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# Morphology and Growth Behaviour of Myelin Figures of Lecithin

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Growth behaviour and morphological features of myelin figures in egg-yolk lecithin/hydrophilic liquid systems were investigated by microscopic observation at room temperature. The morphological features were conveniently classified into three steps according to the time of growth.

In the first step, myelin figures with a simple rod-like form rapidly grew all together perpendicularly to the circumference of a lecithin block into the medium. In the second step, more complicated forms, such as helical and coiling ones, occurred. In the third step, the parts where a contact between surfaces of neighbouring rods had occurred were associated, fused and transformed into a mosaic structure. The representative growth mechanism of each of these three steps was dependent mainly on a diffusion of aggregates of lecithin molecules towards myelin figures near their roots, on a lateral diffusion in the bilayer along the long axis of a myelin figure and on a lateral diffusion in the bilayer around the long axis, respectively.

## INTRODUCTION

Myelin figures are a peculiar structure found in systems of an amphiphilic molecule and an aqueous liquid medium. They are a multilamellar rod-like structure in which bilayered lamellae of amphiphiles are concentrically wrapped around the rod-axis with the liquid medium between every other lamellae. The structural features of this system are essentially similar to those of the nerve myelin sheath and a bilayer bears a resemblance to a biological membrane. They are observed to grow on the surface of bulk amphiphiles in smectic A liquid cystalline phase in contact with a suitable aqueous liquid medium.[1–3] A typical example of them is found in lecithin/water system. Lecithin, consisting of a hydrophilic polar head group and a hydro-

phobic group with two hydrocarbon chains, is one of the major components of biological membranes. Thus the basic knowledge of its assemblage into myelin figures will afford us an understanding of the process of self-assembly in well organized biological tissues.

In this paper the growth behaviour and morphology of the myelin figures in egg-yolk lecithin/water and lecithin/ethyleneglycol systems will be reported, which are obtained by observations under a polarizing microscope at room temperature. The growth process of myelin figures is easily affected by the change in surrounding conditions giving to them many structural variations. We will propose here three steps of morphological features which change with the time of growth.

## SPECIMEN

The sample of lecithin was commercially available one from Sigma Chem. Co. supplied as a chloroform solution. It was prepared from hen-egg-yolk by the method of Singleton *et al.*, [4] and was used without further purification. $Ca^{2+}$ and other ionic residues may remain in the lecithin. The egg-yolk lecithin is a mixture in the sense that its two hydrocarbon chains show various length and degrees of unsaturation. The two chains of a representative molecule are oleic and palmitic acids. [5]

## MICROSCOPIC OBSERVATIONS OF GROWTH PROCESS

After evaporating chloroform in air, small lumps of lecithin with a waxy appearance, were placed on a slide glass. Then a cover glass was put on the lumps and pressed slightly until the thickness of lecithin lumps was reduced to about 50 μm. Water and ethyleneglycol were used as the hydrophilic medium. Observations of growth of myelin figures were made by use of a polarizing microscope.

A drop of a dispersive liquid medium was contacted with the edge of the cover glass of the preparation, so that the excess liquid medium spread over into the gap of the slide and cover glass. As soon as the surface of a lump of lecithin contacts with the medium, myelin figures of a simple rod-like form were observed to start growing all together [1] (Figure 1). The appearance of myelin figures was changed from the simple rod-like form to forms with various complex structures as they went on growing. We can conveniently classify the growth process into the following three steps by morphological features appearing successively during the growth.

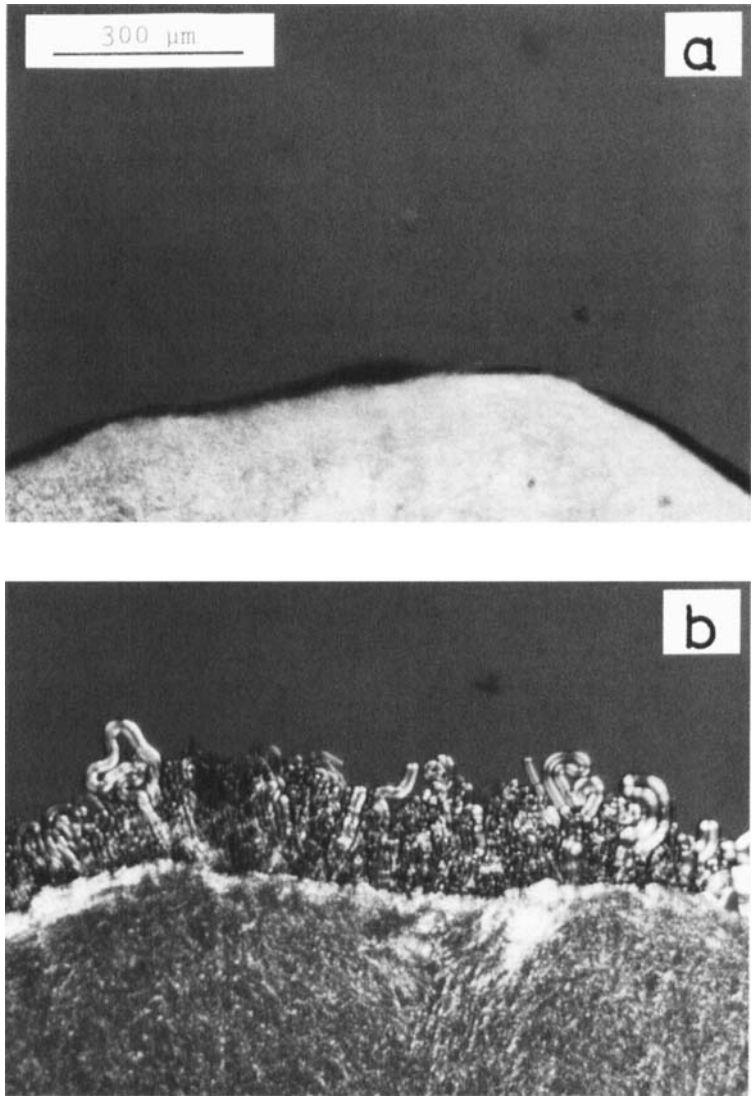


FIGURE 1 A series of photographs of the initial growth pattern of myelin figures in egg-yolk lecithin/water system taken by a polarizing microscope; (a) before introducing water, (b) 1 min and (c) 5 min after introducing water, respectively. Soon after the medium spreading over and being brought into contact with the edge of the lecithin lump, myelin figures of a simple rod-like form are observed to start growing all together. The average length $l$ of myelin figures was measured as the distance between contours of the edge of the lecithin lump and the growth front of myelin figures. The plot of $l$ against $t^{1/2}$ ($t$: time measured from the contact of the medium and the lump), suggests that this initial growth process is limited by a diffusion process. See Ref. 1 for further details.

