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THE ACTION OF ANAESTHETICS ON PHOSPHOLIPID MEMBRANES

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SUMMARY

The permeability of 4 % phosphatidic acid–96 % phosphatidyl choline liposomes to K^+ was studied over a temperature range with and without the anaesthetic compounds diethyl ether, chloroform and *n*-butanol at approximately the concentrations at which they are effective *in vivo*; the results were compared to the same system when the potassium ionophore, valinomycin, was also present in the liposomes. In all cases the anaesthetic increased the permeability. Arrhenius plots were made to find the enthalpy of activation ΔH^* and differences in the entropy of activation. After correction for anaesthetic partition coefficients at the various temperatures, the enthalpy of activation ΔH^* (15.4 ± 1.1 kcal·mole^{-1}) was the same whether or not anaesthetics or valinomycin was present. The ΔH^* of a system containing valinomycin and anaesthetic was lower, depending on the anaesthetic used. It was deduced that: (1) The cation permeability barrier was located at the aqueous–lipid interface. (2) The anaesthetics increased the freedom of movement of groups in the lipid molecule near the interface. (3) The increase in permeability to K^+ in the presence of valinomycin was due to an entropy increase in the activated state of approx. 35 cal·mole^{-1}·degree^{-1}. (4) The increased freedom of movement in the interface when the anaesthetic was present allowed the valinomycin to adopt a more favourable orientation in the interface for the exchange of K^+.

INTRODUCTION

Such a wide variety of chemicals of diverse structure are known to produce an anaesthetic effect that specific chemical interaction with nerve membranes can be regarded as highly improbable. Early in the century OVERTON AND MEYER* drew attention to the correlation between the anaesthetic potency of a compound and its olive oil solubility. FERGUSON[1] improved this correlation by taking into account the thermodynamic activities. The empirical law can be stated as 'Equal thermodynamic activities cause equal degrees of narcosis'. MULLINS[2] discussed the advantage of making an allowance for the partial molar volume of the anaesthetic. On the other hand PAULING[3] pointed out that there was a general correlation between the anaesthetic potency of a compound and the Van der Waals interaction and argued a case for there being an aqueous site for anaesthesia. He suggested that the anaesthetic molecules stabilized aqueous clathrates among the protein side chains. MILLER *et al.*[4], studying the behaviour of the fluorine-substituted hydrocarbons, which have anomalous solubilities in hydrocarbons, decided that the evidence favoured the hydrocarbon rather

* For reviews of early work see refs. 1 and 2.

than the aqueous phase as the site of anaesthesia. BANGHAM *et al.*[5] showed that general anaesthetics such as the *n*-alcohols from butanol to octanol, diethyl ether and chloroform increased the K^+ permeability of liposomes (a protein-free system) at anaesthetic concentrations. With the development of a simplified liposome system[6] it was felt that a study of the permeability variation with temperature might reveal information about the nature and energy of the cation permeability barrier in these membranes and indicate the way in which general anaesthetics affect it.

The Arrhenius method of plotting the logarithm of a rate variable against the reciprocal of temperature can yield valuable results. Straight lines are often obtained from which values of ΔH^*, the activation enthalpy, and sometimes ΔS^*, the activation entropy can be calculated; however, in most biological systems the difficulty lies in the interpretation of the process. ΔH^* can be a combination of several temperature dependent functions and these must all be considered. In this work only changes in ΔS^* were obtained as insufficient information was available to calculate absolute values for ΔS^*.

The equations showing the relation between the liposome permeability and temperature derived below are based on a paper by ZWOLINSKI *et al.*[7], who treated the problem of diffusion across membranes by means of the absolute rate theory[8].

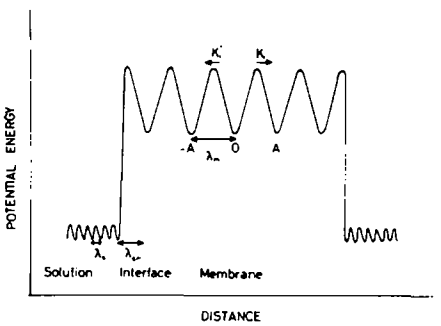

Fig. 1. Diagram showing the potential energy barriers across a bilayer membrane.

The theory regards the flow of molecules as a series of successive jumps from one equilibrium position to the next. Fig. 1 shows a possible potential energy profile for a molecule diffusing through the membrane. λ refers to the distance between potential energy minima, such as OA, and the molecule jumps k_t times from O to A or k_t' from O to —A in 1 sec. The number of potential energy barriers in the solution on the fore side of the membrane is s, the number on the back is s_1 and the number in the membrane is m.

