



# Giant membranes of swollen phosphatidylethanolamines and glycolipids

M. Mutz, R.-M. Servuss, W. Helfrich

## ► To cite this version:

M. Mutz, R.-M. Servuss, W. Helfrich. Giant membranes of swollen phosphatidylethanolamines and glycolipids. Journal de Physique, 1990, 51 (22), pp.2557-2570. <10.1051/jphys:0199000510220255700>. <jpa-00212553>

**HAL Id: jpa-00212553**

**<https://hal.science/jpa-00212553v1>**

Submitted on 4 Feb 2008

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Classification  
Physics Abstracts  
68.15 — 87.10

## Giant membranes of swollen phosphatidylethanolamines and glycolipids

M. Mutz (\*), R.-M. Servuss and W. Helfrich

Fachbereich Physik, Freie Universität Berlin, Arnimallee 14, D-1000 Berlin 33, F.R.G.

(Received 16 May 1990, accepted 25 July 1990)

**Abstract.** — The swelling of phosphatidylethanolamines and biological glycolipids is studied by phase contrast microscopy. In their fluid phase the bilayers of all the lipids separate in water and, with one proven exception, in NaCl solutions. The very rare mutual adhesion of giant membranes which normally separate is apparently always induced by lateral tensions. Its average contact angles are larger than in the case of egg phosphatidylcholine but, as there, do not depend on tension within the one or two powers of ten covered by the observations. Certain cerebroside form needles and fibres when the bilayers are in the solid phase.

### Introduction.

Over many years our group has collected evidence for the spontaneous separation of fluid phosphatidylcholines (= PC) membranes. The zwitterionic PC's swelled in pure water and salt solution ( $\leq 0.5$  M NaCl), forming vesicles and other giant membranes vesicles which did not stick to each other when they met [1-3]. The swelling of well-ordered egg PC multilayer systems could be followed to membrane spacings of 20 nm and seemed unlimited [4, 5]. Recently, investigations of sphingomyelin, which is another neutral phospholipid, and charged phospholipids in ionic solutions have demonstrated that giant vesicles always form if the bilayers are in their fluid phase [6].

Mutual adhesion of giant phosphatidylcholine membranes was very rare and apparently induced by the lateral tensions accompanying it [3, 5, 7, 8]. For egg PC, tensions between  $3 \times 10^{-6}$  and  $3 \times 10^{-4}$  dyn cm<sup>-1</sup> could be estimated from a rounding of the membrane next to the contact area. The contact angles of induced adhesion, 40° to 70° depending on geometry, displayed some scatter but no dependence on tension or salt content. Spontaneous adhesion as opposed to induced adhesion was observed with a natural digalactosyl-diacylglycerol (DGDG). Its membranes undergo a reversible unbinding transition in NaCl solution as the temperature is raised, but separate at any temperature in pure water [9, 10].

All these observations were explained in terms of a competition between van der Waals attraction and steric repulsion of fluctuating bilayers. Lateral tension weakens the out-of-plane fluctuations and this induces mutual adhesion of membranes that normally separate.

---

(\*) *Present address* : Ecole Normale Supérieure, Laboratoire de Physique Statistique, 24 rue Lhomond, 75231 Paris Cedex 05, France.

Measuring by X-ray diffraction the repeat distances in lipid dispersions, other authors found only a limited uptake of water with phospholipids, such as phosphatidylcholines and phosphatidylethanolamines, and glycolipids [11-14]. This may be taken to imply spontaneous adhesion, in conflict with our findings in egg PC. Therefore it is of interest to extend our optical observations to phosphatidylethanolamine (= PE) and glycolipids, materials which like the PC's are electrically neutral at pH = 7. Phosphatidylcholines and phosphatidylethanolamines are common in animal membranes. The glycolipids to be studied are DGDG, frequent in vegetable membranes and some natural cerebroside. The latter amphiphiles contain sphingosine instead of glycerol and one of their two hydrocarbon chains is 24-carbon-long.

Although PE is structurally very similar to PC and is also zwitterionic at pH = 7 there are important differences. The head groups of PE are smaller than those of PC and interact within the monolayer *via* hydrogen bridges [15]. As a result, membrane hydration is less pronounced for PE than PC. The main transition from the crystalline to the liquid crystalline ( $L_\alpha$ ) phase of the multilamellar systems of some phosphatidylethanolamines displays a pronounced hysteresis, absent in PC, which has been studied by different methods such as calorimetry, X-ray analysis, and infrared spectroscopy [16-22]. When the material is heated for the first time, the transition takes place at a temperature  $T_m^1$ . If the multilamellar system is cooled again it stays fluid down to a temperature  $T_m^2 < T_m^1$  where a readily reversible transition into a gel phase takes place. After waiting long enough at  $T < T_m^1$ , one finds  $T_m^1$  again in the first heating run. Phosphatidylethanolamines with unsaturated fatty acid hydrocarbon chains have a single main transition temperature  $T_m$ , but transform at a higher temperature  $T_h$  into the inverted hexagonal ( $H_{II}$ ) phase [23-26] which does not occur in the corresponding PC-water systems. The  $H_{II}$  phase has also been found with monogalactosyl-diacylglycerol water systems, whereas digalactosyl-diacylglycerol remains lamellar at high temperatures [11].

