LECITHIN BILAYERS

DENSITY MEASUREMENTS AND MOLECULAR INTERACTIONS

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ABSTRACT Density measurements are reported for bilayer dispersions of a series of saturated lecithins. For chain lengths with, respectively, 14, 15, 16, 17, and 18 carbons per chain, the values for the volume changes at the main transition are 0.027, 0.031, 0.037, 0.040 and 0.045 ml/g. The main transition temperature extrapolates with increasing chain length to the melting temperature of polyethylene. Volume changes at the lower transition are an order of magnitude smaller than the main transition. Single phase thermal expansion coefficients are also reported. The combination of X-ray data and density data indicates that the volume changes are predominantly due to the hydrocarbon chains, thus enabling the volume v_{CH_2} of the methylene groups to be computed as a function of temperature. From this and knowledge of intermolecular interactions in hydrocarbon chains, the change in the interchain van der Waals energy, ΔU_{vdW}, at the main transition is computed for the lecithins and also for the alkanes and polyethylene at the melting transition. Using the experimental enthalpies of transition and ΔU_{vdW}, the energy equation is consistently balanced for all three systems. This yields estimates of the change in the number of gauche rotamers in the lecithins at the main transition. The consistency of these calculations supports the conclusion that the most important molecular energies for the main transition in lecithin bilayers are the hydrocarbon chain interactions and the rotational isomeric energies, and the conclusion that the main phase transition is analogous to the melting transition in the alkanes from the hexagonal phase to the liquid phase, but with some modifications.

INTRODUCTION

Some time ago it was realized that measurements of heats of transition, ΔH, and volume changes, ΔV, at the main transition in lipid bilayers can be combined with simple theories to elucidate the molecular interactions in biomembranes and to quantify the relative state of disorder resulting from the main phase transition (1,2), often called the gel-to-liquid crystal transition (3). In particular, estimates were made of the increase in the number of gauche rotamers Δn_g at the phase transition by balancing the energy equation

$$\Delta H = \Sigma \Delta U_i + P\Delta V. \quad (1)$$

For molecules as complicated as lipids there are many different internal energies U_i. The largest sources of ΔU_i at the transition seem to be from the increase in the number of gauche rotamers, ΔU_{rot}, and from the van der Waals interactions between the hydrocarbon tails of the lipids, ΔU_{vdW}. Much is known about hydrocarbon chain interactions, so ΔU_{vdW} is calculable from measured volume changes, as we show in detail for the first time in this paper.

To obtain the required volume changes more accurately, we have designed and constructed a new differential dilatometer especially appropriate for lipid dispersions in excess water (4). With this more sensitive apparatus we also obtain coefficients of thermal expansion in the single phase regions while the use of simple D_2O-H_2O flotation measurements (5,6) yields absolute values of density. This gives extra information helpful in understanding lipid bilayers. Although a number of dilatometric studies have been performed (2,6–10), the results for ΔV from different workers have only recently converged for dipalmitoyl phosphatidylcholine (DPPC)[1] and agreement is not yet complete for other lipids. In this paper results for a sequence of lecithins with saturated hydrocarbon chains are reported, with an emphasis on the effects of varying the hydrocarbon chain length.

Throughout this paper comparisons are made between the state of the hydrocarbon chains in lecithins and in polyethylene and the long, single-chain alkanes. Of all analogues of the lipid phase transition, the melting transition in polyethylene and the alkanes is certainly the best one, and new support comes from our novel extrapolation with increasing chain length of the transition temperature T_M to the melting temperature of polyethylene. However, it is also important to emphasize the differences between these systems, such as the fact that the magnitude of the transition changes is smaller for the lipids than for the bulk hydrocarbon transitions. Nevertheless, the same kind of calculation of ΔU_{vdW} and the subsequent energy balancing works as well for the melting transition in polyethylene and the alkanes as for the lipid transitions, thereby lending support to these theoretical calculations.

MATERIALS AND METHODS

All the lecithins used in this study were commercial preparations. Ditetradecanoyl phosphatidylcholine (DMPC)[1] was purchased from Calbiochem (San Diego, Calif.) and Sigma Chemical Co. (St. Louis, Mo.); dipentadecanoyl ($DC_{15}PC$) from Supelco, Inc. (Bellefonte, Pa.); dihexadecanoyl (DPPC) from Calbiochem; diheptadecanoyl ($DC_{17}PC$) from Avanti Biochemicals, Inc. (Birmingham, Ala.) and Supelco, Inc.; and dioctadecanoyl (DSPC) from Supelco, Inc., and Sigma Chemical Co. The DSPC from Sigma showed lysolecithin impurities on thin-layer chromatography plates, but after purification on a silicic acid column, it had the sharp transition reported in this paper. The $DC_{17}PC$ lecithin from Supelco had no transition between 30° and 55°C and was deemed unsatisfactory.

Two experimental techniques were used in this study. First, relative volume changes were determined with a differential thermal dilatometer with a sensitivity of 2×10^{-5} ml and tem-

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[1] *Abbreviations used in this paper:* DLPC (C_{12}), dilauroyl phosphatidyl choline; DMPC (C_{14}), dimyristoyl phosphatidyl choline; $DC_{15}PC$ (C_{15}), dipentadecanoyl phosphatidyl choline; DPPC (C_{16}), dipalmitoyl phosphatidyl choline; $DC_{17}PC$ (C_{17}), diphepatadecanoyl phosphatidyl choline; DSPC (C_{18}), distearoyl phosphatidyl choline; DBPC (C_{22}), dibehenoyl phosphatidyl choline.

perature measurements accurate to ± 0.01°C. The dilatometer is a closed system employing metal bellows whose movement is measured by laser interferometry. This device has been described in detail elsewhere (4). Lecithin dispersions were prepared by vigorously stirring the lipid in distilled water above the main transition temperature. For accuracy in this type of work, two aspects of sample preparation must be carefully attended to. All water of hydration must be removed so that the mass of lipid can be accurately determined. To this end, samples were heated *in vacuo* at 90°C until constant weight was achieved (typically 2–3 h). Second, after an aqueous suspension has been prepared, it must be thoroughly degassed to avoid spurious values for the coefficient of expansion. Between 40 and 70 mg of lipid in a 10-ml flask was typically used for examination of the main transition. To measure the coefficients of thermal expansion in the single phase regions and the size of the lower transition, it was necessary to use 200–300 mg of lecithin. Heating and cooling rates of about 1°C/h in the region of the transitions and 4°C/h in the single phase regions were employed for the larger samples. Rates of 3°–4°C/h for the small samples were used even in the region of the main transition; no significant effect resulted from using these different rates. Samples were constantly stirred mechanically during the experiments to avoid settling of the dispersions.

