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## POLYMORPHISM OF PHOSPHOLIPID MONOLAYERS

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**Résumé.** — Des monocouches de lécithines synthétiques ou d'acide phosphatidique à différents degrés d'ionisation ont été étudiées par la technique de la balance à des pressions supérieures à la pression latérale de vapeur. À partir des courbes isothermes pression-surface et des courbes isobares surface-température on étudie la compressibilité latérale  $\chi$  en fonction de la densité superficielle de lipides ( $a^{-1}$ ) et le coefficient d'expansion thermique ( $\alpha$ ) en fonction de la température ( $T$ ).

La transition entre les états fluides et condensés ( $T = T_M$ ) est caractérisée par une grande hystérésis et apparaît être du premier ordre. On rencontre deux autres transitions à  $T > T_M$  et  $T < T_M$ . Les discontinuités de  $\chi$  et  $\alpha$  montrent qu'il s'agit de transitions du second ordre. Une expérience d'écoulement montre que la transition à  $T > T_M$  dans la région fluide est aussi caractérisée par une discontinuité de viscosité. Pour les monocouches d'acides gras la transition à  $T < T_M$  dans la région condensée est faiblement du premier ordre.

Les discontinuités de  $\chi$  et  $\alpha$  ont été analysées en utilisant la théorie de Landau des transitions de phase. Ceci fournit des informations sur la symétrie des états de monocouches. Cette symétrie est décrite d'une part en fonction du vecteur d'elongation  $\mathbf{J}$  caractérisant l'orientation locale dans le milieu hydrophobe et le milieu hydrophile et, d'autre part, en fonction de l'onde de densité  $\rho$  caractérisant la symétrie de l'organisation latérale. Le diagramme de phase de  $\alpha$ -DPL est établi. Pour une pression supérieure à 15 dyn./cm les états rencontrés, à surface décroissante, sont : le fluide isotrope (I) où les chaînes sont normales à la surface, le fluide anisotrope (II) où les chaînes sont inclinées, le cristallin incliné (III) et le cristallin droit (IV). Les états II et IV disparaissent du diagramme pour des pressions inférieures à 10 dyn./cm.

La chaleur de transition ( $Q_M$ ) à  $T = T_M$  a été obtenue par l'équation de Clausius-Clapeyron.  $Q_M$ , constante à basse température, tend vers 0 à plus haute température suivant  $Q_M = Q_M^0(T - T_c)$  définissant ainsi une température critique. On propose qu'il s'agisse d'un point tricritique au-delà duquel la transition devient du second ordre. Ceci rend compte de la rupture de symétrie à la transition phase fluide-phase condensée. Le comportement des isothermes dans la région de coexistence de la transition  $T - T_M$  est expliqué en fonction de la coopérativité limitée de la transition ; puisque les monocouches sont des systèmes à deux dimensions, on s'attend à d'importantes fluctuations de l'ordre à grande distance.

On établit une relation entre les transitions de phase dans les monocouches et les bicouches. Il s'ensuit qu'une monocouche soumise à une pression extérieure de 12,5 dyn./cm est équivalente à une double couche à son maximum d'hydratation. La pression extérieure déplace aussi la température de transition. La prétransition observée dans les bicouches ne peut être reliée à aucune transition des monocouches, elle résulte plutôt d'un couplage entre couches.

**Abstract.** — Monolayers of synthetic lecithins as well as phosphatidic acid at different stages of ionization were studied with the film balance technique at pressures above the lateral vapour pressure. Pressure ( $\pi$ ) versus area ( $a$ ) curves (isotherms) and, by application of a special technique, area ( $a$ ) versus temperature ( $T$ ) curves (isobars) were recorded. From these data, plots of the lateral compressibility,  $\chi$ , as a function of lateral lipid density ( $a^{-1}$ ) and of the thermal expansion coefficient,  $\alpha$ , as a function of temperature ( $T$ ) were obtained.

The well known transition (at  $T = T_M$ ) between the expanded (fluid) and the condensed (crystalline) states of the films, called the *main transition*, is characterized by a non-horizontal deflection of the isotherms (and isobars) in the coexistence region. A pronounced hysteresis shows that it is a first order transition. Two additional phase transitions, one at  $T > T_M$  (fluid region) and one at  $T < T_M$  (crystalline region), were revealed by breaks in the slopes of the isotherms and the isobars. Discontinuities in the compressibilities and in the expansion coefficients show that these transitions are of second order. A flow experiment showed : that the fluid  $\rightarrow$  fluid transition is also characterized by a pronounced discontinuity in viscosity. In fatty acid monolayers the transition (at  $T < T_M$ ) between two crystalline states is of weak first order ( $-10$  J./Mole).

The signs of the discontinuities, in  $\chi$  and  $\alpha$ , were analysed using the Landau theory of phase transitions. This provided information on the symmetry of the polymorphic states of the monolayers. The symmetry is described 1) in terms of the de Gennes stretching vector  $\mathbf{J}$ , characterizing the orientation within both the hydrophobic and the hydrophilic region of the monolayer and 2) in terms of the density wave,  $\rho$ , characterizing the symmetry of the lateral organization of the lipid molecules. For  $\alpha$ -Dipalmitoyllecithin a phase diagram is established. For  $\pi > 15$  dyn./cm the polymorphic states of phospholipid monolayers observed at decreasing areas are : fluid isotropic (I) (hydrocarbon chains normal to water surface) ; fluid anisotropic (II) (tilted chains) ; tilted crystalline (III) ; non tilted crystalline (IV). At  $\pi \leq 10$  dyn./cm phase II and IV are not observable.

Heats of transition ( $Q_M$ ) of the main transition were obtained from the Clausius Clapeyron equation.  $Q_M$  is constant (37 kJ./Mole) at low temperatures while it converges towards zero at higher temperatures, according to  $Q_M = Q_M^0(T - T_c)$ , thus defining a critical temperature. It is proposed that this is a tricritical point above which the main transition becomes of second order. This allows for a symmetry break at the fluid-to-crystalline (main) transition above this critical temperature. The behaviour is explained by the Landau theory in terms of the strength in coupling between the lateral order (density wave  $\rho$ ) and the chain orientational order (stretching vector  $\mathbf{J}$ ). In analogy to the Rodbell-Bean effect in magnetism a decrease in coupling leads from a first to a second order transition.

The non-horizontal behaviour of the isotherms in the coexistence region of the main transition is explained in terms of the limited cooperativity of the transition. Since monolayers are quasi two-dimensional systems, large fluctuations in the long range order are expected. By considering the phase transition as a *chemical reaction* the areas ( $\delta a$ ) of the cooperative units have been estimated. For  $\alpha$ -DPL they vary from  $\delta a \sim 5 \times 10^3 \text{ \AA}^2$  (at  $\pi = 1$  dyn./cm) to  $\delta a = 1.2 \times 10^4 \text{ \AA}^2$  (at  $\pi = 40$  dyn./cm). As expected the area  $\delta a$ , that is the coherence length of the phase fluctuations, diverge upon approaching the tricritical point.

A relation between the monolayer and the bilayer phase transitions is established. This is done by equalizing both the transition heats and the areas of transition of monolayers and bilayers. For L- $\alpha$ -Dipalmitoyllecithin it then follows that monolayers at an external pressure of 12.5 dyn./cm correspond to bilayers at maximum hydration. A discrepancy in the corresponding transition temperatures,  $T_M$ , is explained in terms of a shift in transition temperature by the external pressure. The bilayer pretransition cannot be related to any monolayer phase transition but is due rather to the monolayer coupling as suggested previously.

**1. Introduction.** — Two-dimensional layers of amphiphilic chain molecules with polar end groups attached to a charged interface represent a class of physical systems of growing interest. Such monolayers bridge the gap between two-dimensional and three-dimensional systems and, for that reason, are of interest for physicists interested in cooperative phenomena and phase transitions. In the field of molecular biology monolayers of phospholipids have gained great attention as potential model systems of biological membranes [1]. Monolayers are used in a variety of technical applications [2] such as the technology of surfactants (e.g. soaps, detergents, foams), the inclusion of foodstuffs (pharmaceuticals, dyes etc.), environmental technology (e.g. purification of water and recycling of rare organic substances). The classic method of monolayer study is the Langmuir-Adam film balance by which the behaviour of monolayers may be studied over a wide range of temperatures and pressures. A number of papers on phospholipid monolayers studied with the film balance have been published (cf. reference [1] and [3] for literature).

In the present work we restudied and compared monolayers of a number of synthetic lecithins as well as phosphatidic acids at different stages of ionization. Besides the familiar pressure-*versus*-area curves (isotherms) we recorded also area *versus* temperature curves (isobars) with a technique described in the method section.

