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## Adhesion in egg lecithin multilayer systems produced by cooling

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**Abstract.** — Slightly hydrated egg lecithin was aligned mostly parallel to the glass plates of the sample cells by squeezing it to a thickness of 20  $\mu\text{m}$ . The rest of the cell was filled with water and the lipid was left to swell at elevated temperatures to bilayer mean spacings between 3 and 17 nm. Cooling by a few  $^{\circ}\text{C}$  gave rise to induced adhesion in the semicylindrical border (and myelin cylinders) where the mean spacing was larger than in the bulk. From the contact roundings of membranes adhering to stacks of other membranes we obtained the lateral tension ( $\text{dyn cm}^{-1}$ ) inducing the adhesion as a function of the equilibrium mean spacing. An extensive analysis of the data in terms of van der Waals attraction and undulatory repulsion leads to serious contradictions. They suggest the intervention of a new, submicroscopic roughness of unstressed lecithin membranes absorbing plenty of membrane area.

### 1. Introduction.

It seems to be a general experience that egg and other lecithins in a large excess of water form giant vesicles and that the vesicles do not stick to each other when they come in contact. The absence of mutual adhesion between lecithin membranes may surprise in view of the van der Waals attraction and adhesion energies measured by several authors [1-4]. On the other hand, unstressed fluid membranes perform strong thermal undulations which are associated with a repulsive force. Theory predicts this kind of steric interaction to be possibly strong enough to overcome van der Waals attraction [5-7].

The adhesive behaviour of egg lecithin membranes has been investigated in our group for many years, the method of observation being light microscopy. Very early it was checked that also in salt solution vesicles emerge, though very slowly at 0.5 M NaCl, and do not adhere to each other [8]. This was first evidence against separation by electrostatic repulsion which would have to originate from impurities because of the zwitterionic nature of lecithin.

Adhesion was observed later on when membranes happened to be stretched during the swelling of lecithin in water [9-11]. A rounding of the membrane in the immediate vicinity of the adhesive contact was used to compute the lateral tension. Being controlled by the

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membrane's bending rigidity and lateral tension, contact rounding is optically resolvable only at extremely low tensions. The contact angles made by the contact area and the asymptotic direction of the free membrane were found to be practically constant in the range of measured tensions extending from ca.  $10^{-4}$  to  $10^{-6}$  dyn  $\text{cm}^{-1}$ . They were ca.  $40^\circ$  for symmetric adhesion, i.e. two single membranes under equal tension, and ca.  $70^\circ$  for adhesion of a single membrane to a bundle of several others. In another study, thin unilamellar tubes with radii of 1  $\mu\text{m}$  or less were seen to adhere to the glass slides of the sample cell and there to themselves while wider tubes did neither [12]. All these instances of induced adhesion may be regarded as indirect evidence for the decisive role of steric interaction in membrane separation. They show van der Waals attraction to dominate whenever lateral tension or geometric constraints reduce the amplitude of the fluctuations.

In work basic to the present one, we studied the orderly swelling of slightly hydrated egg lecithin in contact with excess water [13, 14]. Orderly swelling takes place if the lecithin spans the thickness of the sample cell (which should not exceed 40  $\mu\text{m}$ ) and the membranes are aligned mostly parallel to the slides, forming a well but not perfectly ordered planar phase with a semicylindrical border to the water. It starts with the formation of myelin cylinders and water channels reaching from top to bottom of the cell which, in effect, increase the length of the border. We measured over days or weeks the gradual increase of mean membrane spacing, covering the range of 1 to 15 nm in the planar phase. There were no signs in our samples of an equilibrium spacing, located by others in egg-lecithin multilayer systems at 2.75 nm [1, 2], or of a phase separation. For the maximum mean spacing we could estimate the pressure between bilayers in the planar phase, using the bending rigidity of the bilayers and the radius of very thin tubes protruding from the semicylindrical border into the free water. The pressure conformed with the predicted strength of the undulation forces within experimental and theoretical uncertainties. We inferred from the result that electrostatic forces, even if they would have been predominant at 15 nm, were certainly negligible near 3 nm. Like vesicle formation in salt solution the experiments suggest that stacked egg lecithin membranes can be driven apart by steric interaction alone.

It is agreed today that undulations play a significant role in the interplay of forces between fluid membranes [6, 15, 16]. Undulation forces have been measured and found to be of the predicted strength in multilayer systems of very flexible membranes where they clearly dominate [17, 18]. Whether unstressed lecithin membranes adhere to or separate from each other is still considered a controversial issue. It is therefore of interest to investigate the interaction of these membranes by novel experimental techniques.

In the following we report on adhesion induced by lateral tension in multilayer systems of known mean membrane spacing. The tension was produced by cooling the egg lecithin samples after orderly swelling at elevated temperatures. Adhesion was inferred from a separation of the multilayer system into domains of higher and lower lecithin density. This « phase separation », as it will be called for short, was seen almost exclusively in the semicylinders and cylinders where the mean membrane spacing is larger than in the planar phase. Unless the lecithin density was extremely high, single membranes became visible after some cooling, making contact angles again of ca.  $70^\circ$  with extended domains of adhesion. Lateral tensions between  $4 \times 10^{-5}$  and  $2 \times 10^{-3}$  dyn  $\text{cm}^{-1}$  were obtained from contact roundings and other estimates. They seemed to saturate quickly with decreasing temperature, approaching but not exceeding the strength needed to induce adhesion in the planar phase. The data are compatible with an inverse square relationship,  $\sigma \sim 1/\bar{z}_{\text{eq}}^2$ , between the lateral tension  $\sigma$  and the equilibrium mean spacing  $\bar{z}_{\text{eq}}$  of the membranes in domains of adhesion.

To interpret the data we propose a model for the restructuring of the multilayer system during cooling. It predicts a self-limitation of the lateral tension, in agreement with

experiment, and permits an estimate of intermembrane repulsion in the absence of tension which conforms with the theoretical strength of undulation forces. The scaling law  $\sigma \sim 1/z_{eq}^2$  is shown to be part of a simplified model of induced adhesion which also predicts  $g_a \sim \sigma$ ,  $g_a$  being the adhesion energy, and constant contact angles. The validity of the scaling law seems to confirm that the steric interaction is due to undulations. However, the tensions associated with the equilibrium spacings of adhesion are smaller by a factor of at least 100 than appears permissible theoretically. A similar and probably related contradiction was inferred previously from the largeness of the contact angles of induced adhesion [11] and will be discussed again. Now as then, it appears necessary to postulate an optically unresolved roughness of the membranes which absorbs much more area than do the undulations and possibly enhances steric interaction.

## 2. Experimental.

The samples were prepared as previously [13, 14]. Pure egg lecithin (research grade, 99 %) was used as purchased from Serva (Heidelberg). Water was twice distilled. After hydrating the lecithin (10 to 20 vol.% H<sub>2</sub>O), we deposited it on the bottom glass of the sample cell and pressed it flat which the cover glass to a final thickness of typically 20  $\mu\text{m}$ . The squeezing aligned the bilayers mostly parallel to the glasses. Water sucked in subsequently by capillarity filled the rest of the cell.

The samples were placed under a Leitz Ortholux microscope and observed directly or on a monitor equipped with a recorder. Photographs were made with a camera attached to the microscope. The standard mode of observation was phase contrast which is well suited to bring out single membranes. The numerical aperture was in general 0.75 and the depth of field about  $\pm 1 \mu\text{m}$ . All pictures were taken of the central plane of the sample cells.

In contrast to our earlier work, we controlled and varied the temperature with a thermostat. Swelling was done at elevated temperatures, typically 55 °C, where it took about half as long as at room temperature. After reaching the desired lecithin density in the planar phase of the multilayer system, we lowered the temperature to induce adhesion. At the usual heating and cooling rates of 0.5 °C min<sup>-1</sup> the temperature was known with an accuracy of  $\pm 1$  °C while being varied.

The stages of swelling of the slightly hydrated egg lecithin were the same as at room temperature. Swelling began with the rapid generation of myelin cylinders growing from the border of the planar phase into the free water. (We did not study here the less frequent water channels penetrating the planar phase.) The cylinders consisted of concentric bilayers, spanned the thickness of the sample cell, and tented to fold into patterns of parallel, equidistant sections that were in contact with each other. The cylinders may be regarded as an extension of the semicylindrical border of the planar phase. After their growth slowed down to become quasi imperceptible they started developing visible water cores. Cylinders and border began to widen noticeably, their cross sections turning from nearly circular to elliptical. Subsequently, some of the cylinders began to expand, forming secondary planar phase. The lecithin density of the latter tended to be slightly lower than that of the primary planar phase. The width of border and cylinders had roughly doubled at this point. It tripled in places before the growth of cylinders in length and width ceased completely. Secondary planar phase continued to develop and occasionally the border of the planar phase and the cylinders became circular again. Finally, the observations were discontinued at a lecithin density of ca. 20 vol.% in the planar phase.

