Dilatometry of Dilute Suspensions of Synthetic Lecithin Aggregates†

Donald L. Melchior*,‡ and Harold J. Morowitz

ABSTRACT: Using a sensitive automatic dilatometer the volume *vs.* temperature behavior of dilute (0.5–1.5%) aqueous suspensions of synthetic dimyristoyl-, dipalmitoyl-, and distearoyl-L-α-lecithins in the form of dispersions and thin-walled vesicles was studied. The volume increase due to the gel-to-liquid crystal transition as well as the "melting" temperature were found to be the same in both types of lipid aggregates, although in vesicles the transition was broader. The change in apparent volume for the transition with the previously mentioned lecithins was 0.023, 0.026, and 0.036 ml per g, respectively. An increase in bilayer surface area of about 25% accompanies the transition. The mass coefficient of expansion for dispersions and vesicles is the same after as before the transition. For dispersions, regardless of the lipid, this value was about 0.004 ml/g per deg. Dilatometric values for the mass coefficient of expansion for vesicles lie in the range of 0.009–0.020 ml/g per deg. In contrast to these extremely large values, X-ray diffraction studies of vesicles gave a mass coefficient of expansion of 0.0013 ml/g per deg. This discrepancy in values is attributed to a water effect more marked in vesicles than in dispersions because of the much greater amount of charged surface presented to bulk water by vesicles than by dispersions. An alteration in the charge of the surface of vesicles by adjustment of the suspension pH from neutral to 2.0 causes the dilatometric mass coefficient of expansion for the vesicles to be reduced to 0.002 ml/g per deg. This is consistent with the proposed explanation for the magnitude of the mass coefficient of expansion for vesicles.

Recent studies on thermal phase transitions in lipid bilayers of biological membranes (Engelman, 1971) indicate substantial changes in the bilayer dimensions as well as in the molecular spacings of the aliphatic chains. In order to assess further the effect of these changes on membrane geometry we decided to investigate the volume *vs.* temperature behavior of various suspensions of amphiphilic lipids. The first step in this study was the construction of a scanning dilatometer with sufficient precision to determine the lipid-associated volume changes in dilute aqueous suspensions. The technical aspects of this instrument have been reported elsewhere (Rothman *et al.*, 1972). The present study reports on the application of these dilatometric techniques to a variety of biologically related model lipid systems.

Materials and Methods

The lipids used in these experiments were obtained from Calbiochem, Los Angeles, Calif. Lipid dispersions were prepared by repeatedly heating the lipid in deionized water to about 15° above its phase transition and agitating the suspension vigorously on a Cyclo-Mixer (Clay-Adams, Inc., New York, N. Y.) for approximately 10 min. Vesicles were prepared by the method of Reeves and Dowben (1969). In order to obtain a good yield of thin-walled vesicles, it was found necessary to spread the lipids on a Teflon surface and swell them at a temperature above their phase transition. After formation of both lipid dispersions and vesicle preparations, the pH was adjusted to 7.0 with very dilute NaOH or HCl. In experiments where vesicles were studied at pH 2.0, the pH was adjusted by addition of H_3PO_4.

In all experiments the homogeneity of the lipids was confirmed by silica gel thin-layer chromatography (using chloroform–methanol–water, 65:25:5, v/v) both after sample preparation and after the completion of an experiment. Dry weights were obtained by heating samples at 90° *in vacuo* (10–15 mm of Hg) for approximately 5 hr. Visualization of lipid dispersions and vesicle preparations was accomplished by phase-contrast microscopy and electron microscopy using phosphotungstic acid as a negative stain. X-Ray diffraction studies were carried out by a point-focusing camera using Cu Kα radiation.

The automatic dilatometer used in these studies has been previously described (Rothman *et al.*, 1972). Before loading, samples were degassed *in vacuo* at room temperature. Volume points were taken at either 0.5 or 0.25° intervals. Temperature scan rates were usually 8°/hr although for some experiments rates as low as 4°/hr were used.