Our main concern was the possibility of polymorphism in phospholipid monolayers as found by Adam [4] and Derichvian [5, 6] for monolayers of aliphatic esters. A second point of interest was concerned with the nature of the well known transition from the so called *liquid expanded* to the *condensed* state, that is whether it is a *Van der Waals* type or a *fluid-to-crystal* type of transition. Discontinuities in the lateral compressibility *versus* area density and in the thermal expansion coefficient *versus* temperature indicated the existence of several liquid and crystalline phases separated by first or second order transitions. The data were analysed on the basis of the Landau theory of phase transitions. This analysis led us to conclusions on the symmetry properties of these phases. As an order parameter for the liquid phases we used the stretching vector,  $\mathbf{J}$ , proposed by de Gennes [7] for the description of layers of amphiphilic chain molecules. For the crystalline phases the lateral density,  $\rho$ , was used as an additional order parameter. The most prominent transition <sup>(1)</sup>, the so called liquid expanded  $\rightarrow$  condensed transition, is first order at low pressures. We show that this is due to a strong coupling between the two order parameters. We provide evidence that the main transition goes over into a second order transition at a tricritical point, in analogy

<sup>(1)</sup> In the following this transition will be called *the main transition*.

to the Rodbell-Bean effect in magnetism [8]. This allows for a break in symmetry at the liquid-to-crystal transition even at very high temperatures. At the main transition a phase change from a fluid phase (either non tilted (I) or tilted (II)) to a tilted crystalline phase (III) takes place. The transition between the fluid phases (I, II) is of second order and is accompanied by a discontinuity in viscosity. The tilted crystalline phases goes over into a non-tilted one (IV) at another second order transition.

Judging from our experimental data the main transition does not behave as an ordinary three-dimensional phase change. It is understood rather as a cooperative transition of cooperative units containing of the order of 150 molecules. The monolayer and the bilayer main transitions were compared on the basis of the respective changes in entropy as well as in area per molecule. This comparison showed an intrinsic lateral pressure in bilayers of about 10 dyn./cm.

**2. Method and materials.** — **2.1 MEASURING TECHNIQUE.** — Both the isotherms (pressure *versus* area curves) and the isobars (area *versus* temperature plots) were taken on a commercial Langmuir balance (Meßgerätewerk Dr. R. Wobser KG, Lauda) equipped with a continuous measuring system. The measuring bar, however, was endowed with a heater so that it could be kept at a constant temperature (of 60 °C) in order to reduce temperature fluctuations. In addition, the bar was constructed in such a way that the application of any glue was avoided. This provision was necessary in order to minimize contamination effects. After purchase the trough was cleaned by running several hundred pressure *versus* area curves using fatty acids in order to remove any spurious amphiphilic contaminations and ions.

All isotherms were run twice in the direction of increasing pressure. Each run was performed with a freshly prepared film. If the trough had been carefully cleaned, the two isotherms taken successively were completely identical. The speed of compression for most measurements was 5 Å<sup>2</sup> per molecule per minute. Below this limit a change in the speed of compression did not affect the isotherms. Kinetic hysteresis effects could thus be excluded. The balance was calibrated mechanically with weights.

The area *versus* temperature curves (isobars) were taken by adjusting the pressure to a preselected value using the automatic pressure control of the film balance. All curves were run at both increasing and decreasing temperature at a rate of 2 °C per minute. The pressure could be kept constant to  $\pm 2.5$  dyn./cm during each run. The accuracy of the area was 5 Å<sup>2</sup>.

**2.2 MATERIAL.** — **2.2.1 Water-substrate and spreading solvent.** — The water was prepared by one of the following two methods :

1. Demineralized water was first distilled from potassium permanganate and was subsequently dis-

tilled twice in a quartz still. Spurious amounts of contamination (amphiphilic molecules and ions) of a fresh water substrate were removed by running at least two isotherms with the lipid under investigation.

2. Very pure water could be prepared from demineralized water with a Millipore filtering system (Mille Q<sup>TM</sup>). The pH of the water substrate was varied with a Britton-Robinson II buffer. Besides phosphoric acid, boric acid and sodium hydroxide, the buffer contained only acetic acid as an organic acid.

All lipids were applied to the substrate using a mixture of n-hexane and ethanol in a 9 : 1 volume-to-volume ratio. The n-hexane was spectroscopically pure, the ethanol was *pro analysis* grade (Merck).

**2.2.2 Lipids.** — L- $\alpha$ -Dipalmitoyllecithin was purchased from Fluka (purissimum). Selected batches could be used without further purification. The purity was checked by thin layer chromatography. A very good indication of purity was the sharpness of the main phase transition of the monolayer. Since each isotherm was run with a freshly prepared film, contamination of the lipid by oxidation during the measurements was minimized. DL- $\alpha$ -Dimyristoyllecithin (Fluka) was purified by thin layer chromatography.  $\beta$ -Dipalmitoyllecithin (Fluka) was used without further purification since the thin layer chromatogram showed only one sharp spot. Dimyristoyl phosphatidic acid was prepared in our laboratory from Dimyristoyllecithin by application of phospholipase A by Dr. Galla. The lipid was purified several times by preparative thin layer chromatography on silica gel.

**3. Experimental results.** — **3.1 ISOTHERMS OF UNCHARGED AND CHARGED PHOSPHOLIPIDS.** — In figures 1-5 we compare the pressure-*versus*-area curves of some uncharged zwitterionic lecithins (e.g.  $\alpha$ -Dipalmitoyllecithin ( $\alpha$ -DPL);  $\alpha$ -Dimyristoyllecithin ( $\alpha$ -DML);  $\beta$ -Dipalmitoyllecithin ( $\beta$ -DPL)) and charged phospholipids (e.g.  $\alpha$ -Dimyristoyl phospho-

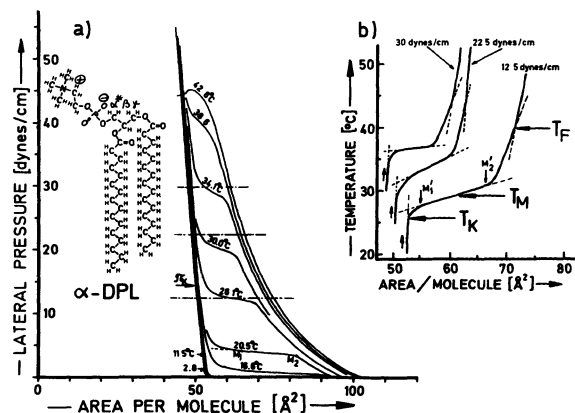


FIG. 1. — a) Continuously recorded isotherms of L- $\alpha$ -Dipalmitoyllecithin (DPL) on pure water substrate. Note the arrow at  $\pi_K$  which indicates a break in the isotherm 2.8 °C. b) Continuously recorded isobars of monomolecular film of L- $\alpha$ -DPL. The curves were recorded at increasing temperatures.

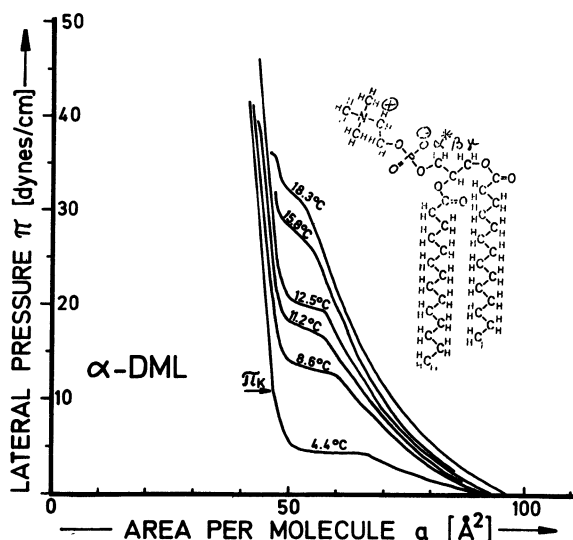


FIG. 2. — Isotherms of L- $\alpha$ -Dimyristoyllecithin. The arrow at 4.4 °C indicates a break in the isotherm.

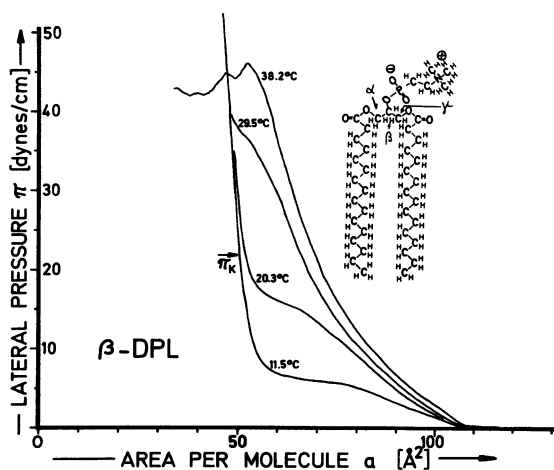


FIG. 3. — Isotherms of L- $\beta$ -Dipalmitoyllecithin.

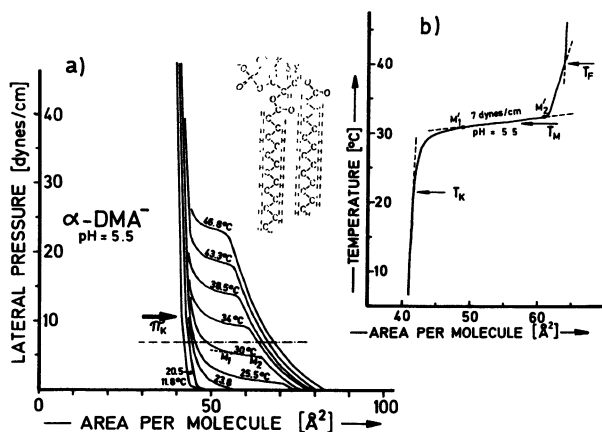


FIG. 4. — a) Isotherms of L- $\alpha$ -Dimyristoyl phosphatidic acid (DMA). Substrate : pure water of pH 5.5. At this pH the lipid has a single negative charge. The arrow indicates a break in the isotherm 11.8 °C. b) Isobar taken at constant pressure of 7 dyn./cm.