The lecithin density was evaluated on the basis of a few representative measurements of its evolution. The method, previously used at room temperature [13, 14], and now at 55 °C, utilizes the fluorescence of egg lecithin by measuring its intensity emanating from a region of

the planar phase. Since the decrease of the density with time varied considerably among and within samples, we simultaneously watched the structural features and the halo effect of the curved regions and related them to the measured densities. Fluctuations in the curved regions were used for the same purpose, being visible at lecithin densities of 40 vol.% and less. The density in the middle of the curved region was taken to be that of the adjacent planar phase multiplied by the ratio of the principal axes of the (nearly) elliptical cylinder. Quantitative data for the planar phase are listed in table I.

Table I. — *The lecithin density in the planar phase as a function of swelling time for the typical sample thickness of 20  $\mu\text{m}$ . The values at 20 °C, i.e. room temperature, are taken from reference [13]. A and B simply mark swelling times ; C and D denote the inception of the growth of secondary planar phase and the cessation of cylinder growth, respectively ; E marks a concentration.*

	A	B	C	D	E	
vol.% lecithin	60	55	40	28	20	20 °C
swelling time in days	1/2	1	2-3	5-7	10-12	
vol.% lecithin	55	48-45	40-35	30-25	20	55 °C
swelling time in days	1/2	1	1 1/2-2	3-3 1/2	5-6	

### 3. Results.

3.1 PHASE SEPARATION. — Cooling by a few °C or more after swelling at elevated temperatures gave rise to deformations of the multilayer system which were in general restricted to the curved regions, unless the temperature was lowered very much, e.g. by several tens of degrees. They were identical or similar in the semicylindrical border of the planar phase and in the myelin cylinders, in accordance with the fact that cylinders and planar phase always remained connected. All the structural changes indicate a separation, to be called « phase separation », of the multilayer system into domains of higher and lower lecithin density. They followed the temperature without noticeable time lag and were reversible within very wide limits, again without delay. However, complete healing often required a temperature up to 5 °C higher than that of swelling. If a sample was not reheated, the deformations vanished by themselves in a day or two, provided the temperature had not been lowered excessively. The fluctuations seen in the curved regions were usually not much affected by cooling.

The deformation patterns observed after cooling depended on the lecithin density and the temperature change. The effects of cooling on the myelin cylinders in a sample of high density are shown in figures 1 to 3. After swelling for a day at 55 °C down to a density of ca. 45 vol.% in the planar phase and ca. 40 vol.% in the center plane of the cylinders, the temperature was lowered to 45 °C. In all three pictures one sees bright stripes running through the cylinders at oblique angles. They are faint in figure 1 taken at 51 °C, but become wider and brighter with decreasing temperature as displayed for 48 and 45 °C by figures 2 and 3. The stripes are irregularly subdivided in all the figures by strokes which are about as dark as the uniform

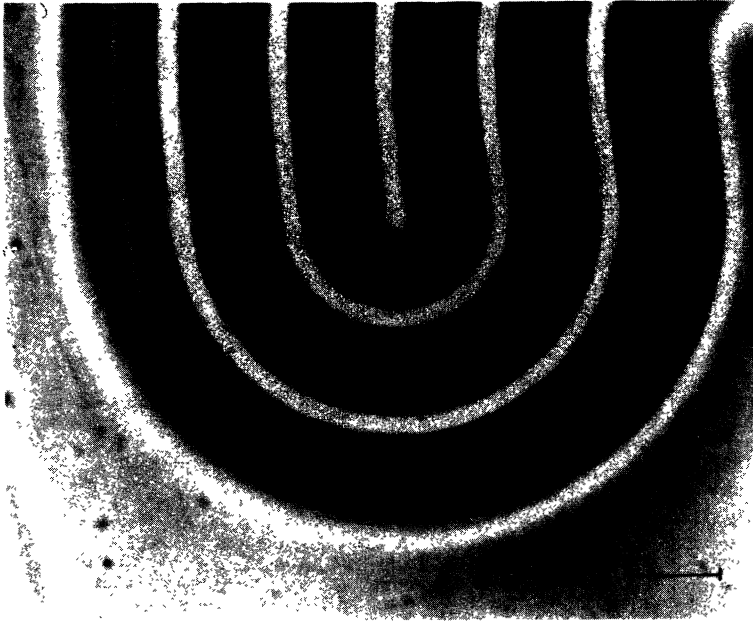


Fig. 1. — Myelin cylinders in a sample being cooled after swelling at 55 °C, viewed in phase contrast microscopy. The three curved cylinders span the sample cell from top to bottom and are seen in a longitudinal section, the thin white center lines being their water cores. Lecithin density in planar phase (not shown) ca. 45 vol.%, sample thickness ca. 20  $\mu\text{m}$ . The bar represents 20  $\mu\text{m}$ . The instantaneous temperature is 51 °C.

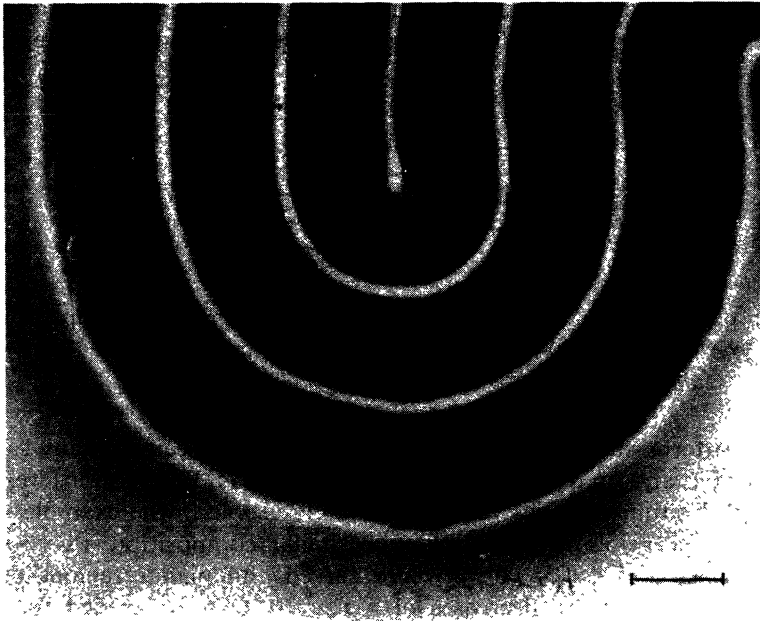


Fig. 2. — Same experiment as in figure 1, but the instantaneous temperature is 48 °C.

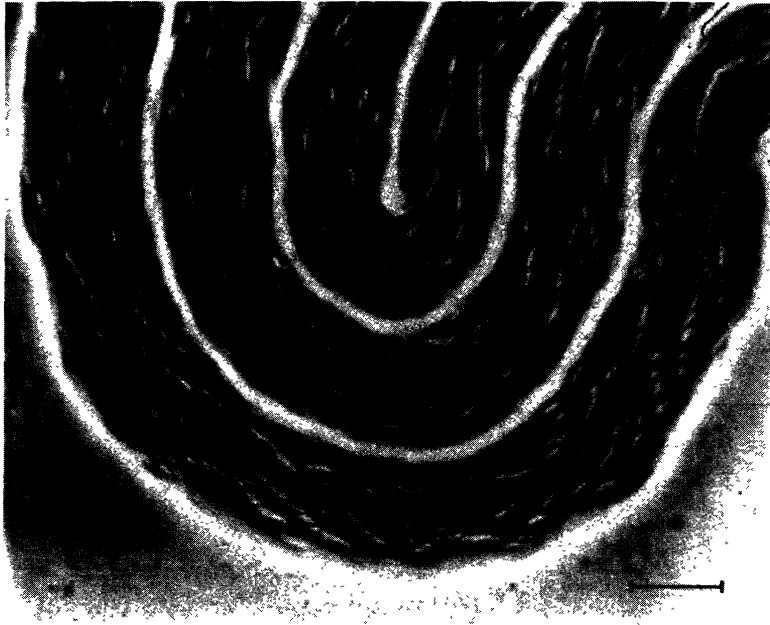


Fig. 3. — Same experiment as in figure 1, but the instantaneous temperature is 45 °C.

surroundings. Since the bright lines in the middle of the cylinders represent water cores [13, 14], the stripes may be regarded as domains of low lecithin density. Changing the focus, we had the impression that they were ribbons of some width in the direction of viewing. Their surroundings look as before cooling, including the fluctuations. A possible arrangement of the bilayers in and outside a stripe is sketched in figure 4. As a cylinder comprises about 1 000 bilayers, the few dark strokes subdividing the bright stripes cannot be single membranes.

