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MOLECULAR INTERACTIONS IN MIXED LECITHIN SYSTEMS

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SUMMARY

The effects of mixing synthetic 1,2-diacyl lecithins having different chain lengths have been studied by differential scanning calorimetry and monolayer techniques, and their phase behaviour in excess water investigated. It is shown that, when the chain lengths of the two components are similar, co-crystallisation and ideal mixing occurs. When the chain length or unsaturation of the two components is very different monotectic behaviour is observed. In the latter case the liquid crystalline transition of the higher melting component becomes broader and is at a reduced temperature. This effect is due to the highly disordered liquid hydrocarbon chains of one component causing an increase in the kinetic motions of the ordered chains of the second component. Monolayer results indicate that the molecular area occupied by the higher melting component becomes larger because of the increased configurational freedom of its chains. Such effects occur within natural lipid extracts and the significance of this is discussed, particularly with respect to the effect of temperature on lipids and membranes.

INTRODUCTION

During the course of two recent studies[1,2] of the thermotropic and lyotropic mesomorphism of pure synthetic lecithins, it was observed that natural lecithins which contain a mixture of chain lengths gave a much broader endotherm at the solid to liquid crystal transition than those of a single discrete chain length. Generally biological systems have a heterogeneous distribution of fatty acyl chains associated with each phospholipid class. An understanding of the properties of mixtures of different hydrocarbon chains and of the chain motions in such cases is therefore relevant to the structure of biological membranes.

The only previous thermal studies of the effects of chain mixing have been for soaps[3], while the conditions for the formation of liquid crystals in mixed systems have been discussed by DERVICHIAN[4]. There have been no such studies on membrane lipid systems. This paper describes the behaviour of lecithin mixtures of controlled composition when spread at the air–water interface and when dispersed in water. The lecithins are dispersed in excess (50 wt. %) water since this corresponds more closely to the biological situation and also the lyotropic mesomorphism is less complicated under such conditions.

EXPERIMENTAL

Materials

Pure 1,2-diacyl L-phosphatidylcholines (lecithins) were synthesised in this laboratory. Details of the preparation and physical properties of the saturated homologues have been given elsewhere[1,5]. The thermotropic behaviour of 1,2-dioleoyl L-lecithin has also been reported[2].

Differential scanning calorimetry

A Perkin–Elmer differential scanning calorimeter DSC-1 was used. The general procedure has been described elsewhere[5]. A scan speed of 8°/min was chosen as giving the best compromise of resolution, attenuation and temperature accuracy.

Preparation of mixtures

Weighed amounts of the two lipid components were first dissolved together in chloroform. The solution was evaporated under N_2 and last traces of solvent were removed *in vacuo*. The intimate mixture of solids was then sealed into a glass ampoule together with a weighed amount of water and mixed by centrifuging the material backwards and forwards through a narrow constriction in the centre of the tube at a temperature above the liquid crystalline temperature (T_c) for the highest melting component.

Interpretation of differential scanning calorimetry curves

Transition temperatures. The point of departure from the baseline (onset temperature) is normally taken as the temperature of transition and is quoted to the nearest degree. It is, however, necessary to distinguish between an isothermal transition and a change taking place over a finite temperature range. The peak maximum corresponds to the temperature at which the change is occurring at maximum rate, whilst the point at which the curve returns to the baseline is partially determined by instrumental factors; therefore, neither of these points is useful in determining the temperature range of the transition. In this study the difference between the onset temperature on heating and on cooling is taken as defining the transition region. For a pure lecithin this difference is less than 1°, indicating a truly reversible isothermal transition.

Heats of transition. For a sharp, well-defined transition of a crystalline compound, peak areas are reproducible to within 2 % and for the more complex case of a lipid dispersed in water to ± 5 %. However, an absolute error must be considered arising from uncertainty in the correct choice of baseline. This error will, in general, tend to make the measured area too small and could be as much as 10 % for very broad peaks.

Mixed monolayers. The techniques used for measuring surface pressure (π) as a function of molecular area (A) have been described in detail elsewhere[5]. The mixed monolayers were obtained by volumetric mixing of solutions containing known concentrations of each component in an appropriate spreading solvent. These solutions were then spread at the air–0.1 M NaCl interface at 22°. With mixed monolayers the errors were ± 1.5 Å²/molecule and ± 0.75 dyne/cm.