From equations derived by ZWOLINSKI *et al.*[7], the permeability coefficient P may be written as:

$$P = \frac{k_s\lambda_s}{\left[s + s_1 + \frac{2k_s\lambda_s}{k_{sm}\lambda_{sm}} + \frac{mk_s\lambda_s\lambda_{ms}}{k_{sm}k_m\lambda_m}\right]} \quad (1)$$

All jump distances and barrier heights in the solution are regarded as equal as are those inside the membrane.

λ_{sm} is assumed to equal λ_{ms}. For membranes which provide appreciable barriers,

k_s is far greater than all the other rate constants, and because all the jump lengths are of the same order of magnitude,

$$P = \frac{k_{sm}\lambda_{sm}k_m\lambda_m}{2k_m\lambda_m + mk_{ms}\lambda_{sm}} \tag{2}$$

The diffusion coefficient D_i in any medium can be expressed as $k_i\lambda_i^2$. The membrane thickness $\delta = m\lambda_m + \lambda_{sm}$. The diffusion substance has a partition coefficient

$$\rho = \frac{k_{sm}}{k_{ms}} \tag{3}$$

Substituting the above three expressions in Eqn. 2 and rearranging

$$\frac{\rho}{P} = \frac{2\lambda_{sm}}{D_{ms}} + \frac{\delta}{D_m} \tag{4}$$

Three distinct circumstances can be deduced from this equation.

(1) Both the diffusion through the interface and through the membrane are rate limiting, so P is given by Eqn. 4.

(2) The diffusion through the membrane is rate limiting,

$$D_{sm} \text{ and } D_{ms} \gg D_m \text{ and } P = \rho\frac{D_m}{\delta} \tag{5}$$

(3) The diffusion through one of the interfaces is rate limiting, so

$$D_m \gg D_{ms} \text{ or } D_{sm} \text{ and } P = \frac{\rho D_{ms}}{2\lambda_{ms}} = \frac{D_{sm}}{2\lambda_{ms}} \tag{6}$$

According to the theory of rate processes, any rate constant k_i is given by the relation

$$k_i = \frac{\tau \mathbf{k}T}{h} e^{-\Delta G_i^*/RT} \tag{7}$$

where τ is the transmission coefficient and is normally taken as 1, $\mathbf{k}$ is Boltzmann's constant, h is Planck's constant, R the universal gas constant and T the absolute temperature. ΔG_i^* is the free energy of activation, the energy difference between the potential maximum and minimum. But

$$\Delta G_i^* = \Delta H_i^* - T\Delta S_i^* \tag{8}$$

where ΔH_i^* and ΔS_i^* are the molar enthalpy and entropy of activation. Combining Eqns. 7 and 8,

$$k_i = \frac{\mathbf{k}T}{h} e^{\Delta S_i^*/R} \times e^{-\Delta H_i^*/RT} \tag{9}$$

Case 2

Substituting for D_m in Eqn. 5 and multiplying by $298.2/T$ yields

$$\frac{298.2P}{T} = \frac{\rho\lambda_m^2}{\delta}\frac{298.2\mathbf{k}}{h} e^{\Delta S_m^*/R} \times e^{-\Delta H_m^*/RT} \tag{5a}$$

ρ must usually be regarded as a function of temperature such that

$$\rho = e^{(\Delta S_{sm}^* - \Delta S_{ms}^*)/R} \times e^{-(\Delta H_{sm}^* - \Delta H_{ms}^*)/RT}$$

and Eqn. 5a becomes

$$\frac{298.2P}{T} = \frac{\lambda m^2}{\delta} \frac{298.2k}{h} e^{(\Delta S_m^* + \Delta S_{sm}^* - \Delta S_{ms}^*)/R} \times e^{-(\Delta H_m^* + \Delta H_{sm}^* - \Delta H_{ms}^*)/RT} \quad (5b)$$

Case 3

Substituting for D_{sm} in Eqn. 6, and multiplying by $298.2/T$

$$\frac{298.2P}{T} = \frac{\lambda_{sm}}{2} \frac{298.2k}{h} e^{\Delta S_{sm}^*/R} \times e^{-\Delta H_{sm}^*/RT} \quad (6a)$$

in both Case 2 and 3 a plot of log $298.2P/T$ against $1/T$ will give a straight line and the net enthalpy of the process can be calculated from the gradients.

Case 1

If similar substitutions are made in Eqn. 4, it will be seen that the plot of log $298.2P/T$ against $1/T$ will be curved, since the equation contains the sum of two exponential functions.

A study of the experimental results allows a distinction between the three cases.

EXPERIMENTAL

Reagents

Phosphatidyl choline was extracted from egg yolks and phosphatidic acid prepared from it by enzymic hydrolysis according to PAPAHADJOPOULOS AND MILLER[9]. Valinomycin was a gift from Dr. J. C. Mac Donald, Prairie Research Laboratory (Saskatoon, Saskatchewan, Canada). With the exception of decane (Hopkin and Williams), all other reagents were analytical grade. Water was twice distilled, the second time from $KMnO_4$ in borosilicate glass apparatus.

