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Invited Lecture. Hats and saddles in lipid membranes

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Invited Lecture

Hats and saddles in lipid membranes

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Extrinsic or intrinsic defects in membranes may produce deformations resembling hats or saddles. It is shown that hats cannot absorb much area without reducing the bending rigidity of the membrane below 10^{-12} erg. Estimates indicate that intrinsic saddles are promoted by the stress profile of electrically neutral biological model membranes. The saddle structures might warp the membrane if they cooperate.

1. Introduction

Fluid lipid bilayers are normally regarded as smooth surfaces deviating from an approximately planar configuration only through their thermal undulations. This applies in particular to the rather stiff biological model membranes made of the phospholipids and glycolipids occurring abundantly in animal and vegetable cell membranes. However, it was noted some time ago by Gruler [1] that cone-like objects represented by proteins may produce conical deformations in otherwise flat bilayers. More recently, Leibler [2] contemplated theoretically a curvature instability of membranes which may arise if conical objects interact attractively with each other.

Today there is reason to believe that three classes of biological model membranes phosphatidylcholines [3], phosphotidylethanolamines [4] and digalactosyldiglycerides [5], are much rougher than they appear under the light microscope. A number of measurements suggest that the ratio of real to visible membrane area is substantially larger than that expected on the basis of their undulations. Any additional area would have to be accommodated by an as yet unknown submicroscopic roughness.

It is therefore of interest to examine the deformations associated with extrinsic and intrinsic defects in the membrane. The word defect is employed here in a very broad sense. It includes extraneous molecules, structural defects and regions of very high curvature whose formation is significantly promoted by a breakdown of regular elasticity. Considering disturbances of the surface that resemble a hat or a saddle, we discuss which defects they need for their formation and how they affect membrane properties. Before that, thermal undulations are newly treated in terms of bending fluctuations of slightly curved discs. The new description, which gives the same results as the standard description, is advantageous in dealing with hats. We also look into the possibility of extending the concept of hats and saddles to lyotropic and thermotropic smectic liquid crystals.

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2. Slightly curved discs

The curvature-elastic energy per unit area of a symmetric fluid membrane may be written as [6]

$$g = \tfrac{1}{2}\kappa(c_1 + c_2)^2 + \bar{\kappa}c_1c_2, \tag{1}$$

where c_1 and c_2 are the principal curvatures (inverse principal radii of curvature), κ is the bending rigidity and $\bar{\kappa}$ the elastic modulus of Gaussian curvature. We use as abbreviations two classical denotations of differential geometry: mean curvature

$$H = \tfrac{1}{2}(c_1 + c_2) \tag{2}$$

and Gaussian curvature

$$K = c_1c_2. \tag{3}$$

The second term in equation (1) is generally omitted in dealing with bending fluctuations, since the integral of K depends only on the genus of the surface (Gauss–Bonnet theorem).

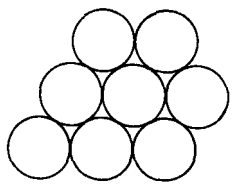

Figure 1. Artificial division of nearly flat membrane into discs as seen from above.

Thermal undulations are usually analysed by means of a Fourier expansion of the displacement $u = u(x, y)$ of the membrane from the xy plane [7]. The modes are coupled with respect to their bending energies if everywhere $|\text{grad}\, u| \ll 1$. Here we take an entirely different route. The membrane is mentally divided into pieces, e.g. a lattice of hexagons or, approximatively, circles as shown in figure 1. (The construction helps our reasoning; it is not really possible on a curved surface.) The discs are characterized by a radius r_d and a uniform mean curvature H_d fluctuating around zero. In the absence of Gaussian curvature the disc is identical to a segment of a sphere. Its mean curvature can be expressed through the polar angle ϑ_d of its edge and its radius r_d:

$$H_d = \vartheta_d/r_d \tag{4}$$

provided $|\vartheta_d| \ll 1$. We assume that any given disc produces only Gaussian curvature in the rest of the membrane (see below for details). The equipartition theorem for the single disc is then

$$\tfrac{1}{2}\kappa\langle(2H_d)^2\rangle\pi r_d^2 = \tfrac{1}{2}k_BT, \tag{5}$$

where k_B is Boltzmann's constant and T is temperature. Because of equation (4) we may rewrite this in the form

