



HAL
open science

On defects in different phases of two-dimensional lipid bilayers

D. Ruppel, E. Sackmann

► **To cite this version:**

D. Ruppel, E. Sackmann. On defects in different phases of two-dimensional lipid bilayers. *Journal de Physique*, 1983, 44 (9), pp.1025-1034. 10.1051/jphys:019830044090102500 . jpa-00209687

HAL Id: jpa-00209687

<https://hal.science/jpa-00209687>

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.

Classification
 Physics Abstracts
 61.30J

On defects in different phases of two-dimensional lipid bilayers

D. Rüppl (*) and E. Sackmann

Physik Department der Technischen Universität München, D. 8046 Garching/München, F.R.G.

(Reçu le 26 janvier 1983, accepté le 19 mai 1983)

Résumé. — La bicouche phospholipidique structure de base des membranes biologiques est un modèle approprié de système bidimensionnel. Les défauts au sein de ces bicouches sont étudiés par microscopie électronique par la méthode de cryo-décapage. On peut différencier des défauts topologiques et non topologiques. Le premier type est analysé par la théorie de l'homotopie. Deux systèmes de défauts, un abélien et un non-abélien, ont été trouvés, ce qui est la conséquence d'une brisure de symétrie. L'effet de petites quantités d'impuretés sur la structure des défauts a été étudié. Un aperçu de l'intérêt biologique possible des défauts est donné dans la dernière partie.

Abstract. — The phospholipid bilayer is the basic structure of biological membranes as well as a suitable model of a two-dimensional system. Defects within these bilayers are studied electron microscopically by application of the freeze fracture method. Topological and non-topological defects can be distinguished. The first type is analysed in terms of the homotopy theory. Two defect systems, an Abelian and a non-Abelian, are found which are a consequence of a symmetry breaking. The effect of small amounts of impurities on the defect structure is studied. An outline of the possible biological relevance of defects is given in the last part.

1. Introduction.

Phospholipids are amphiphatic molecules which together with water organize in different lyotropic liquid crystalline phases as shown by Tardieu *et al.* [1].

The most often observed structure at high water content is the bilayer which is the basic building unit of biological membranes. From a physical point of view the lipid bilayer exhibits typical properties of two-dimensional systems.

The present work deals with a freeze fracture electron microscopy study of the defect structure of isolated phospholipid bilayers at very high water content (> 99 wt % of water). Under these conditions the bilayers close up forming structures of nearly spherical shape, so-called vesicles. Vesicles of pure dimyristoylphosphatidylcholine (abbreviated as DMPC in the following) are studied. Bilayers of this lipid exhibit the following thermotropic phases :

Above a transition temperature of $T_t = 23$ °C the bilayer is in a fluid state (called L_α -phase) which is quite analogous to smectic A layers. Below T_t a first crystalline phase (denoted as P_β by Tardieu *et al.*) appears which exhibits a corrugated surface

profile. Due to this characteristic superstructure it is called the ripple phase. At a temperature of $T_p = 14$ °C, the bilayer undergoes a second first order solid-solid transition, into the so-called L_β phase while loosing the superstructure. X-ray diffraction studies suggest that the lipid chains exhibit a hexagonal lattice in the P_β -phase and an orthorhombic lattice in the L_β -phase. According to Janiak *et al.* [2] these two solid phases are characteristic for lipids the chains of which exhibit a tilt with respect to the normal of the bilayer. The freeze fracture electron microscopy technique allows the observation of defects which lead to a change in the surface profile, such as defects in the P_β superstructure. Defects within the two-dimensional lattice formed by the hydrocarbon chains cannot be detected by this technique. The defects of the P_β phase are discussed in terms of the homotopy-theory introduced recently by Toulouse and Kléman [3] into the analysis of defects.

In the second part of the work we describe the generation and stabilization of defects in phospholipid bilayers by the incorporation of small amounts of impurities. Finally the possible role of defects in biological membranes is discussed.

The defect structure of the lyotropic smectic phase consisting of stacks of bilayers separated by water has already been studied by Kléman *et al.* [4].

(*) Present address : Abteilung für Biophysik, Universität Ulm, D-7900 Ulm/Donau, R.F.A.

2. Materials and methods.

The phospholipid dimyristoylphosphatidylcholine was a product from Fluka (Neu-Ulm). Thin-walled vesicles were prepared by rinsing water over a thin lipid layer deposited on the glass wall of a flask. For the freeze fracture preparation a 20 μm thick layer of the vesicle suspension was brought between thin gold sheets (thickness 100 μm , diameter 5 mm). Rapid freezing was achieved by dipping these sandwiches into liquid freon 23 kept at -160°C . The samples were fractured at -120°C and at 10^{-7} mbar in a Balzers BAF400D freeze fracture device. Without additional etching (water sublimation) the fracture faces were shadowed under 45° with platinum/carbon up to a thickness of 30 \AA . For stabilization a 300 \AA thick carbon layer was subsequently deposited by sputtering. The lipids were removed in a 1 : 1 methanol water mixture. Electron micrographs were taken in a Phillips EM301 electron microscope.