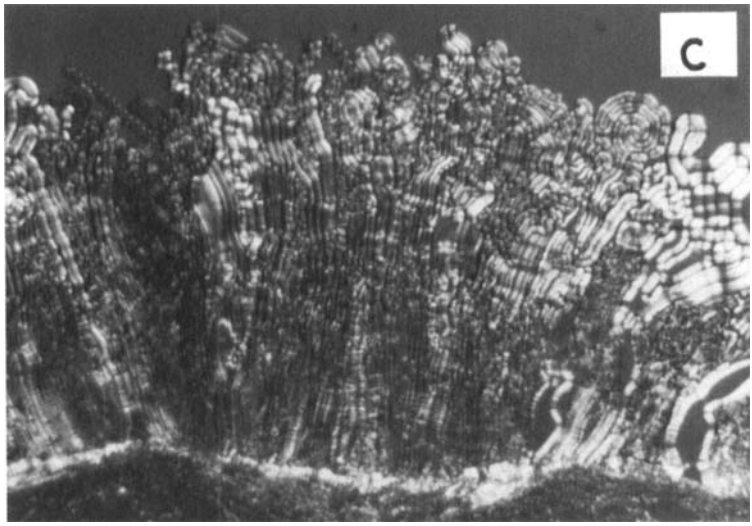


FIGURE 1 Cont.

In the first step, myelin figures which showed a simple rod-like form began to grow from the surface of the lumps into the aqueous medium. In the second step, while the simple rod-like form lasted unchanged, various new forms were observed to appear, such as folding form, helical form, coiling form, tadpole form and so on. These figures seemed to be apparently stable for scores of hours. They showed very small growing rate if the surrounding conditions of the system such as temperature or the ratio of aqueous medium and lecithin were not changed. After the saturation of the second growth step, the third step occurred, where the transformation of the myelin figures into mosaic structures was observed. In the following these steps will be described in turn

### 1. First step

The myelin figures observed in the initial growth step (*i.e.* within the first 10 min in the case of water as the medium, and a longer time in the case of a more viscous liquid medium) have a simple form of rod which is basic to all myelin figures. This simple rod-like form is also seen even in the second step (Figure 2). The rods can grow into very long ones as known by tracing the contour of a rod, even if the rods show many foldings. It is worth noticing that the rods keep their initial diameter all along their length. The average diameter of them

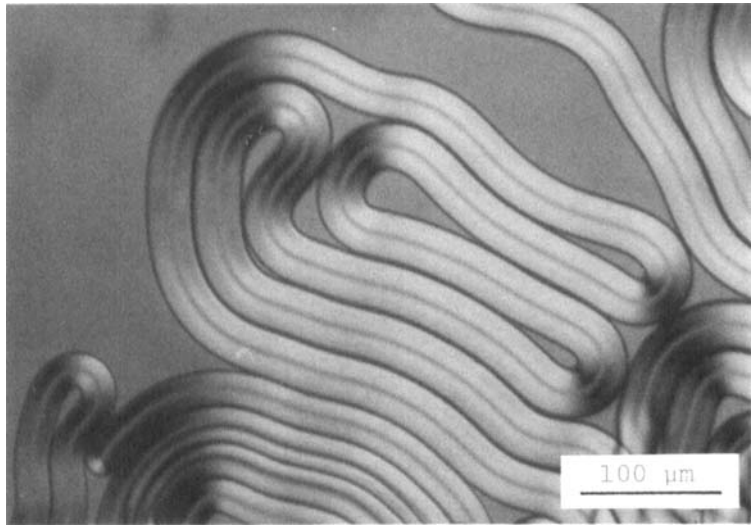


FIGURE 2 A folded myelin figures of the simple rod-like form in egg-yolk lecithin/ethyleneglycol system, which is characteristic to the late first step and especially to the second step. The long myelin figure shows many irregular foldings, but it runs without changing its diameter.

in the present experimental conditions was nearly the same ranging between 20 and 40 μm, irrespective of the difference in the medium.

These rods have a strong uniaxial optical anisotropy and the optic axis is perpendicular to the long axis of the rods and lies centripetally on a cross section perpendicular to the long axis. From X-ray diffraction measurements it was shown that the direction of the hydrocarbon chain axis was parallel to that of the thickness of bilayers constituting the rods in consistent with the optical observation. When a large tension was applied along the axis of myelin figure by a thin pin edge, it was fractured giving rise to a stepwise fractured surface of rod, implying that sets of stacked bilayers were torn off as shown in Figure 3. Observations of the simple rod parallel to the long axis by a polarizing microscope showed the so-called "Maltese cross," implying again radially arranged optic axes (See Figure 7). These features prove that the myelin figure consists of stacked multilamellae winding coaxially around a thin cylindrical core of medium.

Along the axis of the myelin figures, the thin cylindrical core of medium was observed. Figure 4 shows the appearance of such a core for lecithin/ethyleneglycol system, which has a clear boundary with the stacked bilayer part as revealed by use of a phase contrast microscope. The cross-sectional view of the medium core is also ob-

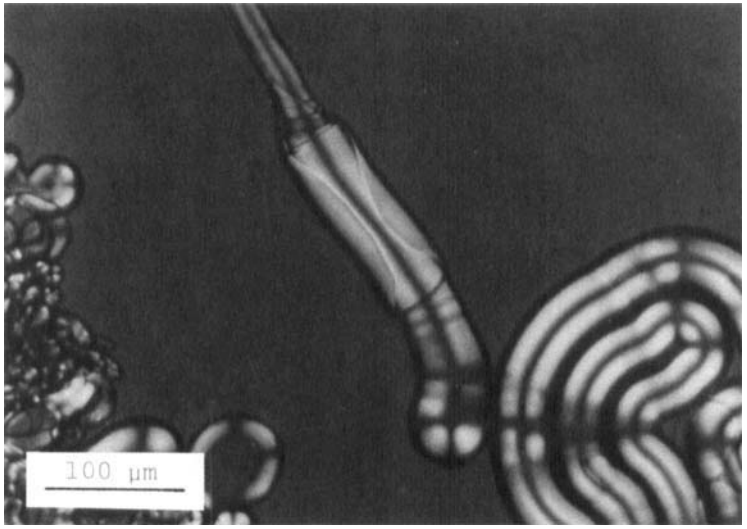


FIGURE 3 A myelin figure of simple rod-like form in egg-yolk lecithin/ethyleneglycol system broken by an external force. The terracedly broken rod implies that the original rod is made up by many stacked bilayers of lecithin.

served when the myelin figures are observed along the long axis as shown by the arrows in Figure 4. The cross-section of medium core is circular, having a diameter of about ~ 2 μm in this photograph. The core radius seems to be constant in spite of the variation in the magnitude of the thickness of myelin figures around 15 ~ 40 μm, when observed at least in the same growing stage.

