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THE EFFECT OF ANAESTHETIC-LIKE MOLECULES ON THE PHASE TRANSITION IN SMECTIC MESOPHASES OF DIPALMITOYLLECITHIN I. THE NORMAL ALCOHOL UP TO C = 9 AND THREE INHALATION ANAESTHETICS

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SUMMARY

The effect of the normal alcohols (up to C = 9) and three clinically used anaesthetics, on the crystalline–liquid crystalline phase transition in 1,2-dihexadecyl-*sn*-glycero-3-phosphorylcholine have been studied A one-degree depression was produced by a 4.4% concentration in the membrane of *n*-octanol and *n*-nonanol agreeing well with the value calculated from the temperature and enthalpy of the transition. It is also shown that the relationship between the partition coefficient P and the water solubility S ($P \cdot S = 2$), holds for the solutes investigated here. The experimental method described offers a simple way of assessing the anaesthetic potency of a wide range of compounds.

INTRODUCTION

It has now been well established by proton relaxation [1], electron spin resonance [2, 3] and by permeability studies [4] that the effect of anaesthetic molecules on membranes, either natural or model, is to increase the fluidity, disorder or entropy of the membrane. None of these techniques, however, gives a direct measure of the changes in the thermodynamic state of the membrane induced by the presence of the anaesthetic agents, and it is difficult and often confusing to attempt to derive thermodynamic data from ill-defined systems. The present work therefore is aimed at studying the effect of anaesthetics using a well-characterised model system for, although this might only approximate to a real membrane, the thermodynamic data obtained will have some clear meaning. A good model in this case is a smectic mesophase of a pure single lipid species in water, the liposome [5]; this extensively studied system also exhibits a phase transition at reasonable temperatures when dispersed in water and therefore it offers an easy way of measuring the change of the chemical potential of the membrane, by observing the depression of the temperature at which the transition occurs. Three clinically used inhalation anaesthetics were studied, chloroform, diethyl ether, and halothane, and the homologous series of the normal aliphatic

alcohols from C = 3 to C = 9 The lipid used was synthetic 1,2-dihexadecyl-*sn*-glycero-3-phosphorylcholine

Rotational phase transition

At maximum hydration dihexadecylglycerophosphorylcholine exhibits a phase transition at 41 °C with a change of enthalpy of 8 66 Kcal $mole^{-1}$ and entropy of 27 6 $cal \cdot mole^{-1}$ $degree^{-1}$ [6] This corresponds to the crystalline to liquid crystalline phase transition and is due to an increase in the rotational motion of the hydrocarbon portion of the lipid molecule [9] The transition can be observed by calorimetric methods [7], volume changes [8] and optical methods [9–11], and the simplest way is to observe the change in turbidity of a suspension of the lipid in excess water at 450 nm in an ordinary spectrophotometer with a heated sample holder

The effect of non-ionic substances on the phase transition of the tetradecyl homologue of dihexadecylglycerophosphorylcholine has been studied calorimetrically [12], but only overall concentrations of lipid, water and solute (which were very large) were noted and no attempt was made to deduce the amount of the solute that was in the lipid

This work showed that the presence of relatively low amounts of *n*-butanol and ethanol had little effect, *n*-hexanol lowered the temperature and *n*-octanol and those with longer chains raised it. The present work has concentrated on the effects of low membrane concentration of foreign molecules, as this is more relevant to general anaesthesia (the relevant membrane concentration is commonly thought to be of the order of 1 %) and where there is justification in applying simplifying approximations to the thermodynamics

Freezing point depression

In bulk systems, the temperature at which a pure liquid freezes can be modified by the presence of foreign molecules. If the solid which initially freezes out consists of the pure solvent, then the transition temperature will be maximally lowered for a given amount of foreign solute and the amount of the lowering will be proportional to the concentration of the foreign molecules in the liquid solution For dilute solutions, the change in the chemical potential of a solvent on adding solute molecules is also proportional to the concentration and so the depression of the freezing point is a direct measure of the change in the chemical potential of the solvent It is not obvious that this theory of bulk solutions should be applicable to what is essentially a two-dimensional structure but the evidence of the present work is that indeed this is the case It should be stressed that whereas the depression of the freezing point is a useful measure of the thermodynamic state of a model membrane, it is not suggested that the phenomenon is itself the mechanism of anaesthesia. The possible relevance of the present results to the theory of anaesthesia is published elsewhere [13]

