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OPTICAL OBSERVATION OF ROTATIONALLY SYMMETRIC LECITHIN VESICLE SHAPES

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Résumé. — On a photographié, avec un microscope de contraste de phase, des vésicules de lécithine possédant une symétrie axiale. Leurs formes ont été comparées à celles calculées en supposant qu’elles sont gouvernées par l’élasticité de courbure de la membrane. Dans tous les cas on a obtenu un accord excellent par la variation de la courbure spontanée.

Abstract. — The contours of some rotationally symmetric lecithin vesicles were photographed under a phase contrast microscope. On the assumption that the shapes are controlled solely by the curvature elasticity of a fluid membrane, theoretical shapes were fitted to the observed vesicle contours. In all cases an excellent fit was possible by varying the spontaneous curvature.

Lecithin in excess water tends to separate spontaneously into membranes [1, 2]. They consist of one or more bilayers and are closed to form vesicles. Each bilayer is thought to be a two-dimensional fluid when it is above the temperature of chain melting.

The area of the membrane around a nonspherical vesicle is assumed to be independent of vesicle shape. This and the assumption of membrane fluidity permit a theoretical explanation of vesicle shapes which is based on the concept of minimum curvature-elastic energy. The elastic energy per unit area of membrane is given by [3]

\[ g_e = \frac{1}{2} \cdot k_e \cdot (c_1 + c_2 - c_0)^2 , \]

where \( k_e \) is an elastic modulus, \( c_1 \) and \( c_2 \) are the two principal curvatures, and \( c_0 \) is the spontaneous curvature.

In a previous article [4] curvature elasticity was used to explain some simple red blood cell shapes. In the following we show the photographed contours of some rotationally symmetric lecithin bilayer vesicles of different types. Also presented are theoretical curves fitted to the experimental shapes, the spontaneous curvature being the only adjustable parameter.

The material used for all samples was egg yolk lecithin as purchased from Merck. About \( 10^{-4} \) g of lipid was spread on a glass slide, the covered area being approximately 1 cm\(^2\), and about \( 10^{-2} \) cm\(^3\) of twice distilled water added. The lecithin-water mixture was covered by another glass slide and sealed to prevent evaporation.

The vesicles were obtained by simple swelling at room temperature over a period of one or more hours [1, 2]. Observing our samples under a phase contrast microscope we found vesicles of various shapes and different membrane thicknesses. From vesicles possessing rotational symmetry we selected those showing lowest optical contrast. (From our experience we judge that the number of bilayers forming the membrane was in the range of one to three lamellas.) Some of these vesicles were photographed and enlargements of high magnification were made. They permitted an exact evaluation of vesicle size and membrane area and easy fitting of theoretical shapes. The pictures shown were taken when the axis of rotation was in the plane of viewing. A line is seen only where the membrane is parallel to the direction of viewing and at the same time in the plane of focus. The vesicles slowly but constantly changed their orientation; they had to observed for some time in order to recognize the shape and to photograph them in the right position.

The pictures (negatives) of some typical vesicles are shown in figures 1 to 4. The denotations of the shapes as given in the captions follow the terminology proposed by Bessis [5] for red blood cells. The black lines drawn in the negatives represent the theoretical fits.
Comparison shows that in general a very good fit can be obtained. Some of the small deviations such as the slight bending of the dumbbell shown in figure 4 are obviously caused by thermal shape fluctuations and indicate a thin membrane.

The characteristic data for all four vesicles are given in table I. $S = 4 \pi R_0^2$ is the membrane area and $V$ is the vesicle volume, $V_0 = \frac{4}{3} \pi R_0^3$ being the maximum volume possible at the constant surface area $S$. Only the area was determined directly from the photographs. $R_0$ is the radius of the sphere which the vesicle would form at constant area. The pressure difference between the outside and the inside of the vesicle is denoted by $\Delta p$. The critical pressure difference $\Delta p_c$ is that at which the spherical shape would become unstable if the volume could vary freely. While the ratio $\Delta p/\Delta p_c$ is independent of the elastic modulus, the absolute values are not. To obtain $\Delta p$, we calculated $\Delta p_c$ with the formula [3]

$$\Delta p_c = (12 - 2 c_0 R_0) k_c R_0^{-3}$$

inserting for the elastic modulus $k_c = 2.3 \times 10^{-12}$ erg which was determined earlier from the shape fluctuations of presumably unilamellar tubelike vesicles [2]. For a membrane consisting of more than one bilayer the result has to be multiplied by a factor which should be roughly equal to the number of lamellas.

The last column of table I gives the spontaneous curvature $c_0$. Since we did not know the exact number of lamellas making up a membrane the listed values do not necessarily give the spontaneous curvature of
TABLE I

<table>
<thead>
<tr>
<th></th>
<th>S/μ²</th>
<th>V/μ²</th>
<th>R₀/μ</th>
<th>V/V₀</th>
<th>Δρ/Δρₑ</th>
<th>Δρ/dyn cm⁻²</th>
<th>c₀/μ⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codocyte I</td>
<td>1092</td>
<td>253</td>
<td>9.32</td>
<td>0.37</td>
<td>1.20</td>
<td>0.032</td>
<td>0.13</td>
</tr>
<tr>
<td>Codocyte II</td>
<td>934</td>
<td>234</td>
<td>8.82</td>
<td>0.43</td>
<td>0.48</td>
<td>0.017</td>
<td>0.08</td>
</tr>
<tr>
<td>Stomatocyte</td>
<td>1777</td>
<td>4074</td>
<td>11.89</td>
<td>0.58</td>
<td>0.18</td>
<td>0.003</td>
<td>0.008</td>
</tr>
<tr>
<td>Dumbell</td>
<td>843</td>
<td>1774</td>
<td>8.19</td>
<td>0.77</td>
<td>1.15(*)</td>
<td>0.057</td>
<td>0.00</td>
</tr>
</tbody>
</table>

(*) It was checked that dumbbell solutions occur for Δρ > Δρₑ.

The calculated spontaneous curvature c₀ is a function of the spontaneous curvatures c₀ᵣ of the individual lamellas and given by

\[ c₀ = \frac{1}{n} \sum_{r=1}^{n} c₀ᵣ, \]

where n denotes the number of lamellas. This formula includes the case that c₀ is stored in the outer or inner bilayer only, as well as any given distribution of c₀ among all the bilayers. Provided the chemical composition of the aqueous media inside and outside the vesicle is equal, a vanishing spontaneous curvature may be expected. In contrast, our calculations yielded c₀ ≠ 0 in three cases. It is known [6] that the time constant of lipid exchange between the two monolayers in dipalmitoyl lecithin bilayers can be of the order of several hours at 30°C. This relaxation may thus be longer than the time of vesicle swelling. The virtual absence of lipid exchange would allow for a self-induced spontaneous curvature [7] dependent on the detailed history of formation of a particular vesicle. However, it has to be remarked that the flip-flop time of lipid molecules is a function of membrane purity and may have been of a much lower order in our material. Apart from self-induced spontaneous curvature which is of mechanical origin there may have been unaccountable differences between the outer and inner aqueous media causing a chemically induced spontaneous curvature. (They could result from the glass effect [8].)

Our results seem to leave no doubt that the shape of vesicles formed by a fluid lecithin membrane can be completely explained by means of curvature elasticity. The perfect agreement of theory and experiment confirms the validity of previous theoretical calculations [9] predicting shapes similar to those shown here and many others. It was assumed both in the previous and present calculations that there is no cohesion where the membrane is in contact with itself.

References