

## Magneto-orientation of lecithin crystals

(lipid single crystal/diamagnetic susceptibility/polar head)

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Communicated by H. Richard Crane, September 5, 1980

**ABSTRACT** For the purpose of clarifying the magneto-orientation mechanism of several biological membrane systems, xylene-suspended thin-layered single crystals of lecithin, which is a major component of membranes, were subjected to magnetic fields of various strengths, and the orientation behavior of the crystals was analyzed. Lecithin crystals oriented in such a way that the directions of both the hydrocarbon chains and the phosphorylcholine group of the lecithin molecule were perpendicular to the magnetic field, suggesting that the crystals were diamagnetically biaxial. In contrast, hydrocarbon chains of phospholipids in many biological membranes are known to orient parallel to magnetic fields. The value of the volume diamagnetic susceptibility anisotropy,  $\Delta\chi$  (i.e. the difference between susceptibilities parallel and perpendicular to the direction of the hydrocarbon chain in the lecithin crystals), estimated from the magneto-orientation behavior was about  $-9 \times 10^{-8}$  cgs. This is distinctly larger than that of vesicles of egg yolk lecithin measured by Boroske and Helfrich [Boroske, E. & Helfrich, W. (1978) *Biophys. J.* 24, 863-868] and also larger than that of single crystals of polyethylene, which was measured for comparison by the same technique as that used for lecithin in this study. The differences between these values of  $\Delta\chi$  seem to result from the contribution of the polar head (i.e., the part of the lecithin molecule other than the two hydrocarbon chains), which is ordered, less ordered, or deleted in the respective cases above, although mainly from the contribution of the hydrocarbon chains, which are ordered in crystals and disordered in vesicles.

Suspensions of various biological membrane systems such as retinal-rod outer segments (1, 2), chloroplasts (3-6), and purple membranes from *Halobacterium halobium* (7) have been known to show magnetic field-induced orientation. All these systems align with the membrane normal parallel to the field direction, and it seems that this orientation effect is a common feature of biological membrane systems. In this effect, however, lecithin molecules, which are a major component of membranes, orient with their hydrocarbon chains parallel to the field; this orientation is contrary to the reported anisotropy of the magnetic susceptibility of hydrocarbon chains (8). The orientation effect, therefore, is assumed to be primarily caused by the anisotropy that results from oriented membrane proteins (9-11). The aim of this paper is to clarify the role of lecithin molecules in the magneto-orientation effect of biological membranes and to put it on a more quantitative basis. In the course of structure analysis of lecithins by x-ray and electron diffraction methods, we succeeded in making single crystals of lecithin, and we studied the effect of a magnetic field on lecithin crystals suspended in xylene. In the magnetic field, the crystals oriented in such a way that hydrocarbon-chain and phosphorylcholine-group directions, which are orthogonal to each other, are perpendicular to the field direction. We report our results on dipalmitoyl-L- $\alpha$ -lecithin.

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## MATERIALS

Dipalmitoyl-L- $\alpha$ -phosphatidylcholine (Pam<sub>2</sub>PtdCho) was obtained from Fluka (Buchs, Switzerland) and was used as received. The single microcrystals with thin-layered structure were prepared as follows (12): the xylene suspension of the lipid (which had been dried under reduced pressure for a few days) was prepared below 50°C and was kept at room temperature for a day. In this procedure, the greater part of the lipid seemed to dissolve and recrystallize into thin single microcrystals. Each microcrystal was a thin rectangular platelet (Fig. 1) in which bilayers of lecithin molecules were stacked parallel to each other, and the plane of the bilayer was parallel to that of the platelet. Average dimensions of the Pam<sub>2</sub>PtdCho crystals used in the present study were about 36  $\mu$ m long, 18  $\mu$ m wide, and 0.1  $\mu$ m thick (evaluated from the contrast and appearance of images on electron microscopic photographs). The x-ray diffraction pattern of crystals suspended in xylene was that of a typical single crystal of rectangular lattice with spacings 8.7 Å and 8.9 Å. Details of the x-ray study of the crystals will be reported elsewhere.

