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Bending rigidities of some biological model membranes as obtained from the Fourier analysis of contour sections

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Abstract. — We have measured the bending rigidities k_c of egg lecithin (EYPC), dimyristoyl-phosphatidyl-ethanolamine (DMPE) and digalactosyl-diacylglycerol (DGDG) membranes in the fluid state, subjecting the fluctuations of large nearly planar bilayer sections to computerized Fourier analysis. For EYPC we found $k_c = 0.8 \times 10^{-12}$ erg which is less than half the value obtained earlier for egg and other lecithins from the bending fluctuations of tubular vesicles but in the range of more recent measurements on spherical lecithin vesicles. For DMPE we found $k_c = 0.7 \times 10^{-12}$ erg at $T = 60^\circ\text{C}$, while we derived $k_c = 1.7 \times 10^{-12}$ erg from the bending fluctuations of tubular vesicles of dilauroyl-phosphatidylethanolamine (DLPE) at $T = 46^\circ\text{C}$. As in the case of lecithins the different results seem to be mostly due to the different methods. Very low values of $k_c = (0.12 - 0.27) \times 10^{-12}$ erg were measured for DGDG. The scatter was less but also large for the other materials.

Introduction.

The bending rigidities k_c of the fluid bilayers of some lecithins have been measured by various methods. The first value, $k_c = 2.3 \times 10^{-12}$ erg, was obtained for egg lecithin (EYPC) membranes from the fluctuations of the angle made by the two ends of long tubular vesicles [1]. Later on, the same method was applied, apart from EYPC, to dimyristoyl-lecithin (DMPC), dipalmitoyl-lecithin and distearoyl-lecithin, yielding bending rigidities in the range of $(1.8 - 2.4) \times 10^{-12}$ erg [2]. Studying fluctuation amplitudes and relaxation times of tubular and spherical vesicles, Schneider *et al.* found $k_c = (1 - 2) \times 10^{-12}$ erg for the bending rigidity of the EYPC bilayer [3, 4]. The variations in rigidity among the vesicles were $\pm 30\%$ or larger in most of those studies, being at least as large as the typical statistical error of the value computed for a single vesicle. Very recently, Bo and Waugh determined the radii of (invisibly) thin vesicular tethers and the forces acting on them for stearyl-oleoyl-lecithin (SOPC) vesicles manipulated by a micropipette, which permitted them to calculate, with a rather large uncertainty, $k_c = 1.8 \times 10^{-12}$ erg [5]. Distinctly lower values of the bending rigidities of lecithin membranes have been reported by other authors. From a computerized Fourier analysis of the contours of fluctuating spherical vesicles Engelhardt *et al.* and later Duwe *et al.* derived 1.1×10^{-12} erg for DMPC [6-8]. Bivas *et al.*; also studying spherical

vesicles, expanded the radial fluctuations in Legendre polynomials [9]. Their preliminary experiments on EYPC were continued by Faucon *et al.* who corrected the mean square fluctuation amplitudes for lateral tension, white noise and video sampling time, arriving finally at a very low value of the bending rigidity of egg lecithin bilayers, $k_c = (0.4 - 0.5) \times 10^{-12}$ erg [10].

Theoretical estimates of the bending rigidity may be obtained from the formula

$$k_c = \lambda b^2/48. \quad (1)$$

Containing the bilayer thickness b and the area stretching modulus λ , it is based on the assumption that the constituent monolayers behave like homogeneous sheets of thickness $b/2$ with a neutral surface in the middle of both of them [11]. Insertion of $b = 4$ nm and $\lambda = 140$ dyn cm $^{-1}$, the area stretching modulus measured by Evans and coworkers for the EYPC and DMPC membranes [12, 13], leads to $k_c = 0.5 \times 10^{-12}$ erg. Similar values can be obtained by other model calculations [14], including the mean-field statistical mechanics of fluctuating hydrocarbon chains [15].

