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Statistical Mechanics of the Fluidity of Phospholipid Bilayers and Membranes

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Summary. A statistical mechanical treatment of the fluidity of lipid hydrocarbon chains in phospholipid bilayers is presented, which explicitly takes some account of interchain steric restrictions. With an effective energy separation of 750 cal/mole between *gauche* and *trans* conformations, it is found possible to account both for the chain dependence of the entropy and enthalpy change at the liquid crystalline transition of saturated lecithins, and also for intensity data in the laser raman spectra of dipalmitoyl lecithin. The method is used to calculate conformational probabilities in the lipid chains, in particular those for $2g1$ kinks. The calculated kink concentrations are found to be in agreement with the molecular permeability theory of H. Träuble (*J. Membrane Biol.* **4**:193, 1971).

It is now well-established that the fluidity of phospholipid bilayers, and of the bilayer regions which are known to exist in biological membranes, results from rotational isomerisms in the hydrocarbon chains (Luzzati, 1968; Hubbell & McConnell, 1971; Levine & Wilkins, 1971; Lippert & Peticolas, 1971, 1972; Seelig, 1971; Träuble, 1972). This enables the construction of molecular models of the dynamic structure of the hydrocarbon chains (Träuble & Haynes, 1971; Bothorel, Belle & Lemaire, 1973; Nagle, 1973; *also* H. Hahn & R. Klose, *in preparation*) and in particular has led to the proposal of a specific molecular model for bilayer permeability (Träuble, 1971). Statistical mechanical calculation of those physical properties of the bilayer hydrocarbon chain region, which are expected to have a strong influence on structure and function in biological membranes, requires a knowledge of the statistical weights of the various dynamic chain configurations. The problem in such an approach is to give proper account of the restrictions which the molecular packing in the bilayer places on the possible chain conformations.

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In the following, a simple assumption is made about the way in which the intermolecular steric hindrance between hydrocarbon chains in the bilayer restricts the possible conformations about adjacent bonds in the chain. An exacting experimental test of this assumption is provided by the entropy increase at the liquid crystalline phase transition in phospholipid bilayers (Chapman, Williams & Ladbrooke, 1967; Phillips, Williams & Chapman, 1969; Hinz & Sturtevant, 1972). This entropy change will have considerable sensitivity to the hydrocarbon chain conformations in the fluid state. It is attempted to give a consistent interpretation of the calorimetric data (both transition entropy and transition enthalpy) on saturated lecithins, with an effective *gauche* conformational energy within the range found with liquid hydrocarbons, for which intermolecular effects can be expected to be small. With this same value it is also attempted to account for intensity data in the laser raman spectrum of dipalmitoyl lecithin (Lippert & Peticolas, 1971). This gives an independent experimental check of the assumptions made. The method can then be used to predict conformational probabilities in the hydrocarbon chains. Kink concentrations are predicted which are in agreement with the Träuble (1971) theory of bilayer permeability.

Conformational Restrictions on the Lipid Hydrocarbon Chains

The C–C single bonds in a free alkane chain can take up three stable conformations of which the two *gauche* conformations $g^{\pm}$ are higher in energy than the *trans* conformation t by an amount E_g. In addition, intramolecular steric repulsions limit the possible conformational sequences in the chain. The molecular packing in lipid bilayers places further, intermolecular, steric restrictions on the lipid chain conformations (*see* Fig. 1).

The intramolecular effects introduce a cooperativity into the system, in the sense that the conformations about the various bonds are not independent. This effect is approximated to high accuracy in the theories of polymer liquids and solutions, by assuming that $g^{\pm}\, g^{\mp}$ conformations about any two adjacent bonds are forbidden (Volkenstein, 1963; Birshtein & Ptitsyn, 1966; Flory, 1969). (Even so this assumption does not eliminate all chain conformations which are not self-avoiding.)[1] In the case of lipid bilayers such

1 Long range interactions can be corrected for in dilute polymer solutions, however, by extrapolating to ideal theta conditions, in which the effective covolume for the pair of interacting chain elements is zero. This is not possible in the case of hydrocarbon chains in lipid bilayers, and reliance must be put on the long range effects of the explicit conformational restrictions introduced, and on a degree of cooperativity in the chain motions, as discussed below.