The relative concentration of the number of aligned lecithin molecules in the bilayers was estimated from the optical density obtained by use of a micro-area spectrophotometer system with crossed-Nicols. On this step, the optical density is larger for a micro-area at the root part than for that at the top part along the long axis of a myelin figure. The difference in the optical density between the two parts divided by the distance between two points is proportional to the concentration gradient along the long axis of myelin figure, and it gradually decreases with the growth time.[6]

The average length of rods is in proportion to the square root of the growth time implying a diffusion limited growth mechanism for this step as described in the previous paper.[1] Following to the first step growth, there appears a more complicated growth behaviour where more complicated forms of rods prevail to grow.

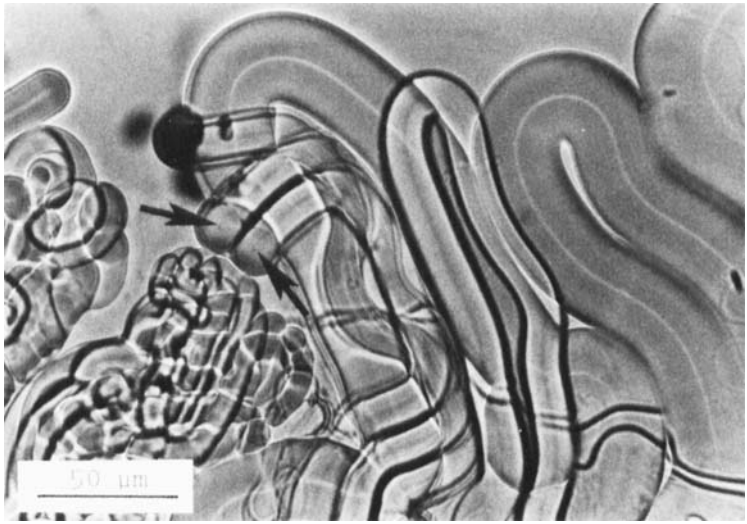


FIGURE 4 The cross-sectional view of medium core (arrows) in the centers of myelin figures in egg-yolk lecithin/ethyleneglycol system. The core part was made clearly distinguishable by doping a fluorescent dye. The medium core is also observed at the folding parts of myelin figures and it is known to be a thin cylindrical rod from the cross-sectional view (arrows). The photograph is taken by use of a phase contrast microscope.

## 2. Second step

The second step of growth is characterized by a growth rate of a few orders of magnitude smaller than that in the first step. The growth took place at both the root and top parts of myelin figures, the rate of which was faster at the root than at the top in the initial period of this step. Many morphological features of myelin figures observed in this step were able to be classified roughly into the following forms; simple rod form, helical form, coiling form, tadpole form, *etc.*

The characteristic feature of the second step of growth is the appearance of helical and coiling forms. Both right-handed and left-handed structures were observed in the present system of myelin figures obtained from egg-yolk lecithin consisting of only L-type molecules. Hence the sense of winding direction of their helical or coiling forms have no relation with the chirality of molecule, whereas the sense of helical structures of some crystals of molecules with similar chemical structures to lecithin depends on the chirality of molecules.[7]

The helical form observed in this step is generally the double-helix of two simple rods having the same diameter (Figures 5 and 6). The

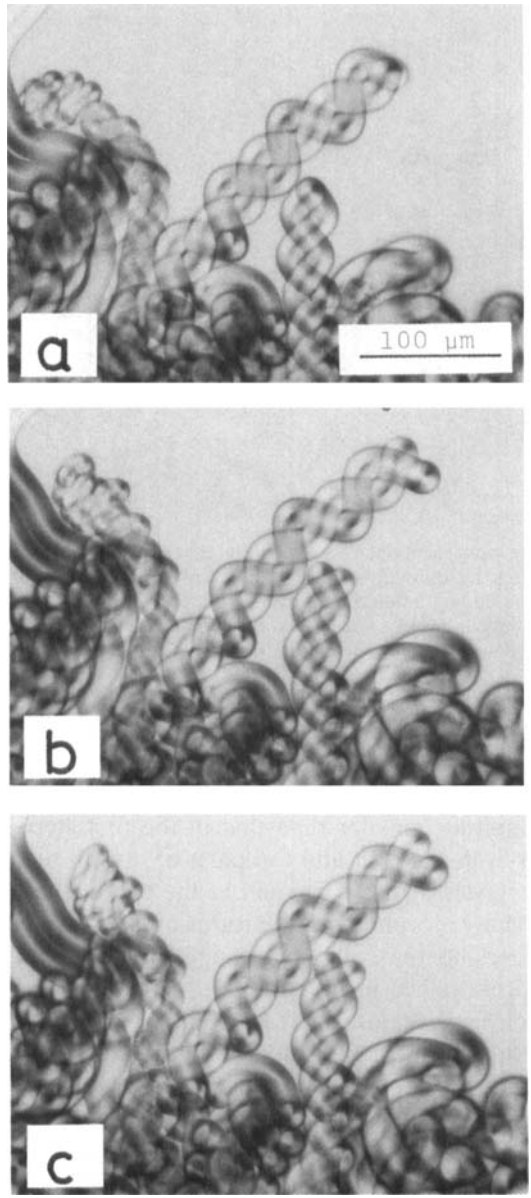


FIGURE 5 A series of photographs of the double helical growth process taken by a polarizing microscope. Two simple rods in egg-yolk lecithin/water system make a double helix. In this series, two separate rods make a double helix. It grows through the addition of a new pitch at the top of the helix, where the two separate rods with the same growth rate make a new twist with each other.