The aim of the present work was to develop a further method of measuring the membrane bending rigidity. Nearly straight sections of fluctuating membrane contours are recorded and Fourier analysed. They belong to extended single bilayers which seem to turn in semicircles from the top to the bottom of the sample cells. The materials investigated are EYPC and, for the first time, two other electrically neutral lipids abounding in biological membranes, namely dimyristoyl-phosphatidylethanolamine (DMPE) and digalactosyl-diacylglycerol (DGDG). Preliminary data on DGDG have been used in discussing the unbinding transition found by us with DGDG if the experiment was done in 0.1 M NaCl solution instead of pure water [16, 17]. Specifically, the enormous spread of the transition temperature was attributed to the large scatter of the measured rigidity. In order to check whether the rigidities of PE membranes depend on the experimental method, as seems true of lecithins, we also analyse the angular fluctuations of tubular vesicles of another cephalin, dilauroyl-phosphatidylethanolamine (DLPE).

Theory.

Since the cylindrical curvatures of the membranes investigated are very weak, it is probably sufficient in calculating the mean-square fluctuation amplitudes to consider a square piece of membrane of area A lying essentially in the (x, y) plane [18, 19]. The instantaneous shape of the membrane may be represented by the scalar field $u(x, y)$ describing the local displacement of the membrane in z direction. The displacement can be Fourier expanded in terms of real waves

$$u(\mathbf{r}) = \sum_{\mathbf{q}}' (a_{\mathbf{q}} \cos(\mathbf{q} \cdot \mathbf{r}) + b_{\mathbf{q}} \sin(\mathbf{q} \cdot \mathbf{r})) \quad (2)$$

where $\mathbf{q} = (q_x, q_y) = \frac{2\pi}{A^{1/2}}(m, n)$, with m and n being integers. The prime at \sum indicates that the summation has to be restricted to a half-plane so that pairs of opposite wave vectors are counted only once. The curvature elastic energy per unit area of fluid membrane may be written as

$$g_c = \frac{1}{2} k_c (c_1 + c_2 - c_0)^2 + \bar{k}_c c_1 c_2 \quad (3)$$

where c_1 and c_2 are the principal curvatures. The spontaneous curvature c_0 vanishes if the bilayer is symmetric as will be assumed in the following. The last term can be omitted since

the integral of Gaussian curvature depends only on the genus of the surface. For the weakly deformed membrane, i.e. $|\nabla u| \ll 1$, we may express the sum of curvatures by

$$c_1 + c_2 = - \left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2} \right) = \sum_{\mathbf{q}} ' q^2 [(a_{\mathbf{q}} \cos(\mathbf{q} \cdot \mathbf{r}) + b_{\mathbf{q}} \sin(\mathbf{q} \cdot \mathbf{r}))]. \quad (4)$$

The curvature elastic energy associated with a pair of modes is then readily seen to be

$$\frac{1}{4} A k_c q^4 (a_{\mathbf{q}}^2 + b_{\mathbf{q}}^2). \quad (5)$$

In the presence of lateral tension σ , a further contribution to the deformational energy results from the fact that the thermal undulations absorb membrane area. The extra area per unit basal area being $\frac{1}{2} (\nabla u)^2$, for $|\nabla u| \ll 1$, one obtains as absorbed area per pair of modes

$$(\Delta A)_{\mathbf{q}} = \frac{1}{4} A q^2 (a_{\mathbf{q}}^2 + b_{\mathbf{q}}^2). \quad (6)$$

The addition of deformational energy

$$\sigma (\Delta A)_{\mathbf{q}}. \quad (7)$$

is taken into account in calculating the mean-square fluctuation amplitudes from the equipartition theorem. The mean energy of deformation per pair of modes being $k_B T$, one has because of (5) to (7)

$$\langle a_{\mathbf{q}}^2 \rangle = \langle b_{\mathbf{q}}^2 \rangle = \frac{2 k_B T}{A (q^4 k_c + q^2 \sigma)}. \quad (8)$$