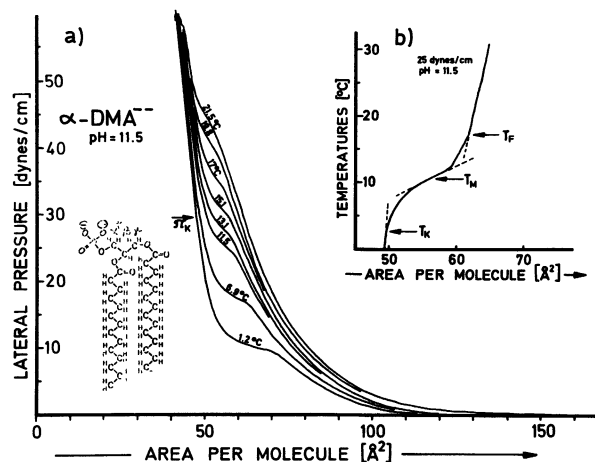


FIG. 5. — a) Isotherms of Dimyristoyl phosphatidic acid at pH 11.5. At this pH the lipid has two negative charges. Substrate : Water + Britton-Robinson II buffer. The arrow indicates a break in the isotherm. b) Isobar.

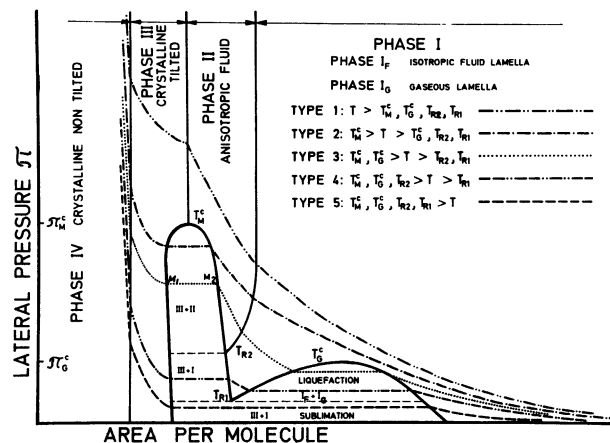


FIG. 6. — Schematic representation of pressure-area phase diagram of phospholipid monolayers as suggested from figures 1-5 (small area region) by reference [10] (low density region) and by reference [11]. Two critical points ( $T_M^c$  and  $T_G^c$ ) and two triple points ( $T_{R1}$ ,  $T_{R2}$ ) are shown. The isotherms may be classified according to 4 different types : Type 1 (—)  $T > T_M^c, T_G^c, T_{R2}, T_{R1}$ ; type 2 (---)  $T_M^c > T > T_G^c, T_{R2}, T_{R1}$ ; type 3 (.....)  $T_M^c, T_G^c > T > T_{R2}, T_{R1}$ ; type 4 (-.-.-)  $T_M^c, T_G^c, T_{R2} > T > T_{R1}$ ; type 5 (-----)  $T_M^c, T_G^c, T_{R2}, T_{R1} > T$ . The critical pressure  $\pi_G^c$  is much smaller than  $\pi_M^c$ .

tidic acid at two different states of ionization (pH)). All the isotherms may be classified according to the 5 different types of curve in figure 6. This clearly shows that phospholipid monolayers exhibit a pronounced polymorphism.

As an example consider the isotherms for Dimyristoyl phosphatidic acid at pH 5.5 (cf. Fig. 4). It shows all the features which are also characteristic for the other lipids :

At low temperatures (below about 25 °C) all curves are of type 5. The pressure starts to increase at a relatively small value of the area ( $< 60 \text{ Å}^2$ ) per lipid. At higher pressures (some ten dyn./cm) the curves of type 5 exhibit an abrupt change in the slope. For

the 11.8 °C-isotherm the break in slope is indicated by an arrow in figure 4. Remarkable is the relatively smooth increase in pressure just above the abscissa  $\pi = 0$ . The break in the slopes of the isotherms indicate a phase transition between two mesomorphic states of the monolayer (see discussion below). At higher temperatures, that is between 25 °C and 46 °C, curves of type 2 and (or) of type 3 are observed. They may be divided into three regions : At large areas the curves seem to increase smoothly <sup>(2)</sup> up to a critical pressure (cf. point  $M_2$  in the 30 °C isotherm) where a sharp decrease in area indicates a pronounced phase transition. It is important to note that from the *transition* point  $M_2$  to  $M_1$  the curves follow a straight, but not horizontal, line. If the area is decreased further, the curves show the same behaviour as those at low temperature (type 5 curves). The transition region between  $M_2$  and  $M_1$  is the well known phase transition between the so called *condensed* and the *expanded liquid* film postulated by Derichvian [5] for fatty acids and by Chapman, Phillips and coworkers [1] for phospholipids. In the following we will refer to this transition as the *main* transition.

**The critical point :** According to figures 1-5 the centre of the main transition between the points  $M_1$  and  $M_2$  clearly shifts to lower areas with increasing temperature. The change in area at the transition seems to converge towards zero upon approaching a critical temperature denoted by  $T_M^c$  in figure 6.

For the charged lipid, DMA<sup>-</sup> (pH 5.5) the corresponding critical isotherm could not be observed within the temperature region accessible to our instrument. However, for DMA<sup>-</sup> at pH 11.5 (Fig. 5) and for the Dialcyllecithins (cf. Figs. 1, 2, 3) the critical region can be observed. For  $\alpha$ -DPL the critical isotherm is located at about 43 °C. Now, the critical point  $T_M^c$  is often considered as analogous to the critical point of a Van der Waals gas <sup>(3)</sup>. The following discussion of our experiments will provide strong evidence that  $T_M^c$  is in fact a tricritical point forming a junction between a first order and a second order transition curve. Accordingly, the two phase region in figure 6 degenerates into a second order transition line at temperatures above  $T_M^c$ .

**3.2 ISOBARS OF UNCHARGED AND CHARGED PHOSPHOLIPIDS.** — Further information about the monolayer polymorphism can be obtained from the area *versus* temperature curves (isobars). Some typical isobars are also presented in figures 1, 4, 5. Consider as an example the isobars of  $\alpha$ -DPL (Fig. 1b). Most pronounced is again the main transition characterized by a straight line connecting the two transition points  $M_1'$  and  $M_2'$ . Since this straight line is not hori-

zontal we define the transition temperature,  $T_M'$  as the midpoint between  $M_1'$  and  $M_2'$ . The isobars exhibit breaks in the slope in the small area region at the transition temperatures  $T_K$ . The phase transition indicated by this break corresponds to the transition observed in the isotherms at the transition pressure  $\pi_K$  (cf. Fig. 1). The corresponding transition line is drawn in the schematic phase diagram of figure 6.

The isobars show, however, a further pronounced phase transition at large areas as indicated by the breaks in the slopes at the temperatures  $T_F$ . It will be shown below that this transition may also be found in the isotherms. The corresponding transition line has also been drawn in figure 6.

**3.3 COMPRESSIBILITY AND THERMAL EXPANSION COEFFICIENT.** — In order to learn more about the nature of the phase transitions indicated by the isotherms and the isobars we determined : 1) the lateral thermal expansion coefficients,  $\alpha$ , as a function of temperature from the isobars and 2) the lateral compressibilities,  $\chi$ , as a function of area density from the isotherms.

In figure 7 the thermal expansion coefficient is plotted as a function of temperature for different lipids and pressures. The very strong central peak is due to the coexistence of the two phases separated by the main phase transition. The two discontinuities in the  $\alpha$  *versus*  $T$  plots observed at the temperatures  $T_K$  and  $T_F$  are due to breaks in the slopes of the isobars. This clearly demonstrates that they correspond to second order phase transitions.

In figure 8b, the compressibility for several lipids is plotted as a function of the area density,  $\rho = a^{-1}$ . Again three phase transitions are observed. The coexistence region which is hard to determine from

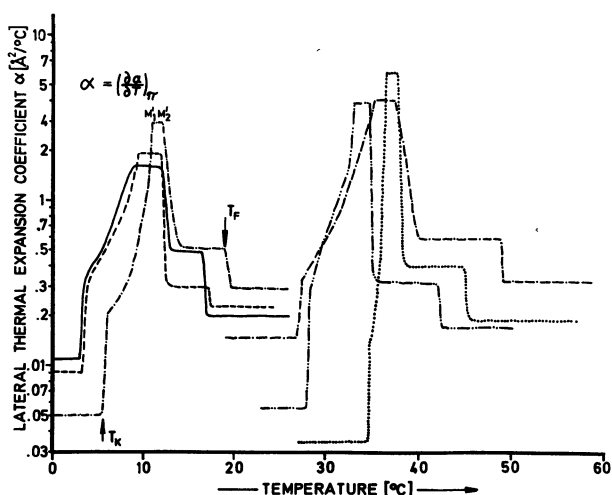


FIG. 7. — Lateral thermal expansion coefficient,  $\alpha$ , as a function of temperature as obtained from the isobars for the following lipids : (—) Dimyristoyl phosphatidic acid at pH 11.5 and  $\pi = 25$  dyn./cm; (---) same lipid at pH 5.5 and  $\pi = 2.5$  dyn./cm; (- · - · -)  $\alpha$ -Dimyristoylleceithin at  $\pi = 37$  dyn./cm; (·····)  $\alpha$ -Dipalmitoylleceithin at  $\pi = 22.5$  dyn./cm; (- - - -) same lipid at 30 dyn./cm; (- - - -)  $\alpha$ -Distearoylleceithin at 6 dyn./cm.