RESULTS

Thermodynamic parameters

The saturated lecithins dispersed alone in excess water give sharp well defined isothermal reversible transitions from gel to liquid crystal (dotted peaks in Fig. 1) with characteristic heats. For such processes the change in entropy $\Delta S = q_{rev}/T$ where q_{rev} is the heat absorbed or latent heat and T is the temperature. In this way the thermal data of CHAPMAN *et al.*[1] have been used to compute the thermodynamic parameters for pure lecithins (Table I). Dioleoyl lecithin gives a large endothermic transition at $-22°$. The reproducibility with this compound was affected by difficulties encountered in removing traces of impurity. Thus the heat associated with the transition for our material is only good to 6.7 ± 0.8 kcal·mole^{-1} (ΔS approx. 27 cal·degree^{-1}·mole^{-1}).

The differential scanning calorimetry curves for equimolar mixtures of dioleoyl lecithin with various saturated lecithins are shown in Fig. 1. The transition for this lecithin is not significantly affected by the presence of an equimolar amount of

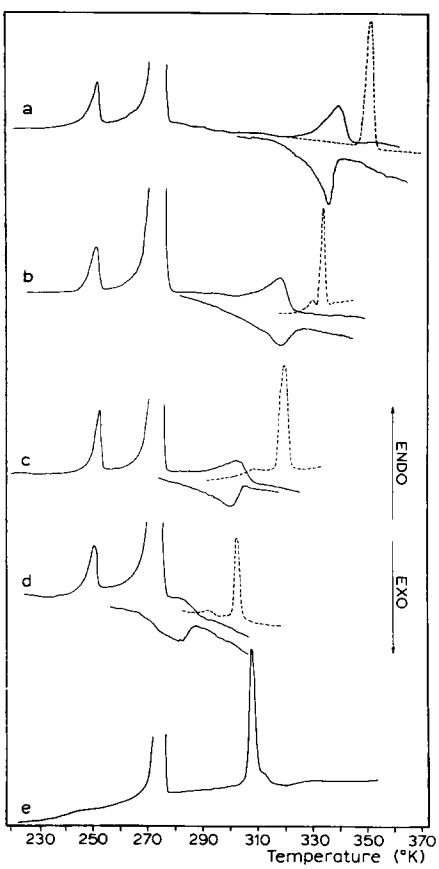

Fig. 1. Differential scanning calorimetry curves for 50 wt.% aqueous dispersions of equimolar mixtures of dioleoyl lecithin with (a) dibehenoyl, (b) distearoyl, (c) dipalmitoyl and (d) dimyristoyl lecithins. Dotted peaks show behaviour of saturated compounds in absence of dioleoyl lecithin. (e) 50% dispersion of 1-stearoyl-2-elaidoyl lecithin.

saturated compound. However, in the mixtures these latter compounds exhibit a broad peak and the transition occurs at a reduced temperature and over about a 20° range. Now for an infinitesimal reversible process $dS = dq/T$ so for a non-isothermal but finite process $\Delta S = \int dq/T$. Since the saturated lecithins undergo a non-isothermal transition when mixed with equimolar amounts of dioleoyl lecithin, it is necessary to employ the latter equation to compute ΔS. The broad peaks, shown in Fig. 1 were therefore analysed by dividing the area under the curve into thin strips so that integration yielded the heats associated with a series of "infinitesimal" processes. The temperature axis was divided into a series of strips 1° wide which were the smallest widths which could be integrated accurately by the graphical method employed. Summation of the entropy changes for each small process yielded a total entropy change which was essentially the same as that obtained by dividing the total heat by the temperature of fastest melting (T_m). The entropy changes listed in Table I for the lecithin mixtures were therefore computed using T_m. In fact the calculated entropy changes are not very sensitive to variations of 20° in T.

TABLE I

THERMODYNAMIC DATA FOR SATURATED 1,2-DIACYL PHOSPHATIDYLCHOLINES DISPERSED IN EXCESS (50 wt. %) WATER

Acyl chain length	*Pure lecithins*			*Equimolar mixtures with dioleoyl lecithin*		
	Liquid crystalline transition temp. (T_c) (°C)	*Enthalpy change* (ΔH) (*kcal·mole*$^{-1}$)	*Entropy change* (ΔS) (*cal·degree*$^{-1}$ *·mole*$^{-1}$)	*Temp. of fastest melting* (T_m) (°C)	*Latent heat* (*kcal·mole*$^{-1}$)	*Entropy change* (ΔS) (*cal·degree*$^{-1}$ *·mole*$^{-1}$)
C_{22} behenoyl	75	14.9	42.8	67	12.8	37.7
C_{18} stearoyl	58	10.7	32.3	50	8.8	27.4
C_{16} palmitoyl	41	8.7	27.5	30	6.2	20.3
C_{14} myristoyl	23	6.7	22.4	>9	>4.2	>14.7