3. Characteristic texture of DMPC bilayer phases as observed in electron microscopy.

The bilayer phases may be unambiguously distinguished by freeze fracture electron microscopy. The L_α -phase exhibits a smooth surface devoid of any structure (Fig. 1a). The P_β phase exhibits the characteristic ripple superstructure (cf. Fig. 1c) while the $L_{\beta'}$ -phase has an essentially smooth surface interwoven by defect lines (Fig. 1d). The pattern of this

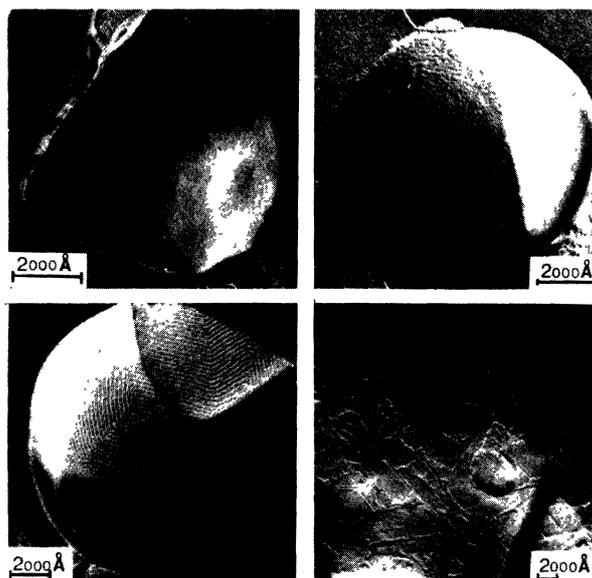


Fig. 1. — Electronmicrographs of DMPC vesicles in different bilayer phases. a) L_α -phase (above 23°C). b) L_α -phase, a slow cooling rate was applied during preparation and the nucleation of the P_β -phase is observed. c) P_β -phase between 23 and 14°C . d) $L_{\beta'}$ -phase. The surface is smooth, but defects need many hours or days for healing out.

network of lines depends on the period of time between phase transition and observation (cf. Fig. 11).

The P_β -phase exhibits two superstructures [5] one with a period of $120 \text{ \AA} \pm 20 \text{ \AA}$ called the $A/2$ -phase and one with a repeat distance of $235 \pm 25 \text{ \AA}$ called the A -phase (cf. Fig. 2). The $A/2$ -phase has an asymmetric (sawtooth-like) profile with an asymmetry of 0.59 while the A -phase is characterized by a symmetric profile with a groove at the maxima (cf. Fig. 2b). As shown in figure 2 the two superstructures may coexist in one vesicle. The surface profiles suggest that the A -phase is generated from the $A/2$ -phase by rotating every second ripple by 180° . The symmetries of the two phases have fundamental consequences for the occurring defects. Note that the tilt of the hydrocarbon chains must change sign if one crosses the grooves of the ripples. However, the resulting tilt of the chains with respect to the average plane of the membrane vanishes [5].

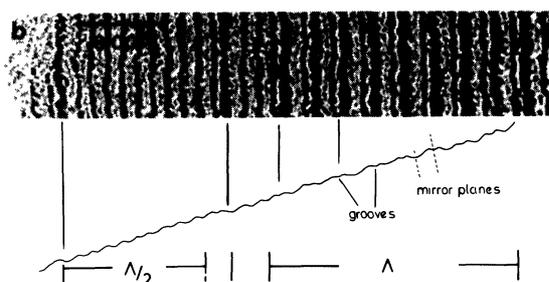
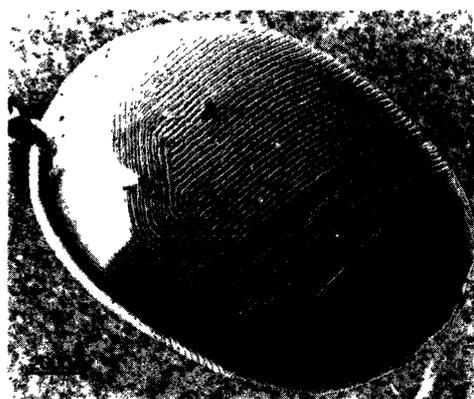


Fig. 2. — P_β ripple-phase (19°C) according to reference 5. a) Vesicle with coexisting A - and $A/2$ -phases. The disclinations $\pm 1/2$ are topologically allowed in the A -phase, whereas they lead to line defects in the $A/2$ -phase. b) Coexisting A - and $A/2$ -phases. A surface profile is reconstructed following a method described in [22]. One can clearly distinguish the asymmetric profile of the $A/2$ -phase and the symmetric profile of the A -phase with grooves at the top.

4. Topologic defects of the P_β phase.

The texture of the A and the $A/2$ -phase are essentially two-dimensional. In two dimensions only point and line defects can be topologically stable. This means,

their cores (the singularities) have dimensions of 0 or 1. The point defects may have the form of dislocations or disclinations. The first have their origin in a translational symmetry of the material whereas the latter are consequences of allowed rotational symmetry operations. (Both types are line defects in three dimensions).

Recently homotopy theory was introduced into the analysis of defects by Toulouse and Kléman [3] and Mermin [6]. The theory yields information about the group of point and line defects and their combination laws when the symmetry group of the material is known.

4.1 HOMOTOPY THEORY OF TOPOLOGICAL DEFECTS OF Λ -PHASE AND EXPERIMENTAL VERIFICATION. — This phase exhibits a continuous translational symmetry along the ripples whereas only translations by integral units of Λ are possible in the perpendicular direction. Moreover the Λ -ripples have a rotational symmetry about a twofold axis C_2 , perpendicular to the membrane plane.

The translation group is therefore $R \times Z$, the direct product of the groups of real and integer numbers. As rotations of π are allowed, the whole symmetry group is

$$(R \times Z) \square C_2 \tag{1}$$

the semidirect product of the translation group $R \times Z$ and the rotational group C_2 (for definitions of the direct and semidirect product see for example, Ref. 7).

The rotation group is isomorphic to Z_2 , the group of integers modulo 2, so that the symmetry group is isomorphic to

$$H = R \times Z \square Z_2. \tag{2}$$

If G is the full Euclidian group, the group $V = G/H$ is the so-called manifold of internal states. Its first homotopy group represents the group of point defects :

$$\pi_1(V_\Lambda) = Z \square Z. \tag{3}$$

According to Kléman, Michel and Toulouse [8] the zeroth homotopy group representing line defects is

$$\pi_0(V_\Lambda) = 0. \tag{4}$$

Equation 3 predicts that the Λ -phase has stable point defects. These are represented by pairs of numbers (z, p) with $z, p \in Z$, corresponding to dislocations of strength $z\Lambda$ and disclinations of strength $p/2$. In order to facilitate the following discussion we provide the second group on the right-hand side of equation 3 with an index H that is $Z_H = \{q \mid q = p/2 \Lambda, p \in Z\}$. Z_H is isomorphic to Z . The point defects are then represented as pairs

(z, q) with $z \in Z$ and $q \in Z_H$. Equation 4 excludes the existence of line defects.