The second volumetric technique was a buoyancy method to determine absolute densities developed by Huang and Charlton (5) for lecithin vesicles and used by Sheetz and Chan (6) for lecithin dispersions. A small amount (10 mg) of lipid was dispersed in approximately 1 g of a D_2O-H_2O mixture and centrifuged. By varying the D_2O-H_2O ratio until the lecithin neither sank nor floated, the density of the lipid was obtained. In this manner, densities were determined at 2°, 20°, 37°, and 50°C. Temperature regulation was ±0.5°C, good enough to obtain three significant figures in the density. The agreement between the two methods in determining the density changes over a range of 50°C for these lipids was excellent.

RESULTS

Our experimental results are shown graphically in Fig. 1 for DMPC, DPPC, and DSPC. Each lecithin has a main transition at T_M associated with disordering in a rotational isomeric sense of the hydrocarbon chains. Also, each lecithin has a lower transition, about 10% as large as the main transition at a lower temperature, T_L. This transition is currently associated with a supramolecular structural change (11). When the lecithin is cooled below T_L for a period of time and then heated slowly, the lower transition occurs regularly at T_L. However, even when the lecithin is cooled through T_L at a rate as slow as 1°C/h, the lower transition occurs several degrees below T_L and can easily be missed altogether if the rate is >6°C/h. This supercooling is indicated in Fig. 1 by dotted lines. In contrast, there is no measurable thermal hysteresis at the main transition.

The two transitions divide the temperature scale into three phases which we shall call the low-temperature phase below the lower transition, the high-temperature phase above the main transition, and the intermediate-phase between the two transitions. In the low-temperature phase, the thermal expansivity $\alpha = (\partial V/\partial T)_p$ (notice that there is no $1/V$ factor in this definition) is nearly constant at about $83 \pm 5 \times 10^{-5}$ ml/g deg for DMPC, DPPC, and DSPC. This value of α is a factor of four smaller than that measured by Melchior and Morowitz (7), see also ref. 10). More recently, Blazyk et al. (8) measured $\alpha/V = 114 \times 10^{-5}$ ml/g deg for DPPC, only about 20% greater than our value. (Actually, taking α from their graph gives a value even closer to ours.)

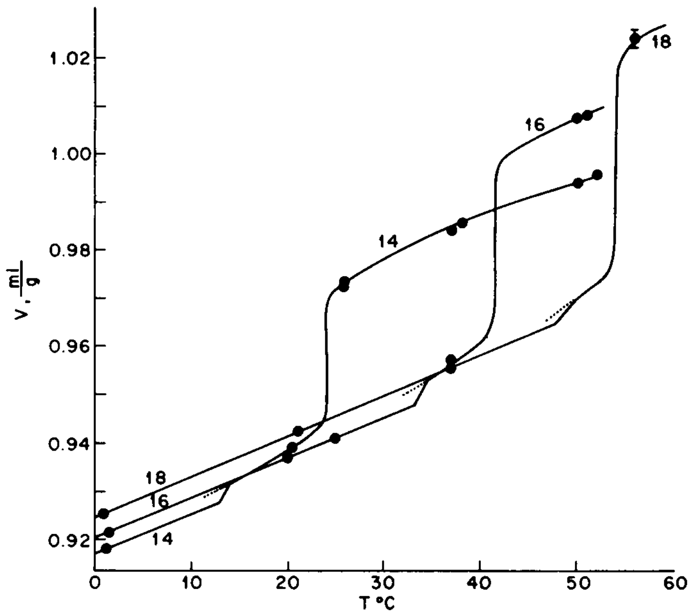

FIGURE 1 Measured specific volumes vs. temperature for DMPC (14), DPPC (16), and DSPC (18). The flotation method gave the heavy dots and the differential dilatometer gave the lines. The dotted lines indicate the metastable continuations of cooling runs at the lower transition.

Thus, there is a reasonable agreement on the measured α. It may be noted that this value of α is large compared to $\alpha = 33 \times 10^{-5}$ ml/g deg for polythylene (12) and $\alpha = 50 \times 10^{-5}$ ml/g deg for long-chain alkanes (13) in their low-temperature phases, but smaller than $\alpha \cong 200 \times 10^{-5}$ ml/g deg for long-chain alkanes in the intermediate phase (13).

In the intermediate phase of the lecithins, the quantity α begins at over 100×10^{-5} ml/g deg just above T_L and increases continuously as T_M is approached. Above T_M, α decreases continuously to about 100×10^{-5} ml/g deg several degrees above T_M. However, this decrease in α apparently continues very slowly as T is raised further. At 50°C, α for DMPC has decreased to about 50×10^{-5} ml/g deg.