The ideal case

The depression of the freezing point of a liquid ΔT due to the presence of a solute is given by

$$\Delta T = \frac{RT^2}{Q}(C^{I} - C^{II}) \tag{1}$$

where C^{I} is the concentration of the solute in the liquid and C^{II} is the concentration of the solute in the solid, Q is the latent heat of the transition and T its temperature in degrees Kelvin. If, as is most likely the case $C^{I} \gg C^{II}$ that is, the solute is squeezed out of the solid on freezing, this simplifies to

$$\Delta T = \frac{RT^2}{Q} C_m = \frac{T}{Q} \Delta\mu_m \tag{2}$$

where C_m is the concentration of solute in the membrane and $\Delta\mu_m$ is the change in the chemical potential of the membrane equal to $RT\,C_m$ (all concentrations are expressed in terms of moles of solute per mole of solvent).

For a system of two phases, e g. membrane and water, let a solute distribute itself between these two phases, then the chemical potential of the solute (μ) in the aqueous phase can be expressed in terms of the chemical potential of the pure solute (if a liquid) (μ^0)) or the chemical potential of water-saturated solution (μ^{sat}) if this is formed

$$\mu = \mu^0 + RT \ln a \tag{3}$$

$$\mu = \mu^{sat} + RT \ln r \tag{4}$$

where r is the fraction of saturation and is given by C_w/S where C_w is the aqueous concentration of the solute and S the concentration at aqueous saturation, a is the activity of the solute and can be expressed as $\gamma_w C_w$ where γ_w is the activity coefficient of the solute in the aqueous phase. For dilute solutions γ_w has a constant value which is nearly equal to the reciprocal of the solubility (this is a result of the concentration of water in the pure water-saturated solute being small).

If Eqn 2 is differentiated with respect to r we obtain the dependence of the depression of the freezing point with the aqueous concentration expressed as the fraction of saturation, that is

$$\frac{\delta\Delta T}{\delta r} = \frac{RT^2}{Q} \frac{\delta C_m}{\delta r} \tag{5}$$

but we also have

$$\frac{\delta C_m}{\delta r} = \frac{\delta C_m \cdot S}{\delta C_w} \tag{6}$$

$$= P \cdot S \tag{7}$$

where P is the partition coefficient of the solute between the two phases. This now gives us

$$\frac{\delta\Delta T}{\delta r} = \frac{RT^2}{Q} \cdot P \cdot S \tag{8}$$

It is possible to predict a value of $P \cdot S$ in an ideal case as follows. At equilibrium the chemical potential of the solute in the two phases will be equal, so

$$\mu^{sat} + \ln r = \chi + RT \ln C_m \tag{9}$$

where χ is the value of the chemical potential of the solute in the membrane when the concentration in the membrane is unity As remarked before the chemical potential of the pure solute and the water-saturated solute need not differ significantly if the concentration of the water in the solute is low, thus

$$RT \ln C_m/r = \mu^0 - \chi \tag{10}$$

the right hand side of the equation is the difference in chemical potential between one mole of the pure solute and one mole of the solute dissolved in one mole of the membrane This will be given by the entropy of mixing only, if the interactions of the solute in the membrane and in the solute are the same The numerical value of the difference in entropy will be $R \ln 2$ hence from Eqn 10

$$RT \ln \frac{C_m}{C_w} \cdot S = RT \ln 2 \tag{11}$$

so

$$P \cdot S = 2 \tag{12}$$

This relation between partition coefficients and water solubilities has been noted previously by Hansch et al. [14] and by the present author in relation to the theories of general anaesthesia [13]