The data were treated in the following manner. ΔV_{obsd} is the change in the volume of the sample contained in the dilatometer from the volume at the start of a run. It is assumed that

$$\Delta V_{obsd} = M_w \Delta \bar{V}_w + M_s \Delta \bar{V}_s \quad (1)$$

where M_w is the mass of the suspending medium in the dilatometer, $\Delta \bar{V}_w$ the change in its specific volume from the start of the run, M_s the mass of the substance suspended, and $\Delta \bar{V}_s$ the change in its apparent specific volume. Since ΔV_{obsd} is obtained from the instrument, M_w and M_s from dry weight determinations, and $\Delta \bar{V}_w$ from either the literature or prior dilatometer runs on the pure suspending medium, $\Delta \bar{V}_s$, the *apparent* change in the specific volume of the suspended substance, can be obtained. In these experiments the suspending medium was water. Values of $\Delta \bar{V}_w$ for water as a function of temperature were taken from the literature (Washburn, 1928) and fitted to a tenth-order polynomial. From this equation values of $\Delta \bar{V}_w$ could be obtained for any temperature with negligible error.

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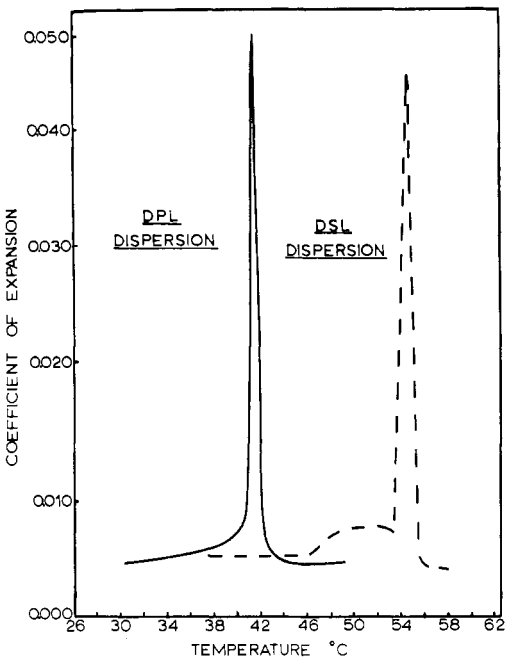

FIGURE 1: The variation with temperature of the mass coefficient of expansion, in units of milliliters per gram per degree, of dispersions of dipalmitoyl-L-α-lecithin and distearoyl-L-α-lecithin at pH 7.0. The spikes represent the gel to liquid crystal transition of the lipids. The points from which the curves were drawn were taken at 0.5–0.25° intervals. For the level portions of the curves the standard error of the scatter from run to run was less than $\pm 10\%$, for the transition the error was substantially smaller. Table I lists some numerical data for these curves.

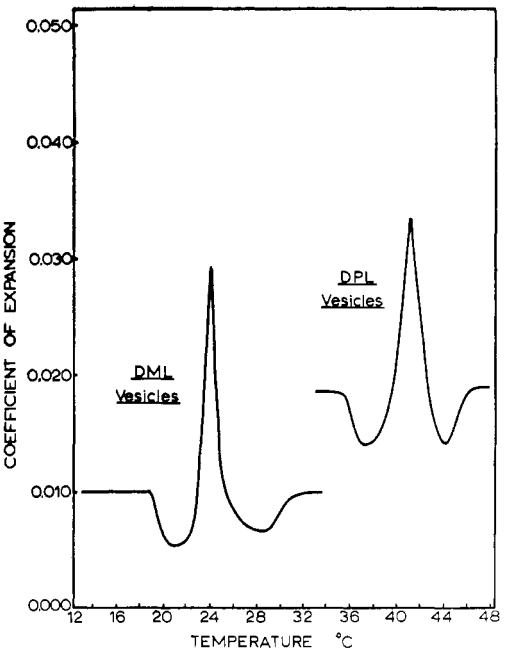

FIGURE 2: The variation with temperature of the mass coefficient of expansion, in units of milliliters per gram per degree, of vesicles formed from dimyristoyl-L-α-lecithin and dipalmitoyl-L-α-lecithin at pH 7.0. The spikes represent the gel-to-liquid crystal transition of the lipids. The points from which the curves were drawn were taken at 0.5–0.25° intervals. For the level portions of the curves the standard error of the scatter from run to run was less than $\pm 10\%$, for the transition the error was smaller. Table I lists some numerical data for these curves.