The gradual changes in α on both sides of the main transition make the determination of volume changes ΔV at the main transition dependent on an arbitrary choice of base line. Inasmuch as we wish to use ΔV_M to compare with calorimetric changes in enthalpy ΔH at the main transition and because ΔC_p appears to be zero except within a few degrees from T_M for pure lipid samples (14–16), we have established our base lines at temperatures 1–5°C above and below T_M, as shown schematically in Fig. 2. In this figure, T_a is typically 1 or 2°C above T_M, and T_b is a comparable amount below. The dashed lines, α_a and α_b, are drawn tangent to the measured curve at T_a and T_b, respectively. The transition temperature, T_M, is taken to be that temperature for which the measured curve bisects a vertical line drawn from the lower dashed line to the upper dashed line. In addition, it is of interest to obtain some measures of the sharp-

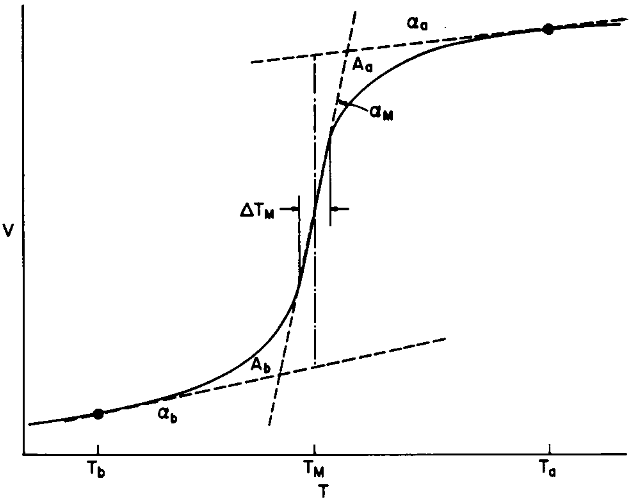

FIGURE 2 Schematic drawing of the main transition on a greatly enlarged temperature scale where T_a–T_b is typically about 2–5°C. α_a, α_b, and α_M are the slopes at T_a, T_b, and T_M, respectively. ΔV is shown by the dashed-dot vertical line at T_M, and ΔT_M is the smallest difference in temperatures for which the volume change equals $\Delta V/2$. A_a and A_b indicate the areas shown on the figure.

ness of the transition. The slope α_M is the maximum slope of the measured curve. The transition half-width ΔT_M is the minimum temperature interval over which a change $\Delta V/2$ is measured. Values for these quantities obtained from the experiments are shown in Table I. The areas A_a and A_b in Fig. 2 are not always symmetrical and vary somewhat from sample to sample. It is interesting that for the alkanes, such as *n*-eicosane, A_a is nearly 0 and A_b is quite large (13). Earlier density measurements on DPPC by Nagle (2) had a nearly zero A_b and an A_a comparable to those found in the present set of measurements.

Our results for T_M and T_L shown in Table I are in good agreement with the calorimetric measurements of T_M and T_L of Hinz and Sturtevant (14) and with the T_M from the dilatometric measurements of Melchior and Morowitz (7). Compared with the more recent measurements of Mabrey and Sturtevant (15), our T_Ls are consistently lower than theirs; agreement is good for T_M for DMPC and DPPC, but their T_M for

TABLE I

EXPERIMENTAL DATA

	α_{below}	T_{lower}	ΔV_{lower}	T_{main}	ΔV_{main}	α_M	ΔT_M
	10^5 × ml/g · deg	*°C*	*10^5 × ml/g*	*°C*	*10^5 × ml/g*	*10^5 × ml/g · deg*	*°C*
DMPC	83 ± 10	13-14	250 ± 50	24.0	2,700 ± 100	9,000	0.13
$DC_{15}PC$		22-23	300 ± 100	33.7	3,100 ± 200	4,400	0.4
DPPC	83 ± 5	33-35	330 ± 50	41.4	3,700 ± 100	12,000	0.15
$DC_{17}PC$		41-43	300 ± 100	48.5	4,000 ± 200	12,000	0.22
DSPC	84 ± 5	48-50	300 ± 100	54.1	4,500 ± 100	13,000	0.24

DSPC is 0.8°C higher than ours. The $T_M = 58°C$ given by Phillips et al. (16) is considerably higher than any of the others. This can be attributed to the much faster heating rates employed for their differential scanning calorimetry runs (cooling runs have not been performed in high-precision differential scanning calorimetry measurements) and to the increased sluggishness of the transition with increasing chain length. On these grounds one should also expect the $T_M = 75°C$ (16) for DBPC to be high. Our results for ΔV for DPPC agree well with the 0.035 ml/g obtained by Nagle (2) and by Blazyk et al. (8) and the 0.04 ml/g obtained by Sheetz and Chan (6), and thus the earlier results of 0.014 ml/g obtained by Trauble and Haynes (9) and 0.026 ml/g obtained by Melchior and Morowitz (7) are superceded. Our results for ΔV for DMPC and DSPC are also consistently larger than the 0.023 ml/g and the 0.038 ml/g, respectively, obtained by Melchior and Morowitz (7), but here the relative discrepancy is smaller. Also shown in Table I are transition measurements on a single sample of dipentadecanoyllecithin ($DC_{15}PC$) and a single sample of $DC_{17}PC$.

In Fig. 3 is shown a graph of T_M vs. $1/n$ and also vs. $1/(n - 3)$, where n is the chain length and where T_Ms for dilauroyllecithin DLPC (C_{12}) (15) and dibehenoyllecithin (DBPC, C_{22}) (16) have been included. Notice that the T_Ms for $DC_{15}PC$ and $DC_{17}PC$ are only 0.3°C higher than a smooth curve drawn through DLPC, DMPC, DPPC, and DSPC. This indicates that there is at most a rather small odd-even chain length effect on the phase transition in lipids. For large n the plot should become linear because the end effects on the transition temperature should scale as $1/n$. But for smaller values of n the $1/n$ plot is curved, so one tries $1/(n - \delta)$ for various values of δ. As can be seen in Fig. 3, the $1/(n - 3)$ plot is much more linear and extrapolates to 137°C at $n = \infty$, very close to $T_M = 138°C$ for polyethylene. (For increased accuracy the extrapolations are actually performed numerically.) One can also extrapolate the plot in the other direction to 0°K. Doing so gives $1/(n - 3) = 0.33$ or $n = 6$. This suggests that the hydrocarbon chains must have at least six carbons to have a sharp cooperative