Let the x axis be in the focal plane of the microscope and the y axis coincide with the direction of viewing. We then see a cut through the membrane in the x, z plane and along the x axis. To calculate the mean squares of the fluctuation Fourier amplitudes A_{q_x} and B_{q_x} of this line, we have to sum over all q_y at fixed q_x . Replacing the sum by an integral,

$$\sum_{q_y} \rightarrow \frac{A^{1/2}}{2\pi} \int_{-\infty}^{+\infty} dq_y, \quad (9)$$

and using (8), we find

$$\begin{aligned} \langle A_{q_x}^2 \rangle &= \langle B_{q_x}^2 \rangle = \frac{A^{1/2}}{2\pi} \int_{-\infty}^{+\infty} \langle a_{\mathbf{q}}^2 \rangle dq_y \\ &= \frac{k_B T}{A^{1/2} \sigma q_x} - \frac{k_B T}{A^{1/2} \sigma q_x (1 + \sigma / q_x^2 k_c)^{1/2}}. \end{aligned} \quad (10)$$

For low lateral tensions $\sigma \ll q_x^2 k_c$ this takes the simple form

$$\langle A_{q_x}^2 \rangle = \frac{k_B T}{2 A^{1/2} q_x^3 k_c}. \quad (11)$$

Equation (10) can also be derived directly and allows, in principle, an estimate of the lateral tension from its effect on low wave vector modes. We used equation (11) to calculate the bending rigidity k_c from the mean-square amplitudes.

The fluctuations of the square have been derived on the tacit assumption of periodic boundary conditions without discontinuities of u and ∇u . As in the experiments we see only a section of the total membrane, we have to check if we can still use (11) to calculate k_c . For this purpose, we examine the contributions of all the basic modes of the total membrane to the Fourier amplitudes of the membrane section seen at $y = 0$ in a window of width L . The total membrane area A is now imagined to be infinite. If the visible section of membrane contour is expressed by

$$u(x) = \sum_{n \geq 0} (A_n \cos(k_n \cdot x) + B_n \sin(k_n \cdot x)). \tag{12}$$

For $x = L/2$ to $x = +L/2$, the amplitude A_n is given by

$$A_n = \frac{2}{L} \int_{-L/2}^{+L/2} \cos(k_n \cdot x) \cdot u(x, 0) dx. \tag{13}$$

Insertion of (2) in (13) yields

$$\langle A_n^2 \rangle = \sum_q \langle a_q^2 \rangle \left[\frac{2}{L} \int_{-L/2}^{+L/2} \cos(k_n \cdot x) \cdot \cos(q_x \cdot x) dx \right]^2. \tag{14}$$

The integral of $\cos(k_n \cdot x) \cdot \sin(q_x \cdot x)$ cancels in the given interval and has therefore been omitted in (14). Replacing the sum over the basic modes by a double integral and integrating over q_y as in (11) results in

$$\langle A_n^2 \rangle = \frac{k_B T}{2 \pi k_c} \int_{-\infty}^{+\infty} \left[\frac{2}{L} \int_{+L/2}^{+L/2} \cos(k_n \cdot x) \cdot \cos(q_x \cdot x) dx \right]^2 \frac{dq_x}{q_x^3}. \tag{15}$$

An analogous expression with sines replacing cosines is obtained for $\langle B_n^2 \rangle$. Figure 1 shows how the basic modes contribute to $\langle A_n^2 \rangle$ of the window mode $n = 5$. The main effect comes from the interval $k_n = 2 \pi / L < q_x < k_n + 2 \pi / L$. The lower q_x modes contribute 22 %, the higher modes less than 3 % as much as the main peak. In accordance with the analysis of

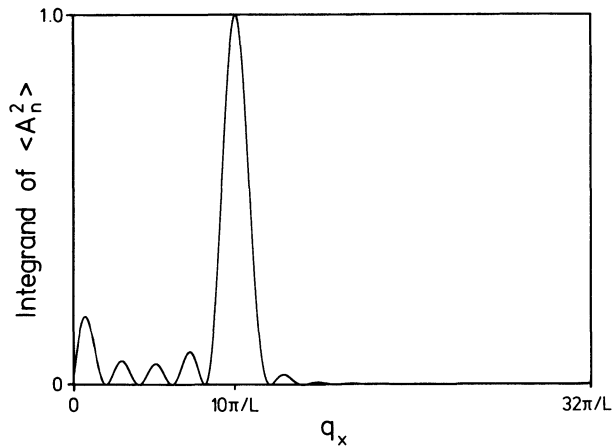


Fig. 1. — The integrand of equation (15) for $n = 5$, representing the contributions of the basic modes to the mean square amplitude $\langle A_n^2 \rangle$ of the window mode as a function of the wave vector component q_x of the former. The maximum is normalized to unity.