$$\langle\vartheta_d^2\rangle = k_BT/4\pi\kappa. \tag{6}$$

It is possible, as long as $|\text{grad}\, u| \ll 1$, to do a Fourier expansion of $2H$ in sinusoidal waves such as $c_q \cos(q_x x, q_y y)$. With the usual periodic boundary conditions and

observing $q < 1/r_d$, one obtains from statistical mechanics

$$\langle c_q^2 \rangle = 2\langle (2H_d)^2 \rangle \frac{1}{N_d}, \tag{7}$$

where N_d is the total number of discs. Insertion of equations (5) and (6) and introducing the total membrane area

$$A = N_d \pi r_d^2 \tag{8}$$

lead to

$$\tfrac{1}{4}\kappa \langle c_q^2 \rangle A = \tfrac{1}{2} k_B T. \tag{9}$$

This is a familiar form of the equipartition theorem, especially with the substitution $c_q = q^2 u_q$, which confirms the validity of the model starting from local fluctuations.

Equation (6) relates the edge angle of the disc to the stiffness of the membrane. For the bending rigidity $\kappa = 1 \times 10^{-12}$ erg, which seems to be typical of biological model membranes [5, 8, 9], and $k_B T = 4 \times 10^{-14}$ erg (room temperature) one obtains $(\langle \vartheta_d^2 \rangle)^{1/2} = 3{\cdot}2°$. It is interesting to note that we are free, in this particular calculation, to choose for the disc any size above the molecular cross section. This demonstrates the well-known scale invariance of the thermal undulations of fluid membranes.

We wish to calculate the membrane area absorbed by fluctuations in the model of curved discs. The ratio of real to projected area is larger than unity whenever the membrane, thought to be unstretchable, is not parallel to its basal plane. The local value of the ratio may be expressed by

$$\left(\frac{A_{real}}{A_{proj}} \right)_{local} = 1 + \tfrac{1}{2}(\text{grad}\, u)^2 \tag{9}$$

as long as $|\text{grad}\, u| \ll 1$. In order to derive the absorbed area associated with a single disc we may start from a formula for axisymmetric shapes with vanishing bending energy, i.e. with $H = 0$, which was derived earlier [10],

$$u = u_0 - r_0 \ln \{r/r_0 + [(r/r_0)^2 - 1)]^{1/2}\}, \tag{10}$$

where u is the displacement in the z direction and r the radius. The height is $u = u_0$ at $r = r_0$ where the membrane turns over, making an angle of 90° with the xy plane. It follows from the general solution (10) that we may write, for $r \geqslant r_d$,

$$\frac{du}{dr} = \vartheta_d \frac{r_d}{r}, \tag{11}$$

which is exact if $|\vartheta_d| \ll 1$. (It may also be seen directly that equation (11) satisfies $H = 0$ sufficiently well.) Apart from the contribution of the disc itself, one has for the mean extra area ΔA_d associated with the disc

$$\Delta A_d = \int_{r_d}^{R} \frac{1}{2} \left(\frac{du}{dr} \right)^2 2\pi r\, dr = \pi \langle \vartheta_d^2 \rangle r_d^2 \ln \frac{R}{r_d}. \tag{12}$$

Like u of equation (10), ΔA_d diverges logarithmically with the outer radius R which serves as a measure for the size of the system. Employing superposition to obtain the total area ΔA absorbed by bending fluctuations, one finds because of equation (6) for the relative increase

$$\frac{\Delta A}{A} = \frac{\Delta A_d}{A_d} = \frac{k_B T}{4\pi\kappa} \ln \frac{R}{r_d}. \tag{13}$$

Again, the formula is identical to that calculated with the standard method [7]. For an estimate we use in addition to the above numbers $r_d = 1\,\text{nm}$ and $R = 10\,\mu\text{m}$, which are a molecular length and a typical vesicle size, respectively. The result is $\Delta A/A = 0{\cdot}03$.

3. Hats

Bending fluctuations of a membrane may also result from conical defects. Let us consider the idealized case where the latter are the only origin of curvature. Extrinsic defects of any desired type may be provided by suitable proteins and other biomolecules embedded in one or both of the monolayers. Intrinsic defects are not so easy to imagine, but are required in a membrane consisting of a single species of molecules. In connection with hats, one can think of thermal pores with a tendency to form funnels [11, 12]. Alternatively, there could be a breakdown of regular bending elasticity at very high spherical curvature. It may be expressible by additional terms of the bending elastic energy which are of higher than quadratic order in the curvature [13]. The defects should not be so dominant as to destroy the planar membrane, e.g. by transforming it into small vesicles. Accordingly, we prefer the idea of thermally excited hats whose energy is much lower than expected on the basis of equation (1), but definitely positive.

