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MONOLAYER CHARACTERISTICS OF SATURATED 1,2-DIACYL PHOSPHATIDYLCHOLINES (LECITHINS) AND PHOSPHATIDYLETHANOLAMINES AT THE AIR–WATER INTERFACE

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SUMMARY

Surface pressure–area data have been obtained for the homologous series of saturated 1,2-diacyl phosphatidylcholines and phosphatidylethanolamines at the air–water interface. The results are compared with data already in the literature and the various physical states of the monolayers are described. The lecithins formed more expanded films than the phosphatidylethanolamines and this is interpreted in terms of differences in the size and orientation of the polar groups. The heats and entropies associated with the transition from condensed to liquid-expanded film were calculated for dipalmitoyl lecithin. The values of these thermodynamic parameters were similar to those observed for the transition from gel to smectic mesophase for this lecithin. This transition occurring in the bimolecular lamellae in water corresponds to the transition from condensed to expanded monolayer.

INTRODUCTION

Due to the growing interest in the properties of cell membranes, there are many studies being made on model membrane systems[1]. One such system is the monolayer at the air–water interface. Since lecithins and phosphatidylethanolamines are important constituents of most cell membranes, it is natural that there should be a great deal of monolayer work being carried out using these compounds.

The early work was done with rather ill-defined materials extracted from natural sources which made the interpretation of the results difficult. In 1955, ANDERSON AND PETHICA[2] made the first study of a synthetic lecithin and since then an increasing number of reports[3–15, 35] on such systems have appeared in the literature. Included in these is an earlier paper[16] from this laboratory on the effect of *trans* double bonds in such films. However, despite this effort there are still uncertainties associated with the force–area curves of pure lecithins. In this paper we shall therefore attempt to explain some of the discrepancies which exist by presenting data for a series of well-defined synthetic lecithins and phosphatidylethanolamines.

EXPERIMENTAL

Apparatus and procedure

The surface pressure (π) was measured as a function of the surface area per molecule (A) on a conventional Langmuir–Adam film balance[16,17]. Where surface potentials (ΔV) were determined, an apparatus similar to that previously described[6] by one of us was used. Particular attention was paid to cleanliness so that with this equipment it was possible to detect changes in π of less than 0.2 dyne/cm. All the films were spread on a 0.1 M NaCl solution (pH 5) when it was found that π could be determined to within $\pm$ 0.5 dyne/cm. The areas could be reproduced to $\pm$ 1 Å^2 per molecule and surface potentials to within $\pm$ 15 mV. The temperature control during a run was better than $\pm$ 0.2°.

Spreading of the phospholipids has been the cause of considerable difficulties. Unfortunately, lecithins and phosphatidylethanolamines are insoluble in *n*-hexane which is normally a good spreading solvent. Chloroform–ethanol was found to give satisfactory results for experiments at 22° and above, but at low temperatures the chloroform was retained in the film. Hexane and methanol are immiscible in suitable proportions so finally 9:1 and 4:1 hexane–ethanol mixtures were used for the lecithins and phosphatidylethanolamines, respectively. For complete solution of the phosphatidylethanolamines warming to 35° was necessary. To prevent evaporation of the solvent, the stoppered flask containing the spreading solution was kept in a desiccator which contained a beaker of hexane. The solutions were stored at 5° when not in use and were renewed every two days. If the solutions were kept much longer than this, expanded π–A curves were obtained due to decomposition of the phospholipid. In order to check that the use of hexane–ethanol as a spreading solvent did not lead to artifacts, certain of the lipids were spread from the crystal. The resulting compression curve was then fitted to a π–A curve obtained by using the spreading solvent. The curves were reproducible within experimental error except when $\pi < 2$ dynes/cm. Therefore at these pressures it appears that there is some solvent retention, so that the areas at which a pressure is first registered ("lift-off") are subject to a greater error ($\pm$ 2 Å^2/molecule) than that quoted above. It was found that if a particular film was initially compressed to remove any retained solvent and then recompressed several times, all the π–A curves obtained were identical which confirms that at all the temperatures quoted in this paper, when $\pi > 2$ dynes/cm, solvent retention is no problem.

A complete isotherm was obtained within about 20 min of commencing the compression. The area/molecule was reduced until the monolayer collapsed or, as was more usual, the water spilled over the edge of the trough.