experimental contours, the effect of the longest wavelength modes is reduced by subtracting a symmetric parabola from $\cos(q_x \cdot x)$. The parabola is taken to coincide with the endpoints of the cosine and determined by the method of least squares. The subtraction of the parabolas results in the curve plotted in figure 2. The effect of the longest wavelength modes is seen to be markedly reduced and the total contribution of all side peaks is now only 13 % of that of the main peak. In the case of $\langle B_n^2 \rangle$, a straight line coinciding with the endpoints of the $\sin(q_x \cdot x)$ is subtracted from the sine, again in agreement with the analysis of the experimental data. After this subtraction, which is even more necessary than the other, the side peaks contribute to $\langle B_n^2 \rangle$ is only ca. 8.5 % as much as the main peak. Side peak contributions are about equally important for $n = 10$ as for $n = 5$, both with $\langle A_n^2 \rangle$ and $\langle B_n^2 \rangle$. The modified integral (15) turns out to be practically identical to $\langle A_{q_x}^2 \rangle$ as given by (11) for the same wave vector, being smaller by only 1 and 2 % for $\langle A_n^2 \rangle$ and $\langle B_n^2 \rangle$, respectively.

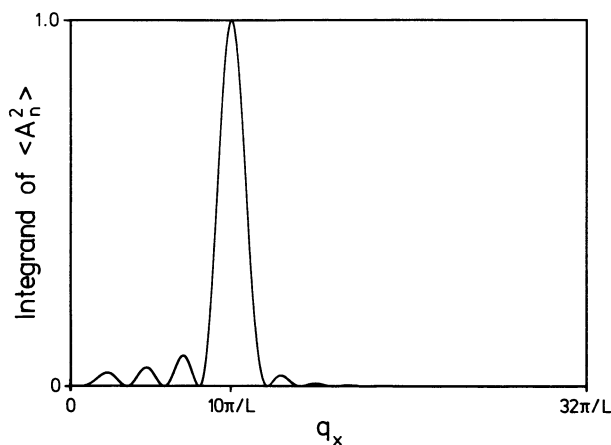


Fig. 2. — The integrand of equation (15) for $n = 5$, modified by subtracting the parabola.

Materials and methods.

Dilauroyl-phosphatidylethanolamine (DLPE), dimyristoyl-phosphatidylethanolamine (DMPE), egg-yolk-phosphatidylcholine (EYPC) and digalactosyldiacylglycerol (DGDG) extracted from wheat flour were obtained from Sigma (Munich). The glycolipid DGDG, again extracted from wheat flour, was also purchased from Serva (Heidelberg).

The materials were used without further purification. The purity of DGDG claimed by the distributor was 95 % (Sigma) and 99 + % (Serva). We checked the purity by thin-layer chromatography on silica gel 60F. The runs were performed in a mixture of chloroform, methanol, and water (65 : 65 : 2.5). DLPE, DMPE and DGDG showed a single spot when developed with iodine vapour. In view of the sensitivity of the procedure, impurity concentrations larger than 1 % can be excluded. We dissolved the lipids in chloroform (10 mg/ml) and stored them below -20°C .

A small amount (some μl) of the chloroform solution was spread on a glass slide and left overnight to evaporate under vacuum. Subsequently 40 μl of ion depleted water from an ion exchanger (Seradest) of pH 5.5-6.0 were added. A cover slip was put on top and the approximately 40 μm high cell was sealed to prevent evaporation. All the lipid was close to the bottom slide at the beginning of swelling. All four lipids swelled spontaneously, forming vesicles and other highly dilute structures, if their bilayers were in the fluid state. EYPC and

DGDG are fluid at room temperature [20, 21]. When heated in excess water, DLPE and DMPE undergo their main transitions at 44 °C and 53 °C, respectively, so that the experiments had to be performed above these temperatures [23]. After several hours of swelling we started to look for extended membrane contours with straight sections and tubular vesicles. The former belonged to huge sheets apparently going semicylindrically from the bottom to the top of the cell. They were seen in the middle plane of some of the samples.