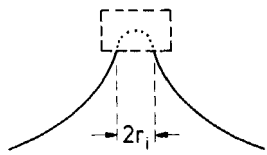

Figure 2. Cross section of an axisymmetric hat of inner radius r_i. The broken box frames the (unknown) central part.

A single conical defect will produce a hat as sketched in figure 2. The top of the hat, being controlled by the defect, is considered a 'black box' and ignored in the following. Outside the inner radius r_i of the hat, the membrane contour satisfies equation (10). The outer radius r_h of the hat is defined by

$$\pi r_h^2 = A/N_h, \tag{14}$$

where N_h is the total number of hats. We choose this definition because we expect the collection of hats to be similar in some respects to an area-filling system of the same number of slightly curved discs. The tilt angle φ of the membrane in the presence of a single hat obeys, because of equation (10),

$$\varphi = \varphi_i r_i / r, \tag{15}$$

where φ_i is the angle at r_i. In this approximation (which becomes poor if $|\varphi_i| \geqslant 1$) the edge angle of the discs belonging to the hats is obviously given by

$$\langle \vartheta_h^2 \rangle = \vartheta_h^2 = \varphi_i^2 (r_i/r_h)^2 \tag{16}$$

If the hats are free to move and point upwards and downwards with equal probability, they make the membrane in effect flexible. Treating them as discs, we write down the inverse of the defect-mediated flexibility which is a rigidity

$$\kappa_{\text{def}} = \frac{k_B T r_h^2}{4\pi \varphi_i^2 r_i^2}. \tag{17}$$

If the membrane is flexible by itself with some rigidity, the total rigidity is, of course, calculated by adding flexibilities. According to equation (13) the membrane area absorbed by a single hat is given by

$$\Delta A_h = \pi \varphi_i^2 r_i^2 \ln \frac{R}{r_i}, \tag{18}$$

which implies for a system of hats the relative increase

$$\frac{\Delta A}{A} = \frac{\Delta A_h}{A_h} = \varphi_i^2 \frac{r_i^2}{r_h^2} \ln \frac{R}{r_i}. \tag{19}$$

In order to have a large relative increase, say $\Delta A/A = 0{\cdot}3$, we start from $\varphi_i = 1$, which seems to be a kind of maximum. Equation (19) and $\ln R/r_i = 10$ (see above) then require $r_h^2/r_i^2 = 30$. Inserting this ratio, $|\varphi_i| = 1$, and $k_B T = 4 \times 10^{-14}$ erg into equation (17) results in $\kappa_{def} = 1 \times 10^{-13}$ erg. It may also be generally seen by eliminating $\varphi_i^2 r_i^2 / r_h^2$ between equations (17) and (19) that any substantial increase of $\Delta A/A$ above the value of 0·03 estimated for undulations would be linked with a corresponding decrease of the total rigidity below 1×10^{-12} erg. This rules out hats as a mechanism to absorb much area in membranes of this rigidity. From a general point of view, however, it is interesting to note that relatively small concentrations of hats can lower the rigidity quite effectively. An estimate based on equation (17) and employing $r_i = 10^{-6}$ cm (the minimum radius of sonicated vesicles), $\varphi_i = 1$, and $\kappa_{def} = 10^{-12}$ erg yields $A_h = \pi r_h^2 = 10^{-9}$ cm^2.

4. Saddles

Saddles caused or promoted by defects and the deformation of their surroundings are difficult to deal with. We can treat them only by way of rather crude estimates. However, saddle structures are potentially very interesting because of their mutual interaction. Saddles could be created by sufficiently large saddle-shaped molecules residing in the membrane. Here we focus on a possible intrinsic mechanism which promotes saddle curvature in fluid membranes of a certain type. A single saddle structure embedded in a flat membrane is sketched in figure 3. The central part is a region of membrane with mostly saddle curvature, i.e. negative Gaussian curvature. For pure saddle curvature we have $K = -c^2$, the principal curvatures being $c_1 = -c_2 = c$. According to the theory of minimal surfaces, pure saddle curvature cannot be constant over extended areas. The formation of the saddle should release a substantial part of the energy needed to form the necessary highs and lows which, in contrast to the centre, have a distinct mean curvature.