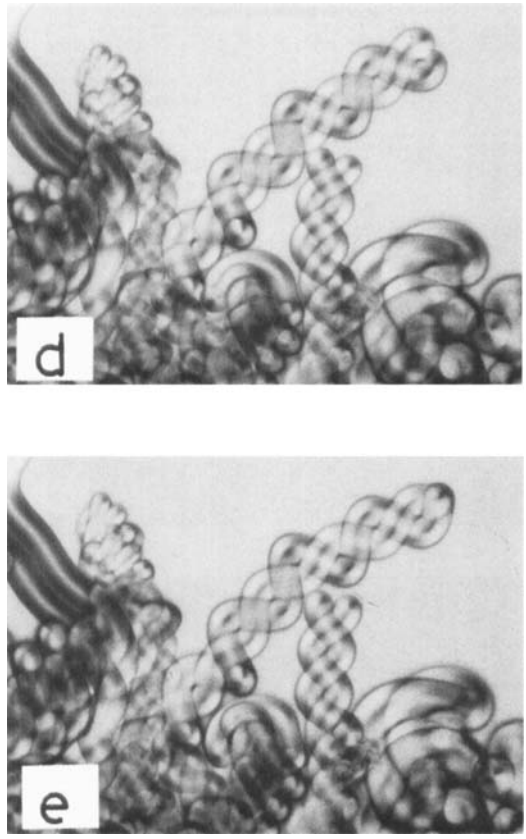


FIGURE 5 Cont.

coiling form is constructed of only looped one rod (Figure 7) or a rod winding around the other coil (Figure 8). The growth of these two forms proceeded by the addition of new pitches at the top of each figure, which took several minutes per pitch. However, the addition of a pitch occurred not only by a helical twisting or coiling of two separate rods of simple form at the top (Figure 5), but also by a twisting deformation of a loop connecting two helically twisted rods at the top of a helix (Figure 6). Therefore, two types of growth procedure at the top of myelin figures are distinguished; one is the helical twisting of two separate rods of simple forms occurring at the top of a growing helix (Figure 5), and the other is a complicated twisting deformation of a loop connecting two simple rods at their

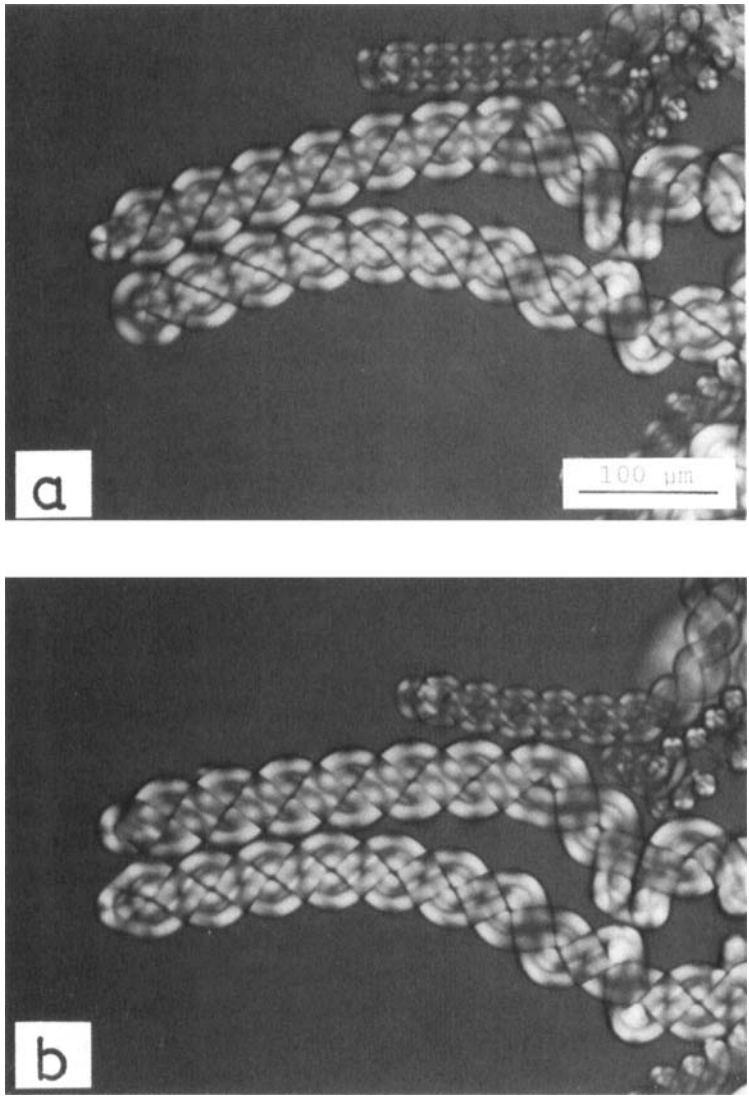


FIGURE 6 A series of photographs of the double helical growth process taken by a polarizing microscope. Two simple rods in egg-yolk lecithin/water system make a double helix. The upper helix is of left-handed winding and the lower one is of right-handed winding. Near the root part of upper helix, a defect in helical winding is observed. Since a translation or a rotation of the defect along or around the helix axis are not observed here and also the addition of new pitches occurs in the part beyond the defect, it is implied that the growth of the double helix occurs at its top part. In this series, however, the growth behaviour at the top of the helical structure is different from those in Figure 5. Two tops of two simple rods are always connected with each other making a loop during the formation of a new pitch.