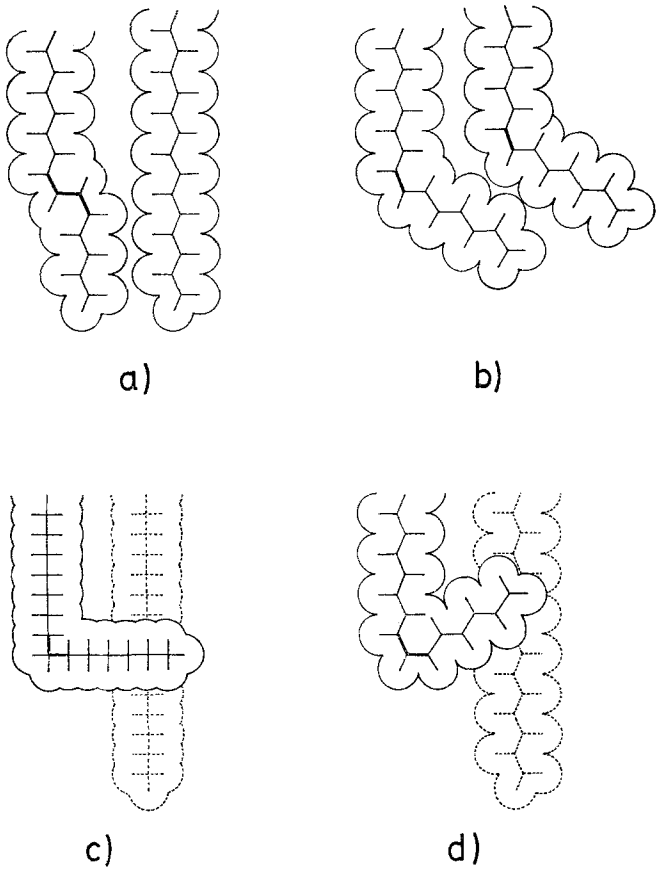

Fig. 1. Sterically allowed and forbidden chain conformations in lipid bilayers. Allowed bond sequences: *a*) $g^{\pm}\, tg^{\mp}(2g1$ kink); *b*) $g^{\pm}$. Forbidden bond sequences: *c*) $g^{\pm}\, g^{\pm}$; *d*) $g^{\pm}\, g^{\mp}$. Bond rotations from the all-*trans* configuration are indicated by the heavy lines. The projections are those for which the chain backbones lie in the plane of the Figure

effects are even more important, since the number of possible intermolecular encounters is much larger than intramolecular ones. The simplifying assumption used here is that intermolecular effects further restrict the possible conformations such that $g^{\pm}\, g^{\pm}$ conformations about adjacent bonds are also eliminated. The rationale for such an assumption can be seen in Fig. 1, which indicates the effects of chain packing.

In Fig. 1, conformations *c* and *d* are of the type which are eliminated in the present model, and can be seen to give rise to rather strong intermolecular encounters because of the large angular deviations (90° and 120°, respectively) from the all-*trans* backbone. Conformations *a* and *b* are of the type which are allowed in the model. Conformation *a* packs very well

into the bilayer structure, since it involves no net angular deviation of the chain, simply a lateral displacement. This is the $2g1$ kink introduced by Pechold (1968) and proposed by Träuble and Haynes (1971) for phospholipid bilayers. Conformation $1b$ is a simple *gauche* conformation, which involves a 60° deviation from the all-*trans* backbone. This is consistent with bilayer packing if some degree of cooperative motion takes place between chains, although this need not be so uniform as indicated in Fig. $1b$. (Cooperative motions are unable to yield better packing in case $1c$ because of the much tighter 90° bend in the chains.) It seems clear from Fig. $1b$ that the chains with single *gauche* conformations will pack more easily into the bilayer when the *gauche* conformation is closer to the end of the chain. This is allowed for in a rather crude manner, by assuming that the first three bonds in the chain are maintained in an all-*trans* configuration (*see below*).

Examination of the higher order allowed bond sequences shows that all allowed conformations with an odd number of *trans* bonds between two *gauche* bonds have angular deviations similar to Fig. $1a$ and $1b$. However, "allowed" sequences with an even number of *trans* bonds between two *gauche* bonds do involve large angular deviations. The success of the approximation relies on these unfavorable sequences being of relatively low probabilty. An estimate for four-bond sequences, using the methods of the following section, bears this out: the unfavorable sequences consist of less than 5% of the total allowed four bond sequences. The contribution to the calculated chain dependence of the transition entropy will thus be of a similar magnitude.

Higher order forbidden bond sequences must involve at least three *gauche* bonds, and so will be of relatively low occurrence unless the intermolecular packing is particularly favorable. There is one "forbidden" sequence which could pack quite well among bilayer chains. This is the helical sequence $g^+g^+g^+g^+$, which causes no net angular deviation of the chain from the all-*trans* backbone. The net statistical weight of these sequences would depend on the relative balance between the unfavorable intramolecular energy and the favorable intermolecular packing. Hahn and Klose (*in preparation*) have calculated that, at maximum, these "Reneker defects" could contribute 26% to the chain dependence of the phospholipid transition entropy. For the reasons stated above, it seems likely that this contribution could be considerably lower; an estimate based on the statistical methods for free polymer chains gives a relative probability of 0.26% for the Reneker defects. Finally in this connection, it is worth noting that Hahn and Klose (*in preparation*) have shown that the maximum possible entropy contribution from all conformations which stay within the first two, vertical,

neighbor shells on a diamond lattice, is insufficient to account for the chain dependence of the saturated lecithin transition entropy. This suggests that contributions from configurations of type 1*b* are probably important.

Since configurations involving the $g^{\pm}g^{\mp}$ sequence are strongly disfavored on intramolecular steric grounds, it seems unlikely that any of these configurations will have an appreciable degree of occurrence because of intermolecular packing considerations. It thus appears that the interchain restrictions introduced in this section could well give a fairly realistic approximation. The crucial test, of course, lies in the agreement with the experimental data.