In the following we describe swelling and its structures as observed in phase contrast microscopy. The fluid membranes of all the lipids turn out to separate. For the first time we measure main transition temperatures of single PE bilayers. Our principal interest is in collecting data on the contact angles and lateral tensions of PE and DGDG membranes in induced adhesion. Adhesive contacts being rare and accidental, a large number of samples has to be searched for them. The results are similar to what was found with egg PC, i.e. the contact angles scatter but appear not to depend on tension. The average contact angles are even larger than with egg PC. Cerebroside membranes become fluid and separate only at rather high temperatures which may be the reason why they show no induced adhesion. However, part of them are found to form needles and fibers at temperatures below the main transition.

### Materials and methods.

1,2-dilauroyl-sn-glycero-3-phosphatidylethanolamine (DLPE), 1,2-dimyristoyl-sn-glycero-3-phosphatidylethanolamine (DMPE), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine (POPE), egg-yolk-phosphatidylethanolamine (EYPE), galactocerebroside from bovine brain (type I, II and mixed) <sup>(1)</sup>, semisynthetic N-nervonoyl-glucocerebroside (prepared from bovine brain) and digalactosyl-diacylglycerol (DGDG) from wheat flour were

<sup>(1)</sup> Type I contains approx. 98 %  $\alpha$ -hydroxy fatty acids, type II contains approx. 98 % non-hydroxy fatty acids, primarily nervonic and lignoceric acid, type III is a mixture of type I (50-60 %) and type II (40-50 %).

obtained from Sigma (Munich) and used without further purification. The saturated PE's and the glycolipids were purchased as crystalline powders, EYPE and POPE as solutions in chloroform (10 mg/ml). Sometimes we dissolved the saturated PE's and the glycolipids in the same way before an experiment. All lipids were stored at  $-20^{\circ}\text{C}$ .

The purity of the lipids was checked by thin-layer chromatography on silica gel 60 F<sub>254</sub>. Runs were performed in a mixture of chloroform, methanol and water (65 : 65 : 2.5). The synthetic lipids and the glycolipids showed a single spot when developed with iodine vapor, which means that their impurities content should be less than 1 %. In some experiments the synthetic ethanolamines were hydrated before swelling. For this purpose, some mg of the crystalline powder was incubated in a water saturated atmosphere above  $T_m^1$  for at least 12 h.

A small amount (some  $\mu\text{g}$ ) of lipid was spread on a glass slide, covering about  $1\text{ mm}^2$  of the available area of  $1\text{ cm}^2$ . Any  $\text{CHCl}_3$  solution was evaporated under vacuum for at least overnight. Subsequently distilled water was added, the pH being between 5.5 and 6 because of some  $\text{CO}_2$ , a cover glass put on top, and the 20-40  $\mu\text{m}$  high cell sealed with a glue to prevent evaporation. The lipid was completely covered with water at the beginning of swelling.

The object stage of the microscope could be heated to the desired temperatures electrically (Sensortek) or by coupling to an external thermostat (Haake). The temperature of the sample was measured with a thermocouple mounted to the object stage and calibrated with a second thermocouple in an empty cell, the accuracy being  $\pm 0.5^{\circ}\text{C}$  [2]. The sample was watched under a phase-contrast microscope (Leitz), the numerical aperture of the objective usually being 0.75. Single membranes are seen in the focal plane where they are parallel to the optical axis of the microscope. A videosystem (Grundig) with monitor and recorder and a camera (Olympus) were attached to the microscope for simultaneous use.

## Results.

As long as fresh PE in excess water was kept at temperatures below  $T_m$  or, in the case of metastability, below  $T_m^1$  the lipid remained crystalline and no significant changes were seen under the microscope. When the temperature was raised above  $T_m^1$  or  $T_m$  membranes started to detach from the compact material, forming many kinds of membrane structures, among them unilamellar vesicles. Our transition temperatures were compared with the following values :  $T_m^1 = 43\text{--}44^{\circ}\text{C}$  for DLPE [16-18],  $T_m^1 = 53\text{--}56.3^{\circ}\text{C}$  for DMPE [20-21],  $T_m = 12^{\circ}\text{C}$  for EYPE [19] and  $T_m = 25^{\circ}\text{C}$  for POPE [24, 25]. We found agreement within  $1^{\circ}\text{C}$ , making no effort to measure more precisely. The first membranes, always attached to compact lipid, used to emerge in a few minutes after the start of swelling. Further membranes formed until after an hour practically all the lipid was converted into vesicles or other dilute structures. Multilamellar walls were found to split within days into unilamellar ones. There was a great variety of membrane shapes and sizes. We saw little difference from the structures obtained with egg PC in identical experiments. In fact, it is impossible to decide after briefly looking at sample cell whether the swollen lipid is PE or PC. However, the two types of garlands shown in figures 1 and 2 seemed to occur only with PE's. In a more systematic way, PC and PE could be distinguished from each other by measuring a large number of contact angles of induced adhesion, if available, and comparing the averages (see below).

If a sample was cooled again after swelling, the membranes started to freeze at a certain temperature. Freezing was revealed by the ceasing of undulations. Especially multilamellar vesicle walls also developed flat facets separated by more or less pronounced ridges. In all samples there were membrane structures that displayed supercooling by up to  $4^{\circ}\text{C}$ . Freezing and melting of complex structures always caused irregular changes of the geometries.