The samples were observed with a phase contrast microscope (Leitz), the numerical aperture of the objective (PH 40×) being 0.75. The object stage could be heated to the desired temperatures electrically or by connecting it to an external thermostated water bath (Haake). Membranes are seen in the focal plane where they are parallel to the optical axis of the microscope. The depth of field was $\pm 1 \mu\text{m}$.

An image analysis system (Imaging-Technology Inc., ITI) with videocamera (Grundig) and PDP-11 computer (DEC) were attached to the microscope. It allowed us to store an image in a frame buffer, composed of 512×512 pixels with a 6-bit resolution for the grey levels and 1 pixel corresponding to about 135 nm. The sampling time of the video images was 40 ms. The contour of the membrane was then determined by a special finding algorithm.

After being oriented horizontally, the length of the straight section of membrane contour within a frame was about 70 μm . In many cases the membranes were not quite horizontal and slightly curved. We eliminated gradient and curvature by subtracting a generalized parabola consisting of a symmetric parabola and a uniform slope. Its ends coinciding with those of the contour, the parabola was determined for every contour with the method of least squares. This procedure has the advantage of reducing the contribution of long wavelength modes of the total membrane to the window mode, as was shown in the theoretical calculation of the mean-square amplitudes. We used a fast Fourier algorithm to calculate the window mode amplitudes from the adjusted contours, after testing it by simulation. The number of pictures entering into each calculation of the mean square amplitudes was in general 200 and never less than 100. The time intervals of more than 10 sec between pictures were longer than the relaxation times of the slowest modes.

Results.

An example of a DGDG membrane showing typical fluctuations is displayed in figure 3. We selected unilamellar structures by looking for contours of lowest optical contrast. Single membranes gave the lowest bending rigidities, but in the cases of EYPC and DGDG the spread between maximum and minimum values was still a factor of two. We analysed only membranes which were fluctuating freely and not attached to other membranes or vesicles, which diminished strongly their number. In the experiments with DMPE particular problems arose from the fact that to keep the bilayers in the fluid phase the measurements were performed at $T = 60 \text{ }^\circ\text{C}$. At this high temperature convection currents in the samples sometimes caused a distortion of the membrane contour.

A typical result of the computerized analysis for a DGDG membrane is shown in figure 4. The bending rigidity k_c was calculated by means of equation (11) from the average of the mean square Fourier amplitudes $\langle A_n^2 \rangle$ and $\langle B_n^2 \rangle$. The calculated bending rigidity k_c forms a plateau up to the 12th mode and then starts to decrease. However, as indicated by apparently higher values of k_c , the first two modes are considerably weakened because of geometric constraints (see below). The average of the values in the plateau was taken to be the definitive value of the bending rigidity.

Table I summarizes the results for the three different lipids investigated with this method which were obtained from 10 DGDG, 11 EYPC and 10 DMPE membranes. The standard

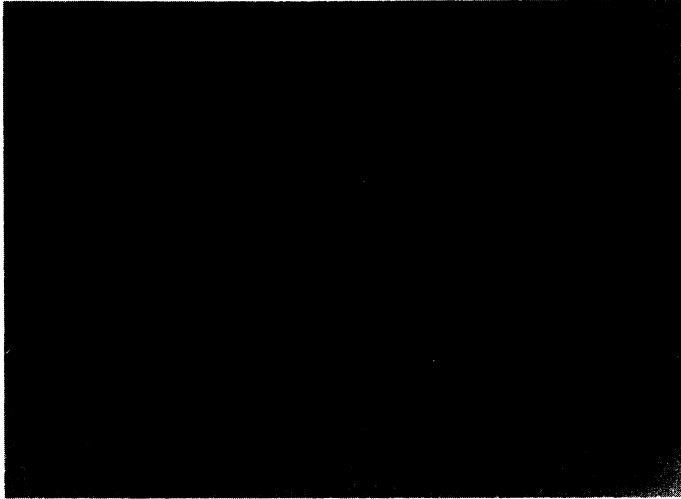


Fig. 3. — Typical fluctuations of extended DGDG membrane (the bar represents 10 μm).