Statistical Mechanical Background

The statistical mechanical calculations have been carried out using the matrix methods employed for free polymer chains, as described by Flory (1969). It should perhaps be emphasized that this treatment applies only to the configuration of the lipid chains in the fluid state, i.e. above the gel to liquid crystalline transition temperature T_c. This is the state of fluidity normally found in biological membranes.

The conformational restrictions introduced in the last section can be summarized in a statistical weight matrix[2] of which the elements $u_{\zeta\eta}$ are the statistical weights of a two-bond sequence, in which the first bond has conformation ζ and the following bond has conformation η:

$$U=[u_{\zeta\eta}]=\begin{array}{c} \\ t \\ g^+ \\ g^- \end{array}\begin{array}{c} \begin{array}{ccc} t & g^+ & g^- \end{array} \\ \begin{pmatrix} 1 & \sigma & \sigma \\ 1 & 0 & 0 \\ 1 & 0 & 0 \end{pmatrix} \end{array} \tag{1}$$

$$\sigma=\exp(-E_g/RT). \tag{2}$$

2 The restrictive assumptions may be elaborated somewhat by using a more general statistical weight matrix:

$$U=\begin{pmatrix} 1 & \sigma\chi & \sigma\chi \\ 1 & \sigma\psi & \sigma\omega \\ 1 & \sigma\omega & \sigma\psi \end{pmatrix}$$

where χ, ψ, ω are factors which allow variation in the relative strengths of the steric restrictions placed on the conformations $g^{\pm}$, $g^{\pm}g^{\pm}$ and $g^{\pm}g^{\mp}$, respectively. For the simplified case above: $\chi=1$, $\psi=0$, $\omega=0$. The factors may be expressed as: $\chi=\exp(-E_\chi/RT)$, $\psi=\exp(-E_\psi/RT)$, $\omega=\exp(-E_\omega/RT)$, where E_χ, E_ψ, E_ω then take on the character of pseudopotentials governing the steric repulsions for the various chain configurations. This approach should prove useful when considering different phospholipids and mixed systems, in which intermolecular separations are likely to vary.

Calculation of the thermodynamic properties of the lipid chains is performed via the configurational partition function. The partition function for a single chain is the sum of the statistical weights over all possible total chain configurations $\{\phi\}$:

$$Z=\sum_{\{\phi\}}\prod_{i=1}^{n'-1}u_{\zeta\eta;i} \tag{3}$$

where n' is the number of skeletal bonds in the chain. The product is taken over all skeletal bonds except the last, since the terminal methyl group is assumed to have free rotation. This sum can be evaluated, subject to the conformational restrictions implicit in Eq. (1), by matrix multiplication (Flory, 1969):

$$Z=(1\ 0\ 0)\,\boldsymbol{U}^{n'-1}\begin{pmatrix}1\\1\\1\end{pmatrix}. \tag{4}$$

The evaluation is facilitated by diagonalizing the statistical weight matrix:

$$\boldsymbol{A}^{-1}\cdot\boldsymbol{U}\cdot\boldsymbol{A}=\boldsymbol{B}\cdot\boldsymbol{U}\cdot\boldsymbol{A}=\boldsymbol{\Lambda}=\begin{pmatrix}\lambda_1 & 0 & 0\\ 0 & \lambda_2 & 0\\ 0 & 0 & \lambda_3\end{pmatrix}. \tag{5}$$

The partition coefficient is then given by:

$$Z=\sum_{\zeta=1}^{3}\Gamma_\zeta\lambda_\zeta^{n'-1} \tag{6}$$

where the expansion coefficients are related to the elements of the diagonalization matrices by:

$$\Gamma_\zeta=A_{1\zeta}\sum_{\eta=1}^{3}B_{\zeta\eta}. \tag{7}$$

The conformational probabilities in the hydrocarbon chains can be expressed in similar terms (detailed derivations will be found in Flory, 1969). The probability of a particular total chain configuration $\{\phi\}$ is given by the statistical weight of that configuration divided by the partition function:

$$p_{\{\phi\}}=Z^{-1}\prod_{i=1}^{n'-1}u_{\zeta\eta;i}. \tag{8}$$

The *a priori* probability that two adjacent bonds occur in a particular conformation $\zeta\eta$ is given by the weighted sum of the probabilities of all the

total chain configurations in which the conformation $\zeta\eta$ occurs, divided by the total number of bond pairs. In the limit of long chains it can be show that:

$$p_{\zeta\eta}=u_{\zeta\eta}A_{\eta 1}B_{1\zeta}/\lambda_1 \qquad n'\to\infty. \tag{9}$$

The *a priori* probabilities of longer bond sequences may be calculated using the conditional probability $q_{\zeta\eta}$ that a bond is in conformation η, given that the preceding bond is in the conformation ζ. Again for long chains it can be show that:

$$q_{\zeta\eta}=u_{\zeta\eta}A_{\eta 1}/A_{\zeta 1}\lambda_1 \qquad n'\to\infty. \tag{10}$$