Fig. 1. — A garland of single membranes swelling from a well-ordered multilayer system of DMPE. The nonzero contact angles, although somewhat smaller than the typical  $45^\circ$ , indicate (induced) adhesion. The absence of visible contact roundings means rather large lateral tensions. The bar represents  $10\ \mu\text{m}$ .

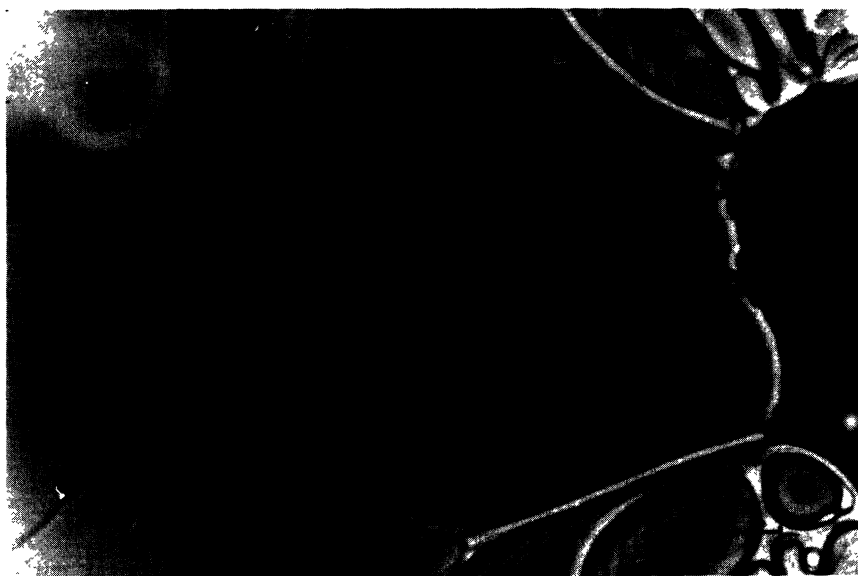


Fig. 2. — Another type of garland swelling from DMPE. The bar represents  $10\ \mu\text{m}$ .

Reheating made all structures resume their undulations at one and the same temperature which was taken to be the main transition temperature. We found  $29.5 \pm 0.5^\circ\text{C}$  in careful measurements of DLPE and  $51 \pm 1^\circ\text{C}$  for DMPE. These transition temperatures agreed within  $1^\circ\text{C}$  with the temperatures  $T_m^2$  as reported for multilamellar systems,  $T_m^2 = 30.1$ - $30.6^\circ\text{C}$  for DLPE [16-18] and  $T_m^2 = 49.0$ - $49.6^\circ\text{C}$  for DMPE [17, 18, 22]. No dependence of

the melting temperatures on the number of lamellae in a wall was noticed within an experimental error of  $\pm 1/2$  °C.

If saturated PE was hydrated immediately before being covered with free water, vesicle formation began somewhere between  $T_m^1$  and  $T_m^2$ . Otherwise, hydration had little influence on swelling process.

In a series of experiments we used salt solutions instead of pure water in order to make sure that the separation of PE membranes was not due to electrostatic repulsion. The speed of swelling did not significantly depend on NaCl up to a concentration of 0.1 M where it was reduced by about a factor of two. Apart from the slowing, NaCl did not change the character of the swelling process, even at 0.3 M NaCl where the Debye length is only 0.5 nm. To rule out any ionization of the PE we did an experiment with PE in 50 mM tris buffer at pH 7.0, i.e. well inside the range of zwitterionicity. The buffer made no difference apart from a slight decrease of the speed of swelling.

In other experiments we looked for the inverted hexagonal phase of the unsaturated phosphatidylethanolamines EYPE and POPE. Since very small amounts of impurities are known to shift the lamellar to hexagonal transition to higher or lower temperatures [27, 28] we used the material immediately after delivery by the manufacturer when its purity should be best. The transition had been located at  $T_h = 25-30$  °C with EYPE [19, 25] and  $T_h = 69-75$  °C with POPE [24-26]. However, the membrane structures of the swollen phosphatidylethanolamines did not change as the transition temperature was passed and no sign of disintegration was detected in tests extending up to 45 °C for EYPE and 81 °C for POPE, the latter temperature being the experimental limit.

The glycolipid DGDG has its main transition at about  $-50$  °C [11]. DGDG at room temperature formed unilamellar vesicles and other membrane structures similar to those obtained with the phospholipids. The only striking difference was the strength of the membrane fluctuations indicating a low bending rigidity. The rigidities of both DGDG and PE membranes were, in fact, determined from the Fourier analyzed fluctuations of contour sections as is reported elsewhere [29].

The cerebroside swelled to form giant single membranes only between 70 and 80° which is in the range of temperatures reported for the main transitions in multilayer systems [31, 30]. Precise comparisons are not possible since the fractions of different hydrocarbon chains may not be equal. When being cooled, galactocerebroside membranes remained in a metastable fluid state down to 10-15 °C below the main transition. Surprisingly, nervonoyl-glucocerebroside put in water at room temperature developed rigid needles projecting into the free water as exemplified by figure 3. The needles vanished and giant membranes appeared when the temperature was increased beyond the main transition at 75-76 °C. Cooling from this state gave rise to a transformation of the membranes into a disorderly array of rigid fibers as illustrated by figure 4. The phase change took place about 10 °C below the main transition and was reversible. One of the galactocerebroside (type II) also formed needles but no fibers. It may be noted that observations at these high temperatures are difficult because of fluctuations and convection currents.