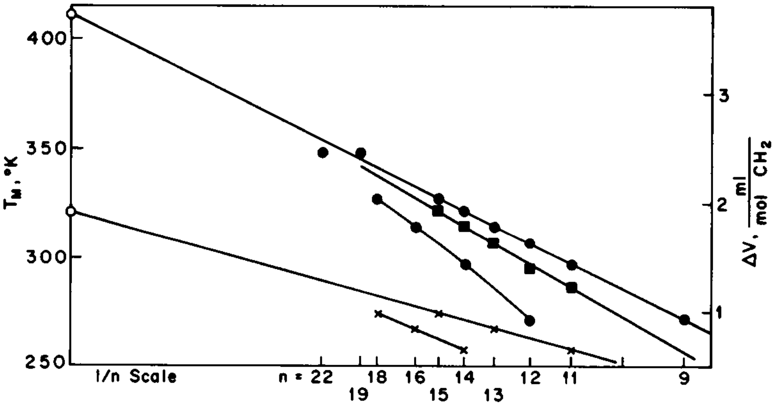

FIGURE 3 Main transition temperatures (dots) in degrees Kelvin (left-hand scale) and volume changes (at the main transition) (Xs) in milliliters per mole CH_2 (right-hand scale) plotted vs. the reciprocals of n (hydrocarbon chain length) and n-3. The lower transition temperatures (squares) are plotted vs. $(n\text{-}3)^{-1}$.

transition, although the freezing of water precludes testing this. Although there is a temptation to read a physical significance into the value $\delta = 3$, such inferences should be drawn cautiously because this is merely a numerical extrapolation scheme. However, the fact that T_M extrapolates to T_M for polyethylene at $n = \infty$ is significant and provides further evidence that the main transition in lecithins is primarily one involving hydrocarbon chain melting disordering.

Also in Fig. 3 is shown a graph of ΔV vs. $1/n$ and vs. $1/(n - 3)$. The $1/(n - 3)$ plot gives a reasonably straight line which extrapolates to about 2 ml/mol CH_2. This is only $\frac{2}{3}$ as large as the crystalline-to-liquid transition in polyethylene and the alkanes. However, many of the alkanes have two transitions, crystal-to-hexagonal solid and solid-to-liquid. The first transition is called a premelting transition and appears to involve rotation of the entire molecule and not involve rotamer disordering (17,18). The second transition in the alkanes from the hexagonal solid to the liquid is the melting transition and has a ΔV close to 2 ml/mol CH_2, which is closely comparable to the extrapolated value for lecithins.

It is also of some interest to consider the lower transition temperature T_L as a function of n. The T_L vs. $1/(n - 3)$ plot shown in Fig. 3 gives a reasonably straight line, except for $DC_{15}PC$ about 2°C below the line drawn through the others. This line crosses the main transition temperature line in Fig. 3 at about $n = 26$, suggesting that lipids with chain length longer than 26 will have no lower transition. The lower transition in lipids is often compared to the pretransition in the alkanes. However, it is noteworthy that there is a marked odd-even effect with chain length (17) in the pretransition temperatures in the alkanes that amounts to about 8°C differences for chain lengths near $n = 20$. Our measurements show that the odd-even effect in the lower transition in lipids is much smaller, if present at all. Furthermore, the volume change at the lower transition in lipids is only about 10% as large as the volume change at the main transition. In comparison the volume change at the pretransition in the alkanes is about 25–60% as large as the melting transition in the alkanes and has a strong odd-even dependence. In absolute terms the volume change at the lower transition in lipids is a factor on 10 smaller than the volume change at the pretransition in the alkanes. The current picture of the lower transition in lipids (11) is quite different from the picture of the pretransition in alkanes (17).[2] The considerable differences in the volumetric properties of these two transitions support the conclusion that these two transitions are not closely related.

THEORY

Elementary Interpretations

Following Tardieu et al. (19), it is useful to partition the lipid volumes into two parts:

$$v = v_H + v_{HC}, \qquad (2)$$

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[2] But see ref. 37 for a different interpretation of the pretransition in lecithins, closer to the picture of the pretransition in alkanes.

where v_{HC} denotes the volume of the hydrocarbon chains, $2[(CH_2)_{n-2}CH_3]$, and v_H denotes the volume of the remainder of the molecule and will be called the head region, which also includes the glycerol backbone. The X-ray work of Tardieu et al. (19), (Appendix, p. 732) gives cross-sectional areas for the hydrocarbon chains of DPPC of 19.2 Å^2 at 4°C and 20.0 Å^2 at 33°C. Because the length per CH_2 group along the hydrocarbon chains is 1.27 Å, this gives the volume of each CH_2 group, v_{CH_2} = 24.4 Å^3 at 4°C. It is customary to suppose that each CH_3 group has twice the volume of each CH_2 group (19), and so for DPPC at 4°C, $v_{HC} = 32 \times 24.4$ Å^3/CH_2 = 781 Å^3. From our volume measurements for DPPC, v = 0.9235 cm^3/g = 1,125 Å^3/molecule. Thus, from Eq. 2, v_H = 344 Å^3/molecule. This may be compared to v_H = 340 Å^3/molecule obtained by Tardieu et al. (19) at 20°C.

A natural assumption is that v_H is nearly constant both as a function of temperature for a given lecithin and for different lecithins. Direct evidence supporting this assumption is that the change in hydrocarbon chain volume for DPPC between 4° and 33°C is 0.8 Å^2 × 1.27 Å × 32 = 32 Å^3 by X-ray measurements (19), whereas our dilatometry measurements give a total change of 29 Å^3/molecule. The difference Δv_H = −3 Å^3/molecule is within experimental error of 0. In passing, it is interesting to note that one can estimate Δv_H from the changes in solvation volumes of water solutions of typical molecules such as ethanol, fructose, glycerol, and phosphoric acid when the concentrations and concentration changes are comparable to the concentrations and the changes at the polar groups in bilayers at the main transition. Such estimates give Δv_H in the range −1 to −4 Å^3/lipid molecule. This amounts to only about 4% of the measured volume change at the main transition, and so it will be neglected in the following calculations. However, it is worth emphasizing that the sign of this change in v_H is always negative so that by neglecting it we are underestimating the change in v_{HC}, as was also discussed by Trauble and Haynes (9).