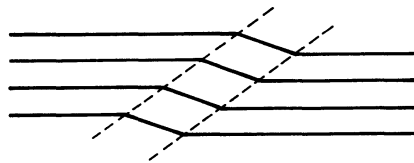


Fig. 4. — Sketch of presumed arrangement of membranes in an oblique stripe. Note the increased membrane spacing within the stripe.

Deformation patterns such as those of figures 1 to 3 were seen in many samples, both in cylinders and border, and used to look equal over large areas. Fluctuations were of a speckled type at these high lecithin densities. They were barely visible in the example shown but well developed or not discernible in others of quite similar composition. Phase separation was found up to a density of ca. 55 vol.% where curved and planar regions differed little in concentration and temperature decreases of a few 10 °C were needed to produce deformations. However, the strong halo effect typical of these very high densities could have prevented the recognition of any pale patterns.

The second series of pictures exhibits a small part of the semicylindrical border of a sample whose lecithin density was ca. 40 vol.% in the planar phase. Swelling had taken place at 65 °C and the cooling rate was 1 °C min<sup>-1</sup>. Figures 5 to 7 show the sample at roughly 55, 40 and 25 °C, respectively. The short white strokes parallel to the border occurring in figure 5 are probably water pockets. They join only rarely to form structures resembling the oblique white stripes seen in the first series. The border widens considerably as the temperature continues to be lowered. Its dark areas in figures 6 and 7 are domains of relatively high lecithin density. They are largely parallel to the direction of the border and multiply connected by short dark lines. The lines represent bundles of membranes of variable thickness. Especially in figure 7 some of them seem to be single bilayers since they have the same minimal strength. Also, they make contact angles of 70° with the dense domains as is typical of single egg lecithin membranes adhering to stacks, i.e. bundles of ten or more bilayers [11]. Being in equilibrium with single membranes, the dense domains of the multilayer system must be subjected to adhesion.

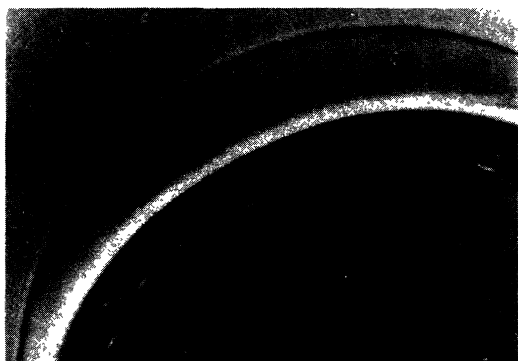


Fig. 5.

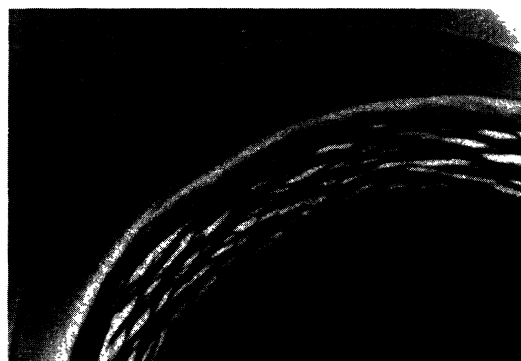


Fig. 6.

Fig. 5. — Semicylindrical border of planar phase. The multilayer system is on lower right, free water (and an accidental single membrane) on upper left. The sample is being cooled after swelling at 65 °C. The sample thickness is 18 μm, the lecithin density in the planar phase ca. 40 vol.%. The bar represents 20 μm. The instantaneous temperature is ca. 55 °C.

Fig. 6. — Same experiment as in figure 5. The instantaneous temperature is ca. 40 °C.

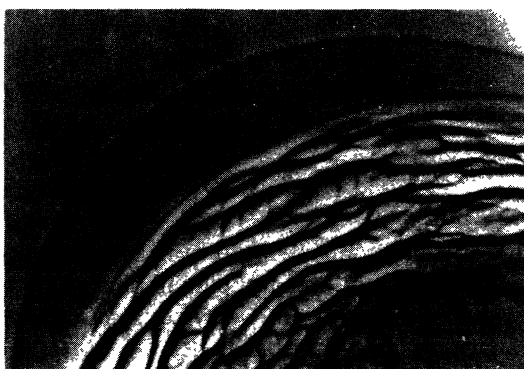


Fig. 7. — Same experiment as in figure 6. The instantaneous temperature is ca. 25 °C.



Many of the lines in figure 7 display a contact rounding where they merge with a dark area. Although barely resolved, the rounding will be used below to evaluate the lateral tension. Figure 7 also exhibits structural changes in the planar phase which started to be visible at 30 °C. Deformations were in general not fully reversible after temperature drops of this size. Fluctuations looking like speckles were well visible before cooling and in the state of figure 5. The single membranes and thin bundles seen at lower temperatures also fluctuated strongly. The fluctuations seemed to be reduced after cooling in the dense domains, but this may have been an optical illusion.

The next two photographs show the border region of a sample swollen at 55 °C. The lecithin density was between 35 and 40 vol.% in the planar phase and 20 % less than that in the center plane of the border before cooling. The initial state is seen in figure 8, while figure 9 displays the same section after lowering the temperature to 45 °C. After cooling, the border has lost its uniform appearance and clearly increased its width. One sees bundles of membranes traversing water-rich domains or water pockets and merging with each other or with broad domains of adhesion. Many of the bundles seem to be bent where they merge, but it is hardly possible to quantify the curvatures. Fluctuations appeared strong before and after cooling. The experiment was not continued to lower temperatures.

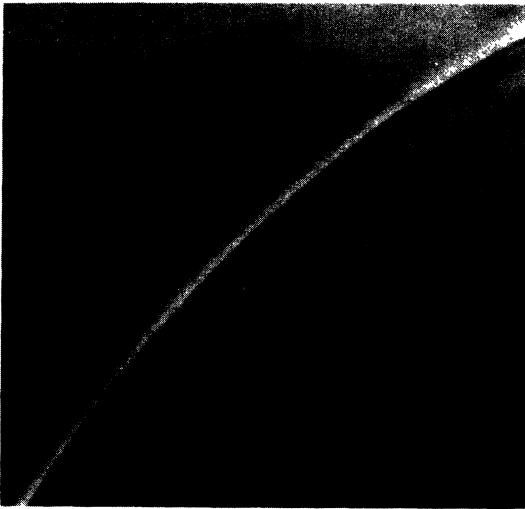


Fig. 8.

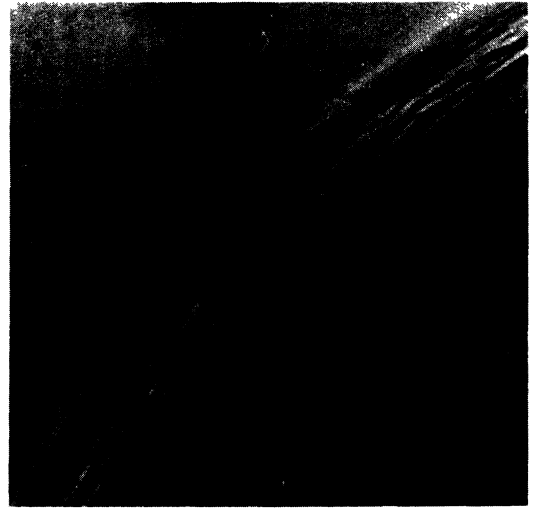


Fig. 9.

Fig. 8. — Semicylindrical border of a sample swollen to a lecithin density of 35 to 40 vol.%. Multilayer system is on lower right, free water on upper left. Sample thickness 20  $\mu\text{m}$ . The bar represents 20  $\mu\text{m}$ . The picture shows the initial state after swelling at 55 °C.

Fig. 9. — Same experiment as in figure 8. The picture shows the sample after cooling to 45 °C.

