Passages in Lecithin-Water Systems

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Large numbers of tunnel-like structures apparently connecting neighbouring bilayers were found in phase-contrast microscopy of certain preparations of egg lecithin in water. These passages either cause a pairing of the bilayers or form threedimensional lattices. They appear to originate while the lecithin swells in water. Some theoretical aspects of passage formation in fluid bilayers is shown to be $4\pi\bar{\varkappa}$, $\bar{\varkappa}$ being the elastic modulus of Gaussian curvature.

I. Introduction

Two adjoining artificial vesicles or biological cells may connect their insides and still remain closed to the outside by forming a small tunnel as shown in Figure 1. These tunnels, which we call passages, are not to be confused with pores, the latter being holes in a single bilayer or membrane. Simple pores, i.e. those not involving extraneous molecules, seem to be in general rare or nonexistent in fluid lecithin bilayers. However, they may be instrumental in their rupture under stress, in certain permeation processes, and perhaps even in fusion [1]. On the other hand, the fusion of vesicles or cells may occur also via passages. In the present paper we wish to report on the optical observation of passages in lecithin water systems.

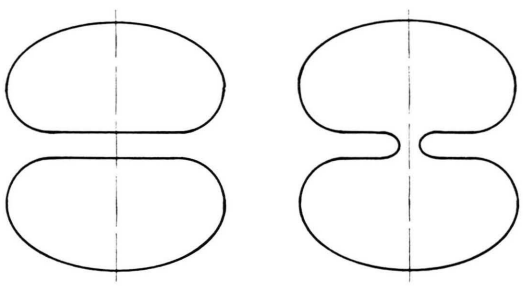

Fig. 1. Scheme of two vesicles (left) and of a passage connecting them (right). The dashed line denotes the axis of rotational symmetry of the passage.

II. Experimental

The experimental setup was the same as in our earlier optical studies of lecithin vesicles [2, 3, 4]. Very little lecithin and a much larger quantity of twice distilled water are put on a glass slide and covered with another slide separated by spacers. The sample is then sealed, kept at a temperature above the main transition and observed under a phase-contrast microscope. The lecithin swells spontaneously in an irregular manner, forming vesicular structures. Previously, the compact lecithin was spread in a very thin layer and it used to disintegrate in water within roughly an hour. In the present work, the same lecithin was spread again rather homogeneously, but in a thicker layer (ca. 0.5 mg cm^{-2} instead of ca. 0.2 mg cm^{-2}). This resulted in a prolonged swelling time of several hours and a strong tendency of the lecithin to form onion-like structures. Generally, egg lecithin was found to be more active in passage formation than dimyristoyl, dipalmitoyl, and distearoyl lecithins. The photographs to be shown here were all obtained at room temperature with egg lecithin as purchased from Merck.

A membrane contour is seen under the phase-contrast microscope where the membrane is parallel to the optical axis, i.e. the direction of viewing. The situation is slightly complicated by the construction of the microscope. In our Zernike-type model (Leitz, Ortholux II) the incident light making a small angle with the optical axis is focussed on the narrow phase ring shifting its phase. It is easy to realize that an infinite flat membrane very nearly parallel to the optical axis may not be visible because both the primary and all the "dif-

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fracted" light pass through the phase ring. In practice, the membranes are generally curved so that either an image is produced by the ordinary diffractions of small objects or two contours appear at different depth of focus, but both very close to the depth characterized by parallelism. We convinced ourselves that even with very large vesicles (ca. 100 μm in diameter) just one contour could be seen in the microscope.

III. Observations

A typical isolated passage in an array of parallel membranes which touch each other in places is shown in Figure 2. Occasionally we saw rows of passages just outside the rim of a large area of contact between two vesicles. In a few cases the number of passages rose in the course of hours or days, but the reproducibility of this phenomenon was very poor. As mentioned above, a dramatic increase of passage density was found if the swelling process and, thus, bilayer separation were slowed. All the following photographs show results obtained in this way. Our observations may be grouped and interpreted under two headings:

1. *Bilayer pairing:* Frequently the lamellar systems displayed a more or less pronounced tendency of pair formation, as seen in Figures 3 and 4. If there was pairing, the first two membranes on the outside of an onion-like structure used to join, then the third and fourth and so on. Each pair seems to be held together by passages. The fairly constant spacing of the two constituent membranes over large regions suggests a fairly dense packing of passages, their separation being of the order of the mean membrane spacing. The blurred appearance of the space inside the pairs points to the absence of a regular arrangement of the passages (see below). Small single passages, even if in focus, are difficult to discern, presumably because of strong membrane curvature. From the existence of passages, the equal contrast of the contours apart from the passages, and the apparent absence of membrane splitting we conclude with confidence that the membranes forming the pairs are single bilayers.