Using the assumption that v_H = 344 Å^3/molecule for all lecithins for 0°C < T < 60°C and the volume data in Fig. 1, one can compute the average volume per CH_2 group in the hydrocarbon chain region. The results are shown in Fig. 4. The first point

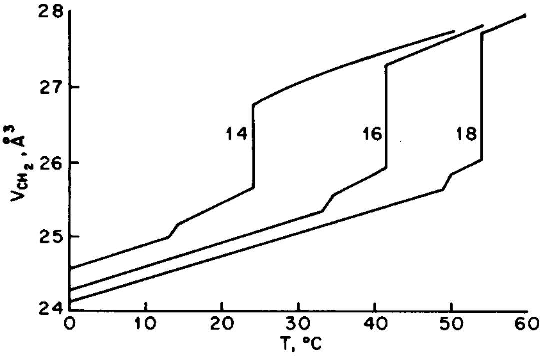

FIGURE 4 Calculated values of v_{CH_2} in Å^3 vs. temperature for chain lengths 14 (DMPC), 16 (DPPC), and 18 (DSPC).

to notice is that $v_{CH_2}^{DMPC} > v_{CH_2}^{DPPC} > v_{CH_2}^{DSPC}$ at any temperature. This is understandable because the total van der Waals forces that laterally compress the bilayer are stronger for longer chains and also because the disordering tendency of the free methyl ends of the chains is greater for shorter chains. The total specific volumes shown in Fig. 1 are in the reverse order, with DMPC most dense. The reason is that the head group, of computed d 1.5 g/cm^3, constitutes a relatively greater fraction of DMPC than of DPPC or DSPC. Also, it appears possible that the v_{CH_2} for all three lecithins may be converging to a single curve in the high-temperature phase, although we do not have enough data to confirm this.

For comparison, v_{CH_2} for the alkanes (from density data [13] with $v_{CH_3} = 2v_{CH_2}$ and X-ray data [20] and correcting for thermal expansion to transition temperatures comparable to those of DPPC) is 23.2 Å^3/CH$_2$ in the low temperature crystalline phase. In the intermediate hexagonal phase of the alkanes, v_{CH_2} is 24.7 Å^3/CH$_2$ and increases to 27.2 Å^3/CH$_2$ in the liquid phase. The alkane crystalline phase value of v_{CH_2} is in excellent agreement with v_{CH_2} for polyethylene at 40°C, and the alkane liquid phase v_{CH_2} is close to the liquid phase value for v_{CH_2} for polyethylene when thermal expansion is taken into account. Turning now to the lipids, Fig. 4 shows that above T_M, v_{CH_2} is nearly equal to v_{CH_2} of liquid alkanes. For lipids just below T_M, v_{CH_2} is larger by about 1.2 Å^3/CH$_2$ than v_{CH_2} in the intermediate hexagonal phase of the alkanes. (One way to reduce this latter difference would be if the lipids below T_M have one kink per molecule as suggested by Raman measurements (21). Then v_{CH_2} is reduced by nearly 1 Å^3/CH$_2$ because the measured X-ray spacing does not change, but the average length of each chain is reduced by 1.27 Å/2.)

Energetics of the Main Transition

The basic energetic equation is given by Eq. 1. At atmospheric pressure the hydrostatic work term $P\Delta V \simeq 6 \times 10^{-4}$ kcal/mol and is negligible compared to $\Delta H \simeq$ 8.7 kcal/mol for DPPC at the main transition. There are many different energy terms ΔU_i. Between head groups there are dipolar interactions, van der Waals interactions, possible weak hydrogen bonding, and finally the head group interaction with water. However, our best estimates (22) suggest that these change by <0.5 kcal/mol at the transition. The first change in energy ΔU_i that is obviously very important is the change in rotamer energy ΔU_{rot} in the hydrocarbon chains, which amounts to about ϵ = 0.5 kcal/mol for each trans-to-gauche isomerization (23,24), although ϵ could be as high as 0.6 kcal/mol (25). Unfortunately, there are no direct measurements of the change in the number of gauche rotations Δn_g at T_M for lecithins. However, an order of magnitude estimate of ΔU_{rot} may be obtained from polyethylene and the alkanes where the low-temperature solid state has very few gauche rotamers and the high-temperature liquid state is thought to be rotamerically completely disordered (26) as in a vapor. With these assumptions, the change in the probability of a gauche rotamer (neglecting the exclusion of g^+g^- pairs) is

$$\Delta p_g = 2e^{-K}/(1 + 2e^{-K}), \tag{3}$$

where $K = 0.5$ kcal/RT. Applying this formula to DPPC gives the first order estimate $\Delta U_{\mathrm{rot}} = 24\,\epsilon\Delta p_g = 5.6$ kcal/mol, where there are 12 rotatable bonds per chain with $\epsilon = 0.5$ kcal/mol. (The OC—CH_2 bond has rotation energy $\epsilon = 0$ as does the adjacent H_2C—CH_2 bond [24; and A. E. Tonelli, private communication]. These bonds lead to increased flexibility but must not be counted in the energy equation.) Furthermore, it seems that the bilayer constraint should not increase the rotamer disorder, so this is a reasonable upper bound on ΔU_{rot}. Thus, ΔU_{rot} should be a major fraction of ΔH but is clearly not all of it. It has been proposed that another major contribution to ΔH is the change in van der Waals energy ΔU_{vdW} between the hydrocarbon chains (1, 2), for which we now proceed to give a detailed calculation.