The sample shown in figure 10 was only 12-14  $\mu\text{m}$  thick. It had swelled for 3 days at 50 °C down to a lecithin density of 20-25 vol.% in the planar phase. Then it was cooled to 40 °C where the picture was taken. Phase separation is very conspicuous in much of the border, but the outermost and innermost parts of the semicylinder were little affected by cooling in this particular case. Many of the black lines running between dense domains look similar. However, as they are blurred we cannot decide if they represent single bilayers. The contours

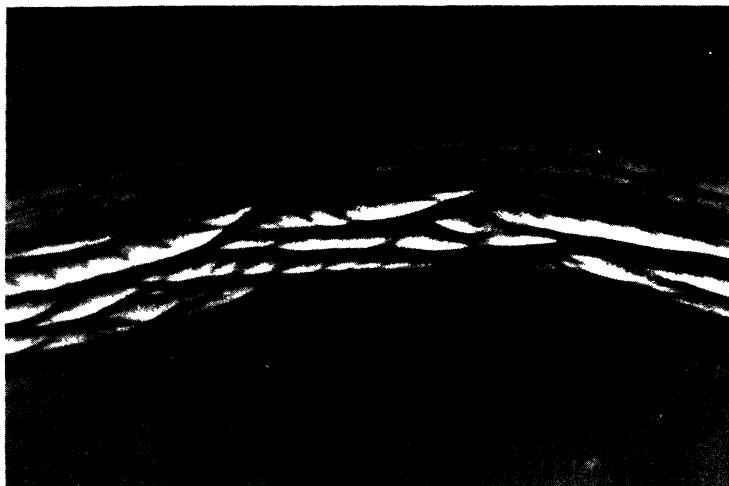


Fig. 10. — Semicylindrical border of planar phase of a sample swollen at 50 °C to a lecithin density of 20 to 25 vol.%. Multilayer system is on the side of the bar representing 20  $\mu\text{m}$ . The sample thickness is 12 to 14  $\mu\text{m}$ . The picture shows the sample after being cooled to 40 °C.

display well visible contact roundings where they merge with domains of adhesion. In addition, they are slightly bulged over their entire length towards the water side of the border. This bias is also noticeable in figure 7; we will return to it in the discussion. The lowest lecithin densities in further experiments were 18 vol.% in the planar phase and, before cooling, 8 vol.% in the curved regions. At these low densities, little cooling produced many single membranes, but the overall appearance of the samples was rather irregular.

**3.2 LATERAL TENSION.** — Membranes in adhesion make rounded contact angles because of their bending rigidity. The gradual turn of the visible contour of the membrane from some other direction into that of the contact area is governed by an angular correlation length which also depends on lateral tension. If optically resolvable, this length can be used to calculate the tension. We repeat here its derivation [9-11] as the method will be applied not only to membranes but also to bundles. Denoting by  $\phi(s)$  the local angle made by the free membrane and its asymptotic direction and by  $s$  the arc length, we may write for the energy per unit area of membrane.

$$g = \frac{1}{2} \kappa \left( \frac{d\phi}{ds} \right)^2 + \sigma_f (1 - \cos \phi) \quad (1)$$

where  $\kappa$  is the bending rigidity and  $\sigma_f$  the lateral tension of the free membrane. (The asymptotic direction is only approximately known because of other deformations of the membrane.) The formula presupposes negligible curvature in the direction of viewing which seems reasonable whenever the angular correlation length is much smaller than the sample thickness. The Euler-Lagrange calculus leads immediately to the one-dimensional membrane shape equation

$$\frac{d^2\phi}{ds^2} = \frac{\sigma_f}{\kappa} \sin \phi \quad (2a)$$

For small angles,  $\sin \phi$  may be replaced by  $\phi$ , which defines the angular correlation length

$$\xi = \left( \frac{\kappa}{\sigma_f} \right)^{1/2} \quad (2b)$$

The last equation gives the lateral tension if the correlation length and the bending rigidity are known. The solution of the differential equation (2) has been plotted elsewhere [11]. It is practically an exponential for  $\phi \leq 30^\circ$ , but turns more sharply into the direction of the contact area at larger angles.

Equations (2) should be equally valid for bundles of membranes adhering to other bundles since both  $\kappa$  and  $\sigma$  have to be multiplied by the number of membranes if more than one is involved. However, the contact angles are smaller with bundles than with single membranes. The bending rigidity of egg lecithin membranes has been measured by several authors [19-23], the values ranging from 0.5 to  $2.3 \times 10^{-12}$  erg, all obtained at room temperature. We use here  $1 \times 10^{-12}$  erg, instead of  $2 \times 10^{-12}$  erg as previously, since we suspect that the smaller and larger values are typical of big vesicles and thin tubes, respectively [23]. The decrease of the rigidity with temperature is probably less than a factor of two in the temperature range of our experiments [24].

Angular correlations lengths almost down to  $1/4 \mu\text{m}$  were read or estimated from the deformation patterns, corresponding to an upper limit of almost  $2 \times 10^{-3}$  dyn  $\text{cm}^{-1}$  for the lateral tensions. Tensions at this virtual limit of optical resolution, which was attained only with bundles, were checked in a simple but approximate way by establishing lower and upper bounds. Regarding one quarter of the width of the thinnest bright stripes seen in figure 1, namely  $1/4 \mu\text{m}$ , as an upper limit to the angular correlation length, one finds from (2)  $\sigma_f > 1.6 \times 10^{-3}$  dyn  $\text{cm}^{-1}$ . The other bound to the tension stems from the observation that fluctuations were not markedly suppressed by cooling. For an estimate, we need the tension  $\sigma_b$  of the membranes where they are bound to one another. The two tensions of a membrane are related by

$$\sigma_b = \sigma_f \cos \psi, \quad (3)$$

where  $\psi$  is the contact angle the free membrane makes with the stack of membranes to which it adheres. It is assumed here that the deformation of the stack by the adhering membrane is negligible. For the typical contact angle  $\psi = 70^\circ$ , one has  $\sigma_b = (1/3) \sigma_f$ . The elastic energy of an undulation mode contains  $\sigma$  and  $\kappa$  in the combination  $\sigma_b q^2 + \kappa q^4$  where  $q$  is the component of the wave vector parallel to the membrane [9]. (We disregard the perpendicular component giving rise to compression and dilation of the stack.) One may expect the first term not to exceed the second for the shortest resolvable optical wavelength  $\lambda$  as long as there is no significant suppression. This leads to  $\sigma_b < \kappa (2\pi/\lambda)^2$  and, after the plausible substitution  $\lambda = 2 \mu\text{m}$ , to  $\sigma_b < 1 \times 10^{-3}$  dyn  $\text{cm}^{-1}$  and  $\sigma_f < 3 \times 10^{-3}$  dyn  $\text{cm}^{-1}$ . Evidently, the bounds are not far apart and conform with the estimate for  $\sigma$  obtained from contact rounding practically within the large experimental inaccuracy.

Looking at samples exhibiting well-resolved contact roundings, we found the angular correlation length not to be entirely uniform over the sample. This may have been due to local variations in lateral tension and to optical perturbations, e.g. the halo effect characteristic of phase contrast microscopy. These difficulties were partially compensated by the large number of contact roundings available for analysis in each sample.

In a few cases we evaluated the angular correlation length at different temperatures. It appeared to increase slightly on the way to room temperature, but the change was not significant in view of the general uncertainties. This has the remarkable implication that the lateral tensions, once measurable, did not change much when the temperature continued to be lowered. The width of the curved regions often increased dramatically with temperature as shown by figures 5 to 7. However, the sum of the widths of the dense domains as opposed to the total width of the border (or half the cylinder) seemed to change little. It is particularly noteworthy that this sum appeared to be half the sample thickness, where the widths were

large enough to be assessable, indicating a lecithin density nearly equal to that of the planar phase. A plot of the lateral tension  $\sigma_f$  versus the mean membrane spacing  $\bar{z}$  in the planar phase is shown in figure 11, each point representing a different sample. We assumed a constant membrane thickness of 3.6 nm [1, 2] when converting volume densities into spacings. The data agree fairly well with an inverse square law,  $\sigma_f \sim (1/\bar{z})^2$ , especially if  $\bar{z}$  is replaced by  $\bar{z} - 2$  nm to account at least crudely for the effect of rather impenetrable hydration layers [4]. The quadratic dependence will be seen to agree with a theoretical model. However, it remains to be verified in further experiments because of the large experimental errors.

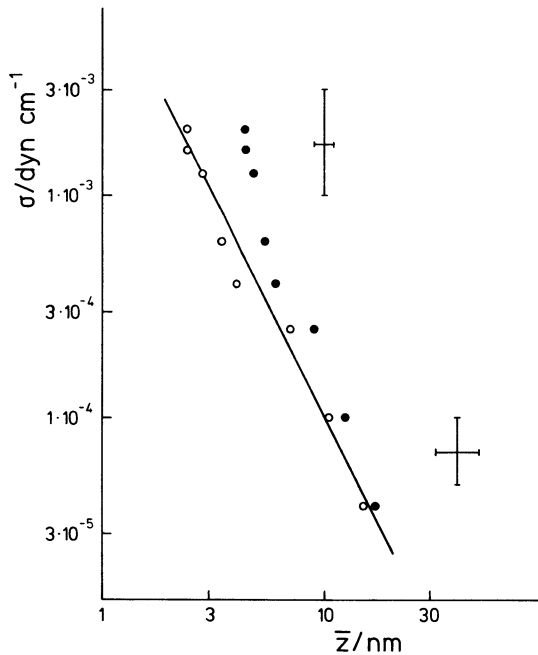


Fig. 11. — Doubly logarithmic plot of the lateral tension  $\sigma_f$  vs. the mean membrane spacing in the planar phase. The solid dots are the raw data, increasing the membrane thickness by 2 nm results in the open dots.