Method

A full description of the experimental method is given in ref. 6. Briefly a stock solution of 4 % phosphatidic acid–96 % phosphatidyl choline in chloroform was evaporated to dryness. For each 100 μmoles lipid, 1 ml of 0.155 M KCl was added, containing a total of 40 μC $^{42}K^+$, half-life 12.4 h. The lipid dispersion was sonicated to clearness under N_2 and allowed to stand overnight. The isotope outside the liposomes was removed by passage of the dispersion over a Sephadex G-50 column equilibrated with 0.16 M KCl. The dispersion was diluted, and 1-ml portions were put into 8/32 Visking dialysis tubing bags.

Valinomycin was incorporated into the liposomes by adding it (in methanol solution) to the lipid in chloroform. After evaporation to dryness, the lipid and valinomycin residue was sonicated in 0.16 M KCl. One half was passed over a Sephadex column equilibrated with 0.16 M KCl and the other half over a column equilibrated with 0.16 M KCl and anaesthetic. Since the K^+ permeability of valinomycin-containing liposomes was much higher than the unmodified preparations, a different experimental procedure was required[6]. Dialysis bags containing about 12 μmoles lipid in 1 ml were allowed to exchange isotope in 20 ml of 0.16 M KCl or 0.16 M KCl *plus* anaesthetic. Samples were withdrawn for counting. The fraction of isotope leaving

the dialysis bag up to the time of sampling was n_2/N, where N was the total number of counts in the flask all of which were initially inside the liposomes and n_2 the number of counts outside the dialysis bag. Only values of n_2/N less than 0.6 were used. v_2/v_0 was the ratio of the mean volume of the solution outside the dialysis bag to the total volume during the sampling period, this varied less than 1 %. The derivation and validity of Eqn. 10 describing fast exchange across one compartment liposomes is discussed in the preceding paper[6]. A computer found the best values of L and K to fit Eqn. 10 to the experimental points $(t, (v_2/v_0 - n_2/N))$

$$\frac{v_2}{v_0} - \frac{n_2}{N} = \frac{0.952}{(K-L)}[Ke^{-Lt} - Le^{-Kt}] \qquad (10)$$

where $K = p_1/0.952$ and $L = p/v_c$, where p was the permeability of the liposomes and p_1 the permeability of the dialysis bag. v_c was the volume of isotope trapped inside the liposomes. $v_c = N \times 0.01$/count rate of 0.01 ml swelling solution.

In the experiments without valinomycin a Forbes bar provided a temperature gradient for a series of test tubes containing 10 ml of 0.16 M KCl or 0.16 M KCl *plus* anaesthetic. After preliminary chemical and thermal equilibration, each dialysis bag was allowed to exchange isotope for 1–3 h (time t). The isotope outside the dialysis bag was then counted (n_2).

The permeability of the liposomes was calculated[6] from the relation $p = (1.1 \times n_2 \times 0.01)/(t \times \text{standard count rate})$.

The amount of lipid in the dialysis bags was found by phosphate analysis, and the surface areas measured by uranyl titration described by JOHNSON AND BANGHAM[6].

The permeability coefficient P is calculated from $P = p(\text{cm}^3 \cdot \text{sec}^{-1})/\text{surface area of liposomes (cm}^2)$. $\log P + \log 298.2/T$ was plotted against $10^3/T$. The K^+ unidirectional flux $= P \times 0.16 \cdot 10^{-3}$ moles$\cdot$cm$^{-2}\cdot$sec^{-1}.

Partition coefficients

1.5 ml of decane were added to test tubes containing a known quantity of anaesthetic in 10 ml of 0.1 M KCl. The tubes were tightly sealed, and allowed to equilibrate in a Forbes bar overnight. Samples of the hydrocarbon or aqueous phase were rapidly withdrawn and kept at 0° in stoppered tubes.

(1) *n-Butanol.* The aqueous phase initially contained 50 and 100 mM *n*-butanol. Approx. 5-μl samples of the decane phase were passed over a modified Pye panchromatograph at 100° using a stationary phase of 20 % (w/w) dinonyl phthalate on 100–120 mesh Gas Chrom Z_1, an argon flow rate of 50 ml/min and a flame detector. The ratio of the area of the butanol peak to a water-insoluble decane impurity peak was calculated, and the amount of butanol found by comparison with the linear calibration curve obtained from samples with 0.4–0.05 % *n*-butanol in decane.

(2) *Diethyl ether.* The aqueous phase contained initially 19 and 25.1 g/l ether, (approx. 0.2 and 0.3 saturated). The refractive index of the ether–decane layer was measured with an Abbé refractometer; and the quantity of ether in the decane found by comparison with a linear calibration curve, using up to 25% ether–decane mixtures.