The disclinations are quite analogous to those of liquid crystals but two-dimensional (Fig. 3). It should be noticed that there are two defect systems a and b with the rotation axes a and b in the core.

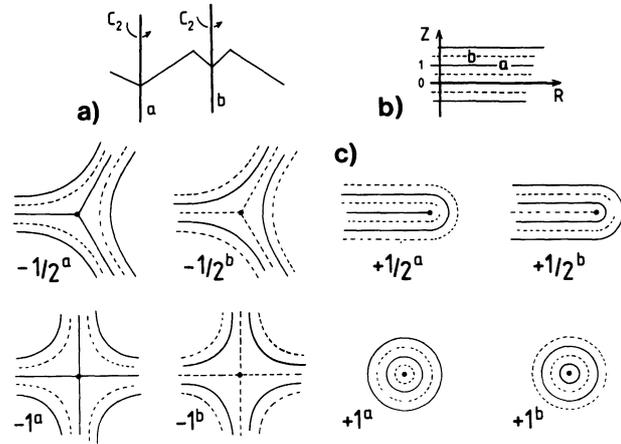


Fig. 3. — Possible disclinations of the Λ -phase. *a*) Profile of a Λ ripple with C_2 -rotation axes a and b . *b*) Translation symmetries of the ripples. Any translation is allowed along the ripples whereas only discrete translations by $z \times \Lambda$ are possible in the perpendicular direction. *c*) Disclinations of systems a and b .

The defects may combine. In consequence of the semidirect product in equation 2 the combination law is given by

$$(z, q) \cdot (x, p) = (z + R_q(x), q + p) \tag{5}$$

for $z, x \in Z$ and $q, p \in Z_H$. R_q stands for a rotation of $2\pi q$. As q corresponds to half integral values this means simply a change of sign for x (see Fig. 6).

As most defect groups, the group $\pi_1(V_\Lambda)$ is not Abelian. The Λ -phase is a physical realization of the example presented by Poenaru and Toulouse in their figure 9 [9].

A consequence of the non-Abelian character of the defect group is that the sum of defects depends on their mutual arrangement. Therefore, one can only distinguish groups of conjugated defects. If one conjugates a disclination characterized by a pair of numbers $(0, p)$ with a general defect (z, q) one obtains

$$(z, q) \cdot (0, p) \cdot (z, q)^{-1} = (2z, p) \tag{6}$$

which is a general representation of a disclination of strength p . This result implies that, depending on their relative arrangement, two disclinations of strength $\pm p$ can combine to form either $(0, 0)$ or $(2z, 0)$. The latter is a double dislocation.

The defects of the system a and b differ in a displacement of their cores by $\Lambda/2$. Conjugating defects of the system b with $((2z + 1)/2, q)$ ($z \in Z$ and $q \in Z_H$)

gives the general representation $(2z + 1, p)$ for the defects of system b . Therefore combinations of disclinations of system a and b of opposite strengths give odd numbered dislocations. Figure 4 shows an example how a simple dislocation can be related to a sum of disclinations.

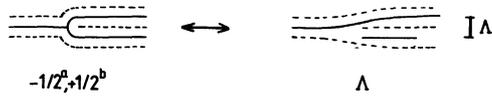


Fig. 4. — Correspondence of a dislocation and a pair of disclinations of different types a and b .

Figures 5 and 6 exhibit some typical experimental verifications of the above considerations.

In figure 5 disclinations of type a and b are observed. Disclinations of half strength prevail. Obviously, they are energetically and entropically favoured. Pure dislocations are rarely observed. The reason for this is that single dislocations are easily transformed into pairs of disclinations of different type (a or b) while double dislocations go over into pairs of disclinations of the same type.

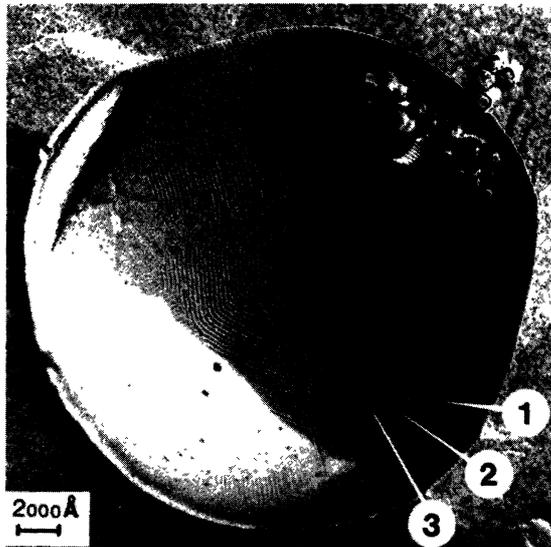


Fig. 5. — Vesicle with a typical A -phase defect pattern. The following combinations are indicated by arrows. 1) A pair of disclinations as presented in figure 4. 2) Defect system shown schematically in figure 6a together with reduction. 3) Defect system as shown and reduced in figure 6b.

Figure 4 illustrates the first pattern. Figure 6 shows schematically examples which can be observed in figure 5. Simultaneously the reduction of such patterns into simple disclinations is illustrated in figure 6.