The *a priori* probability of the bond sequence $\varepsilon\zeta\eta$ is then given by:

$$p_{\varepsilon\zeta\eta}=p_{\varepsilon\zeta}\times q_{\zeta\eta}. \tag{11}$$

Finally, the probability of a single bond conformation η is given by the sum over all *a priori* two-bond probabilities in which the second bond of the pair is in the conformation η divided by the total number of bonds. For long chains the result is:

$$p_\eta=A_{\eta 1}B_{1\eta} \qquad n'\to\infty. \tag{12}$$

The diagonalization of the statistical weight matrix, Eq. (1), is required in the application of Eqs. (6)–(12) to the calculations in subsequent sections. These solutions are given below:

$$\lambda_{1,2}=1/2(1\pm\sqrt{1+8\sigma}) \tag{13}$$

$$\lambda_3=0 \tag{14}$$

$$A=\begin{pmatrix}\lambda_1 & \lambda_2 & 0\\ 1 & 1 & 1\\ 1 & 1 & -1\end{pmatrix} \tag{15}$$

$$A^{-1}=B=\frac{1}{2(-\lambda_1+\lambda_2)}\begin{pmatrix}-2 & \lambda_2 & \lambda_2\\ +2 & -\lambda_1 & -\lambda_1\\ 0 & -\lambda_1+\lambda_2 & \lambda_1-\lambda_2\end{pmatrix}. \tag{16}$$

The coefficients in the partition coefficient expansion, Eqs. (6) and (7), are then:

$$\Gamma_{1,2}=\frac{1}{2}\left(1\pm\frac{1+4\sigma}{\sqrt{1+8\sigma}}\right) \tag{17}$$

$$\Gamma_3=0. \tag{18}$$

For the values of E_g required here it is found that σ is small, and in consequence $\Gamma_1 \approx 1$ and Γ_2, λ_2 are small, thus $\Gamma_2 \lambda_2^{n'-1} \approx 0$. The latter is especially true for long chains. It is thus possible to use the following approximation for Eq. (6):

$$Z = \lambda_1^{n'-1}. \tag{19}$$

It will be seen below that the value of Γ_1 does not, in any case, contribute to the chain length dependence of the thermodynamic properties.

Transition Entropy of Saturated Lecithins

The configurational entropy is a quantity which is clearly sensitive to the allowed conformations of the lipid chains. It thus provides a good test of the proposed statistical method. If it is assumed that the lipid chains are in the all-*trans* configuration below the liquid crystalline phase transition of a phospholipid bilayer, then the increase in entropy at the transition gives a measure of the configurational entropy of the lipid chains in the fluid state. Because of the large number of degrees of freedom in the hydrocarbon chains, it may be assumed that other contributions to the transition entropy are relatively unimportant. By centering attention on the chain-length dependence of the transition entropy, this will certainly be the case.

Since the liquid crystalline phase transition is a first-order transition, the change in free energy (at constant pressure) will be zero; hence:

$$\Delta H = T_c \cdot \Delta S. \tag{20}$$

Phillips *et al.* (1969) have calculated the increase in entropy at the transition ΔS for a series of saturated lecithins, from calorimetric measurements of the transition temperature T_c and enthalpy increase at the transition ΔH. The data of Phillips *et al.* (1969), which is derived from the calorimetric measurements of Chapman *et al.* (1967), is given in Fig. 2. More recent data of Hinz and Sturtevant (1972) is in substantial agreement, except for the case of dipalmitoyl lecithin, and the mean chain-length dependence is similar. The data of Phillips *et al.* (1969) is used here because it refers to a more extensive series of chain lengths.

The chain entropy can be calculated from the configurational partition function, using the customary thermodynamic relationship:

$$S = R\left(\log Z + T\frac{\partial}{\partial T}\log Z\right). \tag{21}$$

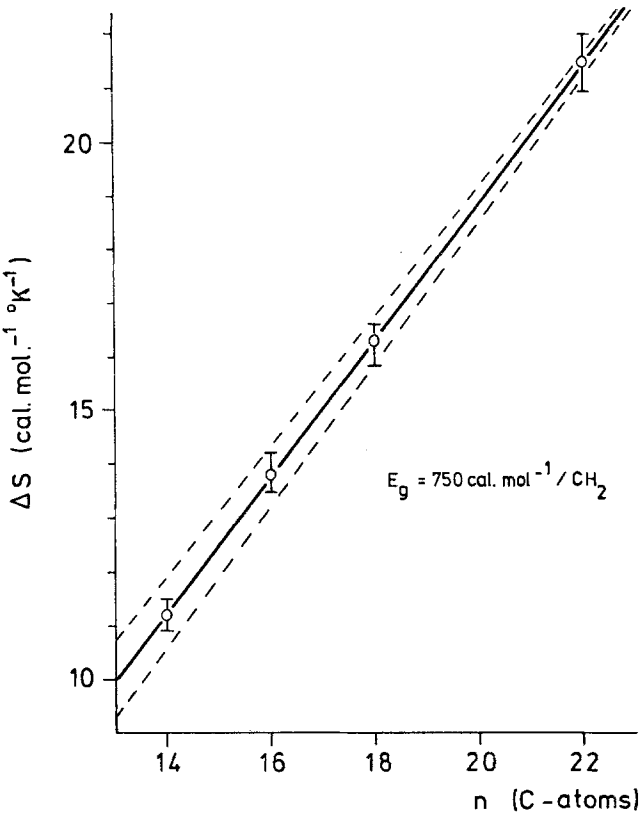

Fig. 2. Variation with chain length of the entropy change, per chain, at the liquid crystalline transition of fully hydrated bilayers of saturated lecithins. I, experimental data of Phillips *et al.* (1969). ——, chain-length dependence calculated for $E_g = 750$ cal/mole; ○, absolute values calculated for $n' = n$(C-atoms)-4. (Broken lines: *lower curve*-values calculated for $E_g = 500$ cal/mole, $n' = n$(C-atoms)-5; *upper curve*-values calculated for $E_g = 900$ cal/mole, $n' = n$(C-atoms)-3.)