<sup>(2)</sup> A more detailed analysis presented below will establish a further phase transition at pressures below the transition point  $M_2$ .

<sup>(3)</sup> Such a type of critical point is observed for the gas-to-liquid transition at low densities at the temperature  $T_G^c$  [10] and pressure  $\pi_G^c$  [10].

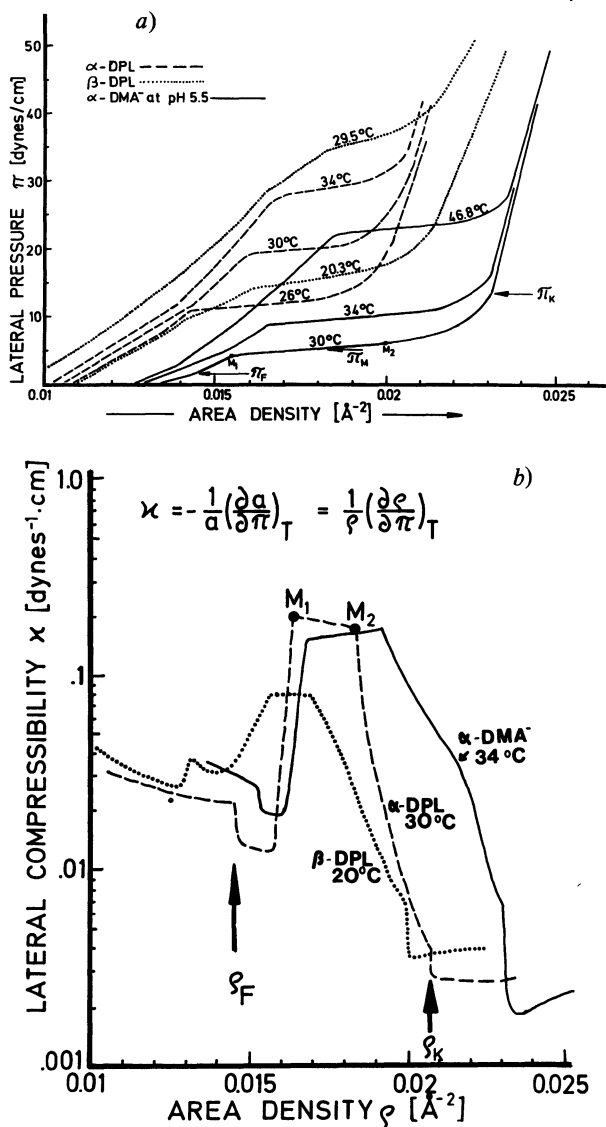


FIG. 8. — a) Pressure plotted as a function of area density ( $\text{\AA}^{-2}$ ) for some lipids at different temperatures.  $M_1$  and  $M_2$  are defined as the end points of the straight line characterizing the main transition. The breaks observed at  $\pi_K$  and  $\pi_F$ , respectively, indicate the two additional phase transitions also found in the isobars. b) Plots of lateral compressibility,  $\chi$ , as a function of area density as obtained from the curves in figure 8a. The main region of the transition is again indicated by  $M_1$  and  $M_2$ . The arrows ( $\uparrow$ ) indicate the two additional low and high density phase transitions for the curve of L- $\alpha$ -DPL.

the  $\pi$ - $\rho$  plots (cf. Fig. 8a) or the isotherms can be rather accurately determined from the end points  $M_1$  and  $M_2$  of the central peak in figure 8b. Two discontinuities at the densities  $\rho_F$  and  $\rho_K$  are due to breaks in the slopes of the pressure *versus* density curves of figure 8a. This again shows that the transitions at  $\rho_F$  and  $\rho_K$  are of second order.

### 3.4 HYSTERESIS, VISCOSITY AND HEAT OF TRANSITION.

— In figure 9 the isobars of L- $\alpha$ -Dipalmitoyllecithin recorded at increasing and decreasing temperatures are compared. At high temperatures the two curves nearly coincide. The absence of a hysteresis supports

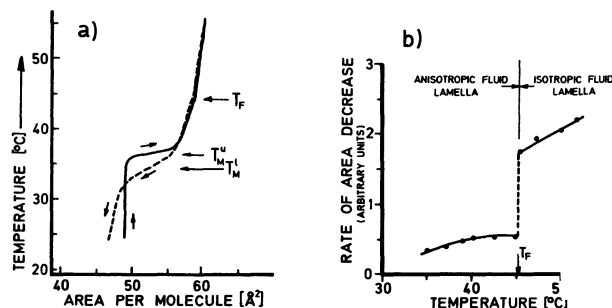


FIG. 9. — a) Isobars of L- $\alpha$ -Dipalmitoyllecithin recorded at increasing ( $\rightarrow$ ) and decreasing ( $\leftarrow$ ) temperature. b) Rate of flow of DPL-monolayer through leak in the trough as a function of temperature. The curve was determined from isobars taken at 30 dyn./cm.

our previous conclusion that the transition at  $T_F$  is of second order. The main transition exhibits a pronounced hysteresis. Recording curves under varying experimental conditions showed that this hysteresis is not determined by the apparatus. The transition temperatures observed at increasing ( $T_M^u$ ) and decreasing ( $T_M^d$ ) temperatures differ by about 2°C. This clearly shows that the main transition is of first order. — An unambiguous conclusion concerning the order of the transition at  $T_K$  was not possible. The reason for this is that the values of  $T_K$  as determined from the break in the slope depend on the experimental conditions. Most probably this is due to kinetic effects caused by the very high rigidity of the high density phases. The comparison of several equal isobars taken under different experimental conditions showed that more reliable results are obtained with increasing temperatures.

In order to get information about monolayer viscosities flow experiments are necessary. Such an experiment can be performed in a most simple way by creating a leak at the rim of the trough. The rate of flow of monolayer through such a leak can be easily estimated by comparison of isobars taken under leaking and nonleaking conditions. A typical result of such an experiment is presented in figure 9b where the rate of decrease in area (that is the flow rate) is plotted as a function of temperature. A sharp increase in the flow rate, at  $T_F$ , observed at increasing temperatures indicates a corresponding discontinuity in lateral viscosity. This again is indicative of the phase transition at  $T_F$ . A rather high flow rate is observed for both phases which shows clearly that the transition at  $T_F$  separates two fluid phases.

The heats of the main transition,  $Q_M$ , can be obtained from the isotherms by application of the Clausius Clapeyron equation ( $^4$ ).

$$\frac{d\pi_M}{dT} = \frac{S_{II} - S_{III}}{a_{II} - a_{III}} \quad (1)$$

( $^4$ ) The derivation of the Clausius-Clapeyron equation is based on a circular process. It does not depend on the slope of the transition curves (cooperativity of transition).



II and III denote the phases separated by the main transition.

$S_{II}$  and  $S_{III}$  are the entropies of phases II and III, respectively. The areas  $a_{II}$  and  $a_{III}$  are defined by the transition points  $M_1$  and  $M_2$  of the isotherms (cf. Figs. 1 and 8a). Using the definition

$$Q_M = (S_{II} - S_{III}) \cdot T_M,$$

the heats of transition plotted in figure 10 for Dimyristoyl phosphatidic acid and  $\alpha$ -Dipalmitoyllecithin are obtained.  $Q_M$  tends to become constant at low temperatures. At higher temperatures  $Q_M$  decreases linearly with the temperature according to

$$Q_M = Q'_0(T_M^c - T). \quad (2)$$

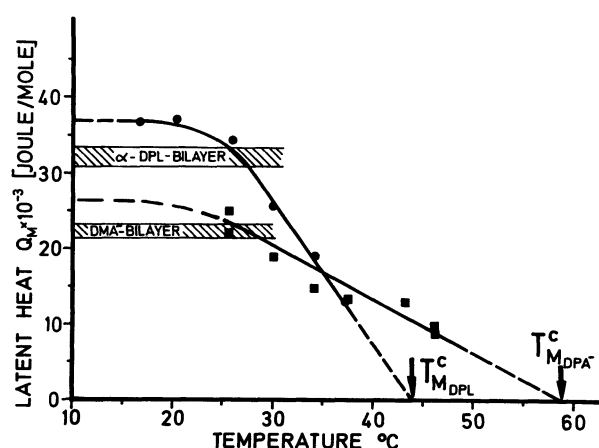


FIG. 10. — Heat of transition of Dimyristoyl phosphatidic acid (DMA, at pH 5.5) and of L- $\alpha$ -Dipalmitoyllecithin determined by application of the Clausius-Clapeyron equation. The temperature where the curves cut the abscissa define the critical temperature  $T_M^c$ .