#### 4. Discussion.

**4.1 INTRODUCTORY REMARKS ON INDUCED ADHESION.** — The experiments have shown that cooling causes deformations in the curved regions of the multilayer system. The structural changes are characterized by the formation of domains of increased water content and by the generation of lateral tensions. Assuming a constant bilayer thickness of 3.6 nm, we compute from the measured lecithin densities mean spacings that range from 3 to 17 nm in the planar phase and to 41 nm in the curved regions before cooling. Phase separation in a multilayer system does not necessarily imply induced adhesion. A coexistence of two lamellar phases of different finite spacings but equal intermembrane net repulsion is in principle also possible [2]. However, we think that in the present study phase separation has always been due to adhesion. The coexistence of single membranes with domains of high lecithin density may be regarded as a direct proof of adhesion. Ideally, the system should split into a stack of membranes in adhesion and free water. The need to conserve the water volumes enclosed by

adjacent bilayers leads to the formation of water compartments. Single membranes (or very thin bundles) were detected for mean membrane spacings down to  $\bar{z} \approx 4.5$  nm in the planar phase. A coexistence of two lamellar phases both with spacings between 5 and 3 nm cannot be excluded on the basis of our microscopic observations. However, it is very unlikely as there is no plausible mechanism to explain it.

Lateral tensions induce adhesion by reducing fluctuations and thus steric repulsion, as will be discussed below in terms of undulations. This can result in a total interaction of the membranes which is still repulsive at small spacings but turns attractive at some distance. A schematic plot of the total interaction energy per unit area,  $g_{\text{total}}(\sigma_b, \bar{z})$ , as a function of mean spacing at fixed  $\sigma_b$  is given for such a case in figure 12. The energy minimum marks the equilibrium value of the mean spacing,  $\bar{z}_{\text{eq}}$ , which according to the experiments and theoretical predictions is a decreasing function of lateral tension. (Any stable deviations of  $\bar{z}$  from  $\bar{z}_{\text{eq}}$  would, of course, require pressure differences for a complete balance of forces.) For the reasons given above, the function  $\bar{z}(\sigma_f)$  plotted in figure 11 should be practically identical to the function  $\bar{z}_{\text{eq}}(\sigma_f)$  which by use of (3) can be converted into  $\bar{z}_{\text{eq}}(\sigma_b)$ .

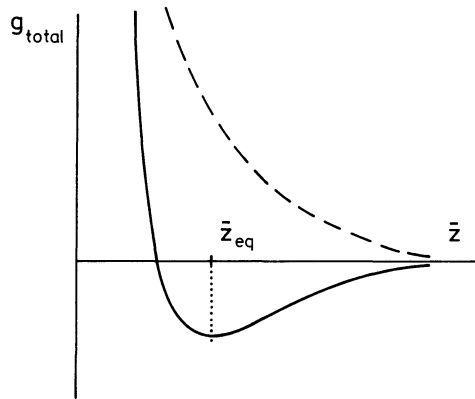


Fig. 12. — Schematic plot of the total interaction per unit area vs. the mean spacing of two membranes (or a stack). The full line represents the interaction at some fixed lateral tension while the dashed line depicts the interaction in the absence of tension.

The minimum equilibrium spacing of induced adhesion as observed in our experiments is 3 nm. We may infer that the membrane separation in egg lecithin multilayer systems would collapse from infinity, i.e. from the dissociated state to 3 nm or less if the membranes were flat rather than fluctuating. The obvious conclusion that electrostatic repulsion has been insignificant in our samples can be drawn with confidence only when we discuss forces and their dependences on membrane spacing. Beforehand, we propose a model to explain how the lateral tension comes about and why it levels off when cooling continuous after the appearance of induced adhesion.

**4.2 RESTRUCTURING OF MULTILAYER SYSTEMS AND SELF-LIMITATION OF LATERAL TENSIONS.** — Lecithin membranes shrink in area when the temperature is lowered. This would lead to very large lateral tensions if the system retained its structure. An estimate of those theoretical tensions may be based on the thermal area expansivity  $\alpha = 2.4 \times 10^{-3}/^{\circ}\text{C}$  and the stretching elastic modulus  $\kappa_s = 140 \text{ dyn cm}^{-1}$ , both measured by Kwok and Evans [25], for egg lecithin bilayers. Neglecting a thermal change of membrane thickness and the bulk

expansivities of water ( $1.3 \times 10^{-4}/^{\circ}\text{C}$ ) and egg lecithin (probably also much less than  $\alpha$  [26]), one has for the tension produced by cooling the simple expression

$$\sigma = -\kappa_s \alpha \Delta T, \quad \text{where } \Delta T < 0.$$

Inserting the above numbers and  $\Delta T = -10^{\circ}\text{C}$  for the change in temperature yields  $3.4 \text{ dyn cm}^{-1}$  which is several orders of magnitude above the actual tensions and at the limit of membrane rupture [25]. Evidently, the multilayer system is capable of restructuring itself, thus avoiding the build-up of high lateral tensions.

To develop a model of restructuring, we begin with the unrealistic example of a long myelin cylinder that is disconnected from the planar phase. When the membranes shrink in area each of the unilamellar cylinders can conserve its enclosed volume by becoming shorter and wider simultaneously. For an estimate of the geometric changes one may choose a convenient cross section of the cylinders, e.g. two semicircles of fixed radius  $r$  connected by two straight lines of variable length  $w$ . Omitting the effect of cylinder ends and starting from circles, i.e.  $w = 0$ , one has the first-order equations

$$\frac{\Delta A}{A} = \frac{\Delta L}{L} + \frac{w}{\pi r}$$

and

$$\frac{\Delta V}{V} = \frac{\Delta L}{L} + \frac{2w}{\pi r}$$

linking the relative changes of area  $A$ , length  $L$ , and volume  $V$ . With  $\Delta V = 0$  one obtains

$$\frac{\Delta L}{L} = 2 \frac{\Delta A}{A}$$

and

$$\frac{w}{r} = -\pi \frac{\Delta A}{A}$$

where the radius  $r$  and the width  $w$  are different for each membrane. Because of  $\Delta A/A = \alpha \Delta T$  one finds with  $\Delta T = -10^{\circ}\text{C}$  and the quoted  $\alpha$  the ratios  $\Delta A/A = -0.024$ ,  $\Delta L/L = -0.05$ , and  $w/r = 0.08$ . As a rule, the widening of the curved regions and the shortening of the cylinders have been a few times larger than the estimates suggest. The amplification should be due to the connectedness of the cylinders to the planar phase, as is shown next.

Shrinkage of the membranes in the planar phase releases water which also has to be accommodated by the myelin cylinders and in the semicylindrical border. The capacity of the curved regions for additional water is small between the innermost but large between the outermost membranes, being proportional to the individual radius  $r$  for the simple geometry introduced above. The planar phase can adjust to the nonuniform capacity by transverse dilation and compression of the multilayer system. In the center plane the membrane spacing has to increase so that no water is squeezed out despite membrane contraction. The dilation has to decrease linearly on the way to top and bottom of the sample cell, changing halfway into compression as the sample thickness is fixed. Accordingly, the increment  $\Delta \bar{z}$  of the mean spacing may be expressed by

$$\frac{\Delta \bar{z}}{z} = -\frac{\Delta A}{A} (1 - 4r/D) \quad (4)$$

where  $D$  is the sample thickness and  $0 < r < D/2$ . The increase of the membrane thickness, at fixed bulk density, is omitted in this equation. It results in a factor 2 on the right-hand side when membranes and water layers are equally thick, but becomes less important with more water.

If the mean membrane spacing in the multilayer system is nonuniform and the intermembrane forces decrease with spacing, the membranes will experience forces. In mechanical equilibrium the net forces per unit area must be balanced by pressure differences between successive water layers. Recalling that especially after cooling the membrane spacing is definitely larger in the curved regions than in the planar phase and dismissing, for the moment, the possibility of adhesion, we expect membrane interaction to be absent or reduced in these regions. As a consequence, all or part of the pressure difference  $\Delta p = p_{in} - p_{out}$  between the water layers on the inner and outer sides of a membrane must be balanced by other means in the curved regions. The additional force can only be brought about by a lateral tension whose largest possible value is

$$\sigma = r \Delta p \quad (5)$$

for cylindrical curvature  $1/r$ . Existing also in the planar phase, the tension may reduce the intermembrane forces. We will return immediately to this important point.