MATERIALS

Substrate materials

Laboratory distilled water was deionised and redistilled from alkaline permanganate under nitrogen. A final distillation was then made in an all-glass apparatus, which gave water free of surface-active contaminants. The ionic content of the water was determined and the results are presented in Table I. Spectrographically standardised NaCl from Johnson Matthey Chemicals Ltd., was dissolved in this water to make

the substrate solution. The NaCl was quoted as containing approx. 4 ppm of iron, 2 ppm magnesium and less than 1 ppm of other multivalent ions. It was not necessary to roast the sample to remove organic contaminants.

TABLE I

IONIC CONTENT OF SUBSTRATE WATER AS DETERMINED IN AN ATOMIC ADSORPTION SPECTROMETER

Metal	*Mn*	*Cu*	*Mg*	*Ca*	*Na*
Parts per $10^9 \pm 50\%$	40	<10	20	25	15

Spreading solvents

Hexane (Hopkins and Williams spectroscopic grade) was further purified by passage through a column of activated alumina. Ethanol (Burroughs abs. alcohol) was shaken with activated charcoal, filtered through alumina and redistilled. Chloroform (AnalaR grade) was treated similarly and redistilled over phosphorus pentoxide.

Monolayer materials

The phospholipids used in this study were synthesised in this laboratory, either under the supervision of Mr. J. S. CHADHA or by Mr. M. D. BARRATT. The lecithins were synthesised as monohydrates from a crystalline L-α-glycerylphosphorylcholine–cadmium chloride complex[18] and very pure fatty acids (99.9 % pure by gas–liquid chromatography). They were purified by special techniques developed in this laboratory[19,40] and found to be optically pure. L-α-Dipalmitoyl lecithin, for example, had an $[\alpha]_D^{22} = +6.9°$ (*c* 4.15, chloroform–methanol, 1:1, v/v) compared with a literature value[20] of $[\alpha]_D^{23} = +6.6°$ (*c* 4.2, in the same solvent). These materials gave single spots on thin-layer chromatographic analysis. Some of the lecithins were examined in a mass spectrometer and found to be free of homologous impurities. It may be noted that these compounds exhibited well-defined mesomorphic transitions[21] which also indicates a high degree of purity. The phosphatidylethanolamine samples were

TABLE II

SELECTED PHYSICAL CONSTANTS FOR SATURATED LECITHINS AND PHOSPHATIDYLETHANOLAMINES

Acyl chain length	*Mol. wt. of lecithins*	*Mol. wt. of phosphatidyl-ethanolamines*	*Krafft point for lecithins (ref. 21)*	*Main endothermic transition temp. for phosphatidyl-ethanolamines in water (ref. 21)*
Behenoyl (C_{22})	920	—	75°	—
Stearoyl (C_{18})	808	748	60°	89°
Palmitoyl (C_{16})	752	—	41°	—
Myristoyl (C_{14})	696	636	23°	77°
Lauroyl (C_{12})	640	580	approx. 0°	62°
Capryl (C_{10})	584	524	—	—

synthesised [40,*] from diacyl L-α-glyceroliodohydrin following the method of MALKIN[22] and co-workers. The products were purified* by chromatography on silica columns (silicAR CC-7, ex. Mallinckrodt) and were finally crystallised from hot dry dioxane. The L-α-distearoyl phosphatidylethanolamine had an $[\alpha]_D^{23} = +6.2°$ (*c* 3.4, chloroform–acetic acid, 9:1, v/v), while the literature[23] value is $[\alpha]_D^{24} = +6.0°$ (*c* 4.4, in the same solvent). The L-α-dimyristoyl phosphatidylethanolamine had an $[\alpha]_D^{22} = 6.54°$ (*c* 2.4, chloroform–acetic acid, 9:1, v/v). The high purity of these samples was confirmed by the same checks as were applied to the lecithins. Selected physical constants for these phospholipids are given in Table II.

RESULTS

Figs. 1 and 2 depict the π-A curves at room temperature for the homologous series of saturated lecithins and phosphatidylethanolamines, respectively. In Fig.1 the curve for dilauroyl lecithin is not shown as it coincides almost exactly with the curve for dimyristoyl lecithin. Data for some of these materials have been published by other workers and it is worthwhile to compare them with the present results. It is clear that when comparing π–A curves from various laboratories, discrepancies arise in the main, either from differences in surface-balance technique or in the purity of the materials used. With regard to the former point, it is rare to find different workers spreading monolayers on the same substrate and compressing them at the same rate.