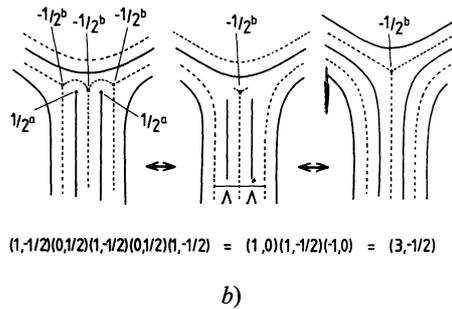
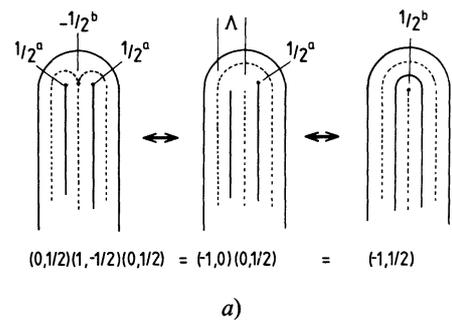


Fig. 6. — Defect systems of the A -phase to be found in figure 5. As the point defects form a group, one can calculate the sum of a defect system. The semidirect product reduces in the case of the A -phase to simple change of sign :

$$(z, q)(x, p) = (z + (-1)^{2q}x, q + p),$$

with $z, x \in \mathbb{Z}$ and $q, p \in \mathbb{Z}_H$. a) Reduction and calculation of system 2 of figure 5. b) Reduction and calculation of system 3 of figure 5.

4.2 HOMOTOPY THEORY OF TOPOLOGICAL DEFECTS OF $A/2$ -PHASE AND EXPERIMENTAL OBSERVATIONS. — The $A/2$ -phase has an asymmetric profile. Therefore only rotations about $2\pi\mathbb{Z}$ ($z \in \mathbb{Z}$) are allowed. As these rotations commute with the discrete translations perpendicular to the ripples and the continuous ones along the ripples, the semidirect product reduces to a direct product and the symmetry group is simply

$$H = (R \times \mathbb{Z}) \times \mathbb{Z}. \tag{7}$$

The group of point defects becomes

$$\pi_1(V_{A/2}) = \mathbb{Z} \times \mathbb{Z} \tag{8}$$

and the group of line defects

$$\pi_0(V_{A/2}) = 0. \tag{9}$$

In analogy to the A -phase no stable line defects exist. The point defects are represented by pairs of integers (z, q) where z stands for dislocations and q for disclinations of integer strength q . As these are elements of a direct product of the Abelian group \mathbb{Z} with itself, the group of point defects $\pi_1(V_{A/2})$ is commutative. Therefore one has a one-to-one correspondence of defects and these pairs [6].

The combination law is for $x, q, z, p \in Z$

$$(x, q) \cdot (z, p) = (x + z, q + p) \quad (10)$$

because in the case of a direct product, $R_q(z)$ of equation 5 becomes zero for all z and q .

The fact that only integer disclinations are allowed is directly related to the fact that $\pi_1(V_{A/2})$ is an Abelian group. Z is just the half of Z_H and $\pi_1(V_{A/2})$ is made of those elements of $\pi_1(V_A)$ which commute, the integer ones.

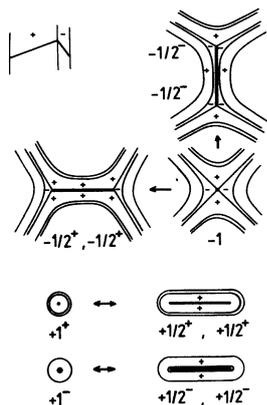


Fig. 7. — Disclinations of the $A/2$ -phase. Only integer disclinations are stable. But they decompose into two quasi disclinations of strength $\pm 1/2$ coupled by a finite line defect. It should be noticed that the system is coupled and the overall strength is always integer. One can distinguish $+$ or $-$ defects depending upon whether the long or the short ripple side is in the core.



Fig. 8. — $+$ defect line in the $A/2$ -phase (only one half is shown).

Figures 7, 8 and 9 present some typical experimental results concerning the defect structure of the $A/2$ -phase of pure DMPC. Examples for impurity containing bilayers of this lipid are shown in figure 12. Generally disclinations of integer strength are observed. But they decompose into quasi half strength disclinations which are connected by line defects. The line defects have finite length, therefore the quasi half strength disclinations are coupled so that the overall strength of the defect is integer. One can distinguish $+$ and $-$ line defects (Figs. 7 and 8). Apparently, the finite line defects with their rather large core are associated with a lower elastic energy than the integer disclinations with a point-like core. Very often the $A/2$ -phase exhibits defect-free regions which are separated by grain boundaries. An example is shown in figure 9.

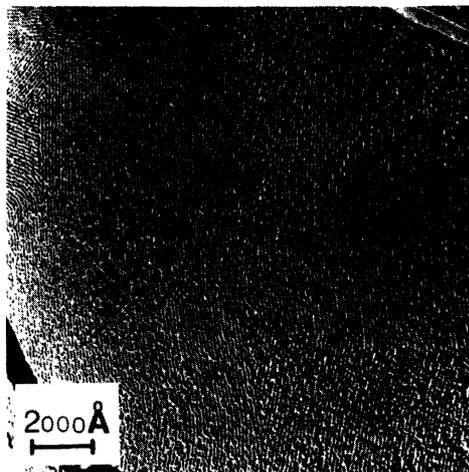


Fig. 9. — $A/2$ -phase with defect free domains adjoining at grain boundaries. Such a pattern is observed very often especially in large vesicles.

5. Defect structure of the L_{β} -phase.

With respect to the symmetry properties the L_{β} -phase is equivalent to a two-dimensional spin fluid. Therefore at our scale all translations as well as rotations about multiples of 2π are allowed. The group of point defects is therefore Z describing the disclinations. There are no dislocations.