We were particularly interested in the adhesive properties of the fluid membranes studied. Floating vesicles, which usually fluctuated strongly, did not stick to each other or to other giant membranes, regardless of the number of membranes in the vesicle wall. However, « branched structures » occasionally observed during swelling and practically stationary exhibited membrane adhesion. Examples of these structures which also occur with egg PC [3] are given for DMPE in figures 5 and 6. The adhesion was generally associated with lateral tension, as could be immediately inferred from the weakened undulations of adhering membranes. Practically the same branched structures were again obtained with DGDG,

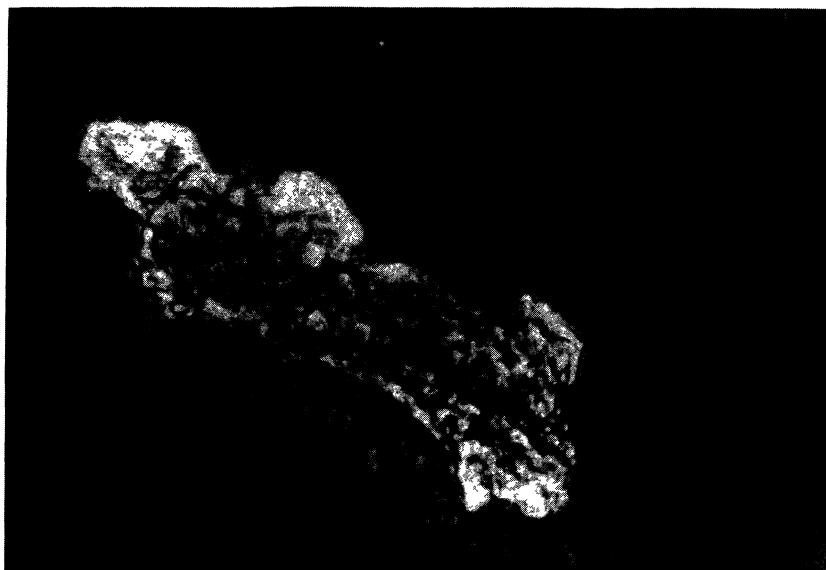


Fig. 3. — Needles growing from nervonoyl-glucocerebroside at room temperature, i.e. far below the main transition temperature. The bar represents 10  $\mu\text{m}$ .



Fig. 4. — Fibers formed in a reversible transformation at ca. 65 °C from single membranes by cooling swollen nervonoyl-glucocerebroside. The bar represents 10  $\mu\text{m}$ .

examples of which have been shown previously [9, 10]. Since DGDG membranes in NaCl solution display spontaneous adhesion, only pure water was used with DGDG in the present work. No branched structures were found with the cerebroside, perhaps because of the high temperatures.

Inspection showed most branched structures to consist of membranes turning semicylindri-



Fig. 5. — Branched structures of DMPE during swelling as used to evaluate contact angles and contact roundings especially of symmetric adhesion. The bar represents 10  $\mu\text{m}$ .

cally from top to bottom of the sample cell. All the membranes turn, of course, in the same way, which explains why one side of a bundle is usually brightened up by the halo effect of phase contrast microscopy. For the purpose of evaluation the branched structures are taken to be two-dimensional. The curvature in the direction of viewing can be neglected as it is generally much smaller than the curvature of contact rounding for a sample thickness of 20  $\mu\text{m}$  or more.

The branched structures furnish the contact angles of adhesion. If there is an optically resolvable contact rounding they also give the lateral tension. Ideally, the free membrane leaving a contact area tends toward an asymptotic direction. The height of the membrane above its asymptotic plane drops exponentially with a characteristic length  $s_0$  wherever the angle which the membrane makes with the plane is  $30^\circ$  or less. For arbitrary contact angles  $\phi \leq 90^\circ$  the height  $y_0$  of the edge of the contact area obeys

$$\frac{y_0}{s_0} = 2 \sin \frac{\phi}{2}. \quad (1)$$

Because of

$$\sigma = \frac{k_c}{s_0^2}, \quad (2)$$

where  $\sigma$  is the lateral tension and  $k_c$  the bending rigidity, the tension can be computed from  $\phi$  and  $y_0$ , if they are measurable and  $k_c$  is known.





Fig. 6. — Another branched structure of DMPE during swelling. The bar represents 10  $\mu\text{m}$ .

Rather than plot contact angle *versus* lateral tension, we use the two quantities to calculate the adhesion energy per unit area,  $g_a$ , by means of the Young equation

$$g_a = 2(1 - \cos \phi) \sigma. \quad (3)$$

The adhesion energy is then plotted *versus* the tension as has first been done for egg PC [3, 8]. The factor 2 on the right-hand side of (3) applies to the special case of symmetric adhesion, i.e. adhesive contact between two single membranes of equal tensions and, thus, equal contact angles. The Young equation comes from the theory of interfaces; it is valid for membranes if their changes in (projected) area due to tension and contact are negligible, i.e. no more than a few percent [3, 5]. The plots are shown in figure 7 for the PE's and in figure 8 for DGDG. The bending rigidities used in computing the tensions are  $0.7 \times 10^{-12}$  erg for PE and  $0.2 \times 10^{-12}$  erg for DGDG [29].