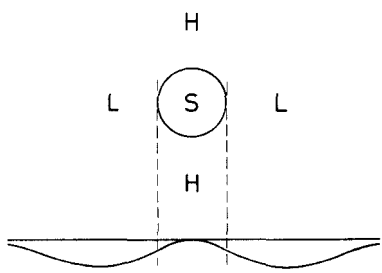

Figure 3. Saddle structure consisting of a central saddle (S), two highs (H) and two lows (L). Top view and cross section through the lows.

The negative deformational energy cannot come from a positive $\bar{\kappa}$ since negative Gaussian curvature in one region is compensated by positive Gaussian curvature elsewhere. However, one may find the desired negative energy by considering the modulus of K^2, i.e. of a fourth power of curvatures, in the elastic energy of membranes with $\bar{\kappa} > 0$. (All of the fourth order terms were given some time ago by Mitov [13].) In order to show this, we start from

$$\bar{\kappa} = \int s(z)\, z^2\, dz, \tag{20}$$

which equates the modulus of Gaussian curvature to the second moment of the stress profile of the membrane [14]. Being the normal stress (dyn cm^{-2}) along a vertical cut through the membrane, $s(z)$ is isotropic in the plane of a fluid membrane. The zeroth and the first moments are assumed to vanish because of equilibrium and symmetry, respectively. It is convenient in the following calculations to have the xy plane locally coincide with the middle surface of the membrane.

The physical meaning of equation (20) is easy to understand: negative Gaussian curvature $K = -c^2$ produces a relative decrease of the membrane area at the height $\pm z$ by $c^2 z^2$ so that $\bar{\kappa}K$ represents the integrated work done against the stresses of the flat membrane. Any horizontal contraction or dilatation of the membrane as a whole to minimize deformational energy contributes in higher order of K (see below).

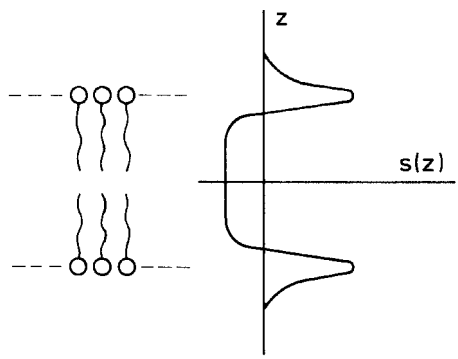

Figure 4. Most widely accepted type of stress profile of electrically neutral biological model membranes.

The stress profile to be expected for electrically neutral biological model membranes such as those initially listed is shown schematically in figure 4. It is characterized by pull in the region of the polar heads, where the interfacial tension between oil and water acts, and by push in the region of the hydrocarbon chains, which are preferentially aligned parallel to the layer normal. A pure saddle curvature $(-K) = c^2$ is likely to thicken the membrane, since the bulk density of the lipid should be practically constant under bending deformations. In order to calculate the thickening we take, at least for the moment, the middle surface of the membrane to be the area of inextension as in figure 5. If the lipid density is indeed constant, we have to move mass originally at $\pm z$ to $\pm \tilde{z}$ according to

$$\tilde{z}(1 + \tfrac{1}{3}K\tilde{z}^2) = z, \tag{21}$$

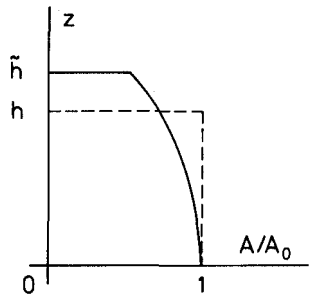

Figure 5. The effect of pure saddle curvature $(-K)$ on the thickness h and the relative cross section $A(z)/A_0$ of the upper monolayer. The broken line represents the undeformed state before deformation. In the complete model A_0 may also depend on K (see text).