For an irreversible change the heat absorbed q_{irrev} will be less than q_{rev}. Thus q_{irrev}/T will be smaller than ΔS. Now when heating an equimolar dimyristoyl–dioleoyl lecithin mixture the dimyristoyl lecithin peak occurs at about 0°C and is obscured by the ice-melting peak. Unfortunately, we were not able to supercool the water in order to distinguish the dimyristoyl lecithin peak. The dimyristoyl lecithin was therefore undercooled so that the peak associated with its transition was separated from the ice peak. Supercooling is an irreversible process and, therefore, the parameters listed in Table I for the dimyristoyl–dioleoyl lecithin mixture can only be taken as lower limits. Similar problems are encountered with lecithins of shorter chain length than dimyristoyl lecithin.

The transition at 0°C in Fig. 1 is due to the melting of ice. Since the amount of water in the system is known it is possible to estimate the total heat for this transition assuming that all the water has formed ice. It has been found[1] that not all the water freezes and this is attributed to the fact that each lecithin molecule has "bound" water associated with it. For pure dipalmitoyl lecithin there are about ten and for dioleoyl lecithin about twelve water molecules bound to each lecithin molecule. The

increased bound water with the dioleoyl lecithin is presumably due to the greater area occupied by the unsaturated molecule. In the equimolar mixtures there are about eleven bound water molecules for each lecithin molecule, so it appears that mixing lecithin molecules of different chain length does not significantly affect the hydration of their polar groups.

Phase diagrams

It is apparent that, in order to understand mixed lecithin–water systems completely, it is necessary to establish the temperature–composition diagrams for these systems. Fig. 2 shows such diagrams, obtained by plotting the onset temperatures from heating and cooling curves, for the dipalmitoyl–distearoyl and dimyristoyl–distearoyl lecithin systems in excess water. The behaviour is completely reversible. The temperatures for the pre-transition peaks are omitted from these diagrams because reproducible values could not be obtained. With the dimyristoyl–distearoyl lecithin system there is some uncertainty in the region of 20 % dimyristoyl lecithin. (A discontinuity in this region has also been detected using a Dupont 900 Differential Thermal Analyser but the effect seems to be too small to be fully resolved by differential thermal methods.)

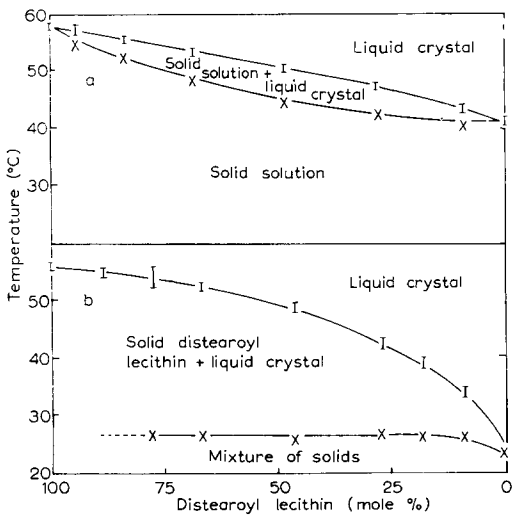

Fig. 2. Temperature–composition diagrams for systems containing distearoyl lecithin mixed in excess water with (a) dipalmitoyl lecithin, (b) dimyristoyl lecithin. I, onset temperature from differential scanning calorimetry cooling curve; ×, onset temperature from differential scanning calorimetry heating curve.

Monolayers

The effect of mixing dioleoyl lecithin with saturated lecithins has been investigated in terms of changes in molecular area at the air–water interface. Fig. 3 shows the π–A curves for dioleoyl–distearoyl lecithin mixed monolayers at 22°C. Similar measurements were made with dioleoyl–dipalmitoyl and dipalmitoyl–distearoyl lecithin mixtures and the results for all three systems are summarised in Fig. 4. The broken line on these plots of mean molecular area *versus* composition represents the behaviour found when the following molecular area additivity rule is obeyed:

$$(1-n)A_1 + n\,A_2 = A_{12}$$

where A_1 and A_2 are the areas per molecule for the pure components, A_{12} is the area per molecule in the mixed film and n is the mole fraction of Component 2. This equation is followed when the two components either form an ideal mixture or are completely immiscible.