The defect structure depends on the interval between the time of the $P_{\beta'} \rightarrow L_{\beta}$ -transition and the time of observation. Up to some 10 min., the surface structure of the L_{β} -phase seems to resemble the ripple-texture. However, a closer inspection shows that the shell of the vesicles exhibits a step-like profile as indicated in figure 10a. The steps involve a shift of the lipid chains by about 30 Å [5] in the direction of the average orientation of their long axes. The steps have a long lifetime in regions of high curvature while they heal out rather rapidly in nearly planar

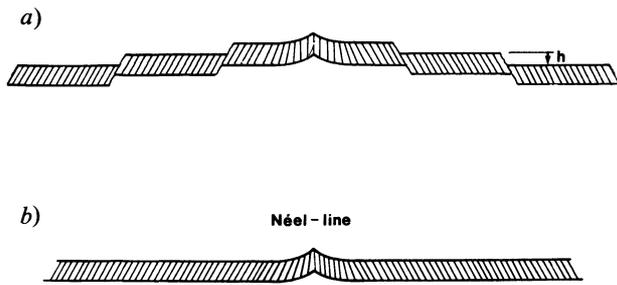


Fig. 10. — Schematic representation of the structure of $L_{\beta'}$ -phase defects. *a)* Profiles typical for healing times of some minutes. Ripples are deformed into steps of height, $h \approx 30 \text{ \AA}$ which corresponds roughly to monolayer thickness. *b)* Extended healing leads to essentially smooth surface. Domains of opposite tilt direction form Néel-lines due to escape in third dimension.

bilayers. The steps may form closed or spiral-like lines (cf. Fig. 11*a* and Fig. 11*b*) within the plane of the membrane. The first type corresponds to the $+1$ disclinations of the $A/2$ -phase whereas the latter is a combination of a $+1$ disclination and a dislocation. After healing times of some hours the dislocations vanish and the two types of defect lines transform into disclinations. Their core may be linear like Néel lines (cf. Fig. 10*b* and 11*c*) as well as point like. The situation is quite analogous to the case of the $A/2$ -phase.

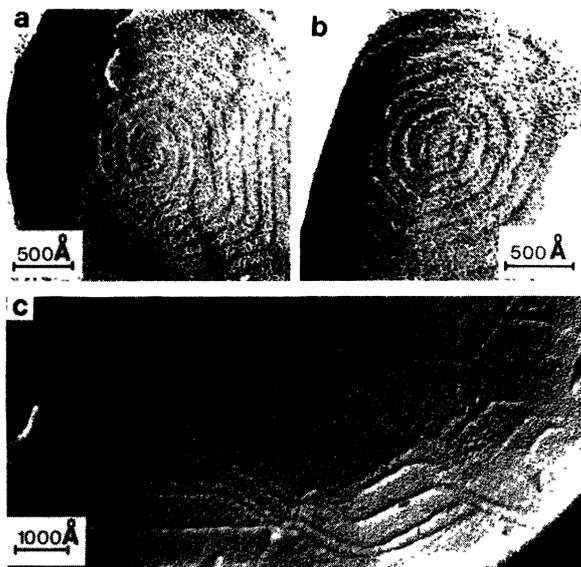


Fig. 11. — Observed $L_{\beta'}$ defect structure. *a, b)* After a healing time of some 10 min. the ripples of the $P_{\beta'}$ -phase are transformed into steps (Fig. 10*a*). They may form closed systems like a disclination $+1$ (Fig. 11*b*) or spirals (Fig. 11*a*) which are a combination of a dislocation and a disclination $+1$. *c)* After many hours to days of healing time the surface is essentially smooth interrupted by Néel-lines with a triangular profile.

6. Generation and stabilization of defects by impurities.

Addition of small amounts of small as well as of macromolecular impurities may have drastic effects on the structure of the topological defects of the $L_{\beta'}$ - and $P_{\beta'}$ -phases. In addition impurities may induce the formation of localized orientational defects in the fluid L_{α} -phase or topological defects in tilted fluid phases.

6.1 EFFECT OF CHOLESTEROL, A DETERGENT AND A MACROMOLECULE (GLYCOPHORIN) ON THE DEFECT STRUCTURE OF $L_{\beta'}$ AND $P_{\beta'}$. — In all cases studied very small amounts of the impurities (≥ 1 mole per cent in the case of the cholesterol or of the detergent and 0.02 mole per cent in the case of the protein) lead to a complete suppression of the A -phase in favour of the $A/2$ -phase. Moreover the line defects of the $A/2$ -phase are considerably shortened. This shortening which is clearly visible in figure 12 may eventually lead to point-like cores of $+1$ disclinations (cf. Fig. 12*b*). The incorporation of the impurities may lead to a high flexibility of the vesicles with crystalline shells. These assume polygonal shapes with the foreign molecules accumulated in the corners and edges. An example is shown in figure 12*c* for the case of a large amphiphatic macromolecule (e.g. glycophorin).

In the case of the $L_{\beta'}$ -phase the impurities lead to a long term stabilization of the step-pattern. A typical example is shown in figure 13. At higher concentrations of impurities (about 5 mole % of cholesterol) the transition from the $P_{\beta'}$ to the $L_{\beta'}$ -phase vanishes which may be related to the stabilization of the defects. The important role of defects for the $P_{\beta'} \rightarrow L_{\beta'}$ transition was stressed out in a previous paper [5].

6.2 INDUCED DEFECTS IN THE FLUID L_{α} -PHASE. — Consider an amphiphatic macromolecule with a conical shaped hydrophobic part. If such a molecule is incorporated in a fluid bilayer with chains oriented perpendicular to the membrane plane (L_{α} -phase), it will induce a tilt of the lipid molecules (cf. Fig. 14*a*). Due to the spontaneous lipid orientation the disturbance is expected to extend over long distances. The splay elastic energy associated with this orientational defect may be relaxed by escape of the bilayer into the third dimension (cf. Fig. 14*a*). This escape is strongly favoured by the quasi two-dimensionality of the bilayer. While the defect induced by the conical protein is localized in the case of the L_{α} -phase it may become topologic if the fluid bilayer exhibits an intrinsic tilt (cf. Fig. 14*b*).

Examples of defects induced by macromolecules in fluid bilayers have not been observed yet. However, an equivalent situation has been observed in fluid bilayers of a charged lipid (i.e. phosphatidic acid) after addition of Ca^{++} to the aqueous phase.