Bearing these points in mind, we find that for the phosphatidylethanolamines

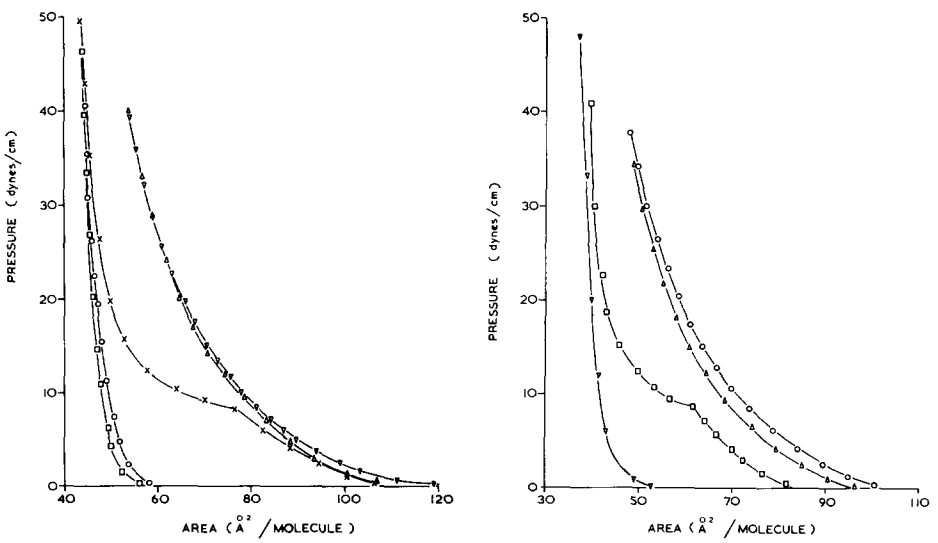

Fig. 1. Pressure–area curves for saturated lecithins on 0.1 M NaCl at 22°. □, dibehenoyl lecithin (C_{22}); ○, distearoyl lecithin (C_{18}); ×, dipalmitoyl lecithin (C_{16}); △, dimyristoyl lecithin (C_{14}); ▽, dicapryl lecithin (C_{10}).

Fig. 2. Pressure–area curves for saturated phosphatidylethanolamines on 0.1 M NaCl at 22°. ▽, distearoyl lecithin (C_{18}); □, dimyristoyl lecithin (C_{14}); △, dilauroyl lecithin (C_{12}); ○, dicapryl lecithin (C_{10}).

* JASWINDER SINGH CHADHA, unpublished results.

our results are similar to those already in the literature. Our isotherm for distearoyl phosphatidylethanolamine compares well with that of STANDISH[7] and is essentially the same as the curve for dipalmitoyl phosphatidylethanolamine recently published by STANDISH AND PETHICA[24] (but see VAN DEENEN *et al.*[3,4]). The only other phosphatidylethanolamine, which has been studied previously is dimyristoyl phosphatidylethanolamine[6] and our result is in excellent agreement with that. The situation with regard to the lecithins is more confused although there now seems to be substantial agreement on the correct fully condensed π–A curve for lecithins (*cf.* ANDERSON AND PETHICA[2] and DEMEL, VAN DEENEN AND PETHICA[4]). However there are minor differences in the various completely expanded isotherms in the literature. Thus the dimyristoyl lecithin isotherm agrees with that of DEMEL and coworkers but is about 8 Å^2/molecule more expanded than that published earlier by one of us[6]. The dicapryl lecithin isotherm of Fig. 1 is about 5 Å^2/molecule more condensed than that already in the literature[4]. There have been a number of studies in which dipalmitoyl lecithin has been utilised[5,8–15,35]. Most of this work has been done with commercial samples and the resultant π–A curves do not show the well defined phase transition of Fig. 3. There is now evidence[5,15] which suggests that these materials are unsatisfactory although we found that the dipalmitoyl lecithin curve of Fig. 1 could be reproduced with a commercial sample that had been further purified.

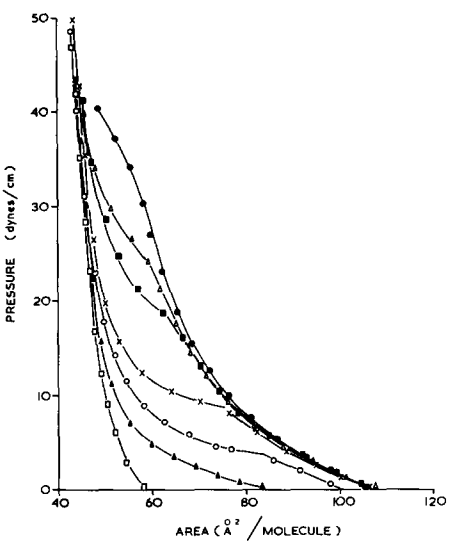

Fig. 3. Pressure–area curves for L-α-dipalmitoyl lecithin on 0.1 M NaCl at various temperatures. ●, 34.6°; △, 29.5°; ■, 26.0°; ×, 21.1°; ○, 16.8°; ▲, 12.4°; □, 6.2°.