Calculation of ΔU_{vdW}

The primary information about van der Waals interactions between hydrocarbon chains comes from Billmeyer's studies (20) which establish that the heat of sublimation of long-chain alkanes, extrapolated to 0°K, is 1.84 ± 0.03 kcal/mol CH_2. For less drastic changes than complete vaporizaion, the r dependence of the energy must be known. Let us begin by considering a formula proposed by Salem (27),

$$U_{\mathrm{vdW}}(r) = (1.84\ \mathrm{kcal}/4)[(r_0/r)^{25} - 5(r_0/r)^5], \tag{4}$$

where r_0 is the nearest neighbor separation between chains at 0°K. The attractive r^{-5} dependence in this formula is exact for infinitely narrow cylinders of polarizable medium. For the case of cylinders of uniform polarizability and nonzero radius, the appropriate formula is given by Langbein (28). Following Salem (27) and assigning the polarizability to the chemical bonds yields a model of the hydrocarbon chain with an inner cylinder of high polarizability density with radius 0.44 Å and an outer cylinder of only $\frac{1}{5}$ the polarizability and radius 1.40 Å. These radii are significantly smaller than the hard-core van der Waals radius or the separations in question. This means that the simple r^{-5} asymptotic dependence is sufficiently accurate for our calculations. The numerical effect of using the more accurate formula of Langbein is to increase our calculated ΔU_{vdW} by 4% for the alkanes and polyethylene and only 1.5% for the lecithins. A much more uncertain feature of Eq. 4 is the repulsive r^{-25} dependence. However, for reasons to be discussed shortly, we do not use this term in our computation of ΔU_{vdW}.

First, the spacing r_0 is estimated from expansion data on polyethylene. Polyethylene has a specific volume of 1.00 ml/g at $T = 251$°K and a coefficient of thermal expansion $\alpha = 30 \times 10^{-5}$ ml/g deg (12). Because α decreases as T decreases, a specific volume of 0.95 cm^3/g is estimated for 0°K. This yields a cross-sectional area per CH_2 group of $A = 17.4$ Å^2. If one assumes a hexagonal packing of chains, one then has $r_0 = [2A/3^{1/2}]^{1/2} = 4.48$ Å. However, polyethylene is not hexagonal and at room temperature there are four nearest neighbors at 4.45 Å and two at 4.93 Å (29). Using a 5% contraction upon cooling to 0°K gives 4.34 Å and 4.81 Å. Neglecting further neighbors this gives an internal energy function

$$U(r) = [(2.03/4)(n/6)][(r_0/r)^{25} - 5(r_0/r)^5], \tag{5}$$

where $r_0 = 4.34$ Å and n is the number of neighbors at distance r. This formula gives a sublimation energy of 1.84 kcal/mol CH_2 because $n = 4$ for $r = r_0$ and $n = 2$ for $r = 1.11\ r_0$ at 0°K. It turns out that calculations with Eq. 4 and $r_0 = 4.48$ Å give nearly the same results for U_{vdW} as Eq. 5 with $r_0 = 4.34$ Å with the latter being about 10% smaller. In this paper the results quoted are for the latter case with $r_0 = 4.34$ Å.

Next, we find the values of r for the lecithins just below the main transition (r_b) and just above the main transition (r_a) from the formula

$$v_{CH_2} = (3^{1/2}/2)(1.27\ \text{Å})\, r^2. \tag{6}$$

For polyethylene and the alkanes the two nearest neighbor distances r_{b_1} and r_{b_2} were obtained from X-ray data and were then scaled to T_M by a factor $(v_b/v_x)^{1/2}$. Similarly, r_a was obtained by scaling the area per CH_2 by the measured volume ratio v_a/v_b (12, 13). The values of r_b and r_a so obtained are tabulated in Table II. Below the transition there are few gauche rotations, and the r_b so obtained should indeed be the interchain spacing. Above the transition there are many gauche rotations so the physical meaning of r_a is not so clear, and the use of r_a in Eq. 5 needs justification. By using r_a in Eq. 5, we are calculating the van der Waals energy for a single state in the high-temperature phase, namely, the one where the parallel chains in the intermediate phase are expanded uniformly to occupy the larger volume of the high temperature phase. This state is obviously unrepresentative of the general high-temperature state from the point of view of the rotamers. But the attractive r^{-5} van der Waals interaction is fairly long range and therefore depends less on the detailed conformations of the hydrocarbon chains than on the overall density of the system. Thus, this calculation

TABLE II

DETERMINATION OF ΔU_{vdW}

	r_b	r_a	$\Delta V/CH_2$	$\Delta U_{vdW}/CH_2$	ΔU_{vdW}/mole
	Å	*Å*	*ml/mol*	*kcal/mol*	*kcal/mol*
Polyethylene	4.54* 5.02	5.13‡	3.08§	0.659	
Alkanes	4.49‖ 4.97	5.02‡	2.93¶	0.632	
DMPC	4.83_0	4.93_3	0.65	0.148	4.1
DPPC	4.85_6	4.98_0	0.85	0.171	5.5
DSPC	4.86_7	5.01_8	0.99	0.203	7.3

*X-ray data (29) extrapolated to T_M using volume data (12).

‡See text.

§Reference 12.

‖X-ray data (18 extrapolated to T_M using volume data (13).

¶For eicosane (13).

neglects detailed fluctuations in the system, in somewhat the same spirit as mean field calculations (30). This results in our simple formula for ΔU_{vdW} based only on density dependence. To do a better calculation of ΔU_{vdW} would require detailed knowledge of the ensemble of states that make up the high temperature phase, and this is unlikely to be available except from model theories.

We have deliberately not extended the discussions of the preceding paragraph to the short range repulsive r^{-25} interaction in Eqs. 4 and 5. Indeed, even though the high temperature phase is expanded relative to the intermediate phase, the entanglement of the chains will lead to many close contacts between chains. Thus, it seems quite likely that the average repulsive energy may be as large, on average, in the high-temperature phase as it is in the intermediate phase. If so, then this repulsive term should be ignored in computing ΔU_{vdW} and in the results presented in this paper we have done so. For the lecithins this makes ΔU_{vdW} about 10% larger than if we had used Eq. 5 without modification.