This shows that  $T_M^c$  is a critical temperature at which the first order phase transition either ends or changes into a second order phase transition<sup>(5)</sup>. The limiting values of  $Q_M$  for both DPL and DMA<sup>-</sup> are close to the values of the heats of transition obtained for the bilayers of these lipids [12, 13]. These calorimetrically determined values of  $Q_M$  are indicated by the bars in figure 10. On a reduced temperature scale  $(T - T_c)/T_c$ , the transition entropies for the charged lipid (DMA<sup>-</sup>) are smaller than for the zwitterionic (uncharged) lecithin. This is also verified by the temperature dependence of the equilibrium pressure  $\pi$ . For DPL we obtained

$$d\pi_M/dT = 2.35 \text{ dyn. cm}^{-1} \text{ degree}^{-1}.$$

For DMA<sup>-</sup> it is

$$d\pi_M/dT = 1.05 \text{ dyn. cm}^{-1} \text{ degree}^{-1}.$$

<sup>(5)</sup> At the present stage of the experimental monolayer technique it is not possible to determine the critical exponents with reliable accuracy. Such data would give better insight into the nature of the phase transition.

The Clausius Clapeyron Equation (1) thus leads to a smaller change in transition entropy,  $\Delta S_M$ , if the surface of the monolayer is charged.

Our experimental data are in contrast to the conclusions obtained in a recent theoretical work by Forsyth *et al.* [14]. These authors calculated the transition entropies for charged lipid layers on the basis of a discrete-surface-charge model. They obtain a higher transition entropy if the lipid surface charges form a discrete ordered lattice. Now, the entropy of a Gouy-Chapman double-layer depends strongly on the Debye length. In our case the Debye length is rather large since pure water was used. The Gouy-Chapman layer is therefore rather diffuse. Accordingly it is very probable that the contribution of this diffuse layer to the transition entropy is rather small compared to the contribution of the rather bulky polar head groups of the zwitterionic lecithin.

**3.5 PHASE DIAGRAM.** — Figure 11 summarizes the results of our monolayer study of L- $\alpha$ -Dipalmitoyllecithin in the form of a phase diagram. Four different phases (characterized by roman numbers I, II, III, IV) are observed :

In the *high temperature* region ( $> 50^\circ\text{C}$ ) the *isotropic fluid* phase prevails<sup>(6)</sup>. In the *low temperature* region ( $T < 15^\circ\text{C}$ ) rigid, and most probably crystalline phases [21], are observed. Our experiments strongly suggest that the rigid phase (III) has tilted chains at low pressure (cf. below).

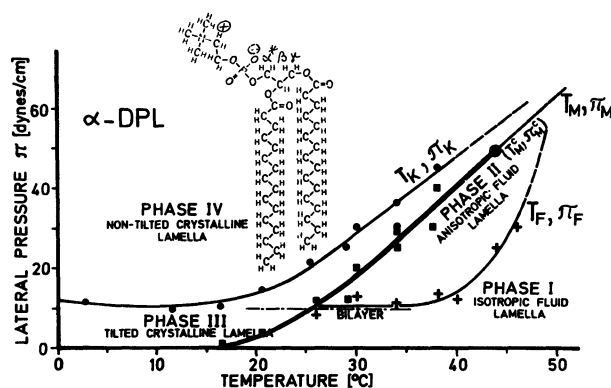


FIG. 11. — Phase-diagram of  $\alpha$ -Dipalmitoyllecithin is obtained from the isotherms and isobars. The transition curves clearly separate 4 different monolayer phases denoted by I, II, III, IV. The thick line corresponds to the main transition in the first order region. • is the tricritical point. The thin lines are supposed to be phase transitions of second order. The line (—) corresponds to the bilayer main transition (cf. Chap. 4. 6).

A transition to a non-tilted phase (IV) takes place along the  $(\pi_K, T_K)$ -equilibrium curve. A nearly constant transition pressure of about 10 dyn./cm is observed between  $0^\circ\text{C}$  and  $20^\circ\text{C}$ . It is this transition

<sup>(6)</sup> The gaseous state at very low pressure (cf. Fig. 6), which was not accessible to our instrument, is not included in the phase diagram.



which is clearly indicated in figures 1-5 by breaks (at pressure  $\pi_K$ ) in the slope of the pressure *versus* area curves taken at low temperatures. The  $(\pi_K, T_K)$ -curve exhibits an upward deflection at  $T > 20^\circ\text{C}$ . The absence of any discontinuity in the area at the III  $\rightarrow$  IV transitions strongly suggests a second order type of transition.

The III  $\rightarrow$  IV transition is more pronounced for fatty acid monolayers. A typical low temperature isotherm showing this transition at the equilibrium pressure  $\pi_K$  has been plotted in figure 12a. In contrast to the phospholipids this transition is accompanied by a small abrupt decrease in area at  $\pi_K$ . Accordingly the compressibility *versus* area plot shown in figure 12b exhibits a sharp peak together with a jump at the transition. From the temperature dependence of  $\pi_K$  we estimate an upper limit of the heat of transition of  $-10$  J./Mol. by application of the Clausius-Clapeyron equation (cf. eq. (1)). The transition separating phase IV and III is thus, at least for fatty acids, of weak first order. In the *intermediate temperature* region ( $15$ - $50^\circ\text{C}$ ) the phase diagram of L- $\alpha$ -DPL is rather complicated. The behaviour is dominated by the main phase transition separating the fluid (I and II) and the crystalline (III) monolayer phases. Up to the tricritical point ( $\oplus$ ) at  $T_M^c, \pi_M^c$  the transition line is of first order. However, the transition line can not end there if phase III exhibits a crystalline phase [21] while phase II is fluid. The transition line propagating from the critical point ( $\oplus$ ) has been included in order to allow for this symmetry break. A crystalline lateral packing of phase III has been observed by Hui *et al.* [21]. Experimental evidence of a crystalline lateral packing of the lipid molecules in phase III has been provided by electron diffraction [21]. Phase III has to be compared with the bilayer phases below the chain melting transition which have been shown to be crystalline by X-ray diffraction. Our conclusion therefore

is : The main transition of monolayers of amphiphilic chain molecules is different from a Van der Waals type of transition.

An outstanding feature of the phospholipid monolayer is the appearance of the mesophase (II) in the intermediate temperature region. The I  $\rightarrow$  II transition is clearly of 2nd order. Surprisingly the mesophase II can only exist at pressures above about 10 dyn./cm. As will be shown below (4.6) it is expected to be absent in bilayers.

At the present state of our study we do not have experimental results concerning the behaviour of the transition lines  $T_K$  and  $T_F$  at pressures above the tricritical point.

#### 4. Discussion. — 4.1 THE ORDER PARAMETERS. —

4.1.1 *The stretching vector.* — Compared to the variety of spectroscopic techniques (ESR, NMR, X-ray) applied in membrane research, the data obtained with the Langmuir balance at first sight seem to yield only rather limited information on the membrane organization on a microscopic level. Nevertheless, an analysis of these thermodynamic data in terms of the phenomenological Landau theory (cf. reference [15]) of phase transitions can yield rather detailed information about the symmetry of the polymorphic states of phospholipid lamellae. A first step in the application of the Landau theory is the search for a suitable order parameter. A convenient order parameter for lipid layers which avoids reference to any specific molecular model is the stretching vector,  $\mathbf{J}$ , introduced by de Gennes [7, 16] which is defined as

$$\mathbf{J} = \rho \langle \mathbf{R}_{m+1} - \mathbf{R}_m \rangle = \rho \cdot \mathbf{J}_0 \quad (3)$$

$\rho$  is the density of the monomeric units of the chain-like molecules. It is isotropic for a fluid phase.  $\mathbf{R}_1$  to  $\mathbf{R}_N$  denote the locations of the monomers numbered from 1- $N$  for each chain of the lipid molecules.  $\langle \rangle$  denotes the statistical average over all configurations. With this definition the order of the hydrocarbon chain (hydrophobic) region ( $\mathbf{J} = \mathbf{J}_h$ ) and of the semi-polar head group (hydrophilic) region ( $\mathbf{J} = \mathbf{J}_p$ ) may be describes separately. For a given density  $\rho$ ,  $|\mathbf{J}|$  is a measure of the length of the projection of either the hydrocarbon chain or of the elongated polar head groups onto the direction characterizing the average lipid orientation.