It is significant that the reproducibility of the phosphatidylethanolamine data is better than that found with the lecithins. This suggests that in the latter case there is an additional problem which is not found with the phosphatidylethanolamines; namely, the purity of the lecithins. In support of this, we find that when using the same lecithin sample we are able to reduce our experimental error due to $\pm$ 0.25 dyne/cm and $\pm$ 0.5 Å^2/molecule. Owing to the hygroscopic nature of the lecithins it is possible to operate with compounds which are satisfactory by chromatographic

standards but which are not completely dry. The lecithins become particularly hygroscopic when they are in the liquid-crystalline phase[21]. This leads to π–A curves which are of the correct form but displaced along the area axis. If this fact is taken into account many of the discrepancies mentioned above would probably disappear.

The data can only be expected to be reproducible while the monolayers are stable. The causes of monolayer instability which are likely to be relevant to lecithin and phosphatidylethanolamine films are solubility and three-dimensional aggregation. With the exception of the distearoyl phosphatidylethanolamine which was found to aggregate quite rapidly even at low π, all the films were stable. A film was judged to be "stable"[6] when π did not decay more than 1 dyne/cm in the first minute after cessation of compression. At high π the monolayers usually spilled over the edges of the trough before they became unstable[15]. It should be noted that the equilibrium spreading pressure[25], which is a measure of the maximum pressure at which one has a monolayer in thermodynamic equilibrium with respect to the bulk crystal phase, is not necessarily the pressure at which the film becomes unstable. It was found that the lecithins would not spread at a measurable rate from the crystal when the substrate temperature was below the Krafft temperature (T_c) (see Table II), but spread rapidly above this temperature. It appears that under these conditions, the equilibrium spreading pressure for lecithins is quite high[26]. The mesomorphic behaviour of phosphatidylethanolamine–water systems is more complicated and in this case the simple correlation with spreading was not found.

It is apparent from Figs. 1 and 2 that lecithin monolayers are more expanded than phosphatidylethanolamine monolayers[3,6,16]. It is dipalmitoyl lecithin which undergoes the transition from liquid expanded through an intermediate to a condensed state*,[28] at 22°, while it is dimyristoyl phosphatidylethanolamine which behaves similarly at the same temperature. The limiting area of the lecithins is about 44 Å^2 per molecule while that of the phosphatidylethanolamine is about 40 Å^2/molecule. This is to be compared with earlier results[3] of 40 Å^2/molecule and 36 Å^2/molecule, respectively. At these areas/molecule, the surface viscosities of the films were generally extremely high[6]. At this point, it is of interest to note that the L and DL stereoisomers of dimyristoyl phosphatidylethanolamine exhibited identical π–A curves which indicates that the configuration of these molecules therefore has no effect on their monolayer (but see VAN DEENEN *et al.*[3]). It was also found that switching the point of attachment of stearoyl and elaidoyl chains within lecithin and phosphatidylethanolamine molecules made no difference to the π–A curves obtained (*cf.* ref. 3). This suggests that molecular packing considerations are not directly relevant to the fact that unsaturated fatty acid chains usually occur in the 2-position of the glycerol moiety of naturally occurring phospholipids.

The surface potentials of some of the materials listed in Table II were also measured. It was found that for fully condensed lecithins ΔV rose from about 500 mV at "lift-off" to around 575 mV at collapse. These values are somewhat higher than those quoted for distearoyl lecithin by ANDERSON AND PETHICA[2]. An expanded lecithin was found to have a ΔV of about 270 mV at "lift-off" which increased to about 450 mV at the highest pressure. This is in good agreement with earlier values[6] quoted for dimyristoyl lecithin. The phosphatidylethanolamines had similar surface

* For a definition of such terms see ref. 27.

potentials and for both series the temperature dependence of ΔV largely reflected changes in state of the monolayer. With all the monolayers studied it was found that at areas greater than "lift-off", ΔV showed random variations of the order of 100 mV which indicated that the films were liquid-expanded and not vapour-expanded. Where the films were heterogeneous, the two-dimensional vapour pressure was seen to be less than 0.2 dyne/cm.