The results of our calculations of ΔU_{vdW} for the lecithins, polyethylene, and the alkanes are given in Table II. Over the last 5 yr the theoretical value of ΔU_{vdW} for DPPC has varied from 5.7 kcal (1) to 6.1 kcal (2) and now to 5.5 kcal. The major systematic change in our present estimate is the realization that r_0 must be extrapolated back to 0°K and this reduces ΔU_{vdW} compared to the earlier calculations. Assuming that no other systematic changes are made, the other uncertainties are estimated to be about ±10%. The completion of these calculations provides a basic ingredient for a discussion of the energetics of the main phase transition.

In passing it may be of interest to note that these van der Waals calculations can be used in the single-phase regions to calculate that part of the specific heat due to the volume expansion. For DSPC in the low-temperature phase, one should have a specific heat from this source of about 0.14 kcal/mol deg or 0.17 cal/g deg.

Balancing the Energy Equation and Computation of Δn_g *for Lecithins*

First, we consider polyethylene and the alkanes. In column 1 of Table III are given the measured enthalpies per mole of CH_2 (12, 18). (Many alkanes have a premelting transition with an enthalpy ΔH_T in addition to the melting transition with enthalpy ΔH_F. The $\Delta H/CH_2$ value presented in Table III is $(\Delta H_T + \Delta H_F)/(n - 2)$ for even n, where $n - 2$ was chosen as a divisor because it yields a ΔH nearly independent of n.) In column 2 of Table III is shown $\Delta H - \Delta U_{\mathrm{vdW}}$. In column 3 is shown ΔU_{rot}, assuming that the liquid state is completely disordered rotamerically (26). To balance the energy equation one needs an additional energy, $\Delta U_{\mathrm{other}}$ which equals $\Delta H - \Delta U_{\mathrm{vdW}} - \Delta U_{\mathrm{rot}}$ and is shown in column 4 of Table III. The fact that $\Delta U_{\mathrm{other}}$ is small compared to ΔH, ΔU_{vdW}, and ΔU_{rot} indicates that the most important interactions have been dealt with.

Turning now to the lecithins, the values per mole of ΔH and $\Delta H - \Delta U_{\mathrm{vdW}}$ are shown in the first two columns of Table III. Substantial agreement for the experimental values of ΔH for DPPC and DSPC seems to have been reached. For DMPC ΔH ranges from 6.6 kcal/mol (16) to 5.4 kcal/mol (15) and 5.0 ± 0.2 kcal/mol (R.

TABLE III

BALANCING THE ENERGY EQUATION

	ΔH	$\Delta H - \Delta U_{\mathrm{vdW}}$	ΔU_{rot}	$\Delta U_{\mathrm{other}}$	Δn_g	Δp_g
Polyethylene/CH_2	0.96*	0.30	0.26	0.04		0.52
Alkanes/CH_2	0.92‡	0.29	0.24	0.05		0.47
DMPC	5.4§	1.3	1.0	0.3	2.0	0.10
	6.6‖	2.5	2.2	0.3	4.4	0.22
DPPC	8.7¶	3.2	2.8	0.4	5.6	0.23
DSPC	10.6¶	3.3	2.8	0.5	5.6	0.20

All energies are given in kilocalories per mole for the lipids and kilocalories per mole CH_2 for the alkanes and polyethylene.

*Reference 12.

‡$(\Delta H_F + \Delta H_T)/(n\text{-}2)$, even n, ref. 18.

§Reference 15.

‖ Reference 16.

¶References 15, 16.

Biltonen, personal communication), so we consider both a high and a low value. Unlike the case of polyethylene and the alkanes, there is no reliable way to estimate ΔU_{rot} for the lecithins independently of the energy Eq. 1. Certainly Δp_g will not be as large as given by Eq. 3 because n_g may not be zero below T_M, consistent with recent Raman results (21,32) and our large v_{CH_2}, and because above T_M the anisotropic bilayer constraint most likely decreases n_g compared to an isotropic hydrocarbon liquid. Therefore, we will make a rough guess for $\Delta U_{\mathrm{other}}$ and then balance the energy Eq. 1 to obtain ΔU_{rot}. In the past $\Delta U_{\mathrm{other}}$ has been set equal to zero (1,2). Previously, the head group conformation was thought to put the zwitterion dipole perpendicular to the bilayer in the lecithins (22), which would give a negative contribution to ΔU_{head}. Now, the head group conformation is thought to be similar to that of the phosphatidylethanolamines with zwitterion dipole parallel to the bilayer (31). This conformation gives a positive electrostatic contribution to ΔH_{head}. Thus, although other contributions are difficult to estimate except that they appear to be small, it now seems that $\Delta U_{\mathrm{other}}$ may be positive, and in this paper it is estimated to be 5% ΔH, also comparable to our estimated errors in our calculation of ΔU_{vdW}. From this estimate of $\Delta U_{\mathrm{other}}$ shown in column 4 of Table III, ΔU_{rot} shown in column 3 is computed by balancing the energy Eq. 1. In column 5 of Table III is shown $\Delta n_g = \Delta U_{\mathrm{rot}}/(0.5\ \mathrm{kcal/mol})$. The number of rotatable C—C bonds with energy $\epsilon = 0.5$ kcal/mol is $2n$-8 (24 for DPPC), so the average change in the probability of a C—C bond being gauche is $\Delta p_g = \Delta n_g/(2n\text{-}8)$ and is given in the last column of Table III. Within estimated errors of ±0.05, the three values of Δp_g for the lecithins are the same, if one takes the higher experimental value of ΔH for DMPC. The fact that the transition is far smaller in the lecithins than in the alkanes or polyethylene, as seen from the ΔV data in Table II or the ΔH data in Table III, is reflected in the smaller Δp_g computed for the lecithins in Table III.