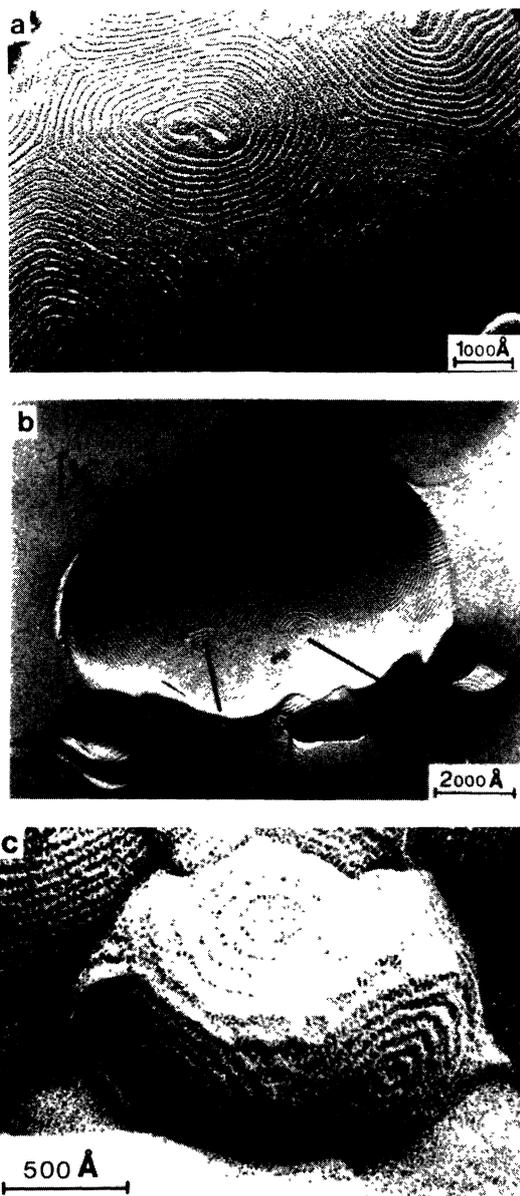


Fig. 12. — Effect of small amounts of impurities on defect structures. *a)* Addition of 6% cholesterol. *b)* Addition of some permille Na-desoxycholate. Generally the A -phase is suppressed. The line defects of the $A/2$ -phase are shortened. One observes + 1 disclinations with point-like cores, but - 1 disclinations always exhibit lines like cores. *c)* Addition of 0.2‰ glycoporphine A (for a general information about this protein and its interaction with lipids see for example [23]). The protein is preferentially accumulated in the point and line defects. These are considerably softened and can serve as edges and corners of polygonal shaped vesicles.

The Ca^{++} leads to a strong contraction of the lipid head groups bound to the ions. As a consequence of this contraction a tilt is locally induced in the bilayer region composed of non-bound lipid. Again an escape in the third dimension is observed (cf. ref. 10 and Fig. 14).



Fig. 13. — Addition of 4% cholesterol. The vesicle is shown at 4 °C where pure DMPC is in the L_{β} -phase. A ripple like structure is observed. Closer inspection shows a step like profile and a repeat distance of (250 ± 20) Å. The cholesterol must be accumulated in the steps blocking their annihilation.

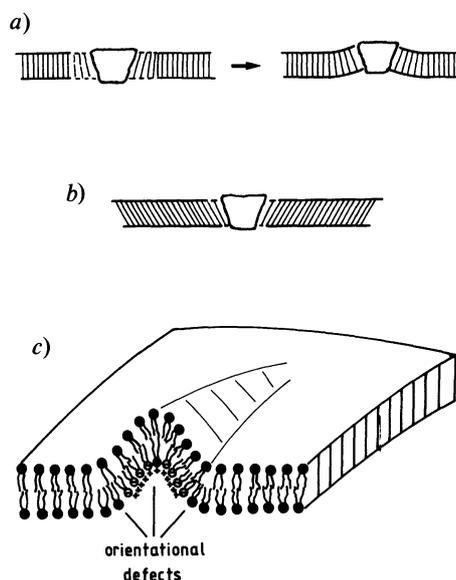


Fig. 14. — Conical shaped protein in a lipid bilayer causing a tilt of adjacent hydrocarbon chains. *a)* The pure phase exhibits no tilt. The induced tilt is only local. The elastic splay energy may be reduced by an escape in the third dimension. *b)* Variation in local curvature induced by the binding of ions to lipids with charged head groups on one side of the bilayer. This leads to a contraction of the head groups and a concomitant spontaneous curvature.

7. General discussion.

7.1 ORIGIN OF THE RIPPLE PHASE AND $A \rightarrow A/2$ SYMMETRY BREAKING. — There is some controversy whether the P_{β} ripple-phase may form in isolated bilayers or whether it can exist in multilayers only. In fact theoretical models of the ripple structure essentially based on the coupling of bilayers have been introduced (cf. Petrov, private communication 1982). Our experiments clearly show that the ripple

superstructure may indeed form in isolated bilayers. However, the P_{β} -phase may be suppressed in very small vesicles of some 100 Å diameter produced by sonication. This is expected since the strong curvature does not allow the formation of the ripple structure. Moreover it has been shown theoretically that a superstructure may be caused in isolated bilayers, either by a ferroelectric order [11] or by spontaneous curvature of the monolayers [12].

An interesting point is the physical background of the symmetry breaking in the $A \rightarrow A/2$ transition. The two phases may coexist in one vesicle. It appears that the A phase prevails in vesicles of high curvature while the $A/2$ phase dominates in only slightly curved lamellae. This may be explained by the fact that the $+1/2$ and $-1/2$ defects of the A -phase are independent of each other. They can arrange themselves more easily in the case of high curvature than the quasi half disclinations of the $A/2$ -phase which are strongly coupled by defect lines. The latter lead to polygonal vesicles which can be stabilized by impurities (Fig. 12c).