Solving for $\tilde{z}$ and expanding to first order in K gives

$$\tilde{z} = (1 - \tfrac{1}{3}Kz^2)z \tag{22}$$

and

$$\tilde{z}^2 = (1 - \tfrac{2}{3}Kz^2)z^2. \tag{23}$$

The physical normal stress $\tilde{s}(z, K)$ in the deformed membrane may be expressed by a fictitious stress $s(z, K)$ through

$$\tilde{s}(z, K)\,\mathrm{d}\tilde{z} = s(z, K)\,\mathrm{d}z \tag{24}$$

and $s(z, K)$ expanded to first order in K

$$s(z, K) = s(z, 0) + \frac{\partial s(z, 0)}{\partial K}K, \tag{25}$$

where $s(z, 0) = s(z)$. The use of $s(z, K)$ means that force per length is associated with mass per area rather than with length $\Delta\tilde{z}$. The energy surface density $\bar{\kappa}K$ may now be replaced by

$$\int s(z)\tilde{z}^2 K\,\mathrm{d}z + \int_0^K\int \frac{\partial s(z, 0)}{\partial K}(z^2 - z_0^2)K'\,\mathrm{d}K'\,\mathrm{d}z + (\bar{\kappa}K)z_0^2K. \tag{26}$$

A possible overall dilatation compensating part of the compression due to $K < 0$ is introduced here. It is taken to be proportional to $(-K)$, so that surfaces of inextension are now at some $\pm z_0 \neq 0$. The last term corrects $\bar{\kappa}K$ for the change of middle surface area due to the dilatation. The argument is easily generalized to include $K > 0$ and compression.

Obviously, with stress profiles of the type shown in figure 4 the first term of equation (26) is more negative than $\bar{\kappa}K$ for pure saddle curvature $(-K) = c^2$. For an estimate of it, we assume the negative stress $s(z)$ in the interior of the membrane to be uniformly

$$s = -\gamma/h, \tag{27}$$

where h is the height of one monolayer, i.e. half the membrane thickness, and γ the tension at the oil–water interface. Expressing the surface tension by a δ-function, we

have for the first term of equation (26)

$$\int_{-h}^{+h} (-\gamma/h)\tilde{z}^2 K \, dz + 2\gamma\tilde{h}^2 K, \tag{28}$$

which because of equation (23) becomes

$$\begin{aligned}\int_{-h}^{+h} (-\gamma/h)z^2(1 - \tfrac{2}{3}z^2K)K \, dz + 2\gamma h^2(1 - \tfrac{2}{3}h^2K)K \\ = \tfrac{4}{3}\gamma h^2 K - \tfrac{16}{15}\gamma h^4 K^2 \\ = \bar{\kappa}K + \bar{\bar{\kappa}}_1 K^2.\end{aligned} \tag{29}$$

The last line serves to identify the modulus $\bar{\kappa}$ of Gaussian curvature and the contribution of the first term of equation (26) to the modulus $\bar{\bar{\kappa}}$ in front of K^2. With $\gamma = 50\,\mathrm{dyn\,cm^{-1}}$ and $h = 2\,\mathrm{nm}$ one arrives at $\bar{\kappa} = 3 \times 10^{-12}\,\mathrm{erg}$ and $\bar{\bar{\kappa}} = -9 \times 10^{-26}\,\mathrm{erg\,cm^2}$.

For a more complete estimate of $\bar{\bar{\kappa}}$, one may use a model of membrane elasticity introduced by Israelachvili *et al.* [15]. Starting from the expression

$$\mu = \gamma\left(a + \frac{a_0^2}{a}\right) + \mu_0 \tag{30}$$

for the Gibbs free energy of an amphiphilic molecule in a flat monolayer, where a is the molecular cross section and a_0 is its equilibrium value, they obtain for the stretching energy per unit area of monolayer

$$g_s^{\mathrm{mono}} = \tfrac{1}{2}(2\gamma)\left(\frac{a - a_0}{a_0}\right)^2. \tag{31}$$

The resulting theoretical stretching modulus of the bilayer, 4γ, agrees quite well with the values near $200\,\mathrm{dyn\,cm^{-1}}$ measured [16] for the biological model membranes listed above. On the basis of equation (31) it seems reasonable in the present context to use the Ansatz

$$\frac{\partial s(z, 0)}{\partial K} = \frac{2\gamma}{h} z^2 \tag{32}$$

permitting a second contribution to $\bar{\bar{\kappa}}$ to be immediately obtained, i.e.

$$\bar{\bar{\kappa}}_2 = \tfrac{2}{5}\gamma h^4, \tag{33}$$

from the z^2K' term of equation (26). In the following we take the total modulus $\bar{\bar{\kappa}}$ to be the sum of $\bar{\bar{\kappa}}_1$ and $\bar{\bar{\kappa}}_2$, so that

$$\bar{\bar{\kappa}} = -\tfrac{2}{3}\gamma h^4. \tag{34}$$

This may be regarded as a conservative formula, since the bending energy cannot rise if the constraint of inextension of the middle surface is lifted.