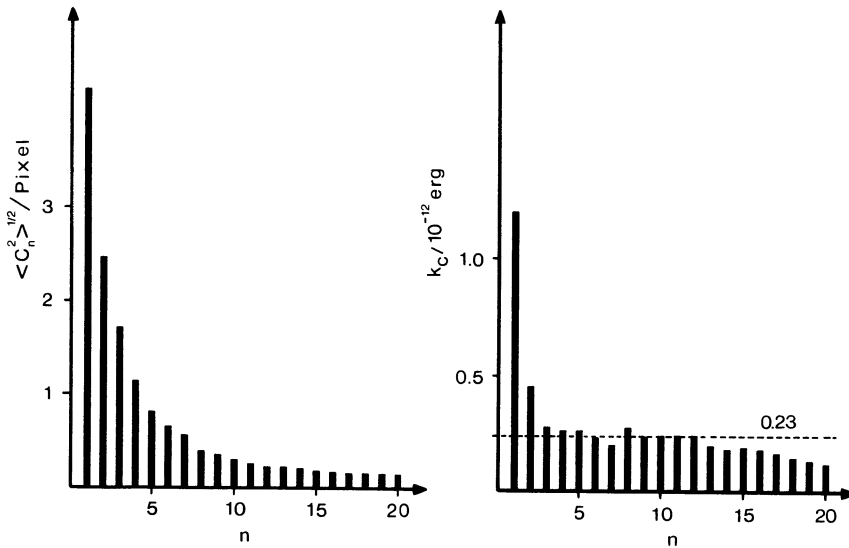


Fig. 4. — Results of the computerized Fourier analysis for a DGDG membrane. Average $\langle C_n^2 \rangle = \frac{1}{2} (\langle A_n^2 \rangle + \langle B_n^2 \rangle)$ of mean-square function amplitudes (left) and rigidities calculated according to (11) (right). The dotted line represents the plateau from where the final value of k_c is calculated.

deviations of k_c for the individual bilayer were less than 15 % for all lipids. However, the standard deviations of the individual values from the mean value for one material were 32 % for DGDG, 25 % for EYPC, and 14 % for DMPE. We also measured the apparent bending rigidities of a few multilamellar contours and obtained multiples of k_c .

DGDG membranes sometimes showed very strong undulations which could not be analysed because parts of the membrane contour were smeared. Figure 5 exhibits such an example. Smearing seems to suggest bending rigidities even lower than 0.12×10^{-12} erg.

Table I. — *Averages and standard deviations of the final values of the bending rigidities as obtained from the undulations of extended membranes.*

lipid	$k_c/10^{-12}$ erg
DGDG	$0.2 \pm 32 \%$
DMPE ($T = 60^\circ$)	$0.7 \pm 14 \%$
EYPC	$0.8 \pm 25 \%$

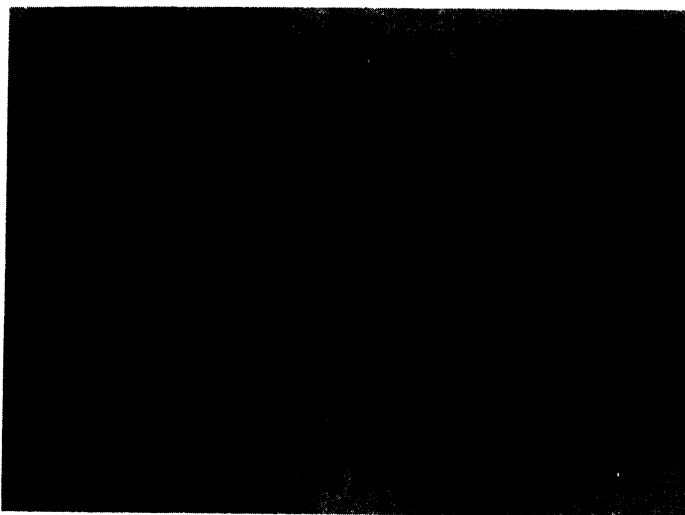


Fig. 5. — DGDG membrane contour smeared in part by very strong undulations (the bar represents $10 \mu\text{m}$).