The presence of adhesion requires minor modifications which, in a sense, simplify the model. Large spacings are now restricted to the small areas where a single membrane runs between water compartments. Intermembrane forces certainly vanish for these pieces, permitting  $\sigma$  or, more precisely,  $\sigma_f$  to assume its maximum value (5). However, the free membrane is likely to form the characteristic contact angle  $\psi$  ( $= 70^\circ$  for egg lecithin) where it hits the stack of outer membranes, which is assumed to be like the planar phase. It follows that the radius of cylindrical curvature should be  $r/\cos \psi$  instead of  $1/r$ . In compensation  $\sigma_f$  has to satisfy

$$\sigma_f = \frac{r \Delta p}{\cos \psi} \quad (6)$$

instead of (5). Because of (3), however, the tension  $\sigma_b$  of the membrane where it is bound should be independent of  $\cos \psi$  and, like  $\sigma$  in (5), obey

$$\sigma_b = r \Delta p. \quad (7)$$

Since the contour length over which a membrane stays single is so small, usually less than the sample thickness, the curvature of the free membrane need not be cylindrical. This might explain the outward bulging of single membranes mentioned above. Spherical instead of cylindrical curvature would lower the tension (7) by half. We disregard the effect of bulging as these curvatures are difficult to determine and do not seem to exceed  $\cos \psi/r$ . We also refrain from discussing the adjustment  $\Delta \bar{z}$  of the mean spacing in the curved regions of adhesion which will differ from that in the planar phase because of a different balance of forces.

Our model of restructuring predicts, to first order, the adjustments of the mean spacings in the planar phase and thus the lateral tensions to be proportional to the decrease of temperature. However, the initially linear rise will level off when the tensions become large enough to reduce the steric repulsion between membranes. The tensions cannot surpass the value at which the intermembrane force vanishes for the mean spacing in the planar phase. To be more precise, they can reach this point only asymptotically because it is the repulsive net force that causes the tensions. The self-limitation of the lateral tension agrees with the observed saturation and explains most naturally why the planar phase displays no adhesion even though its mean spacing is indistinguishable from the equilibrium spacing of adhesion in

the curved regions. The apparent absence of a dependence of  $\sigma$  on  $r$  i.e. on the position of the membrane in the multilayer system, seems to be another consequence of self-limitation.

**4.3 ESTIMATES AND SCALING LAWS.** — For the further discussion, membrane interactions need to be considered in some detail. The energy of direct interaction per unit area,  $g_{\text{dir}}$ , between a pair of flat membranes of uniform spacing may be divided into three parts

$$g_{\text{dir}}(z) = g_{\text{hyd}}(z) + g_{\text{vdw}}(z) + g_{\text{es}}(z). \quad (8)$$

The first contribution,  $g_{\text{hyd}}(z)$ , is the energy of the hydration force. It is repulsive and drops exponentially with  $z$ , the characteristic length being ca. 0.2 nm [1, 2]. It will be neglected in our estimates or simply replaced by a hard core hydration layer of 1 nm on each interface so that the minimum membrane spacing is 2 nm. (Marra and Israelachvili [4] found the minimum of  $g_{\text{dir}}$  to be near 2.4 nm for synthetic lecithins in the fluid state.) The second term of (8),  $g_{\text{vdw}}$ , signifies van der Waals interaction which is always attractive between equal membranes while the other direct interactions are repulsive. It obeys

$$g_{\text{vdw}} = - \frac{H}{12 \pi z^2} \quad (9)$$

in the half-space approximation for small  $z$ ,  $H$  being the Hamaker constant. The range of spacing where (9) is a good approximation may be a few times the membrane thickness of ca. 3.6 nm because of the effect of the very large dielectric constant of water on the zero-frequency term [27]. To take account of the finite membrane thickness  $b$ , equation (9) is often replaced by the ansatz

$$g_{\text{vdw}} = - \frac{H}{12 \pi} \left[ \frac{1}{z^2} - \frac{2}{(z+b)^2} + \frac{1}{(z+2b)^2} \right] \quad (10)$$

resulting from the integration of molecular interactions. This energy goes with  $1/z^4$  for large  $z$ , as it should, but does not reflect the enhanced range of (9). Marra and Israelachvili [4] who measured for lecithins a particularly small value of the Hamaker constant ( $H = 1.3 \times 10^{-14}$  erg instead of the more typical  $7 \times 10^{-14}$  erg) also located the plane of origin of the van der Waals interaction (9) ca. 0.5 nm in front of the theoretical position calculated for flat interfaces, which is equivalent to an increase of the membrane thickness by 1 nm. To improve agreement of theoretical and actual van der Waals interaction Attard *et al.* [28] recently treated the region of the polar heads as a third layer besides lipid and water. The last term of (8),  $g_{\text{es}}$ , represents electrostatic repulsion between charged bilayers. For fixed surface charge density it varies as  $\ln(z/z_0)$  at small enough spacings (ideal gas approximation;  $z_0$  is a reference spacing) and as  $1/z$  for larger spacings [12, 29]. Electrostatic repulsion generally drops with smaller powers of  $1/z$  than van der Waals attraction and undulatory repulsion (see below), but eventually it is cut off by Debye-Hückel screening. As the polar head of the lecithin molecule is zwitterionic in pure water, any appreciable surface charge would have to be due to ionic impurities in the membrane or the water.

We discard the possibility of electrostatic interaction in our samples for the following reasons. No signs of an electrostatic effect were noticed in the study of symmetric adhesion [11] which covered a wider range of tensions than do our data. In particular, the contact angles did not depend on the origin of the egg lecithin and were the same when salt solution was used instead of pure water, as could be checked up to 0.1 M NaCl. Moreover, the age of the sample made little or no difference during the one or two weeks needed for an experiment. The contact angle made by a single membrane with a stack of membranes was



typically near  $70^\circ$ . Just the same angle is characteristic of the present experiments. Its small scatter, now as then rarely more than  $\pm 10^\circ$ , is remarkable as the adhesion energy should be very sensitive to electric charges on the membranes in view of the large Debye length of twice distilled water and the slow decrease of  $g_{es}$  at spacings below this length. Without reference to any other work, it may be argued that electrostatic forces too weak to prevent induced adhesion must be negligible at the smallest spacings around 5 nm, unless the Debye length is very much smaller in the sample cells than the 0.1 to 1  $\mu\text{m}$  of twice-distilled water.

The only steric interaction of fluid membranes that is known to date arises from the mutual hindrance of their undulations. For a stack of membranes regarded as a smectic liquid crystal, the energy of undulatory interaction per unit area of membrane was calculated to be [5]

$$g_{\text{und}} = \frac{3 \pi^2 (kT)^2}{128 \kappa \bar{z}^2}. \quad (11)$$

Here  $k$  is Boltzmann's constant,  $T$  the temperature,  $\kappa$  the bending rigidity of the membrane, and  $\bar{z}$  the mean spacing between membranes. Equation (11) is based on the hypothesis that the direct interaction is that of a hard-core potential at the membrane interfaces. The numerical factor in equation (11) has been confirmed [17, 18] within experimental accuracy (ca. 20 %) by X-ray studies of multilayer systems of very flexible membranes ( $\kappa \approx kT$ ).

The direct interaction of membranes can in general not be approximated by a hard-core potential. So the question arises if the total interaction may be expressed, to a reasonable approximation, by putting  $z = \bar{z}$  and adding direct and undulatory interactions. Whether or not superposition is acceptable as an approximation depends mostly on the power  $(1/z)^n$  to which an attractive direct interaction is proportional in the range of spacings swept by the undulations. Lipowsky and Leibler [6] have shown that superposition must not be used to calculate equilibrium spacings whenever  $n > 2$ , i.e. when direct attraction falls off more rapidly than undulatory repulsion. Assuming a direct interaction consisting of hydration forces and the van der Waals potential (10) with its  $1/z^4$  tail, and employing a renormalization group method, they predicted for a pair of membranes a continuous unbinding transition if the Hamaker constant is lowered to a critical value. The same transition would be discontinuous, the spacing jumping from a finite value to infinity, if superposition were applicable.