The x-ray powder diagrams of crystal aggregates obtained after complete evaporation of xylene from the suspensions were in accord with those of purchased anhydrous powder-like samples used as received, within the probable error in measured spacings, typically of the order of 0.1 Å (12). And from x-ray diffraction studies, both the xylene-suspended samples and anhydrous ones obtained after the xylene had been evaporated have long spacings of about 57-60 Å and about 52 Å, respectively. The long spacing corresponds to the thickness of a bilayer, and the former values for the thickness suggest that the hydrocarbon chains are nearly perpendicular to the bilayer plane. The unit cell of the xylene-suspended crystal has four molecules of Pam<sub>2</sub>PtdCho; its dimensions agree with those of the dihydrated crystals of dimyristoyl-L- $\alpha$ -phosphatidylcholine (Myr<sub>2</sub>PtdCho) reported by Pearson and Pasher (13) as  $a = 8.72$  Å,  $b = 8.92$  Å,  $c = 55.4$  Å, and  $\beta = 97.40^\circ$ , if we consider the increase in the long spacing of Myr<sub>2</sub>PtdCho by about 5 Å, which comes from the two additional carbon atoms in the hydrocarbon chain of Pam<sub>2</sub>PtdCho compared with that of Myr<sub>2</sub>PtdCho. This agreement suggests that the present crystal of Pam<sub>2</sub>PtdCho is in the dihydrated state. From x-ray (13, 14) and neutron diffraction studies (15, 16), the phosphorylcholine groups in the polar heads of lecithin with layered structure have been known to be nearly on the layered planes.

Under a polarizing microscope the crystal is known to show a weak optical anisotropy in the plane of the platelet. The orientation of the optical axis, determined by microscopic observation, was in the direction parallel to the width (shorter side) of the crystal platelet. The main contribution to this optical anisotropy may come from the polar head. Thus, it can be considered that the mean axis of the phosphorylcholine groups

Abbreviations: Pam<sub>2</sub>PtdCho, dipalmitoyl-L- $\alpha$ -phosphatidylcholine; Myr<sub>2</sub>PtdCho, dimyristoyl-L- $\alpha$ -phosphatidylcholine.