Fig. 2. Isolated large passage in an array of parallel lecithin membranes.

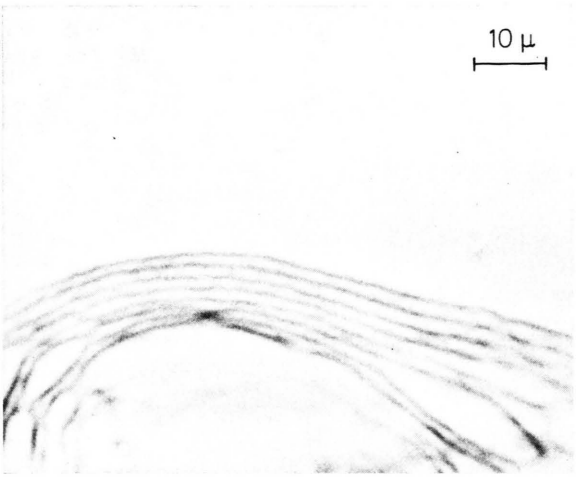

Fig. 3

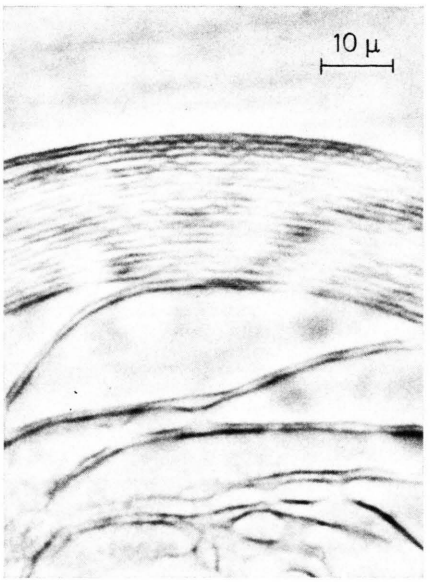

Fig. 4

Fig. 3 and 4. Examples of bilayer pairing. The outer water is at the top.

2. *Three-dimensional lattices:* Inspection of Figs. 2 and 3 also shows that the pairs of bilayers are often held together by other passages ocurring in lower densities than those giving rise to pair formation. In fact, one may even surmise a three dimensional lattice of passages in the upper right corner of the multilayer system of Figure 3. Well-developed lat-

tices were found in some samples. Honeycomb patterns are seen in Figure 5 and 6. We believe them to represent cubic face centered lattices. In any system of bilayers containing enough passages there are two volumes of water, either of them multiply self-connected but, at least in theory, completely separated from the other. Therefore, the specific type of cubic face-centered lattice is probably a dual diamond lattice of water channels, one set of tetrahedral bonds being interwoven with the other. A honeycomb pattern may then appear if one of the four faces of the tetrahedron is parallel to the object plane. As the depth of focus was 5—9 μm, depending on the objective used, the very distinct patterns are likely to be the result of a superposition of the images of a few lattice planes. The visible patterns changed with the depth of focus. For instance, the honeycomb of Fig. 5 became almost a sequence of equidistant layers at a change of several μm, as shown in Figure 7. This can be explained by a tilt of two of the three lattice planes that are parallel to the optical axis in Figure 5. Seemingly perfect layer structures were seen for small spacings of the order of 1 μm. We do not think that the honeycomb pattern and the layered structure result from a hexagonal lattice of passages. One argument is that a view along the hexagonal axis should present a lattice of triangles with faded vortices, in disagreement with observation. Another very regular pattern is displayed in Figure 8. It looks like a lattice