In order to decide whether to expect spontaneous adhesion or separation and afterwards to deal with the effect of lateral tension, we consider in the following the simplified case of « ideal competition » between direct and undulatory interactions, expressing the former solely by the half-space approximation (9) of van der Waals attraction. Since this attraction varies as  $1/z^2$ , we may hope its superposition with undulatory interaction to be (marginally) correct. Adopting superposition, we write for the total interaction in the absence of tension

$$g_{\text{total}} \approx \frac{H_c - H}{12 \pi \bar{z}^2}. \quad (12)$$

Here  $H_c$  is a critical Hamaker constant denoting in the case of ideal competition the limit between total separation ( $H < H_c$ ) and total collapse ( $H > H_c$ ) of the membranes. Apart from a numerical factor not too far from unity we may expect for the stack, because of (9) and (11),

$$\frac{H_c}{12 \pi} \approx \frac{3 \pi^2 (kT)^2}{128 \kappa}. \quad (13)$$

It may be argued that  $H_c$  should be somewhat smaller than given by this equation because  $z$  fluctuates down to zero spacing and, in addition, van der Waals attraction distorts the

distribution function of the spacing. Inserting the measured rigidities into (13), calculating the values of  $H_c$ , and comparing them to the measured Hamaker constants, one ends up on the borderline, unable to predict whether unstressed egg lecithin membranes should adhere to or separate from each other [11]. Likewise, the criterion obtained by Lipowsky and Leibler [6] in their renormalization group calculation permits no decision. This is even more so if the theoretical uncertainties of the two methods are taken into consideration.

The effect of lateral tension on undulation forces is treated as elsewhere [9, 10] by means of an independent-membrane-piece approximation. The approach starts from the mean-square undulation amplitude of, say, a hexagonal piece of single membrane of area  $S$ ,

$$\langle u^2 \rangle = \frac{kT}{4 \pi \sigma} \ln \left( 1 + \frac{\sigma S}{\kappa \pi^2} \right), \tag{14}$$

which is independent of  $\sigma$  for  $\sigma \ll \kappa \pi^2/S$ . The plaquette size  $S$  is chosen such that the undulations just fill the interval between adjacent membranes. For a stack of mean spacing  $\bar{z}$  we may put

$$\langle u^2 \rangle = \frac{\bar{z}^2}{6} \tag{15}$$

as was done for the unstressed membrane between rigid plates [5]. Eliminating  $\langle u^2 \rangle$  between (14) and (15) yields

$$S(\sigma) = \frac{\pi^2 \kappa}{\sigma} \left[ \exp \left( \frac{2 \pi \sigma \bar{z}^2}{3 kT} \right) - 1 \right]. \tag{16}$$

The undulation pressure could now be calculated very directly, but with a poor numerical factor, by treating each piece of size  $S$  as an independent particle of a one-dimensional ideal gas in an interval of width  $2 \bar{z}$ .

We prefer to multiply the undulation forces deriving from (11) by  $S(0)/S(\sigma)$  to obtain those forces in the presence of tension. The effect of  $\sigma$  on the ratio becomes important when the exponent in (16) approaches unity. Any further increase of the exponent by raising  $\bar{z}^2$  or  $\sigma$  leads to a steep drop of  $S(0)/S(\sigma)$  and a corresponding decrease of undulatory interaction. If the exponent is exactly one, i.e. for

$$\sigma = \frac{3 kT}{2 \pi \bar{z}^2}, \tag{17}$$

one has  $S(0)/S(\sigma) \approx 0.58$ . Note that this relationship and the ratio  $S(0)/S(\sigma)$  at small  $\sigma$  in general do not contain the bending rigidity. Incidentally, a relationship like (17) may also be obtained by equating  $g_{\text{und}}$  of (11) and the total stretching energy per unit area of a freely undulating membrane [9, 11],

$$g_f = \frac{kT}{8 \pi \kappa} \sigma_f + \frac{1}{2 \kappa_s} \sigma_f^2. \tag{18}$$

The regular quadratic term containing the stretching elastic modulus  $\kappa_s = 140 \text{ dyn cm}^{-1}$  [25] is negligible at the tensions in question. The numerical factor comes out 12 times larger than  $3/2 \pi$ .

We are interested in adhesion induced by lateral tension which takes place for  $0 < H < H_c$ . The associated equilibrium spacing may be derived from a balance of forces. Following the indicated procedure and replacing  $\sigma$  by  $\sigma_b$ , we write the undulation force per unit area as a function of  $\sigma_b$  in the form

$$f_{\text{und}} = - \frac{\partial g_{\text{und}}}{\partial \bar{z}} \frac{S(0)}{S(\sigma_b)} > 0 \tag{19}$$

where because of (16)

$$\frac{S(0)}{S(\sigma_b)} = 1 - \frac{\pi \sigma_b \bar{z}^2}{3 kT} \quad (20)$$

to lowest order in  $\sigma_b$ . The van der Waals force per unit area is expressed by

$$f_{\text{vdw}} = - \frac{\partial}{\partial \bar{z}} \frac{H}{12 \pi \bar{z}^2} < 0. \quad (21)$$

Rewriting  $g_{\text{und}}$  in terms of  $H_c$  by means of (13) and using the equilibrium condition  $f_{\text{und}} + f_{\text{vdw}} = 0$ , one arrives at

$$\bar{z}_{\text{eq}}^2 = \frac{H_c - H}{H_c} \frac{3 kT}{\pi \sigma_b}. \quad (22)$$

Formally, this equation should be valid whenever  $(H_c - H)/H_c \leq 1/2$ , i.e. for fairly close competition of van der Waals attraction and undulatory repulsion. It has to be kept in mind, however, that the superposition of forces need not be permissible in the marginal case at hand.

Equation (22) has the attractive feature of reproducing the scaling law  $\sigma \sim 1/\bar{z}_{\text{eq}}^2$  suggested by the experimental data compiled in figure 11, thus lending itself to a calculation of  $(H_c - H)/H_c$ . Inserting  $\bar{z} = 10$  nm,  $\sigma = 1 \times 10^{-4}$  dyn cm<sup>-1</sup>, and  $kT = 4 \times 10^{-14}$  erg results in  $(H_c - H)/H_c = 2.6 \times 10^{-3}$ . An even smaller value, ca.  $1 \times 10^{-3}$ , is obtained if the measured tension is replaced by  $\sigma_b = (1/3)\sigma$  to take account of the contact angle  $\psi = 70^\circ$  according to (3). Clearly, so close a competition between van der Waals attraction and undulatory repulsion, i.e. a predominance of the latter by only 0.1 %, appears unlikely if only for practical reasons. Very slight changes, e.g. either a decrease or an increase of temperature, should then give rise to a binding transition. However, spontaneous adhesion has never been observed in our experiments with egg lecithin.

The simple theory underlying the model of restructuring permits another, independent evaluation of the strength of repulsive interaction in the unstressed planar phase. It consists in calculating, for the threshold of induced adhesion, the tensions predicted by the model in the hypothetical case of purely undulatory interaction and comparing them to the measured tensions. The theoretical net force per unit area acting on a membrane of thickness  $b$  may be expressed by

$$\Delta f_{\text{und}} = - \frac{\partial^2 g_{\text{und}}}{\partial \bar{z}^2} \frac{\partial \Delta \bar{z}}{\partial r} (\bar{z} + b),$$

i.e. by the difference of the undulation forces exerted by the two next neighbours. It is directed inward ( $> 0$ ) if the increment  $\Delta \bar{z}$  of mean membrane spacing decreases with  $r$ , i.e. on the way from the middle of the cell to the glass slides. Taking  $\Delta \bar{z}$  from (4) and  $g_{\text{und}}$  from (11), we find

$$\Delta f_{\text{und}} = \frac{9 \pi^2 (kT)^2 \Delta A}{16 \kappa \bar{z}^3} \frac{\bar{z} + b}{A D}$$

which is independent of  $r$ . Use of  $\bar{z} = 10$  nm,  $kT = 4 \times 10^{-14}$  erg,  $\kappa = 1 \times 10^{-12}$  erg,  $b = 3.6$  nm,  $D = 20$   $\mu$ m, and  $\Delta A/A = -2.4 \times 10^{-2}$  as expected for  $\Delta T = -10^\circ$  C, results in  $\Delta f_{\text{und}} = -0.14$  dyn cm<sup>-2</sup>. According to (5), the pressure difference compensating this inward force density produces the tension  $\sigma = 1.4 \times 10^{-4}$  dyn cm<sup>-1</sup> for  $r = D/2$  and progressively

smaller values towards the center of the sample, the average being  $0.7 \times 10^{-4} \text{ dyn cm}^{-1}$ . If correction is made according to (6) for the contact angle  $\psi \approx 70^\circ$ , the tensions  $\sigma_f$  of the membranes where they are single come out three times larger than these numbers, the average being  $2 \times 10^{-4} \text{ dyn cm}^{-1}$ . How to extract from the experimental data an approximate value for the tension in the absence of self-limitation? The measured tension also at  $\bar{z}_{\text{eq}} = 10 \text{ nm}$  is near  $1 \times 10^{-4} \text{ dyn cm}^{-1}$  (see Fig. 11). Obtained at  $-\Delta T \geq 20^\circ \text{C}$ , it is probably close to the saturation value. The tension at  $\Delta T = -10^\circ \text{C}$  is unknown (and unmeasurable), but it cannot be much less than  $1 \times 10^{-4} \text{ dyn cm}^{-1}$  since there is still induced adhesion. On the other hand, it cannot be more than a few times below what it would be in the absence of self-limitation because in order to obtain phase separation the temperature has to be lowered by at least a few  $^\circ \text{C}$ . We may infer that the repulsive forces in the tension-free planar phase differ from the undulation forces derived from (11) by no more than a factor of three. Since  $\Delta f_{\text{und}}$  and the tension calculated from it are essentially proportional to  $1/\bar{z}^2$  like the measured tension, it is enough to do this comparison of theory and experiment for a single spacing.

