an aromatic molecule [12] and assume lower values when the temperature is raised above the T_c of DMPC. Similarities in the contributions of alkyl and halo functional groups are observed when the phospholipid molecules are in the 'frozen' state but these differ markedly when the 'melted' state prevails. In addition, the variation within each series is regular below but irregular above T_c. Thus, interactions between the phenols and DMPC molecules are more pronounced at temperatures above T_c, but also the nature of the interactions of the *p*-alkyl groups and the *p*-halo groups with the DMPC molecules is notably different.

Discussion

The partitioning of eight phenolic compounds into DMPC liposomes as a function of temperature has provided some interesting results for comparison with other model membrane systems.

Firstly, within a given series of functional groups the K of phenol increases with the size of the group. Furthermore, the overall free energies of transfer of solutes from an aqueous phase to a lipid phase (Table I) or the individual group free energies (Table II) demonstrate that the rules of partitioning of non-electrolytes between water and an organic phase [4,5] appear to be valid for the liposome system. Others have found similar behavior for water-soluble alcohols and amides [31].

Secondly, the effect of temperature on K is more pronounced above than

below the T_c of DMPC liposomes and, at least with the *p*-alkylphenols, a reverse in the trend occurs upon crossing T_c. The thermodynamics of the transfer process indicate that partitioning is entropy-driven below and enthalpy-driven above the T_c. Above T_c the fluid state of the phospholipid molecules within the bilayers permits stronger forces of attraction to develop with the phenolic solutes. Entropy losses indicate that transfer takes place into a more ordered environment. However, the inclusion of the phenolic solute in the bilayer may also cause increased structuring of the phospholipid molecules as suggested by the results of the monolayer studies. In comparison, the interaction of the *p*-halophenols with the liposomes above the T_c (except for *p*-fluorophenol) is considerably less, suggesting that these phenols partition in a different region of the liposomal bilayer. Evidence for the apparent 'squeezing out' of the phenols upon cooling through T_c is given by the less favorable $\Delta H_{w\to l}$ values below T_c (Table I). However, the variation of $\Delta H_{w\to l}$ with alkyl chain length of phenol indicates that a hydrophobic type of interaction (i.e., van der Waals interactions) may play the major role in the partitioning process. If this is the case, then gross changes in ordering of molecules within the bilayers are likely to occur. The observed characteristics of the apparent T_c provide evidence of this in each case. Thus, the longer chain *p*-(*n*-propyl)phenol and *p*-(*n*-butyl)phenol result in a more abrupt change in the structure of the membrane at the T_c than do *p*-methylphenol and *p*-ethylphenol which result in transitory increases in fluidity of the bilayer molecules. In contrast, the *p*-halophenols (except *p*-fluorophenol) which participate in hydrophobic interactions to a lesser degree exert only a modest influence on the membrane structure at the T_c. The mechanisms involved in the *p*-fluorophenol interaction with the liposome leading to a gross disordering of the bilayers and a T_c of 10°C are not entirely clear and deserve further investigation. Nevertheless, partitioning is accompanied by a large positive entropy change.

The positive entropies found for the phenols below T_c can in part be attributed to disordering in the liposome. In the *p*-alkylphenol series, the extent of disordering follows the alkyl chain length and presumably outweighs the loss of translational and rotational entropy upon transfer of solute from a water to a lipid phase. It is therefore suggested that this compensation effect gives rise to the observed constant positive change in entropy of the *p*-alkylphenols as a function of chain length. In the *p*-halophenol series, differentiation of the functional group contributions towards the entropy is obtained because compensation due to disordering of the membrane structure is much less. Solubility data of phenols in water could enable a better estimate of the solute-water and solute-lipid interactions involved.