Hence, using Eqs. (13) and (19):

$$S = (n'-1)\left(R \log \lambda_1 + \frac{E_g}{T} \cdot \frac{2\sigma}{\lambda_1 \sqrt{1+8\sigma}}\right). \tag{22}$$

The calculated values are compared with the experimental data in Fig. 2. It is possible to account for the chain-length dependence of the configurational entropy with a value of $E_g = 750$ cal/mole. This is to be compared with values in the range 500 to 800 cal/mole found for liquid hydrocarbons and long-chain hydrocarbon solutions (Birshtein & Ptitsyn, 1966; Flory, 1969). Since steric intermolecular interactions are expected to be small in

the latter case, it seems that the above method of calculation has approximated the steric effects of chain packing in lipid bilayers quite well.

To fit the absolute values of the configurational entropy in Fig. 2, it has been assumed that the first three bonds of the chain remain fixed in all-*trans* configuration, i.e. $n' = n$(C-atoms)-4, and the mean value of the chain-length dependence has been used. This seems a not unreasonable approximation, since the pairs of lipid chains are constrained in this region by both being attached to the same glycerol backbone. It has already been mentioned above that this assumption will also make some allowance for the fact that configurations of the type $1b$ are more likely to occur when the bend is near the terminal methyl end of the chain.

The broken lines in Fig. 2 indicate the effect of varying the two parameters n' and E_g. In these cases the agreement with the experimental data is not so good. However, it can be seen that, on varying n' by ± 1, some measure of agreement can be obtained with values of E_g which still lie within, or close to, the range found for free hydrocarbon chains. This gives some further support for the method of calculation and the restrictive assumptions made. At this point it must be stressed that the value of $E_g = 750$ cal/mole derived above was obtained solely by fitting the chain-length dependence of the configurational entropy. Because of end effects, this is likely to be a more reliable estimate than attempting to fit the individual absolute values of the entropy.

Transition Enthalpy of Saturated Lecithins

There are several contributions to the increase in enthalpy at the liquid crystalline transition:

$$\Delta H = \Delta U + p\Delta V + \Delta H_{\text{int}} \tag{23}$$

where ΔU is the increase in internal energy of the hydrocarbon chains due to rotational isomerism; ΔV is the change in molar volume of the lipid bilayer; and ΔH_{int} is the increase in enthalpy due to the change in the intermolecular cohesive and repulsive forces, arising from the lateral expansion of the bilayer. The fractional increase in bilayer volume at the phase transition has been variously measured as 1.4 to 4.0% (Melchior & Morowitz, 1972; Scheetz & Chan, 1972; Träuble & Haynes, 1972). For $p = 1$ atm, the term $p\Delta V$ is thus found to make a negligible contribution (0.25 to 0.71 cal/mole) to ΔH. The value for ΔU can be calculated using the statistical methods outlined above. The value for ΔH_{int} depends on inter-

molecular potentials, and estimates of this are perhaps rather less reliable. However, given the calculated value of ΔU, it can be estimated whether the value of ΔH_{int} required to account for the measured ΔH is reasonable or not. Again interest centers on the chain dependence of ΔH_{int}, since the calculated ΔU is directly proportional to chain length.

The increase in internal energy of the chains, arising from rotational isomerism, is related to the configurational partition function by the usual thermodynamic equation:

$$\Delta U = RT^2 \frac{\partial}{\partial T} \log Z. \tag{24}$$

Hence using Eqs. (13 and (19):

$$\Delta U = (n'-1) \frac{2\sigma E_g}{\lambda_1 \sqrt{1+8\sigma}}. \tag{25}$$

The values of ΔU, for the various chain lengths and corresponding transition temperatures, have been calculated using exactly the same assumptions as in the entropy calculation, namely that $E_g = 750$ cal/mole, and that the first three bonds remain in the all-*trans* configuration. The values of ΔH_{int} obtained by subtracting this calculated value of ΔU from the measured ΔH are given in Fig. 3.