There was no notable difference between the DGDG from Sigma and that from Serva.

We did not find any tubular vesicles of DGDG and DMPE that were suitable for measuring the distribution function of the angle made by their ends. On the other hand, the swelling of DLPE, which differs from DMPE only by two carbons less in the saturated hydrocarbon chains, produced some suitable tubes but no extended membranes. The measurement of the angular distribution function and the calculation of the bending rigidity from its width have been described elsewhere [1, 2]. The results of our experiments, three with unilamellar and two with multilamellar tubes, are listed in table II. Note that the average bending rigidity obtained with unilamellar DLPE tubes, $k_c = 1.7 \times 10^{-12}$ erg, is more than two times larger than that obtained with extended DMPE membranes.

Discussion.

We have measured the bending rigidity k_c for three different types of lipids. Besides the often studied egg lecithin, we determined k_c also for two synthetic cephalins (DLPE and DMPE) and a natural galactolipid (DGDG). Our new method is similar to the expansion in spherical harmonics used in determining the bending rigidity from the fluctuations of spherical vesicles.

Table II. — *Bending rigidities of individual DLPE membranes and groups of such membranes at $T = 46^\circ\text{C}$ as obtained from the distribution of angular fluctuations of tubular vesicles. The three values with the lowest k_c belong to unilamellar tubes.*

$k_c/10^{-12}$ erg	DLPE (46°C)
1.88 ± 0.20	unilamellar average 1.70 ± 0.26
1.35 ± 0.35	
1.82 ± 0.14	
3.7 ± 0.9	probably bilamellar
5.7 ± 1.1	probably trilamellar

Instead of complete spherical vesicles we studied nearly planar membrane sections which may be regarded as part of a huge vesicle.

Contours with long practically straight sections should be characteristic of vanishing lateral tensions, especially in the frequent case of several of them running parallel. Tensions tend to be associated with circular contours which are often grouped in onion-like structures, as has first been noted with EYPC [24]. The long wavelength fluctuations of our extended membranes were not fully developed. Rather than to lateral tension this seemed to be due to the constraints imposed by the top and bottom of the sample cell where the membranes forming semicylinders in between, are in contact with other membranes or the glass slides. We think that the absence of lateral tension, which can be positive or negative in the case of quasi spherical vesicles, as well as the large radius of the semicylinders of ca. $20\ \mu\text{m}$, are among the advantages of the new method. Also, a possible deformation of the membranes by gravity would not be serious and could be checked by carefully measuring the height of the membrane « equator » where one sees the contour. Many of the extended membranes are enclosed by others and thus shielded from impurities dissolving from the glass slides. Finally, the extended membranes remain in place for long periods, while spherical vesicles must be free to float vertically and horizontally. A disadvantage of the new method is the fact that it mixes modes of different wave vectors, fixing q_x but integrating over q_y , while the wave vectors or, more precisely, angular momenta can be separated in the case of spheres [9, 10]. However, because of the $1/q^4$ dependence of mean square amplitudes most of the contributions to $\langle A_{q_x}^2 \rangle$ and $\langle B_{q_x}^2 \rangle$ should come from basic modes with wave vectors not much larger than q_x . An additional mixing of modes stems from the subsequent integration over q_x as was shown above.

The admixture of other wave vectors also mixes relaxation times and makes it more difficult to deal with the effect of the video integration time which is 40 ms [6, 10]. The long integration time reduces in effect the amplitudes of the high wave vector modes. However, in our experiments the reduction was in general more than compensated by white noise. Limiting ourselves to the plateau modes, we disregard both effects. Estimates of the relaxation times [25] and the direct observation of fluctuations suggest that relaxation can be neglected up to $n = 8$ for EYPC and $n = 15$ for DGDG. Faucon *et al.* [10] corrected for lateral tension, white noise, and video integration time, finding $k_c = (0.4 - 0.5) \times 10^{-12}$ erg for egg lecithin. We doubt that the omission of these effects explains why we obtained a larger value. The large spread of the bending rigidity of DGDG membranes may be the reason for the enormous variation of their unbinding temperature in salt solution [16, 17].