A constant contact angle means a straight line of slope unity in the double logarithmic plots of figures 7 and 8. The scatter of the angle for a given material can, therefore be easily judged from the diagrams. Curiously, the measured contact angles accumulated at upper limits which hardly ever were exceeded. They were  $55^\circ$  for PE's and  $60^\circ$  for DGDG. Contact angles below  $35^\circ$  were not seen with either material. The upper limit had been found to be  $45^\circ$  with egg PC bilayers [3]. The mean contact angles were about  $5^\circ$  less than the upper limits in all three cases.

The contact angles of mutual adhesion were larger than in the symmetric case for a single membrane adhering to a stack of other membranes. In fact, the angle increased with the number of membranes in the stack, reaching (see Fig. 6) on average maxima slightly

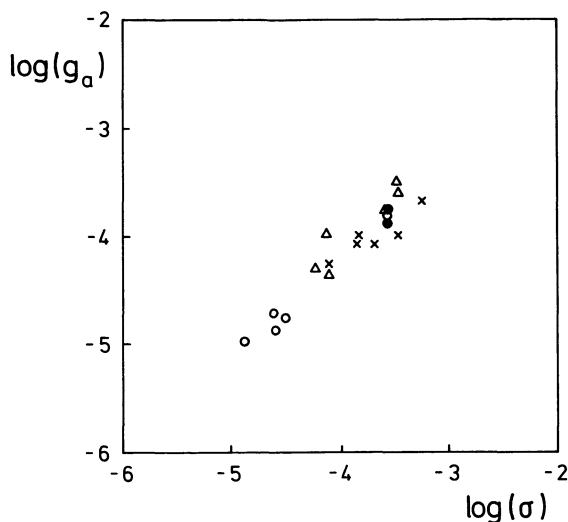


Fig. 7. — The adhesion energy computed by means of the Young equation (3) plotted vs. the lateral tension obtained from contact rounding. The plot collects the data for PE bilayers: (O) DLPE, (×) DMPE, (Δ) DLPE in 30 mM NaCl, (●) DLPE in 100 mM NaCl.

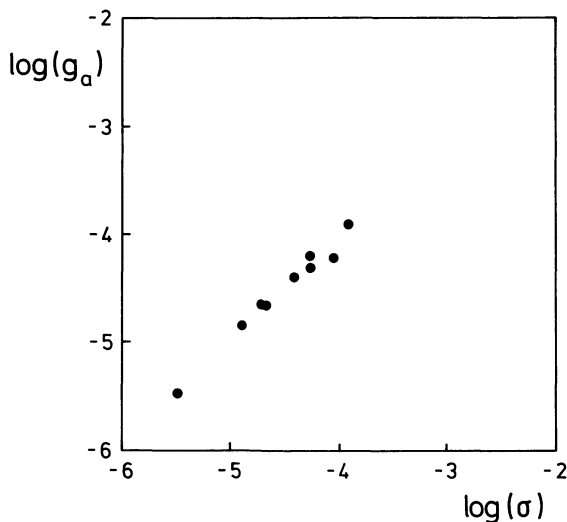


Fig. 8. — Adhesion energy plotted vs. lateral tension for DGDG bilayers. (For details see Fig. 7.)

surpassing 80 and 85° for PE and DGDG, respectively, while 70° had been found for PC [3, 5]. Sequences of measured contact angles for DMPE are plotted in figure 9. The membranes are counted from the side of pair adhesion,  $n = 1$  representing the (symmetric) pair. We did not evaluate the lateral tensions of the membranes seen for example in figure 6 since the contact roundings are barely visible. However, the relative tensions of the free membranes can be derived for the whole sequence of contact angles by means of an equilibrium condition equating the components of the lateral tensions perpendicular to a contact area. Incidentally,

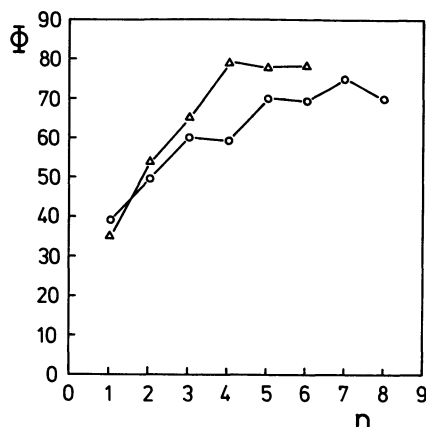


Fig. 9. — Two sequences of contact angles of single membranes adhering to a growing stack of  $n$  other membranes. The case  $n = 1$  represents symmetric adhesion of two membranes. The material is DMPE.

the components of the tensions parallel to the contact areas tended to be much more uniform than the tensions of the free membranes.

Branched structures were also obtained with DLPE in 30 and 100 mM NaCl and with DMPE additionally in 300 mM NaCl. The contact angles of symmetric adhesion as well as the maximum angles of adhesion to a stack remained the same as in pure water.

### Discussion.

Our observations indicate that PE and DGDG membranes, like those of PC, normally separate when they are in the fluid state. The rare adhesive contacts are evidently induced by the lateral tensions accompanying them. In conjunction, separation and induced adhesion imply that steric interaction is necessary for the unlimited separation of fluid membranes. Any electrostatic contributions to the repulsive interaction should be negligible for the PE since, in accordance with egg PC, the contact angles of induced adhesion are the same in NaCl solutions as in pure water. The ring sugars of DGDG being even less likely to be charged [32] than the zwitterionic polar heads of the PE, steric interaction seems sufficient to separate the bilayers of all the lipids in question if there is no lateral tension.