In analyzing results it was found useful to plot the mass coefficient of expansion against temperature. ϵ_T is obtained from the values of $\Delta\bar{V}_s$ in the following manner

$$\epsilon_T = \epsilon_{(T_1+T_2)/2} = \frac{\Delta\bar{V}_{s_{T_2}} - \Delta\bar{V}_{s_{T_1}}}{T_2 - T_1} \quad (2)$$

where T_1 and T_2 are the initial and final temperatures of the interval of interest. ϵ_T is given in units of milliliters per gram per degree. Experiments were carried out on dilute suspensions ranging from 0.5 to 1.5% lipid by weight. Unless otherwise indicated the standard error of the mean for all values of ϵ_T presented lies within $\pm 10\%$ of these values.

Values of ϵ_T calculated from X-ray diffraction were obtained from the percentage difference of the bilayer volume at two different temperatures. The volume per hydrocarbon chain, V, of bilayer at a given temperature is given by

$$V = \left(\frac{d^2}{\sin 60°}\right)D \quad (3)$$

where d is the value of the short spacing (Engelman, 1971) and D is the peak-to-peak spacing (Wilkins *et al.*, 1971). Dividing the volume difference by the temperature interval and using our value of 1.025 g/cm^3 for the density of lecithin at 37° (obtained by pycnometric means) gives the mass coefficient of expansion.

$\Delta\bar{V}_{trans}$ is the change in the apparent specific volume of the lipid over the phase transition of its hydrocarbon chains. This value is the change in volume above that contributed by ϵ_T through the temperature interval of the transition. T_m values given for the transitions are those temperatures at which the volume change is half-completed.

Results

Tables I and II and Figures 1 and 2 give some of the results of our studies.

There was no detectable difference between the T_m of lipid in a dispersion or in vesicle form. The $\Delta\bar{V}_{trans}$ was found to be quite similar for all lipids studied. X-Ray diffraction studies on lecithin dispersions (Chapman *et al.*, 1967), and our measurements on vesicles show that $\Delta\bar{V}_{trans}$ corresponds to the hydrocarbon chains of the lecithin molecules going from a state where they are packed in a two-dimensional hexagonal lattice to a state where they are less ordered.

Volume–temperature curve shapes were the same regardless of the heating rates used, showing that the runs were carried out under equilibrium conditions. Over the concentration range studied there was no correlation between measured quantities and lipid concentration.

Discussion

The dilatometric T_m's for these phase transitions involving the lipid acyl chains is in good agreement with values obtained by calorimetry (Chapman *et al.*, 1967; Hinz and Sturtevant, 1973).

Certain pure long-chain paraffins undergo a thermotropic phase change in which, like the lecithin hydrocarbon chains, they go from a state of hexagonal symmetry to a melted state of lower symmetry (Müller, 1932). The ΔV accompanying this transition (Schaerer *et al.*, 1955) is similar to the value

TABLE I: Dilatometric Data for DML, DPL, and DSL Aggregates in Dilute Aqueous Suspensions.[a]

Lipid	State	T_m (°C) Calorimetric[b]	T_m (°C) Dilatometric	$\Delta\bar{V}_{trans}$ (ml/g)	ϵ_{15}[c] (ml/g per deg)
DML	Dispersion	23.70 ± 0.09			
DML	Vesicle		23.75 ± 0.05	0.023 ± 0.003	0.009 ± 0.001
DPL	Vesicle		41.56 ± 0.03	0.025 ± 0.001	0.0196 ± 0.0026
DPL	Dispersion	41.75 ± 0.06	41.56 ± 0.03	0.026 ± 0.001	0.004 ± 0.0004
DSL	Dispersion	54.24 ± 0.03	54.32 ± 0.08	0.036 ± 0.002	0.0035 ± 0.0002

[a] Concentrations ranged from 0.5 to 1.5 lipid by weight. [b] From the data of Hinz and Sturtevant (1972). [c] These values are for the temperature interval of 12–18°.