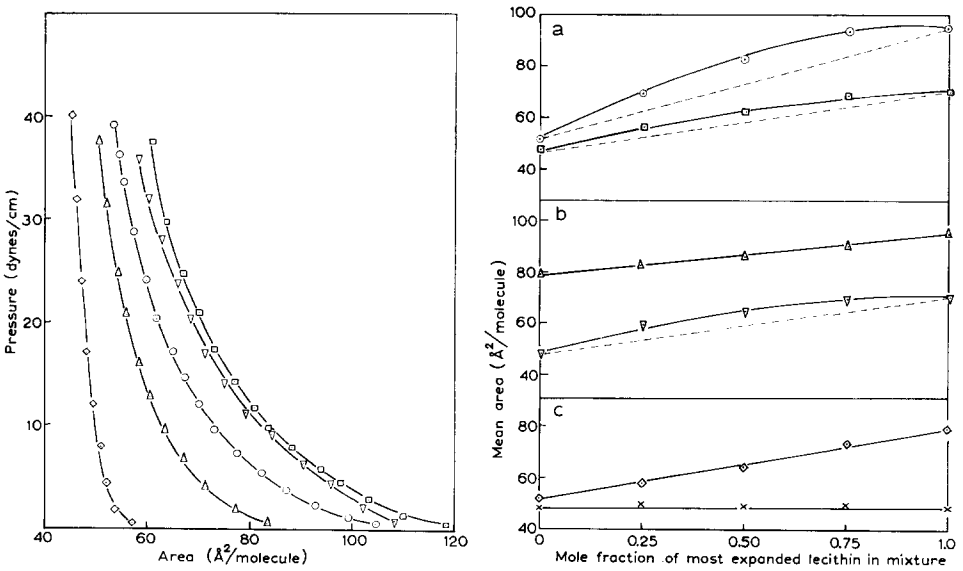

Fig. 3. Surface pressure–molecular area curves for mixed monolayers of distearoyl and dioleoyl lecithins on 0.1 M NaCl at 22°. Mole fraction of dioleoyl lecithin: ⊡, 1.0; ▽, 0.75; ⊙, 0.5; △, 0.25; ◇, 0.0.

Fig. 4. Mean molecular area–composition diagrams for mixed lecithin monolayers on 0.1 M NaCl at 22°. a. Dioleoyl–distearoyl lecithin at 5 (⊙) and 20 (⊡) dynes/cm. b. Dioleoyl–dipalmitoyl lecithin at 5 (△) and 20 (▽) dynes/cm. c. Dipalmitoyl–distearoyl lecithin at 5 (◇) and 20 (×) dynes/cm.

It is apparent from Fig. 4 that, when dioleoyl lecithin is mixed with distearoyl lecithin which forms condensed monolayers[5], an expansion or positive deviation from the additivity line occurs. In the dioleoyl–dipalmitoyl lecithin system ideal mixing occurs at low pressures where the dipalmitoyl lecithin gives liquid-expanded films, whereas an expansion is observed at high pressures where the dipalmitoyl lecithin is condensed. The dipalmitoyl–distearoyl lecithin system appears to be ideal at all pressures.

DISCUSSION

Bulk phase

With lipids dispersed in water it is necessary to consider whether the system is homogeneous with respect to the composition of the individual bilayers, or whether there is a tendency for the lipid components to combine in fixed proportions giving bilayers of different composition. The temperature–composition diagram of the system should enable a distinction to be made. Strictly speaking, since three components are present, the phase diagram of a binary mixture of lecithins in water will

be represented by a triangular prism. Providing the water is present in excess, we need to consider only the two lipid components.

The temperature–composition diagram for the system distearoyl lecithin–dipalmitoyl lecithin–water (Fig. 2a) shows that a continuous series of solid solutions[6] are formed below the liquid crystalline transition temperature (T_c) line. There is no evidence of compound formation or eutectic behaviour and it can be concluded that, for this pair of molecules, with a very small difference in chain length, co-crystallisation occurs.