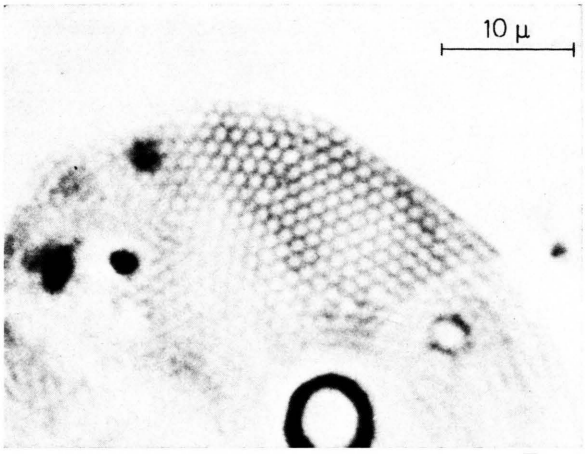

Fig. 5

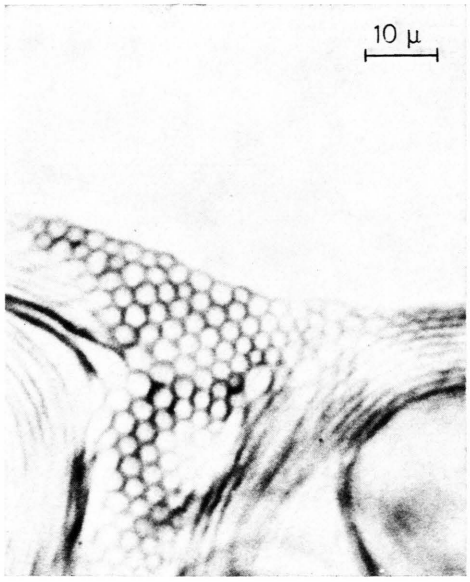

Fig. 6

Fig. 5 and 6. Honeycomb patterns indicative of a twofold diamond lattice of passages.

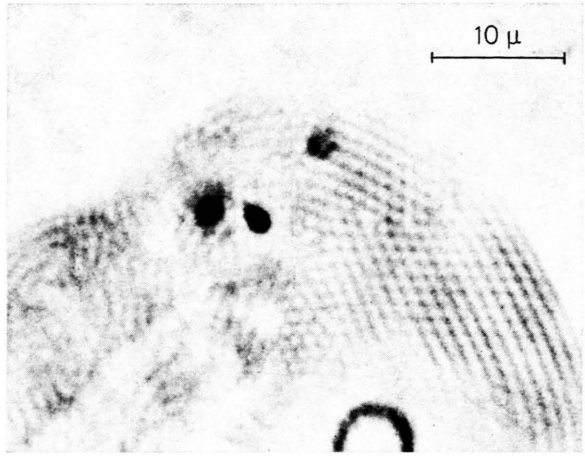

Fig. 7. Same structure as in Fig. 6 at another depth of focus.

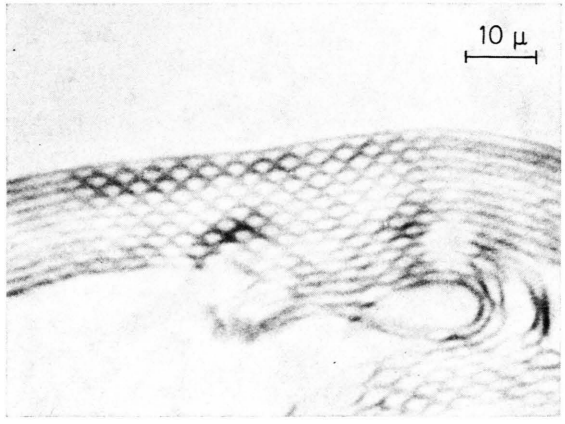

Fig. 8. Three-dimensional lattice of passages between regions of membrane pairing.

of the type just discussed, but distorted into a trigonal structure. The lower symmetry could be caused by mechanical stresses. However, the existence of more than one type of stable lattice in mechanical equilibrium seems also conceivable. At least two parameters play a role in determining which lattice prevails: the number of passages per unit area and the ratio of water volume to unit area of bilayer. Judged from appearance, the two separate quantities of water were equal in the lattices.

IV. Theory

To derive the minimum energy of passage formation we start from the formula for the curvature-elastic energy density g of fluid membranes [5]

$$g = \tfrac{1}{2}\varkappa(c_1 + c_2 - c_0)^2 + \bar{\varkappa} c_1 c_2 , \qquad (1)$$

where $\varkappa$ and $\bar{\varkappa}$ are elastic moduli, c_1, c_2, and c_0 the two principal and the spontaneous curvatures, respectively. Simple calculations based on the mathematics of Ref. [5] show that passages can be constructed between parallel bilayers in such a way that $c_1 + c_2 = 0$ holds practically everywhere. Contours exactly satisfying this condition obey the general formula

$$z = r_0 \ln(r/r_0 + \sqrt{(r/r_0)^2 - 1}) , \qquad (2)$$

where z is the height above or below the equatorial plane and r the distance from the axis of rotational symmetry. Two examples are given in Figure 9. We assume here $c_0 \equiv 0$ although passages satisfying $c_1 + c_2 - c_0 = 0$ can also be formed for $c_0 \neq 0$, as demonstrated by theoretical myelin shapes [6]. Evidently, the minimum elastic energy of a single passage between parallel bilayers is controlled essentially by the second term in (1), i.e. by the Gaussian curvature. The integral of this contribution over a closed shape is known to be invariant under deformations including changes in total area. It is a function only of certain topological properties of the closed surface; e.g. it is different for spheres and tori. We have depicted in Fig. 10 a sphere, two spheres with a passage connecting them, and two separate spheres. The total energy of Gaussian curvature is readily seen to be $4\pi\bar{\varkappa}$ in the first two cases and twice as much in the third. It follows immediately that the minimum elastic energy E_p of a passage is given by