The closing up of the vesicles caused by a reduction of the hydrophobic force at the water-hydrocarbon interface can be considered as a possible external force. The transition from a planar to a curved bilayer corresponds to a reduction of the external symmetry which may be compensated by an increase of the intrinsic symmetry by the $A/2 \rightarrow A$ transition.

Another cause of symmetry breaking is the introduction of impurities. The foreign molecules are not distributed homogeneously but accumulate preferentially in the cores of the defects. It appears that the $A/2$ phase has larger cores and is thus stabilized by the impurities on account of disclinations.

According to figures 11, 12 and 13, the ripples have a polygonal structure and follow the direction of the lattice formed by the hydrocarbon chains. This indicates that P_{β} and L_{β} are true crystalline phases which do not allow for a curvature of the ripples. One consequence of this may be the splitting of the core of the $A/2$ -phase into two half-integer lines. This view is verified by the observation that the defect lines are drastically shortened by the incorporation of small amounts of impurities.

7.2 ON THE POSSIBLE ROLE OF DEFECTS IN BIOLOGICAL MEMBRANE PROCESSES. — The crystalline state is very seldom realized in biological membranes. Outstanding exceptions are (i) the purple membrane of Halobacteria which is essentially a cocrystal of bacteriorhodopsin and lipid or (ii) the cell envelope of bacteria. Electron microscopic studies clearly demonstrate that defects in the cell envelope play an essential role for the growth and division of bacterial cell walls.

In the plasma membranes of cells or in the membranes enclosing cellular subsystems (nucleus, mitochondria) the lipid is in general in the fluid state although crystalline domains cannot be excluded. Therefore only localized defects characterized by a

local variation in the average lipid orientation have to be considered. Localized orientational defects may be induced by the incorporation of proteins into the lipid bilayer (cf. Figs. 14 and 15a) or by a strong local variation in bilayer curvature (cf. Fig. 14c). Such defects may well play a rôle in biological membrane processes such as transport, enzymatic regulation or phagocytosis.

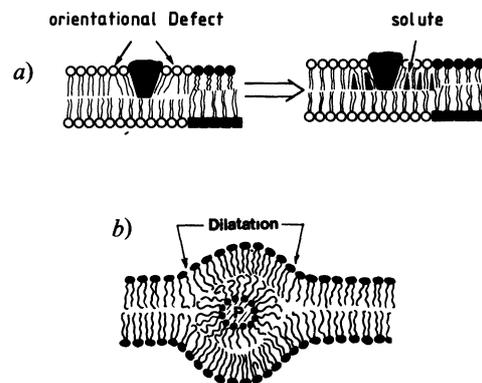


Fig. 15. — Localized defects as attractive traps for drugs or substrates of enzymes in biological membranes. *a*) Orientational defect caused by a membrane bound enzyme with non-cylindrical shape of hydrophobic core. The elastic strain may be relaxed by the incorporation of small solute molecules. This effect is reminiscent of the formation of Cottrell clouds into the strain field of dislocations of metal alloys. *b*) Incorporation of integral protein surrounded by inverted micelle by intercalation between monolayers. A dilatation region forms a long equator of inclosed particle.

According to figure 14a, the incorporation of an amphiphatic protein molecule with a non-cylindrical shape of its hydrophobic part will enforce a tilt of the lipid hydrocarbon chains. As indicated in figure 15a the elastic energy associated with the tilt-deformation may be relaxed by the accumulation of small hydrophobic solute molecules in the environment of the protein. Orientational defects could thus well act as strongly attracting traps for substrate molecules and could thus be involved in the acceleration of enzymatic membrane processes. The elastic strain may also lead to lipid mediated long-range forces between membrane-bound proteins [13]. Another relaxation mechanism is an escape into the third dimension. As postulated in a previous work [14] this could be accompanied by a redistribution of lipid molecules in the monolayer opposing the conical-shaped protein molecules and could thus provide an effective mechanism of trans-membrane coupling. Another type of localized defect arises if particles such as hydrophobic macromolecules or inverted micelles are intercalated between the opposing monolayers (Fig. 15b). The ring of dilatation running around the equator of the particle could again form attractive traps for substrate molecules. Evidence

for such a type of protein incorporation was provided by Stier, Finch and Böstlering [15]. Inverted micelles within bilayers seem to form in membranes containing lipid with charged head groups upon addition of two-valent ions such as Ca^{++} . It has been postulated that the subsequent distortion of the lipid bilayer provides an important step for charge induced membrane fusion [16].

Boundaries at the interface of fluid and rigid domains represent another class of defects with possible biological relevance. The density fluctuations arising at these defects may lead to a drastic increase in passive ion permeability [17, 18] as well as in the activity of enzymes. Examples for the latter are the increase in the rate of lipid-decomposition by phospholipases [19] and the exchange of lipids between membranes by exchange proteins [20].

Examples of localized defects related to curvature are given in figure 16. Variations in the local curvature of bilayers may be achieved by incorporating lipids with different cross sections of the two chains (C) and the head group (H), respectively, into one of the opposing monolayers. A typical example is depicted in figure 14c for a bilayer composed of a lipid-alloy containing one component with a negatively charged

precipitates of this lipid. Simultaneously the binding of the Ca^{++} -ions causes a contraction of the head groups of the charged lipid. The concomitant reduction in the cross section ratio H/C induces a strong spontaneous curvature of the charged lipid precipitates. Consequently (dam-like) protrusions are formed with defects at the base and at the top. The same effect may be triggered by charged polypeptides. Due to the high defect density the protrusions are rather unstable and for that reason they may provide a possible pathway for the fusion of membranes. This process is depicted schematically in figure 16a. Membrane fusion is essential for the transport into and out of cells and it appears that localized defects play an important rôle for this event. It should be noted that fusion may also be greatly facilitated by phospholipids missing one hydrocarbon chain. These so-called lyso-phospholipids are again characterized by a large H/C-ratio and are thus expected to induce localized curvature.