The contact angles of induced adhesion must be less than  $90^\circ$  so that the component of the tension parallel to the contact area remains positive. Interestingly, DGDG membranes which in NaCl solution display spontaneous adhesion make larger contact angles in pure water than PE and PC bilayers, reaching almost  $90^\circ$  for the adhesion of the single membrane to a stack of other membranes.

The interaction of electrically neutral fluid membranes as a function of their spacing may be expressed by two inverse square laws. One of them represents van der Waals attraction whose energy per unit area is

$$g_{\text{vdW}} = - \frac{A_h}{12 \pi d^2} \quad (4)$$

where  $A_h$  is the Hamaker constant and  $d$  the spacing. The half-space approximation (4) may be taken as a good approximation up to  $d = 10$  nm since the dielectric constant of water is so much larger than that of hydrocarbons [33]. There are several measurements of the Hamaker constants of PE and DGDG membranes in pure water. Since the data do not always agree

among each other, we quote two values which seem to us lower and upper bounds for biological model membranes, namely  $A_h = 1.3 \times 10^{-14}$  erg (measured for solid PE [34]) and  $A_h = 5.3 \times 10^{-14}$  erg (measured for fluid DGDG [32]).

Van der Waals attraction is opposed by steric repulsion. If the latter is due to thermal undulations, i.e. the out-of-plane fluctuations governed by the bending rigidity  $k_c$ , the energy per unit area may be written as (Helfrich 78)

$$g_{\text{und}} = \frac{3 \pi^2 (k_B T)^2}{128 k_c d^2} \quad (5)$$

where  $k_B$  is Boltzmann's constant and  $T$  temperature. Note that  $d$  is now the mean spacing of the fluctuating membranes. The numerical factor in the last formula was derived for multilayer systems; it was recently confirmed within 20 % by Safinya *et al.* [36] when they did X-ray studies of multilayer systems composed of very flexible membranes ( $k_c \approx k_B T$ ).

The bending rigidities to calculate  $\sigma$ ,  $k_c = 0.7 \times 10^{-12}$  erg for PE and  $k_c = 0.2 \times 10^{-12}$  erg for DGDG, were obtained from the fluctuations of contour section of very extended membranes of DMPE and DGDG, but  $k_c = 1.7 \times 10^{-12}$  erg was derived for DLPE from the bending fluctuations of tubular vesicles [29]. The lower value for PE,  $k_c = 0.7 \times 10^{-12}$  erg, is thought to be typical of all the PE membranes if the curvatures, apart from local fluctuations, are weak [29]. No attempt was made to measure the bending rigidities of cerebroside.

Inserting the values of  $A_h$  and  $k_c$  quoted for the respective materials into (4) and (5), one finds van der Waals attraction and undulatory repulsion to be nearly equal strong for both PE (with  $k_c = 0.7 \times 10^{-12}$  erg) and DGDG. This seems to imply that all the systems studied are rather close to the borderline between separation and spontaneous adhesion. A renormalization group treatment of the unbinding transition [37] incorporating hydration forces and a more rapid drop than (4) of the van der Waals interaction at larger spacings leads to practically the same prediction. However, the experimental uncertainties are too large to decide unequivocally between spontaneous adhesion and separation.

Lateral tension induces membrane adhesion by reducing the amplitude of out-of-plane fluctuations. It permits van der Waals attraction to dominate over steric repulsion beyond a certain spacing that decreases with increasing tension. A simple theory in terms of thermal undulations predicts the adhesion energy to be proportional to the tension [3, 5]. The constancy of the contact angles of induced adhesion, found first with egg PC, seems to confirm this proportionality, thus looking like a final proof that the membranes are driven apart by their undulations.

However, the theoretical analysis of induced adhesion has shown that the adhesion energy [3] and the sensitivity of the squared membrane mean spacing to lateral tension [5] are both too large by a factor of hundred to be compatible with undulations being the only out-of-plane fluctuations. We do not review here these arguments, which has been done elsewhere [38], but repeat only the most straightforward of them which is also the most pertinent to the present experiments.

Let us begin with an equation that defines in a sense the adhesion energy  $g_a$  of two membranes in symmetric adhesion

$$\alpha_b g_a = 2(\alpha_f g_f - \alpha_b g_b) . \quad (6)$$

The adhesion energy and the energies of stretching  $g_f(\sigma)$  and  $g_b(\sigma)$  of the free and the bound states of the membranes, respectively, are per unit projected in the free and bound state of each of two membranes in symmetric adhesion. (The mean spacings of the membranes in the bound state decreases from infinity as the tension, which actually is  $\sigma \cos \phi$ , increases from

zero.) The factors  $\alpha_f$  and  $\alpha_b$  express the ratios of projected or base area to real area of relaxed membrane in the two states. For instance,  $\alpha = 1$  holds for a flat bilayer of zero lateral tension, out-of-plane fluctuations decrease  $\alpha$  as they absorb area, and a lateral tension ( $\sigma > 0$ ) will increase it by stretching the real area of the membrane. Since in the absence of spontaneous adhesion  $g_b$  must be positive like  $g_f$ , we may derive from (6) the inequality