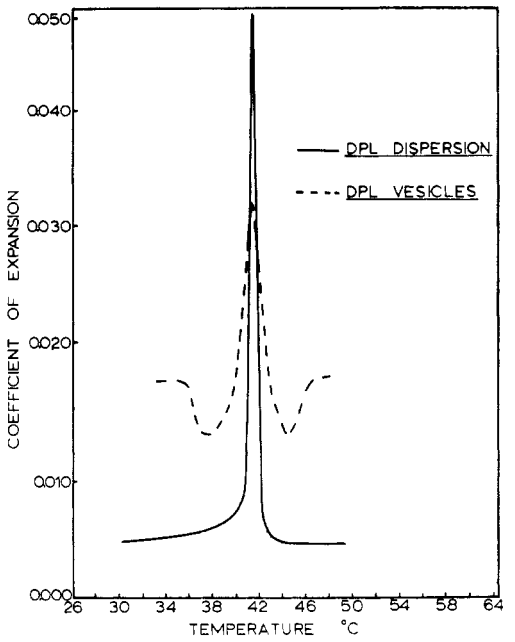

FIGURE 3: A comparison of the variation to temperature of the mass coefficient of expansion, in units of milliliters per gram per degree, of vesicles and dispersions formed from dipalmitoyl-L-α-lecithin at pH 7.0. Although the vesicle and the dispersion T_m and the vesicle and the dispersion $\Delta\bar{V}_{trans}$ for the gel-to-liquid crystal transition are the same, the transition is broader for the lipids in the vesicle state. Most marked is the extreme difference between the mass coefficient of expansion of the dispersion and of the vesicle states in the nontransition region.

we obtain for the hydrocarbon melt in the lecithin systems described. It appears that despite the many differences between these two systems the hydrocarbons melt in a similar manner.

We emphasize that the $\Delta\bar{V}_s$ used in our computations is an apparent volume change of the lipid. Such factors as a possible greater amount of water bound to lipid after the transition than before could contribute to this value. Our value for $\Delta\bar{V}_{trans}$ is in good agreement with that obtained for DPL[1] and DSL dispersions on a conventional glass dilatometer (Scheidler, 1972). Various models have been proposed to explain the hydrocarbon melt in paraffins and lecithins at a molecular level (Träuble and Haynes, 1971).

[1] Abbreviations used are: DML, dimyristoyl-L-α-lecithin; DPL, dipalmitoyl-L-α-lecithin; DSL, distearoyl-L-α-lecithin.

Although the $\Delta\bar{V}_{trans}$ and T_m is the same for a lipid whether it is in the vesicle or dispersion form, the width of the transition is seen to be broader in the vesicle form (Figure 3). If volume change is assumed to be a linear measure of the extent of conversion, α, between the gel and mesomorphic lammelar state of the lecithin, the standard enthalpy change ΔH°_{vH} is given by the van't Hoff equation in the form (Yow Tsong *et al.*, 1970)

$$\frac{d\alpha}{dT} = \alpha(1 - \alpha)\frac{\Delta H^\circ_{vH}}{RT^2} \quad (4)$$

Assuming this formalism to be valid for the systems studied, the ratio of ΔH°_{vH} calculated at $T = T_m$ for DPL dispersions and vesicles is the ratio of the sizes of the cooperative units involved in the transition. The value of this ratio is 2.5. Since the purity and homogeneity of the DPL in vesicle form was the same as that in dispersion, this result indicates that about 2.5 times as many molecules were involved in a cooperative unit in the DPL dispersion as in the vesicle preparation.

X-Ray diffraction studies on phospholipid dispersions (Chapman *et al.*, 1967) and membranes of *Mycoplasma laidlawii* (Engelman, 1971) show that through the transition a lipid bilayer decreases some 10% in thickness. This value together with our value of about a 2.5% increase in bilayer volume during the transition indicates that the transition is accompanied by approximately a 25% increase in bilayer surface area.

TABLE II: Comparison of Values for the Mass Coefficient of Expansion of Dilute Aqueous Suspensions of DPL Aggregates.

State	Mode of Measurement	pH	Temp Interval (°C)	ϵ_T (ml/g per deg)
Vesicle	Dilatometry	7.0	12–18	0.0196 ± 0.0026
Dispersion	Dilatometry	7.0	12–18	0.0040 ± 0.0004
Vesicle	X-Ray diffraction[a]	7.0	3–35	0.0013 ± 0.0001
Vesicle	Dilatometry	2.0	12–18	0.0020 ± 0.0002

[a] X-Ray diffraction spacings for this system were: at 3°, short spacing = 4.24 Å, peak to peak spacing = 45.8 Å; at 35°, short spacing = 4.29 Å, peak to peak spacing = 46.6 Å.