4.1.2 *The density waves.* — In the above definition of the stretching vector,  $\rho$  was introduced as a parameter characterizing the average lateral densities of the monomers of either the hydrocarbon chains or the polar head groups. This is adequate for fluid or glass-like rigid monolayers. For crystalline phases, the density  $\rho$  is that part of the stretching vector which

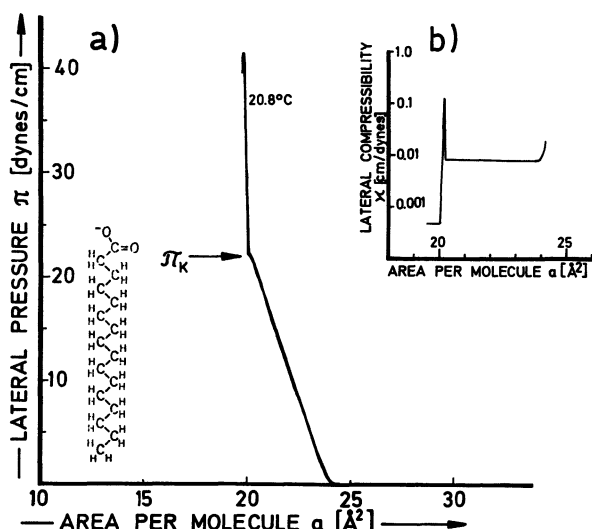


FIG. 12. — a) Low temperature isotherm of palmitic acid showing the transition between phases III and IV at  $\pi_K$ . b) Discontinuity in compressibility of III  $\rightarrow$  IV transition as obtained from the isotherm.

(<sup>7</sup>) This region includes the polar head group and the glycerol backbone with the carbonyl-groups.

describes the symmetry of the lattice.  $\rho$  is completely described by a superposition of a discrete number,  $n$ , of plane density waves with amplitudes  $C_1 \dots C_n$  [17]. The sum of the squares of these amplitudes then define the square of the order parameter

$$\left(\rho^2 = \sum_i^n C_i^2\right).$$

**4.2 CLASSIFICATION OF POSSIBLE MONOLAYER PHASES.** — Now the following lipid phases distinguished by their symmetry are conceivable :

**4.2.1 Gaseous and isotropic fluid phases** (Fig. 13a). — Isotropy of both the stretching vector and of the centres of mass of the lipid molecules. High lateral lipid mobility.  $\mathbf{J}$  is very small and parallel to the normal of the water surface (Fig. 13a). For racemic mixtures, the symmetry is  $C_{\infty v}$ . The fluid and the gaseous phases are only distinguished by their lateral densities and by the length of  $\mathbf{J}$ .

**4.2.2 Anisotropic fluid phases** (Fig. 13b). — Anisotropic orientation of molecular stretching vector  $\mathbf{J}_M$ ; isotropic distribution of molecular centres of mass. High but anisotropic lateral mobility of lipids. Two types of phases are possible :

1) *Smectic C (sm C) states.* — The stretching vector  $\mathbf{J}$  is uniformly tilted in one direction with respect to the normal,  $\mathbf{N}$ , of the water surface. That is,  $\mathbf{N} \times \mathbf{J} \neq 0$ . For racemic mixtures the phase has  $C_{1v}$ -symmetry. This definition also includes rigid smectic lamellae

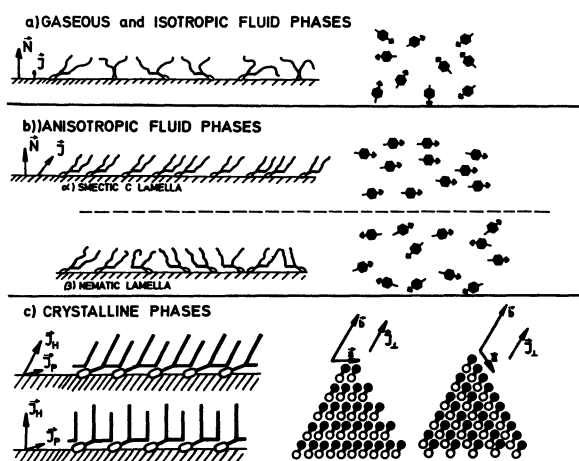


FIG. 13. — Schematic representation of possible phases of phospholipid monolayers : Left side : View of monolayers parallel to water surface. Right side : Bird's eye view of monolayer. In a) and b) the hexagons give the centres of mass of the whole lipid molecules. The arrows indicate the direction of the molecular stretching vector projected onto the water surface. In c) the empty and filled circles indicate the centres of mass of the hydrocarbon chains. The bars between these circles represent the glycerol backbone. The polar head group of one molecule is hidden below the chain of the adjacent molecule (cf. left side). This is indicated by the filled circle. The vectors  $\mathbf{a}$  and  $\mathbf{b}$  are the primitive translation vectors of the two-dimensional lattice.  $\mathbf{J}_1$  is the projection of the stretching vector of the whole molecule onto the water surface.  $\mathbf{J}_h$  stretching vector of hydrophobic part and  $\mathbf{J}_p$  stretching vector of hydrophilic part of the monolayer.

without lateral long range crystalline order of the molecules. The transition between a fluid and a rigid sm C phase would only involve the melting of the hydrocarbon chains<sup>(8)</sup>.

2) *Nematic lamellae.* — The projection of the molecular stretching vector onto the water surface assumes two opposite orientation populated with equal probability. These projections would have the symmetry of a nematic phase. For a racemic mixture one would have  $C_{2v}$ -symmetry.

**4.2.3 Crystalline phases** (Fig. 13c). — The symmetry of these phases may now differ with respect to 1) the lateral lattice formed by the molecular centres of mass, 2) the orientation of the hydrophobic stretching vector ( $\mathbf{J}_h$ ) and 3) the orientation of the hydrophilic stretching vector ( $\mathbf{J}_p$ ). Accordingly, a large number of phases are possible. The X-ray studies of bilayers [19, 20] and electron diffraction studies of monolayers [21] suggest that the hydrocarbon chains form a triangular lattice. The polar head groups may then, however, form two different lattices as shown in figure 13c. A first lattice where the primitive translation vector  $\mathbf{a}$  and  $\mathbf{b}$  form an angle of  $60^\circ$  and a second lattice where  $\mathbf{a}$  and  $\mathbf{b}$  form a  $120^\circ$  angle. In an ideal triangular lattice  $2|\mathbf{a}| = |\mathbf{b}|$ . According to figure 13c the orientation of  $\mathbf{J}_h$  may be varied in both types of lattices. It is thus understood that further types of crystalline phases are possible.

**4.3 THE MAIN TRANSITION.** — The orientation and length of the stretching vector,  $\mathbf{J}$ , and both the density and the symmetry of the lateral molecular packing will change simultaneously at this phase transition. A rigorous description in terms of the Landau expansion of the free energy becomes extremely complicated [16]. However, the essential physical background of the main transition in the neighbourhood of the critical point may be understood by the following simplified model :

At low temperatures the isotherms exhibit a very large jump in the area per molecule (cf. Figs. 1-5). Accordingly, it is the density  $\rho$  which undergoes the most pronounced change, while the direction of  $\mathbf{J}$  changes only slightly. Now, both the value and the symmetry of  $\rho$  change. To a first approximation the difference in the free energies between phases III and II may therefore be expanded in terms of the change  $\rho_1 = \rho_{III} - \rho_{II}$  of the lateral density<sup>(9)</sup> :

$$F_{III}(\pi, T; \rho_1) - F_{II}(\pi, T) = \frac{1}{2} A(\pi, T) \cdot \rho_1^2 + \frac{1}{4} B(\pi, T) \cdot \rho_1^4 + \frac{1}{6} C(\pi, T) \cdot \rho_1^6 \quad (4)$$

<sup>(8)</sup> If the molecules had an average electric dipole moment fixed with respect to the stretching vector  $\mathbf{J}$ , the monolayer would have a resulting macroscopic electric polarization  $\mathbf{P}$  and would be ferroelectric [18]. Since  $\text{div } \mathbf{P}$  is proportional to  $\text{div } \mathbf{J}$  a deformation of the membrane would create a lateral space charge density  $\rho (= \text{div } \mathbf{P})$ .

<sup>(9)</sup> We consider the example between phase II and III. The consideration would also hold between phase I and III.

$\rho_{II}$  is density of the anisotropic<sup>(9)</sup> fluid phase.  $\rho_{III}$  is the density of the crystalline phase III.  $\rho_{II}$  is *isotropic* and consequently phase II has the higher symmetry in the sense of Landau. Since  $\rho_{II}$  is isotropic,  $\rho_1$  may be expressed in terms of a superposition of those plane density waves which determine the lateral density of the phase III alone. For symmetry reasons terms of odd order in  $\rho_1$  have to be omitted in eq. (4). This is evident from figure 13c since both lattices may be approximately described by a single plane density wave with maxima at the position of the heavy polar head groups.