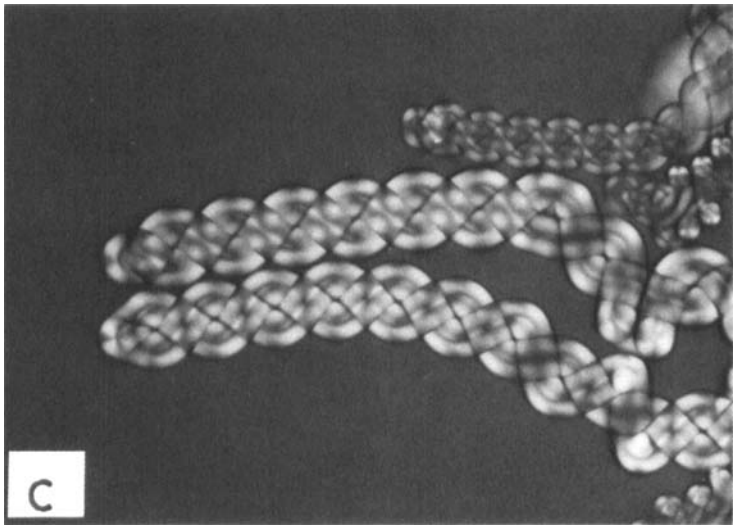


FIGURE 6 Cont.

growth ends (Figure 6). Figure 9 shows more clearly the loop at the top part of a helical myelin figure and in this photograph a thin core of medium is observed also to make a twisted loop. From observation of the growth of new pitch at the top of twisted form, it was recognized that the growth of the myelin figures in this growing step occurred also at the top of them. The growth time necessary for the formation of one new pitch of the twist was several minutes even in the initial second growth step. The helix formation, folding and coiling of the myelin figures in the growth process of the second step all imply that there is a tendency for each rod to decrease the surface area in contact with the surrounding medium.

Another distinctive feature shown in the second step growth of myelin figures was the appearance of a "tadpole" form (Figure 10). As a result of the intake of the excess liquid medium into the central medium core from the root of the myelin figures, the medium core at top part was expanded without changing the thickness of stacked multilayers as shown by arrow A in Figure 10. By the boundaries of the gap of slide and cover glasses, however, the thickness of the expanded part is limited. Thus the expanded part is flattened and shows a disk-like structure, whose thickness is the same as the diameter of its rod part. Thus the structure ressembles the head part of a tadpole. Under a polarizing microscope, the central part of

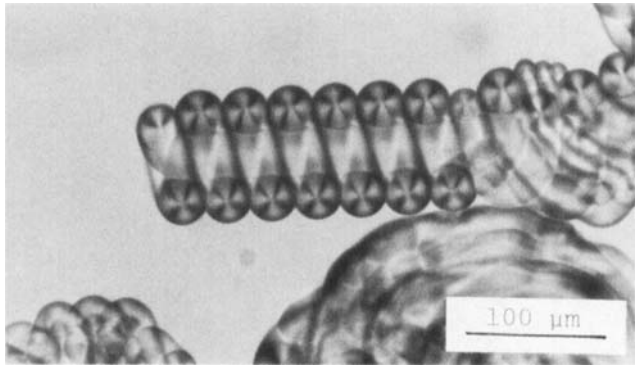


FIGURE 7 A coiling form constructed of only one looped rod in egg-yolk lecithin/ethyleneglycol system taken by a polarizing microscope. The photograph was taken by focusing on the middle part of the coil. Maltese-cross is observed in the cross-sectional view of a rod of myelin figure winding itself over a straight part of the loop.

expanded disk-like structure appears optically isotropic through orthoscopic observation. Through a conoscopic observation, however, an interference pattern, which is characteristic to that expected for a uniaxial system observed along the direction of the optic axis, is obtained (Figure 10-a, inset). It is, therefore, suggested that the central part of disk contains stacked lipid-bilayers within which molecules are oriented normally to the glass surfaces on the average. The ex-

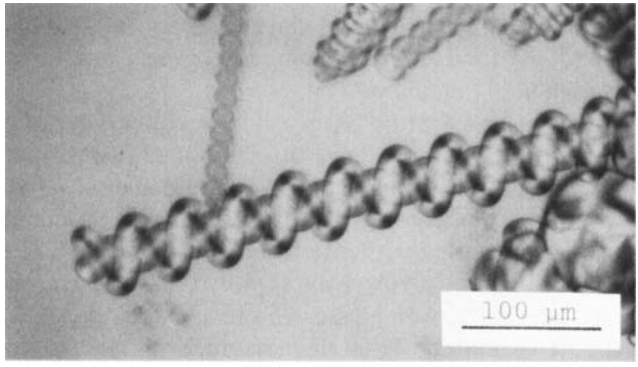


FIGURE 8 The coiling form constructed of two rods in lecithin/ethyleneglycol system. One rod winds coils over around the other.

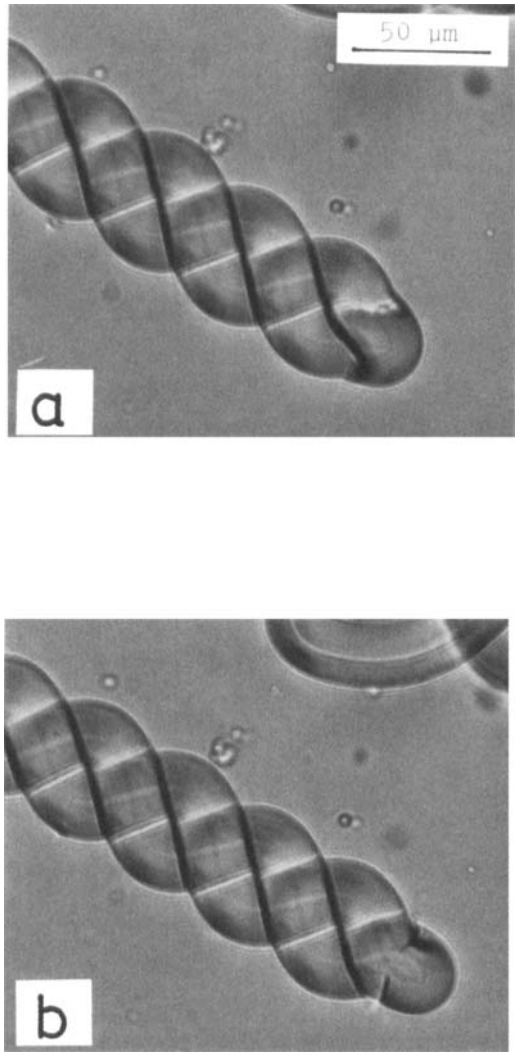


FIGURE 9 The process of the addition of a new pitch at the top of a double helix is shown more clearly by use of a phase contrast microscope. A loop of thin medium core at the top is observed to gradually elongate, twist and make a new pitch.