$$E_p = -4\pi\bar{\varkappa} . \qquad (3)$$

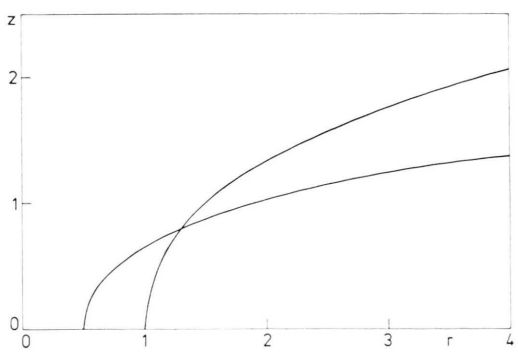

Fig. 9. Theoretical contours of passages derived for $c_1 + c_2 = 0$, $c_0 = 0$.

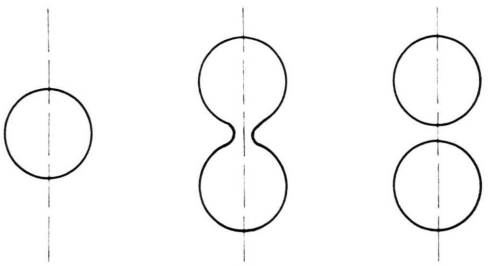

Fig. 10. Three vesicular structures possessing axes of rotational symmetry indicated by the dashed lines (see text).

The occurance of passages indicates that $\bar{\varkappa} > 0$ for egg lecithin bilayers, at least in a certain range of water concentrations. A simple phenomenological formula for $\bar{\varkappa}$ may be derived if one uses the concept of equal but opposite tensions in the polar head (σ_h) and hydrocarbon-chain (σ_c) regions of the monolayers constituting a symmetric bilayer. In the unstrained bilayer the two tensions, measured in dyn cm^{-1}, must be equal. They enter into $\varkappa$ in a complicated fashion including derivatives [7]. However, $\bar{\varkappa}$ can be expressed simply in terms of $\sigma_h = -\sigma_c$ and the bilayer thickness b, provided a uniform stress $2\sigma_c/b$ is assumed for the hydrocarbon region. If only Gaussian curvature is considered, i.e. for

$$c_1 + c_2 = 0 \quad \text{and} \quad c_0 = 0 ,$$

the relative change of area per chain as a function of monolayer depth leads to the energy surface density

$$g = \bar{\varkappa} c_1 c_2 = 2[\sigma_h (b/2)^2 + \sigma_c \tfrac{1}{3}(b/2)^2] c_1 c_2 . \qquad (4)$$

Therefore

$$\bar{\varkappa} = \tfrac{1}{3}\sigma_h b^2 . \qquad (5)$$

Taking $\sigma_h = 18$ dyn cm^{-1} from Marčelja's statistical theory of monolayers and bilayers [8, 9] and putting $b = 40$ Å, one has $\bar{z} \approx 1 \cdot 10^{-12}$ erg. The sign is positive, in agreement with passage formation. It should be noted that the same value was recently estimated by Petrov et al. [10]. They used a similar, but more abstract, theory and obtained a numerical value very indirectly from interpreting electron micrographs by Klêman et al. [11]. Also, they think $\bar{z}$ to be positive only for small water content (less than the saturation value of about 55 weight percent) [12]. On the basis of the present estimate we rather expect passage formation to be energetically favoured at all water concentrations *above* a certain limit below which (1) has ceased to be valid because of nonlinear elastic effects and bilayer interaction.

V. Conclusions

Summing up our result and some other observations, we may propose the following preliminary picture of the process of passage formation of egg-lecithin bilayers in water. There exist probably very few, if any, passages at low water content up to at least 20 weight percent. The conclusion is drawn from the electron micrographs of Ref. [11] which Williams kindly examined for us in this respect. Passages seem to form above an unknown water concentration. Presumably, the energy barrier of formation is high and the collision rate small, which would explain why the lecithin has to swell slowly in order to produce appreciable numbers of passages. As the membrane spacing increases further, the collision frequency and, thus, passage formation decrease again. The passages are blown up together with membrane separation (i.e. they "scale" with mean separation) until they finally obstruct each other and become capable of organizing into lattices. The view that the passages do not preexist in lecithin as taken from the bottle, but originate during swelling is corroborated by the observation of bilayer pairing. As illustrated by Figs. 3 and 4 there are more passages between the first bilayer and the second than between the second and the third, and so on. This may be anticipated if water penetrates into lecithin partly by means of passages, thus expanding the water system connected with the outside more rapidly than the other. Furthermore, it was checked that the exposure of egg lecithin to an atmosphere very nearly saturated with water prior to swelling in water is without noticeable influence on the number of passages found in our samples.

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