(3) *Chloroform.* The aqueous phase contained initially 2.125 and 2.853 g/l (0.3 and 0.4 saturated). 10 μl of the aqueous layer were passed over the panchromatograph under the same conditions as the *n*-butanol sample. The height of the

sharp chloroform peak was measured, and the quantity of chloroform found by comparison with a linear calibration curve of peak height with weight of chloroform up to 4 μg.

Values of log (partition coefficient) were plotted against $10^3/T$ (Fig. 2).

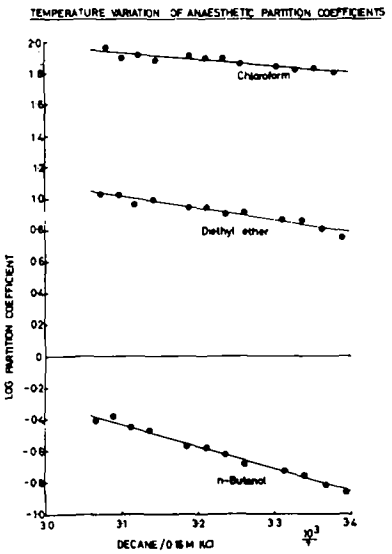

Fig. 2. Temperature variation of anaesthetic partition coefficients.

The partition coefficient of valinomycin between phosphatidylcholine and 0.16 M KCl

50 ng valinomycin were mixed with 0.623 μmole phosphatidyl choline in chloroform. The solvent was evaporated under reduced pressure and the lipid allowed to swell in 3 ml of 0.16 M KCl at 22° overnight. The suspension was centrifuged for 2 h at 1400 × *g* in glass tubes at 22°. The supernatant was quite clear and did not make clean glass surfaces hydrophobic. A measured volume of the supernatant was made up to 2 ml with 0.16 M KCl and mixed with 1 ml of liposome dispersion containing 4 μmoles 4% phosphatidic acid–phosphatidyl choline with trapped $^{42}K^+$. The test tubes were then immediately cooled in ice-cold water. A series of dilutions of valinomycin containing 2–10 ng in 2 ml of 0.16 M KCl were also treated with the dispersion and cooled. Two 1-ml portions were taken from every tube, and each portion was put into a knotted Visking dialysis bag. The bag was dropped into 10 ml of ice-cold 0.16 M KCl. The tubes were then transferred together to a 37° thermostat for 1 h and stirred intermittently. Finally the tubes were plunged again into ice-cold water, and the contents of the bags were placed in 9 ml of fresh 0.16 M KCl containing a trace of Triton X-100 in Panax counting vials. The samples were counted by their Cerenkov radiation on Panax counting equipment Type AC 300/6. The ratios of the counts leaving the liposomes over the total number of counts present were calculated, and the quantity of valinomycin in the supernatants was found from the calibration curve of the standard valinomycin dilutions. The molecular weight of phosphatidyl choline was taken as 800, and its density as 1.016. An approximate value of at least 44000 was obtained for the partition coefficient. This is greater by a factor of 10 than the bulk decane–0.1 M KCl value quoted by TOSTESON *et al.*[10].

TABLE I

EXPERIMENTAL VALUES FOR THE ENTHALPY OF ACTIVATION, ΔH^*

Experiment	ΔH^* (*kcal*)	
	No valinomycin	*+ Valinomycin*
Control 1	15.3 ± 0.75	14.7 ± 0.9
Control 2	14.5 ± 1.0	16.6 ± 0.6
Control 3	16.8 ± 0.8	14.3 ± 1.6
Mean	15.5	15.2

Anaesthetic used	ΔH^* *exptl.*	ΔH^* + *corr. for anaesthetic* ρ	*Valinomycin exptl.* ΔH^*	*Valinomycin* ΔH^* *corr. for anaesthetic* ρ
50 mM *n*-butanol	22.9 ± 1.1	16.4 ± 1.3	16.8 ± 2.1	10.3 ± 2.3
100 mM *n*-butanol	22.7 ± 0.8	16.2 ± 1.0		
0.2 satd. chloroform	15.7 ± 0.6	13.9 ± 0.9	8.3 ± 0.1	6.5 ± 0.4
0.3 satd. chloroform	13.7 ± 1.7	11.9 ± 2.0§		
0.125 satd. diethyl ether	21.0 ± 1.1	17.5 ± 1.4	17.4 ± 2.8	13.9 ± 3.1
0.2 satd. ether	23.0 ± 1.5	19.5 ± 1.8		
Mean	—	15.9		(10.2)

§ This result is rather low but the standard deviation is large. At higher chloroform concentrations ΔH^* does decrease, and it is possible that even at this concentration the bilayer order is beginning to be disrupted.