TABLE III: Values of ϵ_T for Various Substances.

Substance	Description	Temp Interval (°C)	ϵ_T (ml/g per deg)	Reference
Ice	Pure	−20 to −1	0.00013	Hodgman (1962)
Water	Pure	12 to 18	0.00015	Washburn (1928)
DNA	Aqueous solution	10 to 30	0.0003	Chapman and Sturtevant (1968)
Ribonuclease	Native aqueous solution	10 to 30	0.0004	Holcomb and Van Holde (1962)
Ribonuclease	Denatured aqueous solution	55 to 70	0.0018	Holcomb and Van Holde (1962)
Palmitic acid	Pure solid	−7 to 40	0.00028	Singleton and Gros (1952)
Palmitic acid	Pure liquid	63 to 72	0.00097	Singleton and Gros (1952)
Tripalmitin	Pure solid	−38 to −18	0.00022	Bailey and Singleton (1945)
Tripalmitin	Pure liquid	66 to 76	0.00092	Bailey and Singleton (1945)
Cytochrome *c*	Aqueous solution	12 to 18	0.001	Melchior and Morowitz (1972)[a]

[a] In preparation.

It is noteworthy that the extremely high coefficients of expansion for vesicles are so different from those of lipid dispersions (Table I). While the ϵ_T's for the dispersions are similar in value (and in good agreement with those obtained from the data of Scheidler (1972)), the values of ϵ_T for DML and DPL vesicles, although large numbers, differ from one another significantly. The only difference observed between vesicles formed from these two lipids was the greater size of those formed from DML. In order to emphasize the largeness of the vesicle ϵ_T, Table III presents some values of the ϵ_T of different substances from the literature.

The ϵ_T calculated from X-ray diffraction is smaller than values obtained by other means. Since this measures the expansion of the lipids proper, the higher ϵ_T found in dispersions and most strikingly in vesicles must arise from some other process which is included in the apparent change in the specific volume, $\Delta\bar{V}_s$, from which ϵ_T is calculated. The observation that ϵ_T is the same before and after the transition is consistent with ϵ_T being a measure of some process large enough to mask out the ϵ_T of the lipids themselves.

Figure 4 emphasizes the primary differences between vesicles and dispersions: (1) the presence of interstitial layers of water between sheets of dispersion bilayers; (2) the close proximity of lipid head groups in dispersions to others not in the same bilayer. For vesicles the number of head groups adjacent to bulk water is many times greater than for dispersions.

Our results seem to indicate that the large coefficient of expansion observed in vesicle preparations is somehow related to a water effect which is more marked in systems in which larger amounts of charged surface are presented to bulk water. The variations in ϵ_T agree with this. Its value is smallest for a technique which most closely measures the expansion of just the lipid bilayer. When dilatometry is used the values of ϵ_T obtained are always larger than the values obtained by X-ray diffraction. Lipid dispersions in which only the outer and inner side of the lipid particles, many bilayers in thickness, are exposed to bulk water give smaller values of ϵ_T than the very large values given by vesicles which have a much greater surface presented to bulk water.

When vesicles were suspended in water at pH 2.0, a pH at which the charged surface of lecithin bilayers is altered to one of a net positive charge, the ϵ_T obtained by dilatometry drops to a value similar to that obtained by X-ray diffraction and smaller than that of dispersions at neutral pH. Since this pH leads to positively charged head groups with no dipole character, it can be further argued that the large apparent coefficient of expansion at neutral pH involves an interaction between water and the surface charge distributions.