It is now possible to predict the value of the slope of the depression of freezing point as a function of the fraction of saturation,

$$\frac{\delta \Delta T}{\delta r} = \frac{RT^2}{Q} \cdot 2 = 45 \cdot 2 \tag{13}$$

we can also now predict that for a one-degree depression the value of r will be 0 0221 and the concentration of the solute in the membrane 0 0442 The activity coefficient of the solute in the membrane equals a/C_m and will be approximately the same as r/C_m and hence equal to 0 5 in this ideal case

MATERIALS AND METHODS

1,2-Dihexadecyl-*sn*-glycero-3-phosphorylcholine, (L-3-lecithin synthetic pure, Koch–Light) containing greater than 98 % palmitic acid by gas–liquid chromatographic analysis, was used without further purification, its transition temperature found by changes in the optical density of aqueous dispersions was 42 1 °C The normal alcohols, diethyl ether and chloroform were Analar grade or equivalent and were used without purification The Halothane (Fluothane I C I , 2-bromo-2-chloro-1,1,1,-trifluoroethane) was washed three times with water to remove the stabiliser present, immediately before use. The water used was thrice distilled, the last distillation being from alkali permanganate solution

The lipid suspensions were made by shaking a tube containing 7 mg of the lipid and 0.1 ml of water, at a temperature higher than the transition temperature 15 λ of this suspension was pipetted into a 1-cm pathlength glass cuvette with 2 5 ml of water The cuvette was placed in a temperature-controlled stage of a Perkin–Elmer 402 spectrophotometer, and the absorbance at 450 nm noted The output

from the spectrophotometer was connected to the y-axis of a Bryans 22000 X–Y recorder, the other axis monitoring the temperature in the cuvette by means of a copper–constantan thermocouple. The initial temperature of the cuvette was set to about 20 °C and after the system had acquired equilibrium heating was commenced at the rate of about 2 degrees a minute. When the transition temperature of the pure lipid had been established and the system had been returned to 20 °C an appropriate amount of anaesthetic was added to the cuvette and the transition temperature of the lipid plus the anaesthetic found as before. For the very volatile anaesthetics it was necessary to use sealed cuvettes, to stop them evaporating. This was tested by repeating the run a number of times in succession, and seeing if the transition temperature returned to the pure lipid value. After the transition temperature was established for the first anaesthetic concentration, further anaesthetic was added and the process repeated until the run was complete. Three or four lipid samples were used for each anaesthetic The anaesthetic was either added as the pure liquid or as a saturated solution whichever gave the most appropriate volume. In the case of *n*-nonanol and *n*-octanol significant amounts of the anaesthetic partitioned into the lipid resulting in the aqueous concentration not being simply the amount added divided by the volume of the water. It was therefore possible to measure directly the membrane concentration of the anaesthetic that would produce a one-degree depression of the melting point If the aqueous concentration of the anaesthetic assuming that no anaesthetic partitioned was C_T and the number of moles of water is N_W and lipid N_M then if the anaesthetic partitioned

$$N_W \cdot C_w + N_W \cdot C_M = N_W \cdot C_T \tag{15}$$

where C_W is the concentration of the anaesthetic in the water and C_M is its volume in the lipid The partition coefficient is given by

$$P = C_M/C_W \tag{16}$$

so

$$= \frac{C_M}{C_T - C_M N_M/N_W} \tag{17}$$

from Eqn 14 If we assume there is a linear relation between the membrane concentration and the temperature depression, we obtain

$$C_M = C'_M \cdot \Delta T \tag{18}$$

where C'_m is the concentration needed to produce a one-degree depression. Using this in Eqn 16 we obtain

$$\frac{C_T}{\Delta T} = \frac{C'_M}{P} + C'_M \cdot N_M/N_W \tag{19}$$

which means that a graph of $C_T/\Delta T$ against N_M/N_W, which is the aqueous concentration of lipid, gives a straight line with the slope equal to C'_M and the slope divided by the intercept equal to the P partition coefficient.