The sole purpose of the present estimate is to show that $\bar{\bar{\kappa}}$ should be negative for electrically neutral biological model membranes and to obtain an idea of its probable magnitude. It may seem attractive to use the same Ansatz (26) to derive the bending rigidity. Unfortunately, we find this procedure to yield a negative value of κ, which is not permissible and must be regarded as a warning against using any simple model uncritically. It may well be that the model of Israelachvili *et al.* [15] correctly describes uniform stretching and, as we assume, the stretching associated with Gaussian

curvature, whereas it does not account for most of the energy of mean curvature. Its original version with δ-functions at fixed heights for pull and push gives a bending rigidity of the order of $k_B T$.

A negative $\bar{\kappa}$ promotes the formation of highly curved saddles in a membrane. It also facilitates very high spherical curvature, but in the latter case Gaussian curvature is linked with mean curvature. We express the total elastic energy of a single saddle structure in an otherwise flat membrane (see figure 3) by the extremely simplifying Ansatz [17]

$$E_{\text{tot}} = \bar{\kappa} c^4 A_s + \kappa (2c)^2 A_s, \tag{35}$$

which omits the quartic energy terms associated with $(c_1 + c_2)^4$ and $(c_1 + c_2)^2 c_1 c_2$ whose moduli we cannot estimate. Here A_s is the area of the central part with saddle curvature $(-K) = c^2$. The second term represents the bending energy of two sphere segments (curved discs), each of area A_s, and mean curvature $H_d = \frac{1}{2}(c_1 + c_2) = c$. It stands as an approximation for the energy of all four highs and lows belonging to the saddle. A reduction of the energy seems plausible, first, because a positive $\bar{\kappa}$ also helps spherical curvature and, secondly, because on the far side of the extrema $|d^2u/dr^2|$ may be made very small without costing much energy through the curvature resulting from the double oscillation of u on a circle around the saddle. (This has to do with the fact that even with $u \sim r$ in all directions, the curvature-elastic energy would diverge only logarithmically with the size of the system.)

For the very high curvature $c = 1/h$, which is about the maximum reached in the saddle before the onset of geometric constraints, we have, because of equations (34) and (35),

$$E_{\text{tot}} = (-\tfrac{2}{3}\gamma h^2 + 4\kappa)\frac{1}{h^2} A_s. \tag{36}$$

Adopting such a cut-off is a primitive way of dealing with all the contributions to the elastic energy density that are of higher than quartic order in the curvatures. A more realistic cut-off may be defined in terms of a maximum relative decrease in area, X, of the interfaces. Moreover, it is possible to employ the full formula (31) instead of its expansion up to some power of K. An approximate computation along these lines, again under the constraint of fixed middle surface area, results for $X = 0{\cdot}5$ in $K = -0{\cdot}35/h^2$ and a first term of E_{tot} that is about two-thirds of the value used in equation (36). The size A_s of the saddle is expected to be smaller than the square of the membrane thickness if $c \approx 1/h$, since for geometric reasons saddle curvature should become weaker or mean curvature more pronounced as the sides of the saddle bend up and down. On the other hand, very small saddles should be unfavourable because of the energy of the boundary between the saddle proper and the rest of the saddle structure. (This could be described by the gradient terms of Mitov [13].)

Inserting $\gamma = 50\,\text{dyn}\,\text{cm}^{-1}$, $h = 2\,\text{nm}$, and $\kappa = 1 \times 10^{-12}\,\text{erg}$, gives for the energy equation (36) of the single saddle structure

$$E_{\text{tot}} = (-1{\cdot}3 \times 10^{-12}\,\text{erg} + 4 \times 10^{-12}\,\text{erg}) A_s/4\,\text{nm}^2. \tag{37}$$

The very crude formula predicts only a modest reduction by one-third of the energy of the saddle structure through the fourth-order term. However, stable deformations may be possible if we allow for many saddles and cooperativity among them. Regular arrays of saddles that seem energetically favourable at certain membrane configurations in the large are sketched in figure 6. Only one high or low per saddle is needed in the arrangement for the planar case. The most favourable configuration is probably a