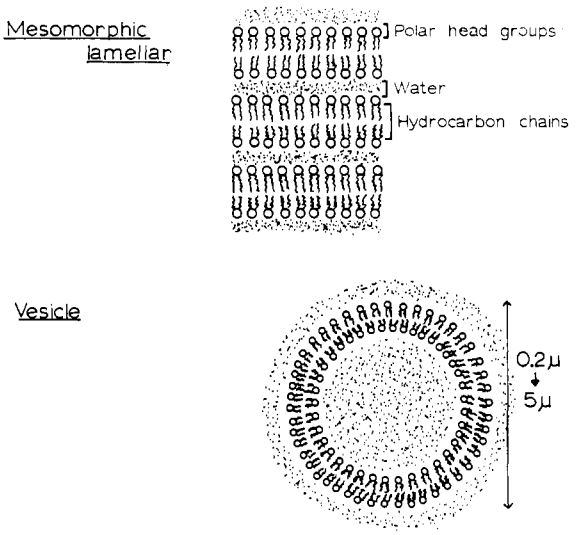

FIGURE 4: A cross-sectional sketch of the two types of lecithin aggregates used in these experiments. The lecithin dispersion is shown in its mesomorphic lamellar phase. The lipid molecules in the vesicle are not drawn to scale. Contrasting characteristics between dispersions and vesicles are: in dispersions the presence of interstitial layers of water between sheets of bilayer as well as the close proximity of lipid head groups to others not in the same bilayer; for vesicles the number of head groups adjacent to bulk water is many times greater than in dispersions.

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Temperature-Dependent Binding of Estrogen Receptor to Chromatin†

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ABSTRACT: Estradiol enters a target cell and rapidly binds to a cytoplasmic protein receptor. The steroid–receptor complex translocates into the nucleus where it binds to chromatin at a presumed regulatory site. It has been reported that the temperature dependence of the nuclear accumulation of estradiol at least partially resides in the entry of estradiol into the cell. We now report that the binding of estradiol-charged receptor to chromatin in a cell-free system is markedly dependent upon the incubation temperature and may require re-examination of results obtained in low-temperature cell-free experiments.

We currently understand that estradiol (E) enters a target cell and rapidly binds to a cytoplasmic protein receptor (R). The R–E complex translocates into the nucleus where it binds to chromatin (C) at a presumed regulatory site (Jensen *et al.*, 1971). Investigators found the nuclear accumulation of E to be temperature dependent with most experiments pointing to the translocation of R–E from cytoplasm to nucleus as the critical temperature-dependent step (Jensen *et al.*, 1968; Shyamala and Gorski, 1969; Giannopoulos and Gorski, 1971). However, Williams and Gorski (1971) recently provided convincing evidence that entry of E into the cell was the initial and perhaps even exclusive temperature-dependent step. We studied R–E behavior in mammary carcinoma tissue (McGuire and Julian, 1971; McGuire *et al.*, 1971) and had preliminary evidence that in a cell-free system the binding of R–E to C was influenced by temperature (McGuire *et al.*, 1972). We now report that the rate of C, R–E interaction is proportional to temperature. Preincubation of the individual components (C, R–E) at 21° in an attempt to induce enzymatic activity or irreversible conformational changes in the components fails to reproduce the marked temperature-enhanced binding observed when all components are simultaneously warmed. Thus, nuclear accumulation of E is influenced by temperature-dependent binding of R–E to C as well as temperature-dependent entry of E into the target cell.

Materials and Methods

Cytosol Preparation. Cytosol was prepared by homogenizing minced uteri of mature recently ovariectomized Sprague Dawley rats in 0.01 M Tris-HCl–0.0015 M EDTA (pH 7.4) in a glass homogenizer (400 mg of uteri/ml of buffer). The homogenate was centrifuged at 105,000*g* for 45 min and the cytosol decanted out from between the floating fat layer and pelleted debris. All procedures were done at 4°. The protein concentration was determined by the method of Lowry *et al.* (1951). The cytosol was incubated with 17β-[^{3}H]estradiol (48 Ci/mmole), final concentration 3.3×10^{-9} M. After 1 hr at 4° the unbound 17β-[^{3}H]estradiol was removed with dextran-coated charcoal (Chamness and McGuire, 1972).

Chromatin Isolation. R3230AC mammary tumors were removed from Fisher rats after a 14- to 21-day posttransplantation growth period. The healthy tissue was minced and homogenized in 5–10 volumes of 0.5 M sucrose in 0.002 M $CaCl_2$–0.25 M KCl–0.05 M Tris-HCl (pH 7.5) (buffer 1) with a 5-sec burst of a Polytron PT-10 at a power setting of 5, followed by five strokes in a motor-driven glass–Teflon homogenizer. The

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