The method used in calculating ΔH_{int} is that with which Salem (1962) successfully estimated the heat of sublimation at 0 °K of long-chain hydrocarbons. In this approach the cohesive dispersion energy between the chains is given by:

$$W_{\text{disp}} = -\frac{1.24 \times 10^3}{D^5} \text{ (kcal/mole per } CH_2) \tag{26}$$

where D is the distance in angstroms between the two chain centers. The repulsive potential between nonbonded hydrogen atoms is given by an empirical expression based on molecular scattering data:

$$W_{H-H} = +\frac{33.2}{d^{6.18}} \text{ (kcal/mole per } CH_2) \tag{27}$$

where d is the distance in angstroms between the centers of the two interacting hydrogen atoms. Then ΔH_{int} is given by the change in these interchain energies at T_c:

$$\Delta H_{\text{int}} = \Delta W_{\text{disp}} + \Delta W_{H-H}. \tag{28}$$

Since these calculations are not of direct relevance to the statistical method only the salient features will be summarized here, and they will be considered

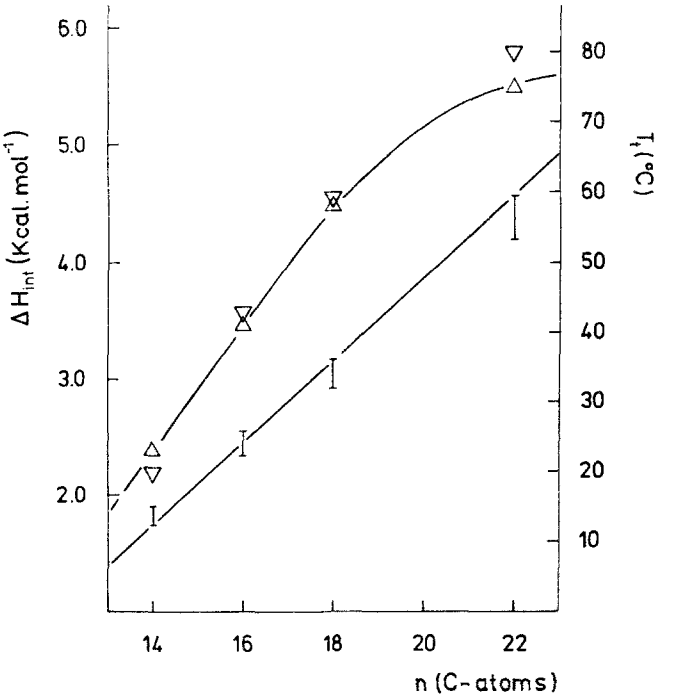

Fig. 3. *Lower curve:* variation with chain length of the contribution from intermolecular interactions to the enthalpy change, per chain, at the liquid crystalline transition of fully hydrated bilayers of saturated lecithins. I, experimental data of Phillips *et al.* (1969), with intramolecular contribution subtracted by calculation with identical conditions to Fig. 2. ——, calculated chain-length dependence according to the method of Salem (1962); the absolute values have been fitted to give best agreement between experimental and calculated chain-length dependences. *Upper curve:* variation with chain length of the liquid crystalline transition temperature of fully hydrated bilayers of saturated lecithins. —△—, experimental data of Phillips *et al.* (1969). ▽, values derived from the calculated values of ΔS and ΔH at the transition

in more detail elsewhere. It is assumed that the hydrocarbon chains are centered on a triangular lattice (both above and below T_c) and that the interchain distance increases from 4.84 to 5.31 Å (calculated from X-ray short spacings) at T_c. Taking the summation of W_{disp} to the third shell of neighbors, the calculated values of the chain dependence of ΔH_{int} are then those given in Fig. 3. A surprisingly good agreement is found in the chain dependence, further supporting the method used to calculate ΔU.

In view of the results, obtained by Salem (1962) it would appear that the estimates for W_{disp} and W_{H-H} below T_c should be rather reliable. It has been assumed in the calculation that $W_{H-H}=0$ above T_c, since the repulsive interaction is very short range, and intermolecular separations above T_c are out-

side the range of applicability of Eq. (27). This assumption will, if anything, tend to underestimate ΔH_{int}. The method of calculating ΔW_{disp} may well overestimate ΔH_{int} since chain motions in the fluid state above T_c will allow chains to approach more closely than their mean separation (though this effect may be to some extent compensated by corresponding larger separations caused by chain motion). It would appear that these effects have cancelled in giving the rather good agreement in Fig. 3.

In Fig. 3 only the chain dependence of ΔH_{int} has been calculated; the absolute value is determined by end effects. This can possibly be accounted for by electrostatic repulsions between the lecithin polar headgroups, and steric effects of the bulky polar headgroups reducing the effective chain length. As a check on consistency the lecithin transition temperatures derived from the calculated values of ΔH and ΔS are compared with the measured T_c's in Fig. 3. The agreement is quite good; the discrepancies come mainly from ΔH, and probably from the ΔH_{int} contribution.

The calculations of transition enthalpy for the saturated lecithins thus support the entropy calculations of the previous section, and also suggest that the method used by Salem (1962) for estimating intermolecular energies may be reasonably applied to lipid bilayers.