The isotherms for dipalmitoyl lecithin over a range of temperatures from 6° to 35° are shown in Fig. 3. These curves clearly demonstrate that dipalmitoyl lecithin can exist in all the classical monolayer states and in this sense shows similar behaviour to myristic acid[29]. There have only been a very limited number of studies[4,6] of phospholipid monolayers over a temperature range. It has however been established that dimyristoyl lecithin and dimyristoyl phosphatidylethanolamine exhibit parallel behaviour. To conclude this section, it is worth mentioning that from a consideration of our data it becomes possible to correlate the effect on these monolayers of change in hydrocarbon chain length and variation of the temperature. Thus subtracting two methylene groups from each chain is approximately equivalent to raising the temperature by about 20°. A consideration of their half-expansion temperatures[30] indicates that the same figures would apply for the fatty acids. Since at room temperature the dipalmitoyl lecithin and dimyristoyl phosphatidylethanolamine isotherms can be taken as roughly the same, it becomes evident that more or less equivalent changes in the physical state of a particular phosphatidylethanolamine monolayer can be induced either by changing to a choline headgroup, or by raising the temperature about 20°.

DISCUSSION

Comparison of π–A curves

Figs. 1 and 2 show that all monolayer states are possible with the saturated lecithin and phosphatidylethanolamine homologues. It is clearly apparent that if the hydrocarbon chains are sufficiently long, condensed monolayers are formed while with shorter chains liquid-expanded films occur. It should be noted that these two limiting states are sufficiently well defined so that at any particular temperature there is only one of the homologues studied that exhibits the transition state between the two. The data also indicate that variations in hydrocarbon chain length which do not give rise to a change in monolayer state do not have a significant effect on the π–A curves. Fig. 3 demonstrates how temperature changes can give rise to the above physical states in a monolayer of a single homologue. Obviously, a sufficiently low temperature causes the film to become completely condensed, while at higher temperatures it is fully expanded. Monolayers in the two limiting states are more or less invariant with temperature and it is only the sensitivity of the phase transition to temperature which leads to the variety of isotherms depicted in Fig. 3.

It can be seen from a comparison of Figs. 1 and 2 that the molecules in a completely condensed phosphatidylethanolamine film are much more closely packed than those in the equivalent lecithin monolayer. In fact, condensed lecithins and phosphatidylethanolamines give coherent films over the surprisingly wide range of about 14 Å^2/molecule. This is much larger than the equivalent range of about 4 Å^2 per molecule for stearic acid and about 7 Å^2/molecule for a condensed diglyceride[6]. It

presumably arises from steric factors associated with the large polar groups on lecithins and phosphatidylethanolamines. The larger limiting area found for the lecithins as opposed to the phosphatidylethanolamines reflects the greater space required by the bigger and probably more hydrated choline groups. Limited comparative studies in this laboratory of lecithins and phosphatidylethanolamines dispersed in water suggest that the lecithins have more bound water associated with their polar groups.

At higher areas/molecule the lecithins also give more expanded π–A curves than the equivalent phosphatidylethanolamines[3,6,16]. There has recently been a detailed discussion[6] of the role of the zwitterion in this effect, so that at this stage we only need to consider further relevant evidence. Previously, it was concluded that both lecithins and phosphatidylethanolamines are isoelectric at pH 5.5 and would exist as essentially neutral monolayers. The validity of this for lecithins is supported by measurements[31] of the electrophoretic mobility of egg lecithin dispersions in 0.145 M NaCl which showed that the isoelectric span ranged from pH 3 to pH 13. In this laboratory we have confirmed that a synthetic lecithin has zero electrophoretic mobility at pH 5.5. In addition we found that a synthetic phosphatidylethanolamine had a small mobility indicating that there is a small net negative charge on phosphatidylethanolamines[32] at this pH. In this context the measurements by HAUSER AND DAWSON[33] of radioactive calcium binding to lecithin and phosphatidylethanolamine films are relevant. They found that at pH 5.5, within the limit of their experiment, calcium did not bind to either film, suggesting that both act as essentially neutral lipids in this situation. Thus it appears that the negative charge on phosphatidylethanolamines is not sufficiently large to significantly affect the monolayer behaviour. In any event, in order to explain the greater expansion of lecithin films over phosphatidylethanolamine films in terms of net charge on the monolayers, it would be necessary to postulate a greater charge on the lecithin rather than on the phosphatidylethanolamine films. We are, therefore, entitled to eliminate explanations based only on net charge of the monolayer from our considerations.