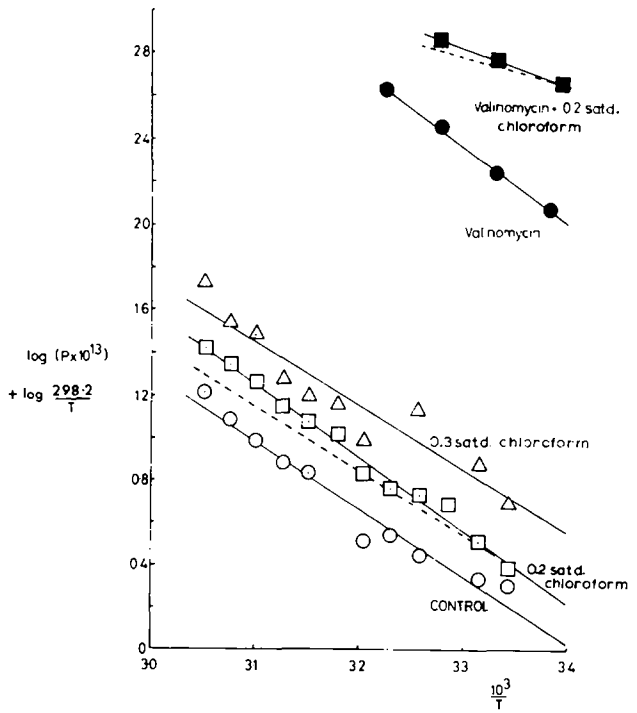

Fig. 3. Liposome permeability with chloroform. P in cm·sec^{-1}. Valinomycin to lipid molar ratio is 1:1·10^6.

RESULTS AND DISCUSSION

Experimental values of $\log P + \log 298.2/T$ are plotted against $10^3/T$ in Figs. 3–5. Since the amount of anaesthetic in the membrane varies with temperature, two types of line were drawn. The solid lines are the regression lines through the experimental points, and the dotted lines combine this experimental information with that of Fig. 2 to show the variation of permeability at constant membrane anaesthetic concentration. Table I summarizes the values of ΔH^* obtained. The errors quoted were calculated from the standard errors of the regression line gradients. Figs. 3–5 show that all the Arrhenius plots of the K^+ permeabilities give straight lines which indicates a single rate limiting step and eliminates the possibility described in Case 1 where the permeability is dependent on the sum of the diffusion through the interface and the diffusion through the membrane (Eqn. 4).

The most surprising feature of the results is that the ΔH^* for the K^+ permeability does not change upon addition of valinomycin. Because a membrane concentration of only 1 valinomycin molecule per $1 \cdot 10^6$ lipid molecules produces a 100-fold increase in K^+ permeability, and as the lipid and valinomycin molecules are of comparable size, a theoretical membrane made of pure valinomycin should have a permeability greater by a factor of approx. $100 \times 10^6/2$ than a lipid bilayer. This is a $5 \cdot 10^7$ change in comparable permeability for no apparent change in ΔH^*. The actual

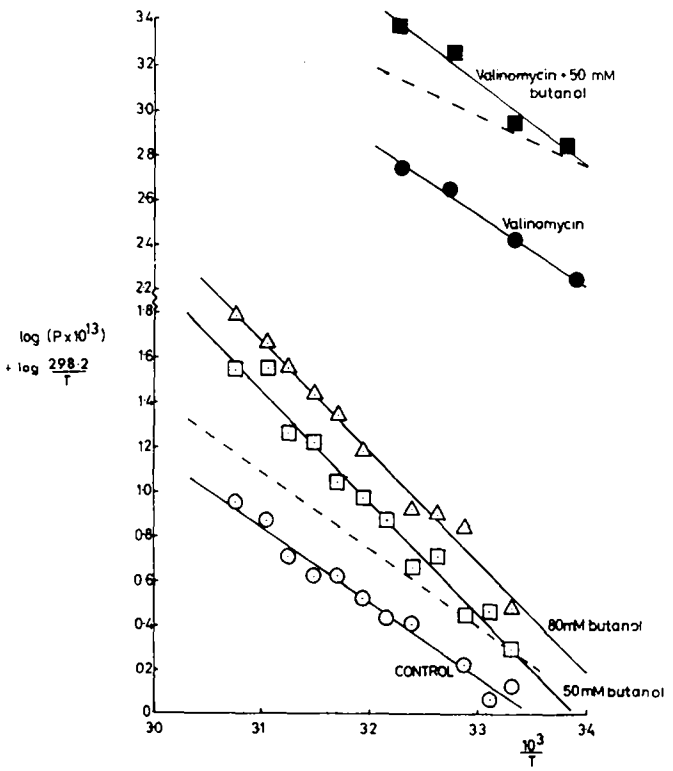

Fig. 4. Liposome permeability with *n*-butanol. P in cm·sec^{-1}. Valinomycin to lipid molar ratio is $1:2 \cdot 10^6$.

change in ΔH^* to account for the differing permeabilities would have to be 11.0 kcal which is far in excess of the experimental error. However, as the rate determining step is a function of ΔG^* rather than of ΔH^* alone, an increase in ΔS^* of 35 cal·degree^{-1} could account for this discrepancy.