DISCUSSION

One of the main goals of physical studies of lipid bilayers is to characterize the state of the membrane. One view expressed by Luzzati's group is: (*a*) Below T_M the chains are stiff, parallel, and fully extended with rotational disorder only (19). (*b*) Above T_M the chain conformations can be visualized as highly disordered like that of a liquid paraffin (19). The kink picture of Trauble and Haynes (9) is in essential agreement with (*a*), below T_M,but in this picture the main transition in lipid bilayers is viewed as analogous to the premelting transition in the alkanes with the conclusion that (*c*) above T_M the chains are still in a relatively ordered state described by kinks (9). Recent Raman measurements disagree with both preceding views below T_M. (*d*) Below T_M there is substantial rotameric disorder (32) with an estimate of $n_g = 2$ for DPPC (21).

The most direct interpretation of our values of the hydrocarbon volumes v_{CH_2} suggests agreement with statements *b* and *d* because our v_{CH_2} above T_M are comparable to fully liquid alkanes and polyethylene and because our v_{CH_2} below T_M are even larger than in the intermediate hexagonal phase of the alkanes. However, we are reluctant to draw such a firm conclusion because the hydrocarbon volume is only one of many factors determining and/or correlated with conformational chain states. It seems likely that the bulky glycerol backbone prevents v_{CH_2} from achieving as small a value in the low temperature phases of the lecithins as in the alkanes in the intermediate hexagonal phase. The tilting of the hydrocarbon chains probably compensates for this (22) but the compensation may not be complete. The fact that lipid bilayers undergo their transitions at higher temperatures than the alkanes of the same chain length implicates the pinning of the head groups to the water interface as an additional constraint that acts as an ordering force. Above T_M this additional ordering force very likely reduces the rotameric disorder below the fully disordered liquid value of $n_g = 11.2$ for DPPC. This is consistent with our estimates of $\Delta n_g = 5.6$ for DPPC and the Raman estimates $n_g = 2$ below T_M. The resulting value $n_g = 7.6$ above T_M is close to the value 8.6 deduced by Schindler and Seeling (33) from nuclear magnetic resonance measurements using Marcelja's model-dependent approximate statistical mechanical calculations (30) and our earlier estimate of 7-8 (1, 2). This picture of the high-temperature phase in lipid bilayers is similar to ones proposed earlier (1, 16, 34). Thus, compared to the alkanes the effect of the head and glycerol groups is to increase v_{CH_2} below T_M thereby permitting some gauche rotamers; the effect above T_M is to decrease n_g due to the interfacial constraint. Both effects reduce the size of the phase transition compared to the alkanes. However, T_M extrapolates with chain length, *n*, as *n* becomes large, to the melting temperature of polyethylene, and ΔV extrapolates to $(2/3)\Delta V$ for polyethylene, which is the fraction of ΔV in the hexagonal-to-liquid transition in the alkanes. These extrapolations clearly identify the main transition in lipid bilayers as essentially a hydrocarbon chain melting transition. These conclusions are not consistent with the kink picture (statement *c* above). It would seem that the kink picture might be more valid below T_M where any disorder would have to be in the compact form of kinks. Above T_M many of the gauche rotamers would be coupled in kinks but, as in a more disordered liquid state, many others would not be.

Nagle (2) previously suggested that the main transition in lipid bilayers may not be a first-order transition but a 3/2 order transition. The evidence for this was that A_b in Fig. 2 was zero in earlier measurements. Even so, A_a in Fig. 2 occurred in too narrow a temperature range to fit the statistical mechanical model calculations. At the present time the relative size of A_a and A_b is not fully resolved, but it is probably best to assume that the transition is first order. However, the observed gradual decrease in α for DMPC over a 25°C range above the transition is consistent with subcritical fluctuations. A possible explanation is that the thermodynamic path followed by lipid bilayers crosses a first-order transition line at a point near a critical point. Such a critical point would be accessible only by changing a different variable, such as lateral surface pressure, which can be controlled in monolayers. This explanation is consistent with more recent statistical mechanical calculations (22).

If the transition is first order, the Clausius-Clapeyron equation,

$$\frac{dP}{dT_M}\left(\frac{\Delta V}{\Delta H}\right) T_M = 1, \tag{7}$$

must hold by basic thermodynamics. This gives an independent check between calorimetry measurements, dilatometry measurements, and T_M vs. pressure measurements. For DPPC using $dP/dT_m = 43 \pm 2$ atm/deg (35 and W. Z. Plachy, personal communication) and $\Delta H = 8.7$ kcal/mol (15, 16) and our values of ΔV and T_M, the left-hand side of Eq. 7 equals 1.006, well within the experimental error of about 10%. For DSPC using $dP/dT_M = 40.1$ atm/deg (36) $\Delta H = 10.6$ kcal/mol (15, 16), the left side of Eq. 7 equals 1.052, which is also satisfactory. For DMPC the values of dP/dT_M range from (*a*) 40 atm/deg (36) to (*b*) 46.1 atm/deg (Plachy, personal communication) and the values of ΔH range from (*c*) 5.4 kcal/mol (15) to (*d*) 6.6 kcal/mol (16). Together with our volume measurements these give four different values of the left side of Eq. 7, namely (*a-c*) 0.962, (*a-d*) 0.787, (*b-c*) 1.11, and (*b-d*) 0.907. Although there is room for improvement for DMPC, the general agreement suggests that measurements of transition changes are close to their true values.

The major theoretical emphasis of this paper has been on the calculation of the change in the van der Waals energy ΔU_{vdW} in hydrocarbon chain melting transitions in lipid bilayers, long-chain alkanes, and polyethylene, using the measured volumes. These calculations show that ΔU_{vdW} amounts to more than half the total enthalpy change ΔH and is therefore a major interaction along with the rotameric disordering energy. The calculated values of ΔU_{vdW} successfully balance the energy equation for the alkanes and polyethylene. This lends support to the use of the energy equation to estimate the change in the number of gauche rotamers at the main transition in lecithin bilayers. These estimates support the picture that the hydrocarbon chains in the high-temperature, biologically relevant phase are highly disordered, but not as disordered as in liquid alkanes or polyethylene.

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