However, the steric factors associated with the choline headgroup which cause the difference in limiting areas of condensed lecithin and phosphatidylethanolamine monolayers could also affect the packing at higher areas/molecule[6]. This is therefore a possible explanation of the greater expansion of lecithin films. It has also been suggested[6] that the polar moieties of the lecithin and phosphatidylethanolamine molecules are undergoing kinetic motions with differing headgroup orientations which lead to dissimilar dipole repulsions. In fact both the steric and dipole factors could be relevant, but proof of this must await further experiment.

Physical states of the monolayers

It has been known for many years[27] that in a liquid-expanded film the hydrocarbon chains are melted[34] and in a highly fluid and mobile state. Also, the chains in a condensed film have been considered to be ordered and arranged somewhat similarly to the chains in a hydrocarbon crystal. The clear distinction between the two types of monolayer is particularly prominent in Fig. 1 and allows us to point out the analogy with the gel and smectic mesophases (bimolecular lamellae) of lecithins dispersed in water[21]. Thus configurations of the hydrocarbon chains in the gel and smectic states are largely the same as those of chains in condensed and liquid-expanded monolayers,

respectively. We suggest that there is an analogy between the two systems and that the transitions occurring in the lyotropic mesomorphism of lecithins correspond to the changes in state occurring in lecithin monolayers.

Consideration of the isotherms in Fig. 3 suggests that the critical temperature (T_c) for the phase transition is close to 41°; the Krafft temperature for dipalmitoyl lecithin. (The substrate temperature could not be raised above 35° due to problems arising from the softening of the paraffin wax which coated the trough.) It should be noted that the transition regions in the π–A curves of dipalmitoyl lecithin never have a zero slope. We have therefore taken the critical temperature as that temperature above which π–A curve no longer shows an inflection, whereas in three dimensions it is taken as that temperature at which the transition region of the pressure–volume isotherms no longer has a zero slope. It is relevant to this discussion to mention that the lung membrane is considered to resemble the dipalmitoyl lecithin monolayer at the air–water interface[15]. Since the temperature in a lung is lower than T_c, the dipalmitoyl lecithin will therefore experience a two-dimensional condensation unless constrained from doing so. This transition may be of significance in the continuous compression and expansion loops that this membrane undergoes during respiration[11,15].

Due to hydrophobic bonding requirements in the dispersed system the bilayer is condensed at all temperatures below T_c. In the monolayer the situation is different in that the surface pressure can be varied and Fig. 3 shows how the transition can be induced at various temperatures within about 25° of T_c. The gel and liquid crystalline states coexist in equilibrium at T_c and the molecular area changes sharply at this point. However at the air–water interface the transition is gradual and measurements of molecular area as a function of temperature (at a given π) do not show a sharp change. The structure of this intermediate region has been the subject of much discussion[27] but it is still not well understood. We have observed that the exact shape of the π–A curve during this transition depends upon the rate of compression of the film. This suggests that some kinetic process is occurring which could well be associated with changes in hydration of the polar groups. It has been found[21] that when dispersed in water each lecithin molecule is associated with about 10 water molecules which do not freeze on cooling the system below 0°. It may be inferred that lecithin molecules at the air–water interface have a similar number of water molecules bound to them.

Heats of transition in monolayers

The data listed in Table III have been calculated by applying the two-dimensional Clausius–Clapeyron equation (1) to the isotherms of Fig. 3.

$$\frac{d\pi_c}{dT} = \frac{Q_c}{T(A_e - A_s)} \qquad (1)$$

In this equation T is the temperature, π_c is the pressure at which the two-dimensional condensation occurs, Q_c is the latent heat of this phase change and A_e and A_s are the molecular areas in the liquid-expanded and condensed phases respectively. A_e is taken at π_c, while A_s is obtained by extrapolating the condensed isotherm downwards to $\pi = \pi_c$. For dipalmitoyl lecithin, A_s has been taken as 47 Å^2/molecule and the linear plot of π_c against T gives $d\pi_c/dT = 1.6$ dynes/cm. deg. It should be noted that the

TABLE III

HEAT AND ENTROPY CHANGES CALCULATED FOR THE TWO-DIMENSIONAL CONDENSATION IN DIPALMITOYL LECITHIN MONOLAYERS AT THE 0.1 M NaCl–WATER INTERFACE