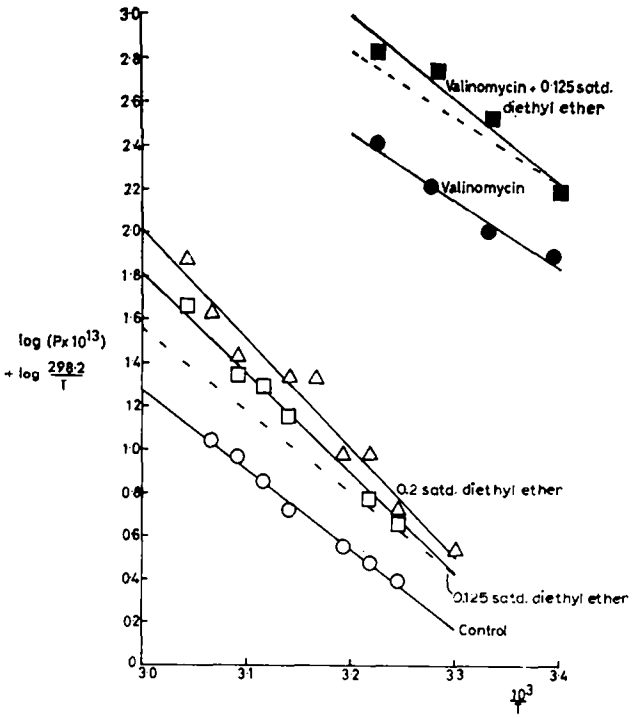

Fig. 5. Liposome permeability with diethyl ether. P in cm·sec^{-1}. Valinomycin to lipid ratio is 1:1·10^6. Liposome lecithin + phosphatidic acid, 4%.

In Case 2 the permeability of the liposomes is controlled by diffusion through the interior of the membranes and is described by Eqns. 5a and 5b. Now the values of ΔH^* for diffusion through bulk non-hydrogen bonded solvents are about 2–4 kcal, and would presumably be less for the K^+, even when hydrated, than for the larger valinomycin·K^+ complex. This means that the ΔH^* value of the partition coefficient ρ would then have to be about 11–13 kcal, and ρ would be markedly temperature dependent.

An attempt was made to measure ρ for $^{42}K^+$ between 0.16 M KCl and decane, but the salt was so insoluble that the results were inconclusive. However, TING *et al.*[11] report that there is no change in the partition coefficient between butanol and 0.08 M KCl over the temperature range 13–39°. Such evidence as there is suggests that there is no large ΔH^* for the partition coefficient, although there is need for caution in extrapolating from a bulk salt partition coefficient to that for an ion into a charged membrane, 60 Å thick.

The behaviour of the valinomycin–phospholipid system is still under study. The partition coefficient measurements showed that virtually all the valinomycin was in

the phospholipid between 22 and 37°, and that the presence of anaesthetic did not change this.

The transport of K^+ by valinomycin across the membrane can be expressed by the steps below:

$$K^+ + \text{valinomycin} \underset{c}{\overset{a}{\rightleftharpoons}} \text{valinomycin}\cdot K^+ \underset{b}{\overset{b}{\rightleftharpoons}} \text{valinomycin}\cdot K^+ \underset{a}{\overset{c}{\rightleftharpoons}} \text{valinomycin} + K^+$$

Solution Interface Membrane interior Interface Solution

a, b, and c are rate constants for the processes indicated. This is clearly analogous to the crossing of the membrane by K^+ alone. A study of the relevant equations obtained by adopting the treatment of BRITTON[12] to the system showed that straight line Arrhenius plots could be obtained only if either a, b, or c were exclusively rate limiting.

In the case of Na^+ transport a is clearly rate limiting as valinomycin so rarely complexes with Na^+. In the case of K^+ which complexes readily with valinomycin, it is felt that c might well be the rate determining step. Since valinomycin is a large molecule with a molecular weight of 1100 it should greatly disorganize the membrane locally. Thus there should be an increase in the entropy of the activated state for K^+ crossing the interface. It is, however, curious that ΔH^* should remain the same.

The experiments with anaesthetics in the absence of valinomycin showed an increase in permeability without a corresponding decrease in ΔH^*. Indeed ΔH^* apparently increased, unless a correction was applied for the temperature-dependent partition coefficient between the anaesthetic and the membrane (Figs. 3–5). A small increase in ΔS^* of about 1.5 cal·degree^{-1} would account for the permeability increase.