An intermembrane repulsion about equal to the theoretical strength of undulation forces agrees with our earlier, completely different determination of the repulsive forces at very large membrane spacings [12]. There is also a purely theoretical argument suggesting a lower limit of ca.  $(1/4) g_{\text{und}}$  to the total repulsive interaction before the membranes collapse with a further increase of the Hamaker constant [7]. It is based on the fact, noted by Lipowsky [30], that the renormalization group flow equations of two-dimensional membranes with a bending rigidity are identical to those of one-dimensional interfaces with a line tension. Since the distribution function of the linear interface can be obtained by solving a Schrödinger-type equation and squaring the wave function, the same procedure may be expected to give adequate results in the case of membranes. Long-range  $1/z^2$  attraction has been considered for one-dimensional interfaces in the context of wetting transitions [31]. These reservations concerning the validity of (22) do not apply to the proportionality  $\sigma \sim 1/\bar{z}_{\text{eq}}^2$  at fixed  $(H_c - H)/H_c$  which also follows from general arguments [11] similar to those leading to (17). However, the theoretical predictions as well as the measurements of the repulsive forces are clearly in conflict with the foregoing conclusion that all but 0.1 % of undulatory repulsion is compensated by van der Waals attraction. In other words, they do not allow for an explanation of the surprisingly small equilibrium spacing at a given tension.

A similar and probably related discrepancy by more than a factor of 100 was noticed in the study of symmetric induced adhesion of a pair of membranes [11]. Being linked to the largeness of the contact angles, it also exists in the present data concerning the adhesion of a single membrane to a stack of others. To show this, we write down two formulas for the adhesion energy per unit area,  $g_a$ . One of them is the Young equation for interfaces

$$g_a = (1 - \cos \psi) \sigma_f \quad (23)$$

relating the adhesion energy to the tension of the single membrane where it is free and to the contact angle. The other equation,

$$g_a = g_f(\sigma_f) - g_b(\sigma_b) \quad (24)$$

links the adhesion energy to the energies per unit area of stretching the free membrane,  $g_f(\sigma_f)$ , and the membranes in the stack,  $g_b(\sigma_b)$ . In the latter case, the membranes are thought to be always at their equilibrium spacing as it goes from infinity to some finite value (we omit here boundary effects for the outermost membranes). Evidently,  $g_b(\sigma_b)$  must not be negative in the absence of spontaneous adhesion, which entails the inequality

$$g_a < g_f(\sigma_f) \quad (25)$$

Putting  $\psi = 70^\circ$  in (24) leads to  $g_a \approx (2/3) \sigma_f$ . On the other hand, substituting the linear term of (18) for  $g_f(\sigma_f)$  in (25) and inserting  $kT = 4 \times 10^{-14}$  erg as well as  $\kappa = 1 \times 10^{-12}$  erg yields  $g_a < 1.6 \times 10^{-3} \sigma$ . The conflict between the two results for  $g_a$  is far beyond any experimental error. Like all other equations invoked as yet, (23) and (24) are founded on the tacit assumption that the area absorbed by out-of-plane fluctuations is no more than a few per cent of the projected area, in accordance with the predictions of undulation theory [9, 11].

**4.4 CONJECTURE OF A NEW MEMBRANE ROUGHNESS.** — There seems to be no obvious way of resolving the two glaring contradictions inherent in the interpretation of our data. They are the more disturbing as the same data are consistent with two scaling laws that seem to follow from undulation theory,  $\bar{z}_{\text{eq}}^2 \sim 1/\sigma$  and  $g_a \sim \sigma$ . The latter is revealed by the constancy of the contact angle and was studied systematically for symmetric adhesion [11]. It is a consequence of the former and of  $g_f \sim \sigma_f$ . Abandoning the simplified model of ideal competition which leads to the scaling laws does not remove the contradictions. They are contained in the set of data belonging to a single mean spacing or lateral tension. As a consequence, we are forced even more than before [11] to postulate that on a submicroscopic scale the membranes are much rougher than predicted by undulation theory. The bilayer area absorbed by an additional roughness could explain the high sensitivity of the membranes to lateral tension as reflected by the small equilibrium spacings and the large contact angles and energies of induced adhesion.

An idea of the amount of extra surface may be obtained, for lack of a better theory, from some formulas of undulation theory [9, 11] by employing a fictitious bending rigidity that is much lower than the measured values. The ratio of absorbed area  $A_{\text{abs}}$  to basal or visible area  $A_{\text{base}}$  may then be expressed for the free membrane by

$$\frac{A_{\text{abs}}}{A_{\text{base}}} = \frac{kT}{8 \pi \kappa'} \ln \left( \frac{\kappa' q_{\text{max}}^2}{\sigma_f} \right) \quad (26)$$

where, apart from  $\sigma_f$ , the « bending rigidity »  $\kappa'$  and an upper cut-off wave vector  $q_{\text{max}}$  of the roughness govern the absorption. Inserting (26) into

$$dg_f = - \sigma_f \frac{dA_{\text{abs}}}{A_{\text{base}}},$$

valid whenever regular stretching can be neglected, one obtains

$$g_f = \frac{kT}{8 \pi \kappa'} \sigma_f \quad (27)$$

in analogy to (18). Because of the inequality (25) the relationship  $g_a = (2/3) \sigma_f$  derived above from the Young equation (23) requires  $\kappa' \leq 2.4 \times 10^{-15}$  erg. The numerical factor on the right-hand side of (27) also appears in (26). It suggests  $A_{\text{abs}}/A_{\text{base}} = 3$  for  $(\kappa'/\sigma_f) q_{\text{max}}^2 = 100$ , i.e. a range of tensions covering two decades (as in the experiments) and just below the tension related to the cutoff,  $\sigma_{\text{max}} = \kappa' q_{\text{max}}^2$ . Incidentally, with  $\sigma_{\text{max}} = 1 \times 10^{-3}$  dyn cm<sup>-1</sup> one has  $q_{\text{max}} = \geq 6.4 \times 10^{-6}$  cm<sup>-1</sup> which is well below optical resolution.

We hasten to point out that the derivation of (26) presupposes  $A_{\text{abs}}/A_{\text{base}} \ll 1$ . If this condition is violated, as in our estimate, one has to take account of  $A_{\text{abs}}/A_{\text{base}}$  in many of our equations. For instance, the Young equation (23) becomes [11]

$$\alpha_b g_a = (\alpha_f - \dot{\alpha}_b \cos \psi) \sigma_f,$$

with

$$\alpha = \frac{A_{\text{base}}}{A_{\text{base}} + A_{\text{abs}}} < 1$$

having different values  $\alpha_f$  and  $\alpha_b$  where the membrane is free and bound, respectively. As  $\alpha_f$  should be smaller than  $\alpha_b$  we may expect  $g_a$  to be smaller than given by the original Young equation. This in turn relaxes the demands on  $A_{\text{abs}}/A_{\text{base}}$  which can now be less than three but not much less than unity.

## 5. Conclusion.

To date there is no clear-cut evidence for a large reservoir of area in unstretched egg lecithin membranes. However, it is also difficult to rule out such a possibility as ultralow tensions of  $10^{-4}$  dyn  $\text{cm}^{-1}$  or less might suffice, according to (26), to consume most of the reservoir by pulling the membrane flat. Some pictures obtained in transmission electron microscopy hint at least at a novel roughness of lecithin bilayers [32]. Also, a theory has been proposed to explain the suspected warping of the membranes, conjecturing highly localized and cooperative saddle deformations in the bilayer [33]. Lecithins abound in biological, especially animal, membranes. It has already been speculated [11] that from an engineering point of view nature, which has selected lecithin, may prefer large contact angles of induced adhesion as they allow large contact areas. Even larger angles have been found to characterize the induced adhesion of two other biological model membranes, namely bilayers of phosphatidylethanolamines [34] and of a natural digalactosyldiacylglycerol [35]. In 0.1 M NaCl solution instead of pure water, the latter membranes display spontaneous adhesion at low temperatures and an unbinding transition when the temperature is raised [35]. The egg lecithin multilayer systems described in the present paper can also be utilized to reproducibly generate multiply self-connected membranes, i.e. sponges or lattices of passages [36].

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