For the system distearoyl lecithin–dimyristoyl lecithin–water (Fig. 2b) monotectic[6] behaviour is observed with very limited solid solution formation. Here the difference in chain length is already too great for co-crystallisation to occur so that, as the system is cooled, migration of lecithin molecules within a given bilayer occurs to give regions corresponding to the two components. The absence of any indication of compound formation precludes the formation of bilayers of specific composition. This behaviour may be contrasted with that of mixtures of saturated sodium soaps in the presence of water[3] for which a plot of the Krafft point against temperature is of meritectic[6] type (stoichiometric mixtures formed) except when the difference in chain length becomes too large. The physical situation is different with the soaps because the transition is from the solid mixture (coagel) to micellar solution and, in this case, there is therefore no restriction on molecular migration.

The differential scanning calorimetry curves for the equimolar mixtures of dioleoyl lecithin and the series of saturated lecithins (Fig. 1) are consistent with monotectic behaviour. With this series of mixtures the problem of ensuring that the components are adequately mixed is enhanced because the dioleoyl lecithin deposited from chloroform at 22°C is already in its liquid crystalline state, whereas the saturated component is below its liquid crystalline transition temperature. A control mixture of dipalmitoyl and dioleoyl lecithins prepared above 65°C so that both lecithins remained in the smectic state at all stages of the mixing, gave the same results as that obtained with mixtures prepared at room temperature. We can, therefore, conclude that the data in Table I for the dioleoyl lecithin systems apply to bilayers containing molecular mixtures. (If the chloroform mixing stage is omitted and an intimate mixture of the dry solids dispersed in water, then essentially two sharp transitions are observed corresponding to the two components. This behaviour is reversible and shows that migration of lecithin molecules between different bilayers once formed in water does not readily occur.)

The temperature–composition diagrams for the systems containing a saturated lecithin and dioleoyl lecithin would be of the form of that shown in Fig. 2b. The gel phase below −22°C consists of bimolecular lamellae containing clusters of crystallised saturated lecithin molecules which are sheathed with crystallised dioleoyl lecithin molecules. As would be expected for a true monotectic system, since the components are crystallised separately, the liquid crystalline transition for the dioleoyl lecithin is the same as found for this compound when studied alone.

In the intermediate region between the transition temperature of the two components, the oleoyl chains are fluid and rotating[7]. As the system is warmed above −22°C a gradual mixing occurs within the bilayers until the saturated compound melts, giving rise to complete miscibility when both components are liquid crystalline. The presence of rotating dioleoyl lecithin molecules influences the melting of the

saturated chains since, in each case, the T_c is lowered and the measured values of ΔH and ΔS are decreased by about 2 kcal·mole^{-1} (0.12 kcal·mole^{-1} per CH_2 group) and 5 cal·degree^{-1}·mole^{-1} (0.15 cal·degree^{-1}·mole^{-1} per CH_2 group), respectively (see Table I). In considering these values it is necessary to remember that, for a monotectic system, the transition of the higher melting component will commence immediately after the lower melting component has transformed. The low-temperature portion of the peak is lost in the baseline, so that the above values represent maximum values of the reduction in ΔH for the saturated compound.

The highly mobile oleoyl chains in the proximity of crystalline saturated chains require an expanded lattice in order to undergo the kinetic motions characteristic of lecithin liquid crystals[7]. This decrease in chain packing density allows increased configurational freedom[8] for the saturated chains. These chains will then undergo co-operative motions with the oleoyl chains and will, therefore, occupy a greater area in the bimolecular lamellae. The interaction energies of the saturated hydrocarbon chains will be reduced leading to the observed decrease in heat of chain melting. The rotational premelting transition[9] in crystals of hydrocarbon chain compounds involves an entropy gain of 0.5 cal·degree^{-1}·mole^{-1} per CH_2 group, so it is apparent that the presence of the melted dioleoyl lecithin molecules does not allow the chains of the saturated lecithin complete freedom to rotate about their long axes since the entropy gain is $<$0.15 cal·degree^{-1}·mole^{-1} per CH_2 group. However, these chains are probably no longer packed at an angle to the plane of the choline groups and are probably undergoing some torsional oscillation.

Monolayers

The above thermal studies on dispersions of mixed lecithins suggest that dioleoyl lecithin causes an increase in the area occupied by the saturated lecithin molecules. Recently, a correlation between bimolecular lamellae and monolayers of lecithins has been pointed out[5], so that it should be possible to obtain confirmation of these changes in area from the results in Fig. 4.