In the presence of valinomycin the anaesthetic clearly produces a decrease in ΔH^*. However, a decrease in ΔH^* of 5 kcal should produce a $10^{3.5}$-fold permeability increase at 37° if ΔS^* remained constant. The increase is only 4-fold, consequently ΔS^* must have now decreased by 13 cal·degree^{-1}. Table II summarises these results.

A possible explanation of these results might be as follows. The membrane immediately adjacent to the interface is normally tightly packed. In the activated state when the interface is disturbed to let through the K^+ there is little freedom of movement of the groups in the interface. When the anaesthetic is present, the ordered packing of the groups in the interface is disarranged, so that in the activated state there is a small increase in ΔS^* due to the increased freedom of movement at the

TABLE II

SUMMARY OF CHANGES IN THE ENTHALPY AND ENTROPY OF ACTIVATION FOR THE K^+ LIPOSOME PERMEABILITY

Experiment	*Compared to*	ΔH^*	ΔS^*
Control + anaesthetic	Control	Probably unchanged	Increase + 1.5 cal·degree^{-1}
Control + valinomycin	Control	Unchanged	Increase + 35 cal·degree^{-1}
Valinomycin + anaesthetic	Valinomycin Control	Decrease	Decrease
e.g. 50 mM *n*-butanol		− 5 kcal	− 13 cal·degree^{-1}

membrane interface. Changes in the activated state are reflecting changes in the ground state.

The unanaesthetised membrane imposes restrictions on the valinomycin molecule so that it is compelled to orientate itself edge on to the interface, which means that the K^+ complexing site in the centre of its ring structure is not immediately accessible. When the packing of the membrane interface is loosened by the anaesthetic, the valinomycin is free to adopt a more favourable orientation for the K^+ complexing process which can take place directly in the interface, so the ΔH^* of the activated state is decreased, as is its entropy.

If increasingly high concentrations of chloroform anaesthetics are used, such as 0.4 and 0.5 saturated solutions, ΔH^* begins to fall and ΔS^* increases. Also one experiment using very freshly prepared lipids gave a value of ΔH^* of 20 kcal although the pattern of the ΔH^* values remained the same, that is $\Delta H^*_{\text{control}} = \Delta H^*_{\text{valinomycin}} = \Delta H^*_{\text{control - anaesthetic}} > \Delta H^*_{\text{valinomycin + anaesthetic}}$. It would appear that ΔH^* is mostly influenced by a structural feature of the lipid at the interface.

TIEN AND TING[13] studied the water permeability of black lipid membranes composed of oxidized cholesterol in *n*-octane over a temperature range, and used the paper of ZWOLINSKI *et al.*[7] in the interpretation of the results. However, the molecular proportions of such a mixed membrane are unknown and may well be temperature dependent, a disadvantage of all black membrane systems.

Recent work by SWEET AND ZULL[14] on the activation energy of the glucose permeability of liposomes made of phosphatidyl choline, cholesterol and dicetyl phosphate, showed that the presence of a protein, bovine serum albumin, also increased the permeability without decreasing ΔH^* very significantly, an indication that ΔS^* had increased in this case.

TOSTESTON[15] studying the K^+ conductance across thick (mm) membranes of sheep red cell lipids in decane, deduced that the primary effect of valinomycin in thick or black lipid membranes was to lower the resistance at the oil–water interface.

METCALFE *et al.*[16] and METCALFE AND BURGEN[17] studied the nuclear magnetic resonance of anaesthetics such as benzyl alcohol in erythrocyte and other membranes. They found that at very low concentrations the rotation of the anaesthetic molecules was much restricted by the membrane both in the protein and the lipid portions, but that the rotation became freer as the anaesthetic concentration increased to clinically useful values. Higher concentrations produced irreversible changes in the protein structure of the membrane.

The thermodynamic activity of volatile anaesthetics is frequently expressed by the ratio $A_{\text{nar}} = p_{\text{nar}}/p_0$ where p_{nar} is the partial pressure of the anaesthetic in equilibrium with its aqueous solution at the chosen biological end point, and p_0 is the vapour pressure of the pure liquid at the same temperature.

In the experiments without valinomycin the change in ΔS^* is at least initially proportional to the quantity of anaesthetic in the membrane, and $\Delta\Delta S^*$ can be calculated from the relation

$$\frac{\Delta\Delta S^*}{2.303R} = \log P_{\text{anaesthetic}} - \log P_{\text{control}}$$

Table III shows the values of A_{nar} for the three anaesthetics calculated to produce a $\Delta\Delta S^*$ change of 1.74 cal·mole^{-1}·degree^{-1}. They are compared with the values of

A_{nar} which produce a 50 % reduction in the axon potentials of synaptic and "non-synaptic" or axonal conduction in the perfused stellate ganglion of the cat. These figures were taken from BRINK AND POSTERNAK's review.[18] It will be seen that there is a correlation with the values of A_{nar} required to block axonal conduction, but little