We have used two methods to measure the bending rigidities of cephalines (PE's), obtaining $k_c = 0.7 \times 10^{-12}$ erg for DMPE from the undulations of extended membranes and $k_c = 1.7 \times 10^{-12}$ erg for DLPE from the angular fluctuations of tubular vesicles. The difference between the two results by factor of two to three is surprising as DMPE differs from DLPE only by two additional CH_2 groups in the hydrocarbon chains. The bilayer thickness b of DLPE is known to be 41.0 \AA [26] and from other data [27] one can estimate $b = 42.5 \text{ \AA}$ for DMPE. The simple model underlying equation (1) predicts the bending rigidity to vary as the cube of the bilayer thickness b if the stretching modulus λ is assumed to be proportional to b . On the basis of this model the bending rigidity should be little larger, not much smaller, for DMPE than for DLPE. The difference is also unlikely to result from the effect of a higher temperature ($\Delta T = 14 \text{ K}$) on chain flexibility and bilayer thickness. Rather, it seems that the bending rigidity depends on the method by which it is measured. The same discrepancy between methods appears to exist in the case of EYPC. Servuss *et al.* [1] and Bebluck *et al.* [2] obtained $k_c = (1.8 - 2.3) \times 10^{-12}$ erg from the bending fluctuations of tubular vesicles, while we derive $k_c = 0.8 \times 10^{-12}$ erg from the undulations of extended membranes.

Evans and coworkers [13], who measured the membrane stretching modulus to be 140 dyn cm^{-1} for EYPC and DMPC, found $\lambda = 190 \text{ dyn cm}^{-1}$ for DGDG. Their data on mixtures of dipalmitoyl-oleoyl-PE and SOPC suggest $\lambda = 240 \text{ dyn cm}^{-1}$ for the pure PE. The thicknesses of all the common biological model membranes are of the order of 40 \AA . Experimental values for PE's have been quoted above; 42 \AA [21] and 36 \AA [22] have been reported for DGPD and EYPC, respectively. Accordingly, one might infer on the basis of equation (1) that the bending rigidities should all fall between 0.5 and 1×10^{-12} erg. The considerable deviations from this narrow range of values are difficult to understand. Equally puzzling is the dependence of the results on the experimental method (and the wide spread of the rigidities measured with the same method). It seems difficult to imagine that the large uncertainties result from an extreme sensitivity to impurities.

All the membranes investigated in the present work separate by themselves in pure water but display mutual adhesion when under lateral tension. Detailed studies of the tension-induced adhesion of egg lecithin bilayers [28, 29] point to a submicroscopic roughness of these membranes much larger than that produced by the usual thermal undulations. One may speculate that such a roughness, if it also exists in the bilayers of the other lipids, might be the origin of some of the inconsistencies.

Conclusion.

The present work shows again that the bending rigidities of electrically neutral biological model membranes are rather elusive quantities. There remains the challenge of determining the true values which may be a function of wave vector and geometry. So far, most of the experiments have been done with the natural materials, especially egg lecithin. Both egg lecithin and wheat flour digalactosyl-diacylglycerol are fluid at all practical temperatures and, moreover, swell very readily to give large unilamellar structures. However, they are likely to differ from batch to batch. Small variation in composition may produce large differences in behavior, if the latter is subject to defects or other singularities in the bilayer. To eliminate extrinsic sources of potential scatter it will be necessary in the future to do more measurements of the bending rigidities of one-component lipids.

A practical problem which remains to be addressed has to do with optical observation. The finite depth of field of $\pm 1 \text{ \mu m}$ may cause a shift of the membrane contour seen under the microscope to positions deviating from the planar cross section. This is because the membrane is seen particularly well where it is exactly parallel to the direction of viewing. Such a shift

produces an additional noise which may result in a too small bending rigidity if it is not taken into account. Clearly, the effect is the less important the larger the investigated objects and fluctuation wavelengths are.

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