Another interesting type of curvature defect is the hydrophilic pore shown in figure 16b. It is essential for the hemolysis of cells. Pore formation is associated with an energy

$$E_p = 2 \pi \cdot r \cdot \gamma$$

where γ is the so-called edge energy per unit length of the inner circumference of the pore which is determined by the elastic energy contained in the orientational defect at the sharp edges of the pores [21]. The edge energy can again be greatly reduced by the incorporation of lipids with large head groups, that is, large H/C-ratios.

Hitherto, defects could only be studied in model membranes while one is still far from understanding the highly complex microstructure of biological membranes which are composed of some hundred different lipids and proteins. However, biological membranes often exhibit sharp protrusions which are expected to contain a high density of orientational defects. Moreover many membrane-bound proteins penetrate the lipid bilayer only partially as indicated in figure 15a. This is expected to lead to orientational defects in the lipid bilayer moiety surrounding the protein.

Finally there is evidence that membranes contain domains of rigidified lipid/protein aggregates. There should be grain boundaries at the interface of these domains and the fluid membrane regions. The investigation of the possible rôle of defects in biological membrane processes is an intriguing and important task of future membrane research.

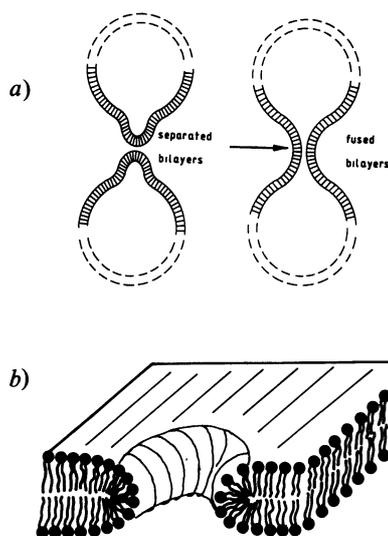


Fig. 16. — Curvature induced defects in bilayers. a) Possible mechanism of fusion of membranes exhibiting strong localized curvatures. b) Formation of hydrophobic pore in bilayer. Note that pore formation may be facilitated by incorporation of lipids with very large head groups into highly defective areas at sharp edge of pore.

head group. If Ca^{++} -ions are added to one side of the bilayer they bind preferentially to the charged component. This leads to the formation of two-dimensional

Acknowledgments.

The financial support of this work by the Deutsche Forschungsgemeinschaft is greatly acknowledged.

References

- [1] TARDIEU, A., LUZZATTI, V. and REMAN, F. C., *J. Mol. Biol.* **75** (1973) 711.
- [2] JANIÁK, M. J., SMALL, D. M. and SHIPLEY, G. G., *J. Biol. Chem.* **254** (1979) 6068.
- [3] TOULOUSE, G. and KLÉMAN, M., *J. Physique Lett.* **37** (1976) L-149.
- [4] KLÉMAN, M., WILLIAMS, C. E., COSTELLO, M. J. and GULIK-KRZYWICKI, T., *Philos Mag.* **35** (1977) 33.
- [5] SACKMANN, E., RÜPPEL, D. and GEBHARDT, C., *Springer Series in Chemical Physics*, W. Helfrich and G. Heppke eds. **11**, 1980, p. 309.
- [6] MERMIN, N. D., *Rev. Mod. Phys.* **51** (1979) 591-648.
- [7] JANSSEN, T., *Crystallographic Groups* (North-Holland Publishing Company, Amsterdam) 1973, Chap. IX.
- [8] KLÉMAN, M., MICHEL, L. and TOULOUSE, G., *J. Physique Lett.* **38** (1977) L-195.
- [9] POENARU, V. and TOULOUSE, G., *J. Physique* **38** (1977) 887.
- [10] HARTMANN, W., GALLA, H. J. and SACKMANN, E., *Fed. Eur. Biochem. Soc. Lett.* **78** (1977) 169.
- [11] DONIACH, S., *J. Chem. Phys.* **70** (1979) 4587.
- [12] GEBHARDT, C., GRULER, H. and SACKMANN, E., *Z. Naturforsch.* **23c** (1977) 581.
- [13] GRULER, H. and SACKMANN, E., *Croat. Chem. Acta* **49** (1975) 379.
- [14] SACKMANN, E., *Light induced charge separation in biology and chemistry*, Gerischer H. and Katz J. J., eds, Life Science Report 12 (Verlag Chemie, Weinheim) 1979.
- [15] STIER, A., FINCH, S. A. E. and BÖSTERLING, B., *Fed. Eur. Biochem. Soc. Lett.* **91** (1978) 109.
- [16] VERKLEIJ, A. J., VON ECHTOLD, C. J. A., GERRITSEN, W. J., CULLIS, P.-R. and DE KRUIFF, X., *Biochim. Biophys. Acta* **600** (1979) 620.
- [17] NAGLE, J. F. and SCOTT, H. L., *Biochim. Biophys. Acta* **513** (1978) 236.
- [18] DONIACH, S., *J. Chem. Phys.* **68** (1978) 4912.
- [19] OP DEN KAMP, J. A. F., BEVERS, E. M. and ZWAAL, R. F. A., in *Structural and Kinetic Approach to Plasma Membrane Function*, Nicolau, C. and Paraf, A., eds (Springer Verlag Berlin) 1977.
- [20] XÜ, Y. H., GIETZEN, K. J., GALLA, H. J. and SACKMANN, E., *J. Biol. Chem.* in press (1982).
- [21] PETROV, A. G., SELEZNER, S. A. and DERZHANSKI, A., *Acta Physica Polonica A* **55** (1979) 385.
- [22] KREBECEK, R., GEBHARDT, C., GRULER, H. and SACKMANN, E., *Biochim. Biophys. Acta* **554** (1979) 1.
- [23] RÜPPEL, D., KAPITZA, H.-G., GALLA, H. J., SIXL, F. and SACKMANN, E., *Biochim. Biophys. Acta* **692** (1982) 1.
-