TABLE III

COMPARISON OF THE THERMODYNAMIC ACTIVITIES OF ANAESTHETICS PRODUCING AN EQUAL INCREASE IN ΔS^* OF 1.74 cal·degree^{-1} WITH THOSE GIVING A 50% REDUCTION IN AXONAL AND SYNAPTIC TRANSMISSION IN THE CAT[18]

Anaesthetic	*Partial molar vol.* (cm^3)	$A_{nar} \times 10^2$		
		K^+ liposome permeability	*Axon block*	*Synapse block*
n-Butanol	98	4.4	4.6	4.6
Chloroform	81	27.2	19.0	6.3
Diethyl ether	105	12.0	12.0	4.2

with the general empirical law quoted earlier, or with the blockage in synapse transmission. As the partial molar volumes are so similar, they cannot explain the discrepancy. From the figures in Table III it would seem that anaesthesia is more probably caused by a reversible reduction in synapse transmission rather than axonal conduction, but that there is a connection between the action of an anaesthetic on liposomes and its effect in blocking the sudden increase in Na^+ permeability initiating the action potential along an axon.

With or without valinomycin, *n*-butanol is much more effective at increasing liposome permeability than chloroform or ether. It is the only one of the three molecules with a hydrophilic head group and a hydrophobic tail, and might well be preferentially situated at the membrane–water interface, behind the choline zwitterions in the region of the carbonyl oxygens of the glycerol ester. The oxygen of the ether might have a similar but smaller effect, whereas the chloroform would be expected to remain largely in the hydrocarbon interior. BANGHAM *et al.*[5] showed that the *n*-alcohols from butanol to octanol were all particularly effective in increasing the liposome permeability to K^+, though the longer the hydrocarbon chain the less effective the molecule. This data further supports the membrane interface as being the site of action.

Finally it must be emphasised that the Na^+ and K^+ permeabilities of these liposomes are so low in comparison to natural membranes, at least in the absence of valinomycin, that the increase in permeability produced by the anaesthetics is in itself trivial, and significant only for what it reveals about the membrane.

This can be summarised as follows. (1) The main barrier to cation movement is in the membrane immediately adjacent to the aqueous–lipid interface. (2) In this region the structure is highly ordered. (3) The presence of general anaesthetics increases the disorder in this region. (4) This disorder is reflected in the behaviour of the ion carrier valinomycin.

It is suggested that the actual cause of the reversible anaesthetic block of the action potential in an axon is due to the presence of the foreign molecules which

sterically impede the rearrangement of the groups near the surface of the lipid which produces the sudden increase in Na^+ permeability.

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REFERENCES

1 J. FERGUSON, *Proc. Roy. Soc. London, Ser. B*, 127 (1939) 387.
2 L. J. MULLINS, *Chem. Rev.*, 54 (1954) 289.
3 L. PAULING, *Science*, 134 (1961) 15.
4 K. W. MILLER, W. O. M. PATON AND E. B. SMITH, *Nature*, 206 (1965) 574.
5 A. D. BANGHAM, M. M. STANDISH AND N. G. A. MILLER, *Nature*, 208 (1965) 1295.
6 S. M. JOHNSON AND A. D. BANGHAM, *Biochim. Biophys. Acta*, 193 (1969) 82.
7 B. J. ZWOLINSKI, H. EYRING AND C. E. REESE, *J. Phys. Colloid Chem.*, 53 (1949) 1426.
8 S. GLASSTONE, K. J. LAIDLER AND H. EYRING, *The Theory of Rate Processes*, McGraw-Hill, New York, 1941.
9 D. PAPAHADJOPOULOS AND N. G. A. MILLER, *Biochim. Biophys. Acta*, 135 (1967) 624.
10 D. C. TOSTESON, T. E. ANDREOLI, M. TIEFFENBERG AND P. COOK, *J. Gen. Physiol.*, 51 (1968) 373 s.
11 H. P. TING, G. L. BERTRAND AND D. F. SEARS, *Biophys. J.*, 6 (1966) 813.
12 H. G. BRITTON, *J. Physiol. London*, 170 (1964) 1.
13 H. T. TIEN AND H. P. TING, *J. Colloid Sci.*, 27 (1968) 702.
14 C. SWEET AND J. E. ZULL, *Biochim. Biophys. Acta*, 173 (1969) 94.
15 D. C. TOSTESON, *Federation Proc.*, 27 (1968) 1269.
16 J. C. METCALFE, P. SEEMAN AND R. S. V. BURGEN, *Mol. Pharmacol.*, 4 (1968) 87.
17 J. C. METCALFE AND A. S. V. BURGEN, *Nature*, 220 (1968) 587.
18 F. BRINK AND J. M. POSTERNAK, *J. Cellular Comp. Physiol.*, 32 (1948) 211.