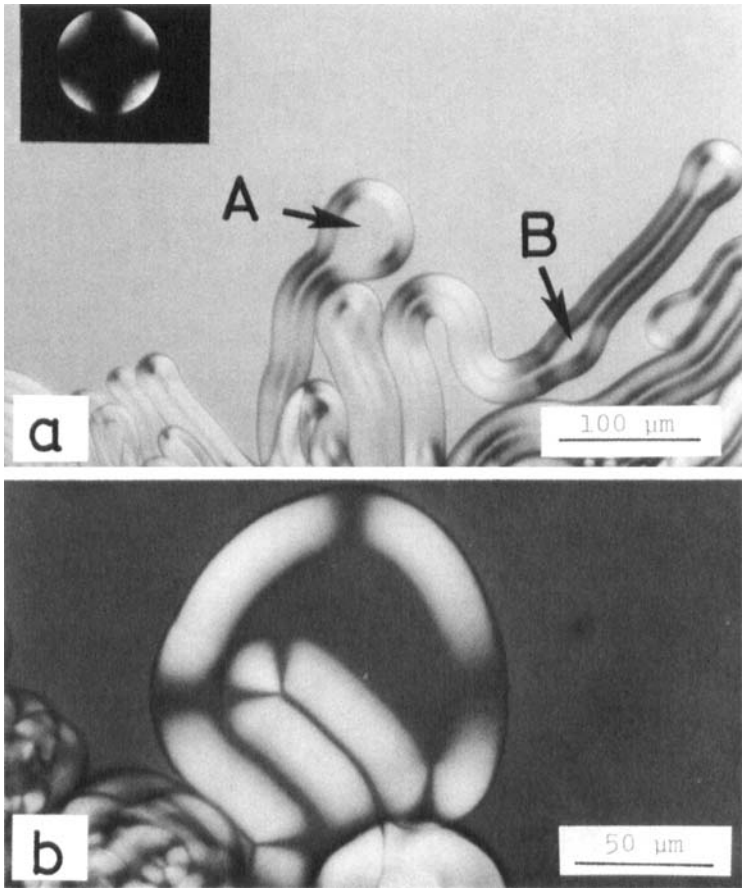


FIGURE 10 The tadpole forms in lecithin/ethyleneglycol system. (a) The expanded part (shown by arrow A) at the top part of simple rod looks like the head of a tadpole. Note that the "wall," *i.e.* the part of stacked bilayers, does not change its thickness. The core of the expanded part, which is restricted in a narrow two-dimensional space of about 50 μm in thickness, is constructed of a disk-like medium having the thickness same as the diameter of the medium core in the rod region. The core is surrounded by the "wall" of stacked bilayers having the same thickness as that of the rod part, and it tends to expand with time resulting in a large expanded core as shown in Figure 11-c. The "head" of a tadpole inside the "wall" is optically isotropic under the orthoscopic observation by a polarizing microscope as shown here. The conoscopic pattern (inset of a) taken from such a part, however, shows a cross figure typical to the uniaxial system observed along the direction of the optic axis implying a sandwich structure, "wall/medium core/"wall," there. An expansion in the middle part of rod also occurs as shown by arrow B. These expanded parts tend to form a structure which looks like an inversed rod with the inside of the original rod out as shown in (b). The contacting outer surfaces in this inversed rod, *i.e.* the central part of it, tend to be deformed giving a structure as will be discussed in the following section for the third step growth.

pansion was also observed at the cylindrical rod part as shown by the arrow B in Figure 10-a. The expanded part increased more and more with a lapse of time and the deformation extended toward the root of the original rod.

The growth in the second step can be thought as an increase of the length of rods supposedly due mainly to the lateral diffusion process along the long axis of the rod, which results in the addition of a pitch to helical or coiling forms, and also due partly to the diffusion process continuing from the first step where a translation of mass of molecules towards the top of the rod should occur even near the root of the rod.

Indeed, an observation by a fluorescence microscope implied the occurrence of lateral diffusion within a bilayer. The observation of the second step of growth by a fluorescence microscope revealed a rapid recovery of fluorescence of the photo-bleached region of myelin figures doped with a fluorescent dye only soluble in the hydrocarbon chain part of lipid molecules. This implies a fast influx of the fluorescent dye into the photo-bleached region, hence the occurrence of the lateral diffusion.

After the saturation of helix formation, it was observed, in some cases, that a helix was untwisted from the top just as if the growth procedure was retraced inversely implying a reversible helix formation at least in such a case. The remainder of helices was transformed to somewhat complicated forms, partly giving rise to a mosaic structure (see Figure 12-b) as is discussed in the next section.

### 3. Third step

The growth of myelin figures in the second step was noticed to be saturated after a growth period ranging from several hours to days depending on the medium used, the size of the original lecithin lump and other surrounding conditions. We call this phase as the third step. The growth in the third step was characterized by the association and fusion of touching outer surfaces of two rods and by the subsequent transformation of these rods to a mosaic structure. Contacting flat areas of the bilayers of phospholipid vesicles tend to be deformed and fused by the interbilayer association at the contact.[8,9] On both sides of the fused contacting outer surfaces of myelin rods, a structure which looked like a regularly twisted mosaic structure appeared as shown in Figure 11. This structure was, in due time, deformed into more complex form similar to "oily streaks" which resulted from regular periodic undulations in the lateral position of the axes of paired disclinations.[10] At first, these regular twists occurred only in