$$g_a < 2(\alpha_f/\alpha_b) g_f. \quad (7)$$

The ratio  $\alpha_f$  of an undulating membrane has been calculated in a first-order approximation as [7, 39]

$$\alpha_f = 1 - \frac{k_B T}{8 \pi k_c} \ln \frac{k_c \pi^2}{\sigma a^2} + \frac{\sigma}{2 \lambda} \quad (8)$$

where  $a$  is a molecular length of ca.  $10 \text{ \AA}$  and  $\lambda$  the lateral stretching modulus which is about  $200 \text{ dyn cm}^{-1}$  for biological model membranes [40]. It follows from (8) and  $dg_f = \sigma d\alpha_f$  that

$$g_f = \frac{k_B T}{8 \pi k_c} \sigma + \frac{1}{2 \lambda} \alpha^2.$$

The second, regular contribution to  $g_f$  can be neglected at the ultralow tensions of interest. Accordingly, one arrives at

$$g_a < \frac{\alpha_f}{\alpha_b} \frac{k_B T}{4 \pi k_c} \sigma$$

which with  $k_c = 0.2 \times 10^{-12} \text{ erg}$  becomes at room temperatures ( $k_B T = 4 \times 10^{-14} \text{ erg}$ )

$$g_a < (\alpha_f/\alpha_b) 1.6 \times 10^{-2} \sigma. \quad (9)$$

This has to be compared with the Young equation (3), now written in the more precise form

$$\alpha_b g_a = 2(\alpha_f - \alpha_b \cos \phi) \sigma.$$

Taking  $\sigma_f = 10^{-4} \text{ dyn cm}^{-1}$ , which is near the largest tension measured with DGDG, one finds from (8) the lower limit to  $\alpha_f$ ,

$$\alpha_f > 0.88.$$

Assuming  $1 > \alpha_b > \alpha_f$ , one may write

$$g_a > 2(0.88 - \cos \phi) \sigma$$

and with  $\phi = 55^\circ$

$$g_a > 0.61 \alpha. \quad (10)$$

Clearly, the two inequalities for  $g_a$ , (9) and (10), are in serious conflict. The discrepancy is even worse, reaching a factor of hundred as with phosphatidylcholines, in the case of DMPE because of its larger bending rigidity.

This and other contradictions led to the postulate of a submicroscopic membrane roughness absorbing much more extra area than the few percent residing in the undulations of PE's and PC's. The 12 percent just estimated for DGDG are exceptional and still not enough to resolve the conflict. We refer two previous work for further discussion of these problems [3, 5, 38], a

model of the conjectured roughness [41], and possible electron-microscopic evidence for it [42].

The main transition temperature of the single membranes of DLPE and DMPE are equal, within  $\pm 1/2$  °C, to the respective main transition temperatures of the metastable hydrated multilayer systems. On the other hand, intermembrane interaction was found to lower the main transition temperature of some synthetic PC's by up to 1 °C [2]. Since PE tends to be much less hydrated than PC, as evidenced by weaker hydration forces [34, 43, 44], one might expect a marked difference in the transition temperature between the single membrane and the multilayer system of PE, but none was observed. It is also remarkable that PE membranes, either single or in stacks, do not transform in our samples into the inverse hexagonal phase at the elevated temperatures where this phase is known to be stable. The pronounced metastability indicates that a high energy barrier prevents intact PE membranes from undergoing the transition.

### Conclusion.

The investigation of the material properties of single biological model membranes is only at its beginning and promises to be full of surprises. The largeness of the contact angles of adhesion induced by lateral tension is one of them. It was the foremost aim of the present work to check whether it is confined to the PC. The finding that large angles are a general feature of electrically neutral membranes suggests their usefulness in nature. The new, submicroscopic roughness of the membranes which they seem to imply is being searched for by electron microscopy [42, 45]. The cerebroside examined differed from the phosphatidylethanolamines and DGDG by very high main transition temperatures which may explain why no induced adhesion was seen. However, they are interesting because of the rigid needles and fibers which they exhibit. We do not attempt to explain these linear structures which seem to have been unknown up to now. An other interesting feature of biological model membranes are the sponges and or lattices of multiply self-connected bilayers which are known from egg PC [3, 46, 47]. They were also seen occasionally with DGDG and with all the phosphatidylethanolamines used in the present work, but remain to be investigated in detail.

### Acknowledgment.

This work has been supported in part by the Deutsche Forschungsgemeinschaft through SFB 312.

### References

- [1] SERVUSS R. M., HARBICH W. and HELFRICH W., *Biochim. Biophys. Acta* **436** (1976) 900-903.
- [2] HARBICH W., SERVUSS R. M. and HELFRICH W., *Phys. Lett.* **57A** (1976) 294-296.
- [3] SERVUSS R. M. and HELFRICH W., *J. Phys. France* **50** (1989) 809-827.
- [4] HARBICH W. and HELFRICH W., *Chem. Phys. Lipids* **36** (1984) 39-63.
- [5] HARBICH W. and HELFRICH W., *J. Phys. France* **51** (1990) 1027-1048.
- [6] SERVUSS R. M., *Z. Naturforsch.* **43c** (1988) 938-947.
- [7] HELFRICH W. and SERVUSS R. M., *Il Nuovo Cimento* **3D** (1984) 137-151.
- [8] SERVUSS R. M. and HELFRICH W., Proc. of the Symposium of Complex and Supermolecular Fluids, Ed. S. A. Safran (Wiley N. Y.) 1987.
- [9] HELFRICH W. and MUTZ M., Random Fluctuations and Pattern Growth: Experiments and Models, Eds. H. E. Stanley and N. Ostrowsky (Kluwer Academic Publisher, Dordrecht) 1988.
- [10] MUTZ M. and HELFRICH W., *Phys. Rev. Lett.* **62** (1989) 2881-2884.