It is clear that when dioleoyl lecithin is mixed with another liquid-expanded film (dipalmitoyl lecithin at 5 dynes/cm) both components occupy the same area as when they are spread alone. Thus, as with the bulk phase, there is no significant change in interaction energy or packing of the chains in a mixture of two liquid components. Below the T_c of both components this is also true for the dipalmitoyl–distearoyl lecithin system, since mixed monolayers give ideal mixing at 20 dynes/cm (Fig. 4), while the dispersed system gives a continuous series of solid solutions.

When lipids giving rise to expanded films are mixed with condensed monolayers an expansion is observed (*e.g.*, with distearoyl–dioleoyl lecithin) which is consistent with the thermal effects observed in the bulk phase. It is probably reasonable to assume that there is no increase in the configurational freedom of the already highly fluid oleoyl chains and to attribute the observed increase in area solely to changes in the stearoyl chains. Making this assumption it is then possible to employ the concept of partial molecular areas[10,11] to compute the areas occupied by distearoyl lecithin in the mixed films. When the mole fraction of saturated component is 0.25, it occupies about 90 Å^2/molecule at 5 dynes/cm, which is essentially the molecular area in a fully expanded monolayer of saturated lecithin at the same pressure. In this case the oleoyl chains appear to have caused the stearoyl chains to gain the maximum possible

fluidity. As the mole fraction of distearoyl lecithin is increased its partial molecular area is reduced. (At an equimolar ratio the distearoyl lecithin occupies about 70 Å^2/molecule, whereas in a pure film at the same pressure the area is 52 Å^2/molecule.) This effect could be due either to each distearoyl lecithin molecule undergoing less expansion, or to incomplete miscibility of the two components. If the latter explanation is correct, then the increase of 18 Å^2 per distearoyl lecithin molecule is a lower limit for the actual expansion experienced and straight lines due to the onset of immiscibility would be expected for part of the mole-fraction plots.

When the experimental temperature is close to the transition temperature of the higher melting compound, the components will be miscible over a large range of compositions. Mixed monolayers of distearoyl and dioleoyl lecithins at 22°C probably fulfil this condition and are largely miscible. The dioleoyl–dibehenoyl lecithin system at the same temperature is, however, a long way below the transition temperature of the saturated compound. Consideration of Fig. 2b would therefore suggest that the dibehenoyl lecithin would only be slightly miscible with the dioleoyl compound. This is confirmed by the mixed monolayer result for this system which gave additivity of molecular areas.

The observed expansions shown in Fig. 4 occur because the dioleoyl lecithin molecules with fluid[7] hydrocarbon chains encourage co-operative movements of the chains of the more crystalline component so increasing its molecular area. It appears that the lipid chains must be highly fluid and well above their T_c before they can cause a significant increase in the area occupied by adjacent crystalline chains. This is the reason why oleoyl chains are so effective in causing expansion. In contrast mixing of melted palmitoyl chains with stearoyl chains does not lead to a detectable expansion. Similar effects can, however, be observed with some fully saturated lipids (*e.g.*, dicapryl lecithin). These effects are not specific to lecithins and can be observed in other lipid systems. Thus we find that mixed monolayers of stearic and oleic acids give significant expansions. Also similar effects have been observed when oleic acid is mixed with cetyl alcohol[12], stearoyl alcohol or amine[13] and when monoolein is mixed with either stearic acid or monostearin (P. R. MUSSELLWHITE, private communication).

Biological systems

Biological membranes have been shown to contain lipids with distributions of hydrocarbon chains of different length. From the results of the present work we see that, if the majority of the components in a membrane have individual transition temperatures considerably below that of the environmental temperature, good miscibility of the components is possible in the bilayer structure. Some lipids having an individual transition temperature above that of the environmental temperature can also be accommodated. In those circumstances where either a lipid mixture or a cell or membrane or lipoprotein system undergoes a cooling process (*e.g.*, as involved in the freeze–etching process required for electron microscopy), there is the distinct possibility of some of the components crystallising first, so giving rise to demixing. This possibility needs to be considered in interpretations of the electron micrographs of lipid mixtures, membranes and lipoprotein systems.

There is a further complication in natural systems because intramolecular mixing of chains also occurs. Fig. 1 shows that 1-stearoyl-2-elaidoyl lecithin gives

an endothermic transition at 26° C with $\Delta H = 8.4$ kcal·mole^{-1} and $\Delta S = 28.2$ cal·degree^{-1}·mole^{-1}. The peak was as sharp as that obtained with a lecithin containing two identical chains which suggests that the two chains melt simultaneously and that the behaviour of inter- and intra-molecularly mixed chains is different.

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