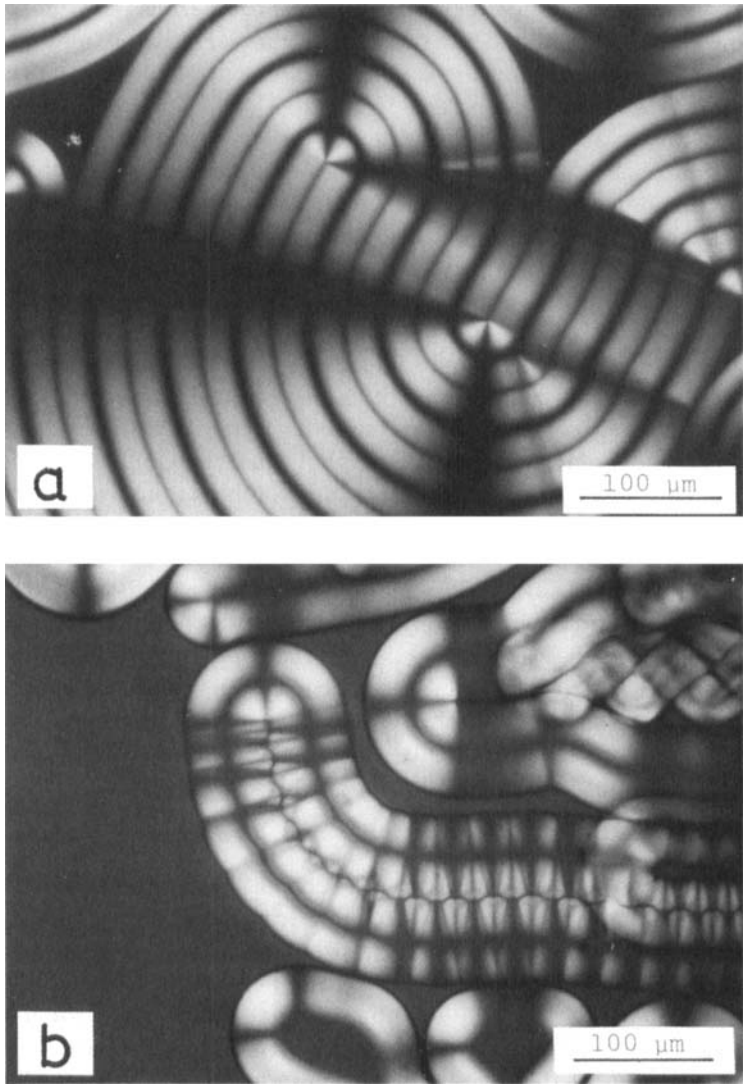


FIGURE 11 Polarizing microscopic observation in the third step growth. (a) The outer surfaces of myelin figures tend to make a contact with each other in the third step. In contrast to the loose fold of simple rod-like myelin figures in the first and the second step, the fold observed here is sharp and the surfaces of rods are closely contacted with each other. (b) On the boundary of contacting outer surfaces of simple-rod myelin figure with a sharp fold back, association and fusion of the two surfaces seem to occur and the boundary is deformed into regularly twisted texture. In this stage, the outer surface of a rod without contacting to the other is not deformed to twist. (c) Two myelin figures with exanded medium cores at the top parts, *e.g.* M1 and M2, come in contact with each other at the outer surface part of each myelin

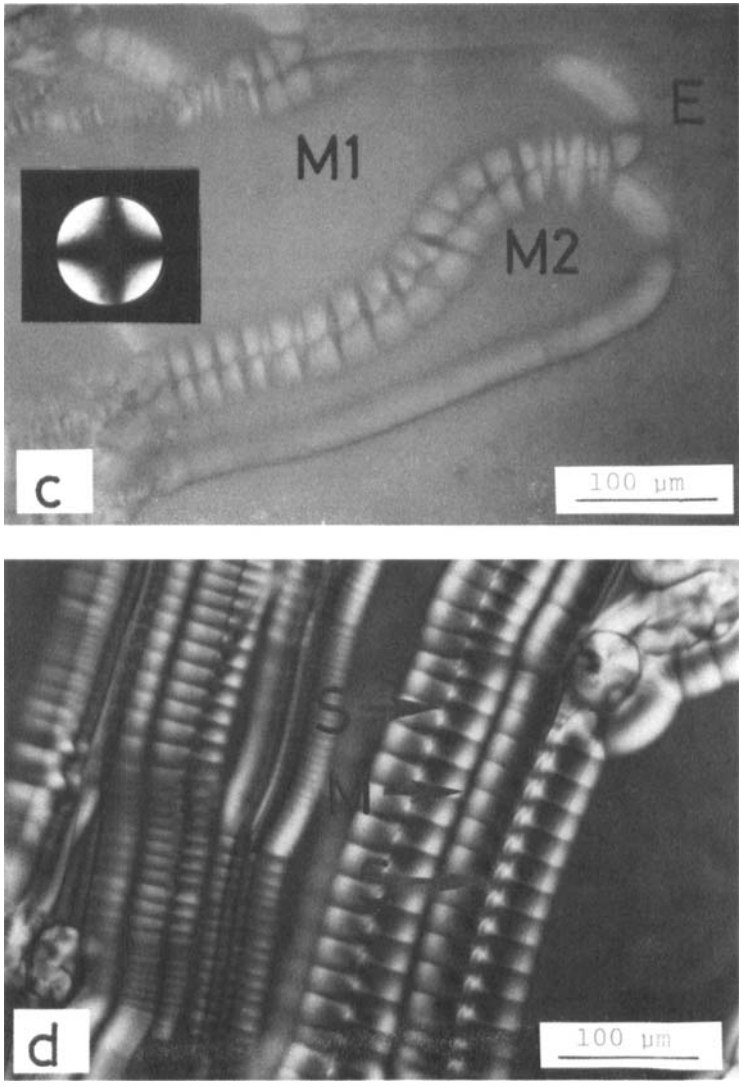


figure. Note that a periodic structure is observed only at the contacting regions, which may be an indication of an association of two myelin figures and a resultant deformation of stacked bilayers. Under the orthoscopic observation by a polarizing microscope, the expanded medium core regions M1 and M2 are optically isotropic just like a region without specimen, E. The conoscopic pattern (inset) taken from the expanded medium region, however, shows a cross figure typical to the uniaxial system observed along the direction of the optic axis just similar to that observed for the tadpole form in the second step (Figure 10-a). (d) The regular twist formation continues steadily. The boundary shown by arrow M was originally the medium core of a rod and that shown by arrow S was the contacting outer surfaces of two rods.

the parts of two myelin figures contacting through their outer surfaces as shown in Figures 11-b and -c. But, as the contacting outer surfaces of the neighbouring rods should interact with each other, they finally associated and fused into an apparently single unit. The boundary of fused neighbouring rods extended along the long axis of them appeared to be a wavy sinusoidal line indicating a periodic orientational change of the optic axis, *i.e.* the normal of stacked bilayers, along the rods as shown in Figure 11-c. This deformation of myelin figures gave a mosaic structure to the rods, and was observed only at the contacting surfaces of two rods as shown in Figure 11. As the deformation proceeded further, the original feature of myelin figures disappeared and the mosaic structure prevailed (Figures 11-d and 12-a). The helical structure still remaining at the end of the third step tended to be deformed into an array of dumbbells as shown in Figure 12-b. During association and fusion occurring at contacting outer surfaces of myelin figures, the two sets of stacked bilayers in the central part of a disk of tadpole were deformed into a mosaic structure (Figure 12-a).