Intensities in the Laser Raman Spectrum of Dipalmitoyl Lecithin

The intensities of laser raman bands from phospholipid dispersions are directly proportional to the probability of occurrence of the configuration to which the particular vibrational mode corresponds. Lippert and Peticolas (1971) have examined the temperature dependence of the laser raman spectrum of a dipalmitoyl lecithin dispersion. Their data for the intensity ratio of the 1089 cm^{-1} band to the 1128 cm^{-1} band is indicated in Fig. 4. The 1089 cm^{-1} band has been assigned as the optical mode of the phosphate diester symmetric stretch; and the 1128 cm^{-1} band as the $k=0$ band of the skeletal optical mode of the all-*trans* hydrocarbon chains (Lippert & Peticolas, 1971, 1972). If the phosphate O–P–O conformation is assumed to remain constant, the temperature dependence of the intensity ratio is given by:

$$\frac{I_{1089}}{I_{1128}} = K \frac{\langle n_{1089} \rangle}{\langle n_{1128} \rangle} \frac{1}{p_{\{\text{all-}trans\}}} \tag{29}$$

where K is a constant depending on the effective oscillator strengths of the transitions, linewidths and lineshapes, etc. This is difficult to calculate reliably and is used as a single, constant, scaling factor to be fitted. $\langle n_\nu \rangle$ is the occupation number of the vibrational mode of frequency (or wavenum-

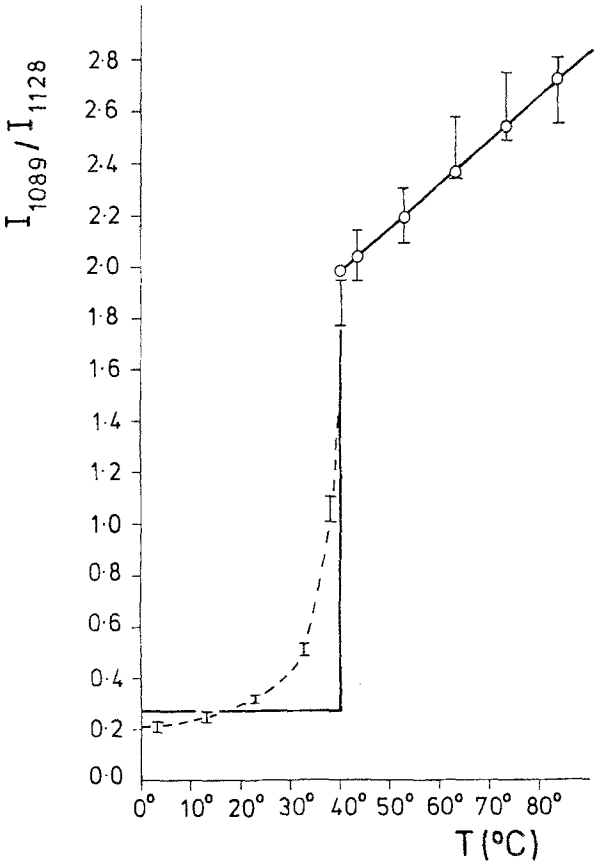

Fig. 4. Temperature dependence of the ratio in intensities of the 1089 cm^{-1} to the 1128 cm^{-1} band in the laser raman spectrum of hydrated dipalmitoyl lecithin bilayers. I and broken line: experimental data of Lippert and Peticolas (1971). ○ and full line: calculated values, assuming the chains remain in the all-*trans* configuration up to T_c and using the same conditions as in Fig. 2 with factor $K=0.03$ for the region above T_c

ber) ν, which is simply given by the Bose-Einstein function:

$$\langle n_\nu \rangle = \frac{1}{e^{h\nu/kT}-1}. \tag{30}$$

The temperature variation of the occupation number ratio is small, since the frequencies are very close.

The probability of occurrence of the all-*trans* chain configuration is given by Eq. (8):

$$p_{\{\text{all-}trans\}} = Z^{-1} u_{tt}^{n'-1}. \tag{31}$$

Since $u_{tt}=1$ [Eq. (1)], and using Eq. (19) for Z, we obtain:

$$p_{\{\text{all-}trans\}}=\lambda_1^{-(n'-1)}. \tag{32}$$

The all-*trans* probability has been calculated assuming again $E_g=750$ cal/mole and $n'=n(\text{C-atoms})-4$.

The results of calculations of I_{1089}/I_{1128} from Eq. (29), for $K=0.03$ are given in Fig. 4. It is seen that both the discontinuity at the phase transition and the temperature dependence above T_c can be accounted for reasonably well with this single value of K, giving further support for the statistical model. The rather complicated pretransitional melting is not accounted for, since it is assumed that the chains remain completely in the all-*trans* configuration up to T_c.

Conformational Probabilities, Kink Concentrations and Bilayer Permeability Coefficients

Various conformational probabilites which characterize the lipid chain fluidity can be calculated, using the value of $E_g=750$ cal/mole derived in the previous sections. The probabilities of *trans* and *gauche* conformations, from Eq. (12), are:

$$p_t=\frac{1}{2}\left(1+\frac{1}{\sqrt{1+8\sigma}}\right) \tag{33}$$

$$p_{g^\pm}=\frac{1}{4}\left(1-\frac{1}{\sqrt{1+8\sigma}}\right). \tag{34}$$

The probability of the conformational sequence g^+tg^-, which corresponds to the $2g1$ kink of Fig. 1*a*, is given by Eqs. (9)–(11):

$$p_{g^+tg^-}=\frac{\sigma}{2}\left(1-\frac{1}{\sqrt{1+8\sigma}}\right)\frac{1}{(1+4\sigma+\sqrt{1+8\sigma})}. \tag{35}$$

Calculated values for the temperature dependence of these conformational probabilities are given in Fig. 5. In all these cases it is of course assumed that the lipid chains are above their liquid crystalline T_c.