The above free energy cannot yet describe the main transition since it neglects any coupling between the simultaneous changes in density order parameter and in the average chain orientation. This coupling may be taken into account by the additional free energy difference [9] :

$$F_c = \frac{1}{2} K(\theta_{III} - \theta_{II})^2 - t |\rho_1|^2 \cdot (\theta_{III} - \theta_{II}) . \quad (5)$$

In this equation,  $\theta_{II}$  and  $\theta_{III}$  are the angles between the stretching vectors and the normal,  $N$ , to the water surface. The first term accounts for the elastic energy involved in the change of the chain orientation upon going from phase II to III.  $K$  is the orientational elastic constant and is positive. The second term is the non-trivial coupling term of lowest order. It can be linear in  $\theta$  since the angle does not undergo a break in symmetry at the phase transition. The correlation between chain orientation and density is obtained by minimizing the total free energy  $F = F_{III} + F_c$  with respect to  $\theta_{III}$ . This yields

$$(\theta_{III} - \theta_{II}) = \frac{t}{K} |\rho_1|^2 . \quad (6)$$

The free energy is then given by

$$F_{III} = F_{II} + \frac{1}{2} A \rho_1^2 + \frac{1}{4} (B - 2 t^2/K) \rho_1^4 + \frac{1}{6} C \rho_1^6 + \dots \quad (7)$$

$t$  and  $K$  are phenomenological coefficients which are expected to depend on both the temperature and on the pressure (or area). For thermotropic liquid crystals the temperature and density dependence of  $K$  is well established and a similar behaviour is expected for lipid layers (see e.g. ref. [9]). From this analogy it then follows that  $K$  must increase with increasing lateral packing density or pressure. The temperature and pressure dependence of the coupling coefficient  $t$  is not known. Now,  $t$  is expected to decrease with increasing pressure since the density jump at the main transition becomes smaller. Accordingly the correction to the 4th order coefficient decreases with increasing pressure. Consequently the transition must become of second order ( $C > 0$ ) and the critical point ( $\oplus$ ) in figure 6 is a tricritical point. The change from first to

second order (at this point) is thus induced by the coupling between the density order ( $\rho_1$ ) and the orientational order ( $\theta$ ). This is completely analogous to the Rodbell-Bean effect in magnetism [8]. Another example is the nematic  $\rightarrow$  smectic A transition [22].

According to figure 10 the heat of transition,  $Q_M$ , decreases linearly with the increasing temperature upon approaching the tricritical point. This behaviour of  $Q_M$  follows directly from the free energy of eq. (7) : The entropy change at the main transition is given by

$$\begin{aligned} \frac{Q_M}{T_M} &= (S_{II} - S_{III}) = \frac{\partial(F_{III} - F_{II})}{\partial T} = \\ &= \frac{1}{2} \frac{\partial A}{\partial T} \Big|_{T_M} \rho_1(T_M)^2 . \quad (8) \end{aligned}$$

Since  $\frac{\partial A}{\partial T} \Big|_{T_M}$  is approximately constant,  $Q_M$  is proportional to  $\rho_1(T_M)^2$ . This change in the density  $\rho_1(T_M)$  is obtained from the condition for the phase transition  $F_{III} - F_{II} = 0$ . Using the usual approximation

$$A = A_0(T - T_M^c)$$

one obtains

$$\rho_1(T_M)^2 \propto (T_M^c - T) . \quad (9)$$

Far away from the tricritical-point,  $\rho_1(T_M)^2$  and therefore  $Q_M$  asymptotically approaches a constant value in accordance with figure 10.

#### 4.4 Second order transition (I $\rightarrow$ II and III $\rightarrow$ IV).

— It is characteristic of these transitions that both the compressibility,  $\kappa$  (cf. Fig. 8) and the thermal expansion coefficient,  $\alpha$  (cf. Fig. 7) exhibit a jump only. This shows that these transitions are of second order. As shown by Landau the direction (sign) of the discontinuities in  $\kappa$  and  $\alpha$  give information about the symmetry break at the transition : By using the Ehrenfest condition for a second order transition ( $\Delta a = 0$  and  $\Delta S = 0$ ) the changes in  $\kappa(\Delta\kappa)$  and in  $\alpha(\Delta\alpha)$  may be related to the change in the specific heat,  $\Delta C_\pi$ , at constant pressure according to :

$$\Delta\alpha = \frac{1}{T} \frac{dT}{d\pi} \cdot \Delta C_\pi \quad (10a)$$

$$\Delta\kappa = \frac{1}{T} \left( \frac{dT}{d\pi} \right)^2 \cdot \Delta C_\pi . \quad (10b)$$

For a second order transition from a more symmetric to a less symmetric phase the Landau expansion leads to

$$\Delta C_\pi = \frac{A_0^2}{2B} T_c \quad (11)$$

where  $T_c = T_F$  for I  $\rightarrow$  II and  $T_c = T_K$  for III  $\rightarrow$  IV. For the 2nd order transition  $B > 0$ . For  $T > 10^\circ\text{C}$  (cf. Fig. 11)  $dT/d\pi > 0$  along all transition lines. Accordingly, both  $\Delta\alpha$  and  $\Delta\kappa$  should undergo an

abrupt increase (positive jump) if one goes from a more symmetric ( $\rho_1 = 0$ ) to a less symmetric ( $\rho_1 \neq 0$ ) phase.

#### 4.4 SECOND ORDER TRANSITION (I $\rightarrow$ II AND III $\rightarrow$ IV).

We first consider the expansion coefficient,  $\alpha$ . At the transition I  $\rightarrow$  II  $\alpha$  exhibits a positive jump and phase II is less symmetric. Since both I and II are fluid phases (cf. Fig. 9b) it follows that II must be an anisotropic fluid phase (cf. Fig. 11). The relatively large decrease in viscosity upon going from I  $\rightarrow$  II (cf. Fig. 9b) strongly suggests that phase II is smectic C rather than nematic (cf. Fig. 13). The viscosity difference between an isotropic and a nematic phase is expected to be much smaller [9].

At the transition from III  $\rightarrow$  IV  $\Delta\alpha < 0$  and phase IV should be more symmetric than phase III. This can be understood by assuming that phases III and IV are both crystalline [21]. According to figures 1-5 the area per molecule in phase III changes continuously upon approaching the phase transition. This strongly suggests that the transition is due to a change in the tilt of the molecules. This leads to the conclusion that phase III is crystalline tilted while IV is crystalline non-tilted. The transition would thus be characterized by a symmetry break of the order parameter  $J_n$ . For palmitic acid, where III  $\rightarrow$  IV is of weak first order the above argument does not apply. This situation is more complicated for the compressibility change.

For the transition III  $\rightarrow$  IV  $\Delta\kappa$  again undergoes a negative jump ( $\Delta\kappa < 0$ ) in agreement with the sign of the change in the expansion coefficients. For  $\beta$ -DPL it is  $\Delta\kappa > 0$  at the transition I  $\rightarrow$  II which again agrees with the behaviour of  $\alpha$ . For  $\alpha$ -DPL and phosphatidic acid, however,  $\Delta\kappa$  becomes negative at this transition as seen in figure 8b.

These difficulties arise due to the fact that the monolayer is a true 3-dimensional system. In the above consideration it was assumed that the changes in lateral pressure and in area are not connected with changes in the third dimension or in the stretching vector  $\mathbf{J}$  <sup>(10)</sup>: the question arises which of the two thermodynamic parameters, the compressibility  $\kappa$ , or the thermal expansion coefficient,  $\alpha$ , gives more reliable information on the symmetry break involved in the second order phase transition. Now,  $\kappa$  is only sensitive to changes in the curvature at the minimum of the free energy.  $\alpha$ , however, is rather dependent on the asymmetry of the free energy curve about the minimum. This suggests that it is  $\alpha$ , rather than  $\kappa$ , which gives more reliable information on symmetry changes connected with changes in  $\mathbf{J}$ . It is for this reason that we come to the conclusion that phase I is anisotropic while II is isotropic. This picture could change if a further mesophase was detected between the gaseous phase and the  $\pi_F/T_F$  equilibrium line.

The second order transition III  $\rightarrow$  IV was interpreted as a change in tilt of the lipid chains with respect to the normal of the water surface. In the isotherms of figures 1-5, phase III started at the transition point  $M_1$ , the end point of the straight line of the main transition. The isotherm of phase III is characterized by a rather soft increase in pressure at decreasing areas. This is interpreted as being due to the change in the tilt angle  $\theta$ . The tilt angle at point  $M_1$  has been estimated by assuming that at the III  $\rightarrow$  IV transition  $\theta = 0$ . We obtain a value of  $\theta \approx 30^\circ$ , which is in reasonable agreement with X-ray data [19].

**4.5 COOPERATIVITY OF MAIN TRANSITION.** — An outstanding feature of the main transition is that both the isotherms and the isobars are non-horizontal in the transition region. This is in striking contrast to the behaviour of ordinary three-dimensional systems in the two phase region.

The first suggestion is to attribute this peculiar behaviour of the monolayers to impurities. In order to test this possibility we took great care in purifying the lipid and the water phase. Although the slope of the transition region was strongly dependent on the purity of the lipid and even more on that of the water, the slope did not change further after a certain degree of purity. The finite slope thus seems to be an intrinsic property of the monolayer.

Some authors attributed the finite slope to an additional surface pressure caused by the electrostatic interaction of the two-dimensional electric dipole layer of the polar head group region [14, 23]. A rough estimate indeed shows that this additional surface pressure amounts to about 10 dyn./cm for a molecular dipole moment of 1 Debye. For an ordinary phase transition such an additional surface pressure could shift the transition temperature but it should not alter the typical behaviour of a true two phase region. Thus, the finite slope has to be attributed to the limited size of the cooperatively transforming units. In fact, it has been pointed out by Landau [17] that in systems restricted in the third dimension the fluctuations lead to a finite size of the cooperative units.