Temp.	π_c (*dynes/cm*)	A_e (Å²/*molecule*)	Q_c (*kcal/mole*)	ΔS (*cal/deg. per mole*)
16.8°	4	84	24.3	84
21.1°	9	76	19.3	66
26.0°	18	64	11.4	38
29.5°	24	59	8.0	27
34.6°	34	57	6.7	22

phase change does not occur at constant pressure* as there is always an upward slope on the π–A curve in the intermediate region. For this reason Q_c cannot simply be equated to the change in heat content (ΔH) as it includes a work term $\int_{A_1}^{A_2} \pi dA$. For dipalmitoyl lecithin this term amounts to about 1 kcal/mole. Now for an isothermal reversible process the change in entropy ΔS is equal to Q/T where Q is the heat adsorbed, so this simple expression yields the entropies listed in Table III. The signs of the heats and entropies are given for the transition from condensed to expanded monolayer. Similar data for myristic acid were calculated from ADAM AND JESSOP's[36] results and are presented in Table IV. In this case $d\pi_c/dT = 1.17$ dynes/cm. deg. and

TABLE IV

HEAT AND ENTROPY CHANGES CALCULATED FOR THE TWO-DIMENSIONAL CONDENSATION IN MYRISTIC ACID MONOLAYERS AT THE 0.01 M HCl–AIR INTERFACE

Temp.	π_c (*dynes/cm*)	A_e (Å²/*molecule*)	Q_c(*kcal/mole*)	ΔS (*cal/deg. per mole*)
7.2°	0.9	43.2	8.2	29
9.1°	2.7	40.9	7.2	26
12.1°	5.5	37.3	5.9	21
14.1°	7.7	35.0	5.1	18
17.0°	10.9	33.2	4.4	15
18.0°	12.7	32.3	4.3	15
22.3°	17.3	30.9	3.9	13
26.2°	21.8	29.1	3.5	12

$A_s = 24$ Å²/molecule. It is interesting to note that $d\pi_c/dT$ for dimyristoyl phosphatidylethanolamine is close to that for dipalmitoyl lecithin and so Q_c and ΔS are also similar. Before going on to discuss the figures given in Tables III and IV, it should be mentioned that the above calculations rest on the assumptions that $d\pi_c/dT$ is an accurate measure of the entropy and that the data are good.

It is apparent that in both systems as A_e increases, Q_c and ΔS rise dramatically. Since the area/molecule is probably the most definitive and unambiguous parameter for describing the physical situation within a monolayer it is best to compare Q_c and

* Eqn. 1 is only completely valid for first-order phase changes which occur at constant pressure. The order of the monolayer transition is not obvious[39]. However, if it is indeed a melting process then it will be accompanied by a latent heat which can be realistically computed from Eqn. 1.

ΔS over the same changes in area/molecule for any transition. Now Table IV shows that to increase the area/molecule from 24 Å^2/molecule in the condensed myristic acid film to 43 Å^2/molecule in the liquid-expanded film requires about 8 kcal/mole and ΔS increases by about 29 cal/deg. per mole (entropy units). By way of comparison, the latent heat of fusion for myristic acid is 10.8 kcal/mole and the entropy gain on melting is about 33 entropy units. BOYD[37] has measured Q and ΔS for the spreading of myristic acid to 24 Å^2/molecule from the crystal. In this case ΔS was approx. 23 entropy units and Q about 6 kcal/mole. These figures allowed BOYD to conclude that the molecules in the film do not possess translational freedom of movement but are able to rotate about their long axes. Now a condensed myristic acid monolayer at 24 Å^2/molecule will obviously permit more chain configurations compared to those possible at the close-packed or crystalline area of about 19 Å^2. This factor and the lack of translational freedom in the film would both help to explain why ΔS and Q for fusion are greater than the values obtained for the condensed to liquid-expanded transition in monolayers. However, it is apparent that the loss of entropy accompanying this transition principally arises from restriction of the configurational freedom of the hydrocarbon chains.