The results of Fig. 5 may be used to estimate the $2g1$ kink concentrations relevant to the molecular model of bilayer permeability given by Träuble (1971). In this theory, the kink isomers are considered as intrinsic carriers. For the case in which the kink diffusion is rate-limiting, the bilayer perme-

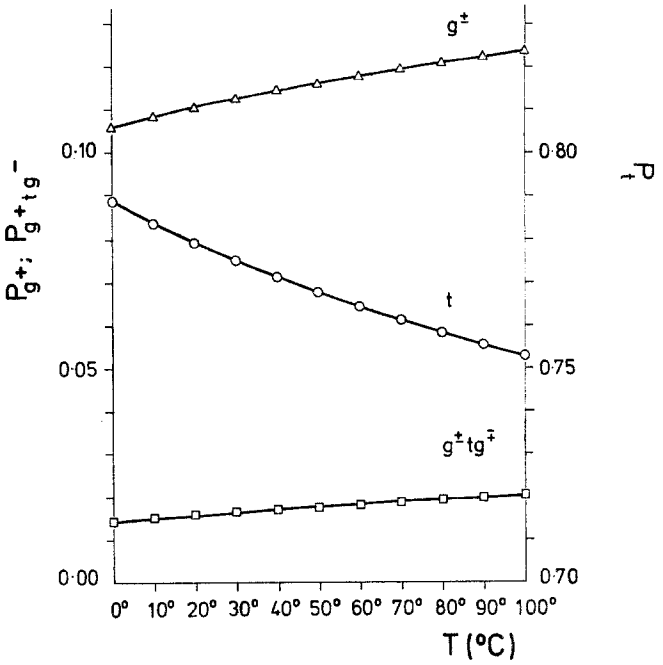

Fig. 5. Calculated conformational probabilities for lipid chains in the liquid crystalline state, at various temperatures. —○—, *trans* conformation; —△—, *gauche* conformation; —□—, $g^{\pm}\ tg^{\mp}$ sequence, corresponding to a $2g1$ kink. The same conditions are assumed as in Fig. 2

ability coefficient is then found to be:

$$P=\frac{D}{\delta}\cdot\frac{nc_k}{K+c''}\cdot\frac{K}{K+c'} \tag{36}$$

where D is the kink diffusion coefficient; δ is the bilayer thickness; c', c'' are the concentrations of permeant molecules on either side of the lipid bilayer membrane ($c'>c''$); K is the dissociation constant for the release (uptake) of permeant molecules by kinks at the bilayer surface; n is the number of permeant molecules per kink; and finally c_k is the kink concentration in the membrane. By considering the limit: $c' \ll K$, the quantity $n\,c_k/(K+c'')$ can be identified with the equilibrium partition coefficient K_p of the permeant between the aqueous phase and the membrane. An estimate for K can be made if K_p is identified with the experimental oil-water partition coefficient, and if the value of c_k is known. Träuble (1971) calculates for a bilayer with 20 CH_2 groups per chain that:

$$c_k=8.5\times10^{-2}\times p(\text{kink}) \tag{37}$$

in moles/cm^3, where $p(\text{kink}) = 2\, p_{g^+tg^-}$ is the kink probability per bond. The factor of two allows for both $g^{\pm}tg^{\mp}$ kink combinations. This estimate is an upper limit for the probability of an isolated kink, since it includes all allowed configurations in which the $g^{\pm}tg^{\mp}$ sequence occurs. This larger value is appropriate, since all $g^{\pm}tg^{\mp}$ conformations will be capable of acting as carriers. Hence at 25 °C, $c_k = 2.72 \times 10^{-3}$ moles/cm^3 (this corresponds to 0.64 kinks per hydrocarbon chain for a chain with 20 CH_2 groups). For osmotic permeability, $K_p = 0.64 \times 10^{-4}$ (Schatzberg, 1963, 1965), thus $K = 0.425 \times 10^2$ mole/cm^3. Using this value for K, together with the estimate made by Träuble (1971) of $D = 1 \times 10^{-5}$ cm^2/sec, gives $P \approx 1.3 \times 10^{-3}$ cm/sec in agreement with both the measured values of osmotic permeability and the theoretical estimate by Träuble (1971).

In conclusion, the steric restrictions on the packing of lipid chains in phospholipid bilayers have been approximated by assuming that $g^{\pm}\, g^{\pm}$ conformations about adjacent skeletal bonds are forbidden. Statistical mechanical methods, such as are used for long-chain molecules in conditions where there are no packing restrictions, have then been able to interpret experimental data which is sensitive to the hydrocarbon chain conformations in phospholipid bilayers. The conformational energies are estimated to be similar to those in the noninteracting systems. The utility of the method in the study of membrane function has been illustrated by application to the calculation of the osmotic permeability of lipid membranes, using a specific molecular model. It is felt that the method can now be applied with some confidence to other such calculations.

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