Values of the K of non-electrolytes between water and various nonpolar organic solvents usually show a systematic relationship [7] which may be described by:

$$\log K_y = a \cdot \log K_x + b \tag{9}$$

for a solute in solvent y compared with solvent x. The slope, a, is indicative of the similarly of the solvent environment in each case with respect to the solute and has been referred to as a selectivity constant [31]. Partition coefficient results of *p*-alkylphenols and *p*-halophenols in DMPC liposomes both below and

above T_c have been substituted as solvent y in Eqn. 9 and bulk organic solvent values as solvent x. Partition coefficients of *p*-alkylphenols in various oil/water systems were obtained from Korenman [32] and those for *p*-halophenols from Korenman [33,34] and Saha et al. [35]. The obtained selectivity constants increase with solvent polarity when compared both below and above T_c. This suggests that the solvent environment of DMPC liposomes is more similar to an alcohol (e.g., oleoyl alcohol) than to a pure hydrocarbon (e.g., cyclohexane). Lower values of the selectivity constant found above T_c indicate that partitioning occurs within a more hydrophobic region of the bilayer when in the 'melted' state. This could possibly be interpreted as meaning that penetration is deeper under these conditions. Comparison of selectivity constants obtained from liposome systems and erythrocytes using the data of Machleidt et al. [36] shows a greater similarity of the partitioning environment of erythrocytes and DMPC liposomes than that of bulk oil phases.

Partition coefficients of compounds in bulk oil/water systems have been

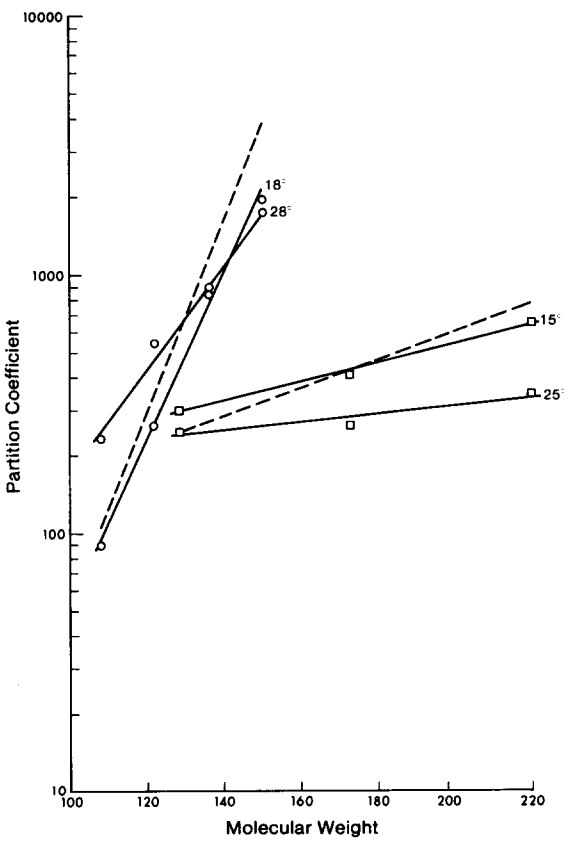

Fig. 4. Linear relationship of the partition coefficient of *p*-alkylphenols and *p*-halophenols in dimyristoyl phosphatidylcholine liposomes and *n*-octanol/water systems. ○, *p*-alkylphenols; □, *p*-halophenols. Curves are presented for experimental data immediately below and above the observed phase transition temperature of the phospholipid. The experimental value for *p*-fluorophenol has not been included. The dotted lines refer to literature data of the *n*-octanol/water partition coefficients (20°C) of the corresponding groups of phenols.

shown by Hansch and coworkers [37,38] as well as others [30,39] to yield correlations with biological activity. Such structure-activity relationships are possible because groups have been found to contribute almost independently and additively to the total activity. In Fig. 4, a plot of the logarithm of the partition coefficient against the molecular weight of various *p*-alkylphenols and *p*-halophenols shows a linear relationship to hold in DMPC liposomes. Using literature results for the *n*-octanol/water system shown for comparison, it may be concluded that partitioning into *n*-octanol more closely resembles partitioning into DMPC liposomes below T_c. Thus, correlations of K with biological activity which are often found suggest that real membranes have a relatively ordered structure. Further examination of the role of the liposome as a model membrane for other solutes is the aim of work in progress.

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