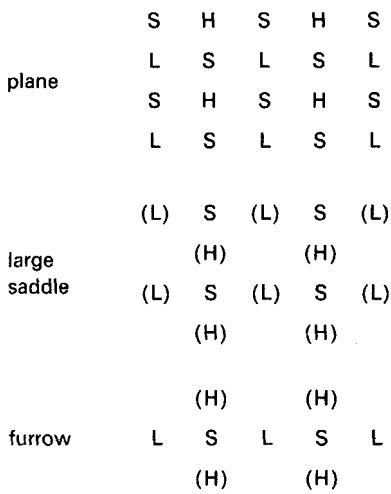

Figure 6. Energetically favourable arrays of saddles for some membrane configurations in the large. Highs and lows in parentheses are thought to be weakly developed.

large saddle, since the highs and lows between the small saddles need not be fully developed if the latter are properly arranged. A line of small saddles forming a furrow or ridge in the large may also be preferred as the saddle structures in a chain can have a lower symmetry than that of figure 3. It appears therefore at least conceivable that saddles in cooperation are strongly enough promoted in biological model membranes to be a physical reality.

Membranes pervaded by saddles may look like a mattress or quilt. If concentration and cooperativity of the small saddles are large enough, the quilted membrane should tend to warp, preferring large saddles or furrows, and thus to increase the ratio of real to projected area. (An appreciable absorption of area by a planar array of closely packed saddles seems less likely.) The saddles might also form crystalline or hexatic superstructures. Depending on their arrangement, the bending rigidity of the membrane may be expected to increase or decrease. For now, we refrain from any further discussion of this most complex and speculative part of the model. It is clear that the high curvatures and small objects predicted are at the limit of applicability of continuum theory. New concepts may be needed to deal effectively with saddle structures if they prove to be real.

A few years ago Beblik *et al.* [8] reported wiggles and knees in tubular phosphatidylcholine vesicles which were too pronounced to be thermally excitable. In the light of the defect model just presented, it is tempting to ascribe these anomalies to the easy formation of furrows or saddles in the large.

One may further suspect that the so-called lipidic particles [18] are related to saddle structures. Occurring typically in rows which often look like ridges or furrows, they are seen as peaks or complementary pits in freeze-fracture electron microscopy of multilayered lipid–water systems. Lipidic particles are usually interpreted as inverted micelles enclosed in a bilayer. They have been found only with lipids forming the inverse hexagonal besides the lamellar phase. The stress profile of those bilayers should be characterized by a strong pull in the region of the polar heads, in accordance with our model. Of the lipids listed above, some phosphatidylethanolamines are known to display the inverse hexagonal phase and lipidic particles [18].

5. Remarks on liquid crystals and conclusion

Hats and saddles may also be envisaged in lyotropic and thermotropic smectic liquid crystals. In order to form three dimensional lattices they would have to be stacked, which implies a loss of translational entropy and raises problems of space filling. Both impediments could be overcome by using rather brittle layers that alone are unstable. Problems of the second kind could be alleviated by a slight tilt of the stacks with respect to the average layer normal, so that along any straight line normal to the layers there can be some relaxation between regions of compression and dilatation of the layers. The same idea is being used to explain smectic $\tilde{C}$ phases made of molecules that form SmA but not SmC (W. Helfrich, unpublished data). SmÃ and SmC̃ are modulated smectic phases consisting of ribbons and ordered in two dimensions. The modulated smectic phases which we propose consist of rods and their order is three dimensional. We note that it may be difficult to find thermotropic smectics that form intrinsic saddles because an overlap of adjacent layers prevents stress profiles of the type shown in figure 4. Three dimensional arrays of 'lipidic particles' in lamellar lipid–water systems have been observed by electron microscopy [18].

The idea of hats and saddles in lipid membranes was developed in the hope of being able to cope with some properties of biological model membranes which cannot be understood in terms of our present simple picture of these bilayers. Although the saddle structures here proposed seem to be rather logical, they are not the only ones to produce locally strong Gaussian curvature. Electron microscopy offers the possibility of detecting these structures. In fact, Klösgen and Helfrich [19] obtained some first results which seem to reveal arrays of defects in single phosphatidylcholine bilayers. Regardless of whether the model of the quilted membrane will be confirmed, one may expect any system selected by nature to be complex in its behaviour. Only delicate systems are capable of responding to subtle influences.

A photograph taken by Dr Beate Klösgen strongly encouraged me to talk and write about the tricky subject of cooperative saddles in membranes.

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