## CONCLUDING REMARKS

We have proposed here a rough classification scheme of the morphology of myelin figures appearing successively in their growth process, and suggested a probable mechanism of structure formation in each growth step.

In the first step, the growth of myelin figures is due to a diffusion process with an apparent diffusion coefficient $D \sim 10^{-6}$ cm$^2$/sec. D can be expressed by a sum of two terms $D_1$ and $D_2$, where $D_1$ comes from the diffusion of an aggregate of lecithin molecules towards the top of myelin figures occurring even within the lump near the root of myelin figures and $D_2$ mainly from the lateral diffusion within the bilayers which constitute the myelin figures. The lateral diffusion in the first step seems to occur towards the top of myelin figures from the roots along the long axis of them on account of the existence of a concentration gradient along the rod axis and the difficulty in diffusion across bilayers.

In the second step, the diffusion process represented by $D_1$ seems to be gradually saturated, and the growth rate of myelin figures becomes a few orders of magnitude less than that in the first step. In addition, a slow growth of myelin figures is observed as the addition of new pitches at the top of helical or coiling structures. From the

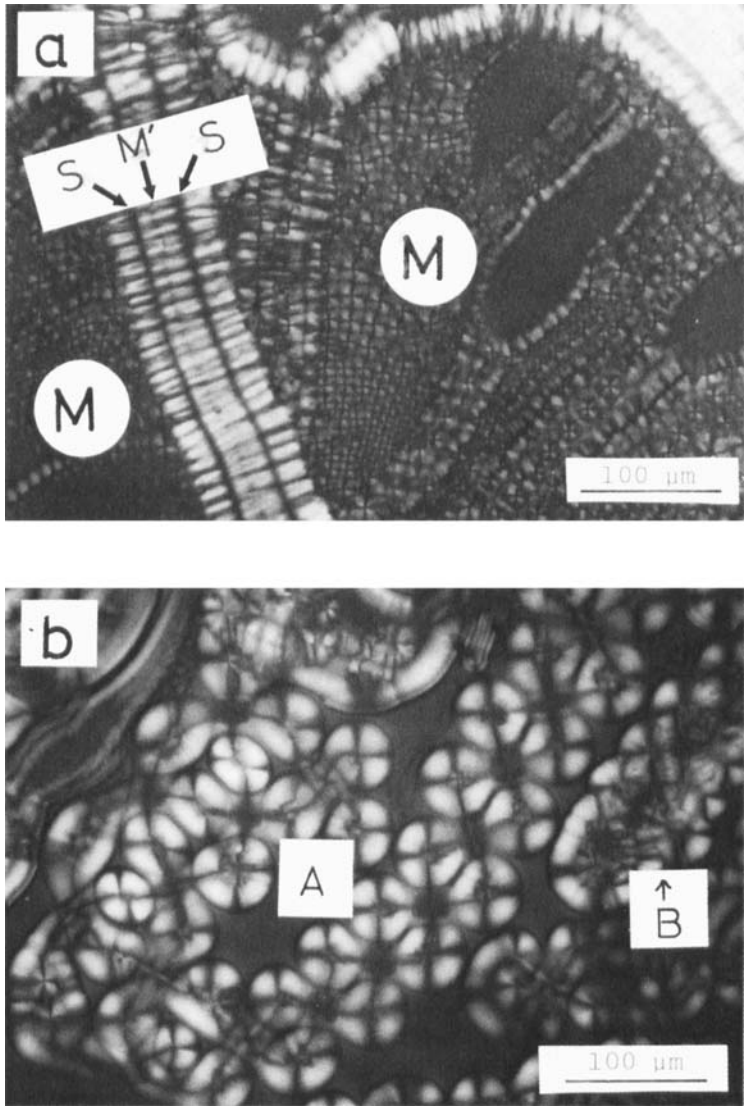


FIGURE 12 (a) The mosaic structure formed by myelin figures whose medium cores fully expanded after the end of third step. M is the expanded medium core which was sandwiched by two walls of stacked bilayers in the second step. M′ is a medium core of a simple form which comes in contact by its two outer surfaces, S, with halves of expanded myelin figures. (b) The helical structure as shown in Figures 5 and 6 in the second step is sometimes transformed into structures appeared in this photograph from the end of the third step and the array of dumbbells, A, gradually transformed into an array of spherulites, B.

growth behaviour and rate, the growth process in the second step is thought to be limited mainly by the lateral diffusion along the long axes of myelin figures.

After the disappearance of the concentration gradient of the system, myelin figures can not show an apparent growth any more and the deformation of them is observed to occur as the third step. The deformation associated with the association and the fusion of myelinic rods, which may be induced by the increasing interaction between outer surfaces of contacting myelin figures, is thought to be mainly driven by lateral diffusion around the long axis of myelin figures.

The present paper only treats an outline of the morphology and growth mechanism of myelin figures on the basis of many microscopically observed features and behaviours of them during the growth process, and many problems remain open such as how and why the molecules can be set in a well organized structure of myelin figures. Further investigations, therefore, would be necessary for the study of self-assembly of molecules in myelin figures, which have an analogue in biological systems.



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## References

1. I. Sakurai and Y. Kawamura, *Biochim. Biophys. Acta*, **777**, 347 (1984).
2. I. Sakurai and Y. Kawamura, *Biochim. Biophys. Acta*, **735**, 189 (1983).
3. M. Kleman, C. E. Williams, M. J. Costello and T. G.-Krzywicki, *Phil. Mag.*, **35**, 33 (1977).
4. W. S. Singleton, M. S. Gray, M. L. Brown and J. L. White, *J. Am. Oil Chem. Soc.*, **42**, 53 (1965).
5. N. H. Tattrie, J. R. Bennett and R. Cyr, *Can. J. Biochem.*, **46**, 819 (1968).
6. I. Sakurai, *Biochim. Biophys. Acta*, **815**, 149 (1985).
7. N. Nakashima, S. Asakuma and T. Kunitake, *J. Am. Chem. Soc.*, **107**, 509 (1985).
8. R. P. Rand, T. S. Reese and R. G. Miller, *Nature*, **293**, 237 (1981).
9. R. G. Miller, *Nature*, **287**, 166 (1980).
10. M. B. Schneider and W. W. Webb, J. Phys. (Paris), **45**, 273 (1984).