RESULTS

Fig. 1 shows that the absorbance of a lipid dispersion, at 450 nm varies with temperature, at various concentrations of *n*-nonanol When there is no anaesthetic present the absorbance decreases twice in the range 20–45 °C The first decrease appears about 32 °C and is thought to be due to either a hydration phenomena or to the aggregation of the lipid vesicles [10, 11]. The second decrease at 42 °C is due to the phase transition under discussion and results from the increase in the volume of the lipid region due to increased rotational motion, lowering the refractive index of the vesicles closer to the value for water and thus decreasing the turbidity of the suspension [10] Low concentrations of anaesthetic shift the first decrement to lower temperatures, and finally is not observed in the temperature range used in the present experiments The phase transition decrement is also shifted to progressively lower temperatures, Fig 2 showing the change in the transition temperature as a function of the percentage saturation of the anaesthetic solution (*n*-pentanol) All the anaesthetics measured gave a straight line passing through the origin up to a value of 15 % saturation or a ΔT of 6 degrees Eqn 19 predicts the effect of the depletion of the aqueous phase of the anaesthetic into the lipid phase, and Fig. 3 shows the results of plotting the results for *n*-octanol and *n*-nonanol in this way The slopes of the graphs which measure the concentration to produce a one-degree depression are the same $4\,4 \pm 0\,5 \cdot 10^{-2}$ and $4\,3 + 0\,5\ \ 10^{-2}$, respectively, which are in good agreement with the theoretical value of $4\,42\ \ 10^{-2}$ predicted above Table I lists the experimentally determined values of $\Delta T/r$ and the derived values of P S (see Eqn 8) These values can be seen to be all within a factor of 2 of the theoretically

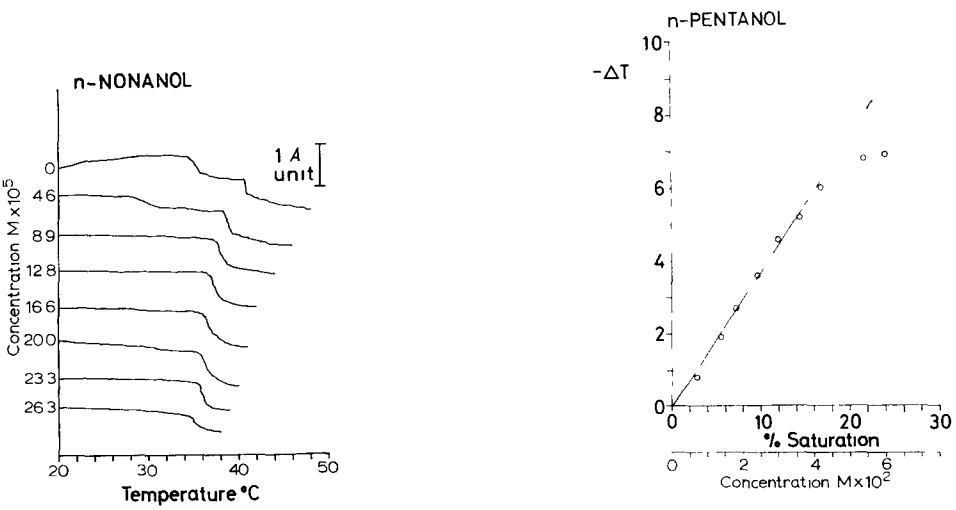

Fig. 1. The effect of increasing temperature on the absorbance at 450 nm of dispersions of dihexadecylglycerophosphorylcholine in water, with and without *n*-nonanol at various concentrations expressed in molarity The lipid concentration was 1 4 μM in 2.5 ml

Fig 2 The depression of the transition temperature of dihexadecylglycerophosphorylcholine in water by *n*-pentanol, as a function of the aqueous concentration of the alcohol Concentrations expressed as percentage of a saturated solution at 20 °C, and molarity