The size of cooperatively transforming units may be estimated from the width of the transition line by considering the phase transition as an all-or-nothing transition between two states (phases) A and B



$K$  is an equilibrium constant. A and B symbolize the clusters of the phases which may transform simultaneously and reversibly into each other. The number of molecules in each cluster is denoted as  $n_A$  and  $n_B$ , respectively. The equilibrium constant is equal to the ratio of the concentration in phase B ( $[B]$ ) and phase A ( $[A]$ ), respectively

$$K = \frac{[B]}{[A]} \quad (13)$$

<sup>(10)</sup> Note that the stretching vector was introduced to extend the monolayer in the third dimension.

The degree of transition is characterized by the order parameter  $\zeta$ ;

$$\zeta = \frac{[B]}{[A] + [B]} \quad (14)$$

Now the Van t'Hoff equation relates the equilibrium constant  $K$  to  $a$ ) the heat of transition  $\Delta Q$  (at  $\Pi = \text{constant}$ )

$$\frac{\partial(\ln K_\pi)}{\partial T} = \frac{\Delta Q}{RT^2} \quad (15)$$

and  $b$ ) the change in area  $\Delta a$  (at  $T = \text{constant}$ )

$$\frac{\partial(\ln K_T)}{\partial \pi} = \frac{\Delta a}{RT} \quad (16)$$

$\Delta Q$  is the heat of transition of the whole cluster undergoing a spontaneous phase transition in the all or nothing process (Eq. (12)): Similarly,  $\Delta a$ , is the change in area of the whole cluster. In the center of the transition ( $\zeta = 1/2$ )  $n_A = n_B = N$  and therefore:  $\Delta Q = N \cdot q_M$  and  $\Delta a = N \cdot \Delta a_M$ .  $\Delta a_M$  is the change of area per molecule and  $q_M$  is the heat of transition per molecule.  $\Delta a$  and  $\Delta Q$ , respectively, may be determined from plots of the order parameter  $\zeta$  versus pressure (from isotherms) and of  $\zeta$  versus temperature (from isobars) according to

$$\Delta a = 4 k T_M \left( \frac{\partial \zeta}{\partial \pi} \right) \Big|_{\zeta=1/2} \quad (17)$$

$$\Delta Q = 4 k T_M^2 \left( \frac{\partial \zeta}{\partial T} \right) \Big|_{\zeta=1/2} \quad (18)$$

Since in the region of the main transition the isobars and isotherms are straight lines,  $\partial \zeta / \partial \pi$  is given by the reciprocal of the pressure difference between the onset and the end of the transition ( $\pi_{M_1} - \pi_{M_2}$  in figure 1).  $\partial \zeta / \partial T$  is obtained in the same way from the isobars.  $q_M$  has been determined from figure 10.  $\Delta a_M$ , the jump in area per molecule is taken directly from the isotherms. For L- $\alpha$ -DPL the size of the clusters determined in this way is given in Table I. Reasonable agreement between the values obtained from the isotherms and from the isobars is observed. The values from the isobars are somewhat smaller due to experimental difficulties (e.g. loss of lipid, fluctuation in pressure). The cooperativity increases upon approach-

ing the tricritical-point. This is consistent with the observed decrease of the heat of transition near the tricritical-point (cf. Fig. 10). This increase in cooperativity is in complete agreement with the Landau theory which predicts a divergence of the coherence length of the fluctuations of one phase in another in the neighbourhood of a tricritical point.

The finite size of the domains undergoing a cooperative phase transition may be caused by other mechanisms. One further possibility is that the density of dislocations and disclinations in the smectic monolayer is rather high and thus may limit the size of the clusters. Secondly, the tendency of lipids to form micelles may lead to fluctuation in the surface curvature which again would limit the size of the cooperative units. Such a model of surface modulation has recently been proposed to explain the ripple structure of lipid bilayers [24].

**4.6 COMPARISON OF MONOLAYER WITH BILAYER MAIN TRANSITION.** — Lipid bilayers undergo two phase transitions at the temperatures  $T_1$  and  $T_2$ , respectively. The so called main transition (at  $T_1$ ) has been attributed to the transition from the isotropic fluid to an anisotropic (tilted) crystalline phase while the two opposing monolayers are supposed to be completely independent. The transition at  $T_2$ , the so called pretransition, has been attributed to the coupling of the two opposing monolayers [24]. It would be most desirable to relate the phase transition of the bilayers with the phase diagram of monolayers, because for monolayers the phase may be studied over a large temperature and pressure range. For two reasons it is not straightforward to obtain such a connection:

(i) The phase diagram is a planar section through the three dimensional pressure-temperature-concentration ( $c_w$ )-diagram ( $c_w$  = molar fraction of water). The most reasonable assumption is that the section is parallel to the  $\pi$ - $T$ -plane near  $c = 1$ .

(ii) More seriously, the surface pressure may be completely different in the bulk and at the water surface. As a way out of these difficulties Phillips *et al.* [13], Nagle [3] and Hui *et al.* [21] propose to equalize the transition temperature of the monolayer,  $T_M$ , with that of the bilayer,  $T_1$ . They therefore conclude that for DPL the main transition of a bilayer is identical to the main transition of a monolayer at a surface pressure  $\pi_B = 50$  dyn./cm. However, this pressure would be about equal to the pressure  $\pi_M^c$  at the critical point of the monolayer. For thermodynamic reasons this cannot be allowed, since at the critical point of the monolayer, both the heat of transition  $Q_M$  and the change in area  $\Delta a_M$  are zero. In DPL-bilayers, however, one observes a rather large transition heat of  $\Delta Q_M = 32.2$  kJ/mole [12] and a large change in area of  $\Delta a_M = 10 \text{ \AA}^2/\text{molecules}$  [20]. In our opinion it is therefore more appropriate to equalize the change in area and the change in entropy of the bilayer with

TABLE I

Number of molecules,  $N$ , in a cooperative unit as a function of temperature and pressure for L- $\alpha$ -dipalmitoyllecithin. The first two columns are obtained from isobars. The other results are from isotherms.

$\pi \frac{\text{dyn.}}{\text{cm}}$	12	30	1	4	12	20	29	40
$T [^\circ\text{C}]$	26	34	17	21	26	30	34	39
$N$	60	100	80	90	130	150	160	190

the corresponding values of the monolayer. From figure 10 and figure 1 it follows that the values of  $Q_M \sim 32$  kJ/mole and of  $\Delta a \sim 10 \text{ \AA}^2/\text{molecule}$  are observed for monolayers at a surface pressure of 12.5 dyn./cm. This seems to be a contradiction, since the transition temperature of 26 °C is much lower than the value of  $T_1 = 42$  °C for the DPL-bilayers.

A way out of this difficulty is again obtained by application of the Landau theory. In order to compare monolayers with bilayers it is necessary to introduce an additional surface pressure  $\sigma$ . The contribution of this surface pressure to the Landau free energy is given by

$$F_\sigma = + \int_a^\infty \sigma(a) da \quad (19)$$

$F_\sigma$  accounts for the fact that in a monolayer the hydrocarbon chain surface is free while in bilayers one has to consider the interaction between two hydrocarbon surfaces. It is important to realize that this additional surface pressure is a function of the area  $a$ . The monolayer interaction depends on the degree of penetration of the hydrocarbon chains of one monolayer into the hydrocarbon region of the opposing monolayer. This penetration depth and therefore  $\sigma$  depends on the packing density  $a^{-1}$ .

In the neighbourhood of the phase transition  $F$  may be expanded in terms of the change in the order parameter  $\rho_1$ :

$$F_\sigma = a_0 + b_0 \rho_1 + c_0 \rho_1^2 + \dots \quad (20)$$

According to the Landau model the free energy cannot contain terms linear in  $\rho_1$  ( $b_0 = 0$ ). At the phase transition the total free energy  $F (= F_{III} + F_\sigma)$  must have a minimum. From  $\partial F / \partial \rho_1 = 0$  it follows

$$2 c_0 \rho_1 + A_0(T - T_c) \rho_1 + B \rho_1^3 + C \rho_1^5 + \dots = 0 \quad (21)$$

The transition temperature of the monolayer is then given by

$$T_M = T_c - \frac{2 c_0}{A_0} \quad (22)$$

$T_c$  is identical to the transition temperature of the bilayer for which  $F_\sigma = 0$ . It is clear that the monolayer indeed has a lower main transition temperature than the bilayer for identical values of change in entropy and area. In the treatment of Nagle [3] the dependence of the additional surface pressure on the area was ignored and therefore  $c_0 = 0$ . Our above conclusion that the DPL-monolayer at about 12.5 dyn./cm has to be compared with the DPL-bilayer is also consistent with the phase diagram (cf. Fig. 11, line - . - .). There the  $(\pi_K, T_K)$ -equilibrium curve is nearly horizontal between 0 and 20 °C and the III  $\rightarrow$  IV transition should be absent in the bilayer. The crystalline phase of the lecithin bilayer is indeed tilted [19, 25]. It is also clear that the III  $\rightarrow$  IV transition cannot be identified with the bilayer pretransition. This follows from the findings that the monolayer III  $\rightarrow$  IV transition is at most of very weak first order. The bilayer pretransition, however, is accompanied by a rather large value of the heat of transition [12]. This is a further proof for our conclusion presented in a previous paper [24]. There we provided strong evidence that the pretransition is due to a decoupling of the two opposing monolayers (at increasing temperatures) rather than to a transition of the polar head group region.

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