Returning now to Table III, it can be seen that Q_c and ΔS are higher for the condensation when it occurs in dipalmitoyl lecithin monolayers. Thus, while a myristic acid film gains about 18 entropy units on "melting" from 24 to 35 Å^2 per molecule, dipalmitoyl lecithin gains about 27 entropy units for an 11-Å^2 increase in molecular area. Now a mole of dipalmitoyl lecithin contains twice as many hydrocarbon chains as a mole of myristic acid, so that from the discussion in the previous paragraph it would be expected that ΔS for the dipalmitoyl lecithin would be roughly twice that found for myristic acid. If it is permissible to assume that the chain configurations in condensed dipalmitoyl lecithin and myristic acid monolayers are the same, then it appears from the above figures that the hydrocarbon chains in a liquid-expanded dipalmitoyl lecithin film possess less configurational freedom than they do in the equivalent myristic acid case. This could well arise from the fact that in dipalmitoyl lecithin two chains are anchored to the same glycerol moiety because, in this situation, it might be expected that the rotational modes would be reduced.

Correlation of monolayers and bilayers

A systematic study of the behaviour of dipalmitoyl lecithin in water has been made in this laboratory by CHAPMAN, WILLIAMS AND LADBROOKE[21]. Their phase diagram of the system indicates that at room temperature, when the concentration of water is less than 20 %, the lipid is in the gel phase. Here the chaines are hexagonally packed in an ordered array and the area/molecule is 48 Å^2. If the temperature is raised above the T_c then the gel changes to the mesomorphic lamellar phase where the lecithin molecules are arranged in bimolecular layers with the hydrocarbon chains in a melted and liquid-like state. X-ray diffraction* indicated that at 20 % water the area/molecule in the liquid crystalline dipalmitoyl lecithin lamellae was about 60 Å^2, while at 40 % water this area had increased to about 70 Å^2/molecule. Clearly at any given temperature above T_c the extent of the hydration of the lecithin molecules determines the area that they occupy in the bimolecular leaflet. SMALL's study[38] of

* R. M. WILLIAMS, unpublished results.

egg lecithin in water lead him to the same conclusion. It is apparent from these studies that the only time that all the water in the system is bound and the hydration requirements of the polar groups are completely satisfied is when the water concentration is 20 %. The heats absorbed during the transition from gel to smectic mesophase for various water concentrations were also determined and if we now express these heats in terms of changes in area per lecithin molecule, we find that to increase the area/molecule from 48 Å^2 to 60 Å^2 (20 % H_2O) requires the supply of 7.9 kcal/mole of dipalmitoyl lecithin. The figure for the transition from 48 Å^2 to 70 Å^2/molecule (40 % H_2O) is about 8.7 kcal/mole of dipalmitoyl lecithin.

The heats calculated for the monolayer transitions in the previous section arise from changes in the physical state of the surface "phase". This region can be taken to include all the insoluble amphipathic molecules and all the water molecules (the 0.1 M NaCl can be approximated to water for this discussion) associated or bound to them. In a surface balance experiment the bulk water phase acts as a reservoir to supply water molecules for the hydration needs of the insoluble molecules. Clearly the heats given in Table III apply for dipalmitoyl lecithin molecules which have their full quota of bound water without the substrate water playing a significant role. Therefore, for the same physical situation in the monolayer and bilayer we must consider the latter at a water concentration of 20 %. When we satisfy this condition and compare the heat required for the transition from about 48 Å^2 to 60 Å^2/molecule in the monolayer and bilayer we see that the values of 8.0 and 7.9 kcal/mole are very close. Since the transition does not occur at the same temperature in the two systems these heats are not strictly comparable. However, the temperature difference and the heat capacities are small so that the comparison is valid. The increase in entropy in both cases is about 27 entropy units. This agreement would seem to offer strong support for our contention that parallel physical transitions occur in the monolayer and dispersed systems. The agreement is only good for the 20 % water situation, and it can be seen from Table III that for the 48–70 Å^2/molecule change the monolayer heat is significantly larger. It is tempting to speculate that a possible cause of this difference is that for the bilayer the measured endothermic heat incorporates an exothermic heat term arising from the occurrence of free water between the parallel sheets of polar groups.

Now that we have established certain similarities between the two systems under discussion it is necessary to outline some of the differences. In the lamellar liquid crystalline phase the degree of hydration dictates the area that each dipalmitoyl lecithin molecule occupies, while in the monolayer the packing is determined by a physical barrier. This mechanical arrangement therefore controls the hydration of the polar groups. Also in the bilayer system the hydration must to some extent be affected by the force-field between the parallel sheets of dipoles. These differences must be borne in mind when considering the two systems. Nonetheless, the above arguments indicate the areas of comparison between monolayers and bilayers and thus should make the monolayer at the air–water interface a more useful model for membrane studies.

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