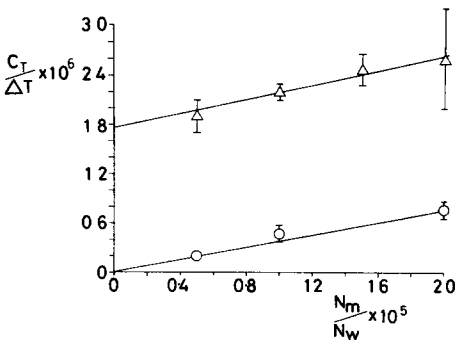

Fig 3 The change in $C_T/\Delta T$ as a function of N_M/N_W the aqueous concentration of lipid for (△–△) *n*-octanol and (○–○) *n*-nonanol For details see text

TABLE I

The aqueous solubilities of the alcohol and anaesthetics used [17–19], together with measured values of $\Delta T/r$, $P \cdot S$ and γ, and the activity a, membrane concentration C_m, the fraction of saturation r, that produces a one-degree depression of the transition temperature of dihexadecylglycerophosphorylcholine and the activity coefficients at infinite dilution f^0 [16]

	Solubility in water (moles/mole)	$\Delta T/r$ (°C)	$P \cdot S$	r for 1 °C	f^0	a for 1 °C	C_m for 1 °C	γ
Propanol	—	—	—	—	14 4	0 028	—	0 63
Butanol	1.78 10^{-2}	46±5	2.08±0 2	0 0217	52 9	0 020	—	0 45
Pentanol	4 50 10^{-3}	37±2	1 64±0 09	0.0270	214	0 026	—	0.59
Hexanol	1 10 10^{-3}	44±2	1 94±0.09	0 0227	903	0 023	—	0 52
Heptanol	2 79 · 10^{-4}	32±3	1 4 ±0 1	0 0313	3560	0 031	—	0.70
Octanol	8 09 · 10^{-5}	46±5	2 03±0 2	0 0217	12 300	0.022	4 4±0 5 10^{-2}	0 50
Nonanol	2 16 10^{-5}	—	—	—	—	—	4.3±0 5 10^{-2}	—
Diethyl ether	1 56 10^{-2}	23±4	1 0 ±0 2	0 0435	69 3	0.047	—	1 06
Chloroform	1 24 · 10^{-3}	27±1	1 19±0 04	0 0370	817	0 037	—	0 84
Halothane	3 15 10^{-4}	27±1	1 2 ±0 2	0.0370	—	0 037	—	0 83

determined values for the ideal case (Eqns 12 and 13) In the series of the alcohols there can be seen a significant difference between the odd- and even-chain lengths, and that the closest to ideal are the even ones. The inhalation anaesthetics are all about a factor 2 away from the ideal values which might be due to the fact that these molecules are less likely to be concentrated in the interface than the alcohols and the fact that the reduction in transition temperature is due more to the disrupting effect of the molecules in the tightly structured interface, than to the effect of the molecules in the more fluid structure of the centre of the bilayer. Finally, it is not possible to use the above analysis for *n*-propanol as it is completely miscible with water hence only the value of the activity to produce a one-degree depression can be determined along with the activity coefficient in the membrane. Nevertheless the value is seen to be in reasonable agreement with the other odd-chain alcohols. The activities of the anaesthetics at infinite dilution f^0 were taken from ref. 16 and the solubilities of the alcohols were calculated from ref. 17 and those of chloroform and ether from ref. 18 and halothane from ref. 19.

DISCUSSION

The general conclusion from this work is that the change in the chemical potential of a bilayer of lipid may be determined, for low concentrations, by the concentration of the dissolved molecule, as would be expected from the use of bulk thermodynamical theory The aqueous concentrations required to produce the appropriate lipid concentration depends on the solubility of the anaesthetic in water as a good rough approximation, thus both the Meyer–Overton rule and that of equal activities can be accounted for in terms of the effect the anaesthetics have on the chemical potential of the membrane [13] The values of the partition coefficients between water and the lipid phases are discussed in detail elsewhere with a comparison between those for organic solvent and natural membranes [15]

The experimental method described offers an attractive way of assessing the anaesthetic potency of a wide range of compounds, further work in this line is at present underway

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