- [11] SHIPLEY G. G., GREEN J. P. and NICHOLS B. W., *Biochim. Biophys. Acta* **311** (1973) 531-544.
- [12] RAND R. P., *Ann. Rev. Biophys. Bioeng.* **10** (1981) 277-314.
- [13] LIS L. J., MCALLISTER M., FULLER N., RAND R. P. and PARSEGAN P. A., *Biophys. J.* **37** (1982) 657-666.
- [14] SEDDON J. M., CEVC G., KAYE R. D. and MARSH D., *Biochemistry* **23** (1984) 2634-2644.
- [15] HAUSER H., PASCHER I., PEARSON R. H. and SUNDELL S., *Biochim. Biophys. Acta* **650** (1981) 21-51.
- [16] CHANG H. and EPAND R. P., *Biophys. Biochim. Acta* **728** (1983) 319-324.
- [17] MANTSCH H. H., HSI S. C., BUTLER K. W. and CAMERON D. G., *Biochim. Biophys. Acta* **728** (1983) 325-330.
- [18] SEDDON J. M., HARLOS K. and MARSH D., *J. Biol. Chem.* **258** (1983) 3850-3854.
- [19] CASAL H. L. and MANTSCH H. H., *Biochim. Biophys. Acta* **779** (1984) 381-401.
- [20] CHOWDRY B. Z., LIPKA G., DALZIEL A. W. and STURTEVANT J. M., *Biophys. J.* **45** (1984) 901-904.
- [21] SILVIUS J. R., BROWN P. M. and O'LEARY T. J., *Biochemistry* **25** (1986) 4249-4258.
- [22] WILKINSON D. A. and NAGLE J. F., *Biochemistry* **23** (1984) 1538-1541.
- [23] CULLIS P. R. and DE KRUIFF B., *Biochim. Biophys. Acta* **513** (1978) 31-42.
- [24] DEKKER C. J., GEURTS VAN KESSEL W. S. M., KLOMP J. P. G., PIETERS J. and DE KRUIFF B., *Chem. Phys. Lipids* **33** (1983) 93-106.
- [25] EPAND R. M., *Biochemistry* **24** (1985) 7092-7095.
- [26] BROWN P. M., STEERS S., HUI S. W., YEAGLE P. L. and SILVIUS J. R., *Biochemistry* **25** (1986) 4259-4267.
- [27] EPAND R. M., *Chem. Phys. Lipids* **36** (1985) 387-393.
- [28] EPAND R. M. and BRYZIEWSKA M., *Biochemistry* **27** (1988) 8776-8779.
- [29] MUTZ M. and HELFRICH W., *J. Phys. France* **51** (1990) 991-1002.
- [30] BUNOW M. R., *Biochim. Biophys. Acta* **574L** (1979) 542-546.
- [31] MAGGIO B., ARTIGA T., STURTEVANT J. M. and YU R. K., *Biochemistry* **24** (1985) 1084-1092.
- [32] MARRA J., *J. Colloid Interface Sci.* **107** (1985) 446-458.
- [33] MAHANTY J. and NINHAM B. W., *Dispersion Forces* (Academic Press London) 1976.
- [34] MARRA J. and ISRAELACHVILI J., *Biochemistry* **24** (1985) 4608-4618.
- [35] HELFRICH W., *Z. Naturforsch.* **33c** (1978) 305-315.
- [36] SAFINYA D. R., ROUX D., SMITH G. S., SINHA S. K., DIMON P., CLARK N. A. and BELLOCQ A. M., *Phys. Rev. Lett.* **57** (1986) 2718-2721.
- [37] LIPOWSKY R. and LEIBLER S., *Phys. Rev. Lett.* **56** (1986) 2541-2544, Erratum **59** (1987) 1983.
- [38] HELFRICH W., *Phase Transition in Soft Condensed Matter*, Ed. T. Riste and D. Sherrington (Plenum Publishing Corporation) 1989, pp. 271-281.
- [39] MILNER T. S. and SAFRAN S. A., *Phys. Rev. A* **36** (1987) 4371-4379.
- [40] EVANS E. and NEEDHAM D. J., *Phys. Chem.* **91** (1987) 4219-4228.
- [41] HELFRICH W., *Liquid Cryst.* **5** (1989) 1647-1658.
- [42] HELFRICH W. and KLOESGEN B., *Fourth Nishinomiya-Yukaya Symposium*, Ed. A. Onuki, *Springer Proc. in Physics* (in press).
- [43] RAND R. P., FULLER N., PARSEGAN P. A. and RAU D. C., *Biochemistry* **27** (1988) 7711-7722.
- [44] MARRA J., *J. Colloid Interface Sci.* **109** (1986) 11-20.
- [45] KLOESGEN B., to be published.
- [46] HARBICH W. and HELFRICH W., *Chem. Phys. Lipids* (in press).
- [47] HARBICH W., SERVUSS R. M. and HELFRICH W., *Phys. Lett.* **57a** (1976) 294-296.