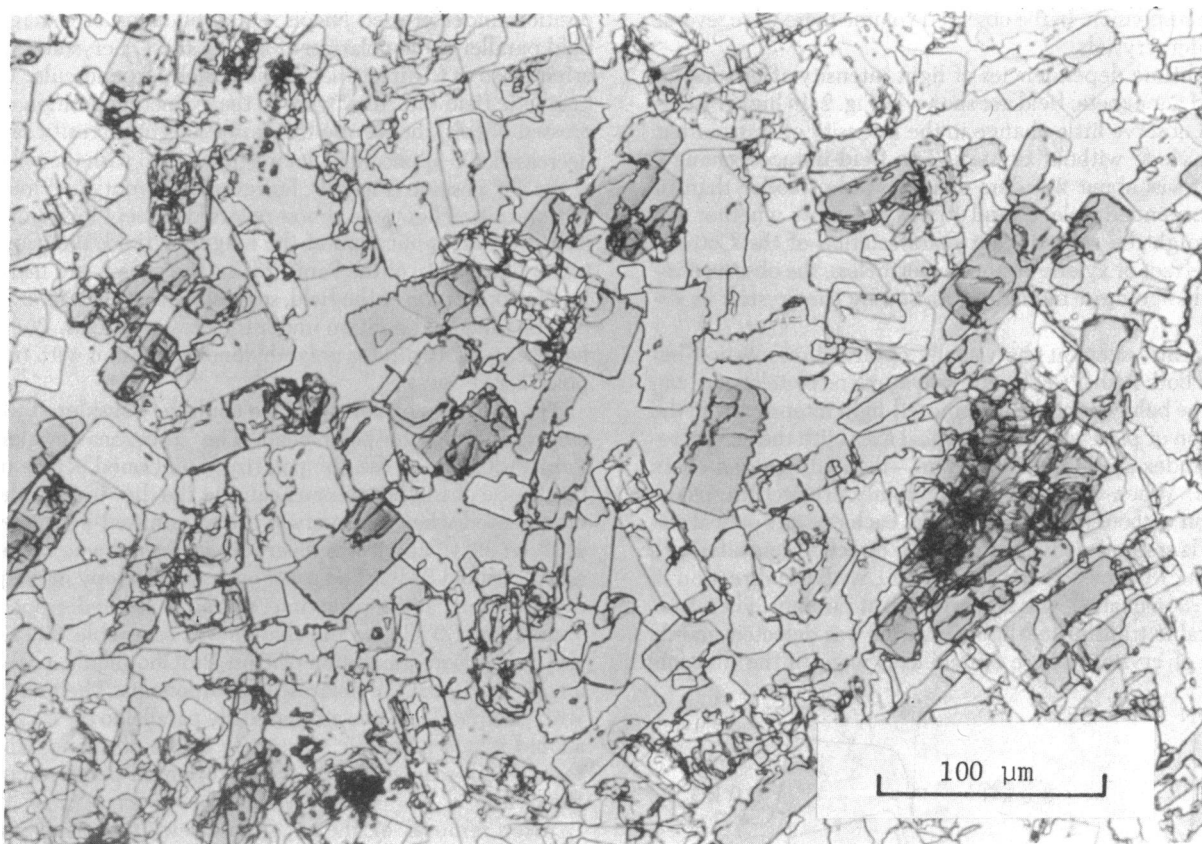


FIG. 1. Photograph of thin-layered single microcrystals of Pam<sub>2</sub>PtdCho taken by a polarizing microscope with a quarter-wavelength plate. The crystals were put on a slide glass, and the photograph was taken after xylene had been evaporated. The thin rectangular appearance of the platelet seen in this photograph was also observed in xylene suspension. The crystals show a weak optical anisotropy in the plane of the platelet. Details of the crystal structure studied by electron diffraction and electron microscopy have been reported elsewhere (12).

of the Pam<sub>2</sub>PtdCho crystal lies nearly on the plane of the platelet and orients to the direction of its width. In this paper, the width and length and thickness were assigned as *a*, *b*, and *c* directions, respectively.

We also used polyethylene single crystals, consisting only of hydrocarbon chains, to clarify the contribution of the polar head groups of lecithin in the magneto-orientation effect. A polyethylene crystal grown from 0.01% xylene solution at 80°C was a thin rhombic platelet with nearly the same dimensions as those of Pam<sub>2</sub>PtdCho crystals (i.e., average dimensions were 16 μm for the longer diagonal, 11 μm for the shorter diagonal, and 0.1 μm for the thickness). It consists of stacked thin rhombic single crystals, in which the hydrocarbon chains are packed perpendicularly to the plane of the platelet (17). In the plane of the platelet of polyethylene, we could not observe any optical anisotropy. The hydrocarbon chain direction was taken as the *c* axis.

These experiments for the observation of magnetic field effects were made at about 22°C with xylene-suspended crystals.

## RESULTS AND DISCUSSION

Under a polarizing microscope, rectangular crystalline platelets of Pam<sub>2</sub>PtdCho suspended in xylene were observed upon application of a magnetic field of about 5 kG to orient with their *b* directions parallel to the field. Furthermore, in the initial stage of orientation, several platelets whose *c* directions were perpendicular to the field but whose *a* directions were not perpendicular showed very fast "rotational motion" before they accomplished the *b*-direction orientation. The rotation was

especially clear for crystals observed from the *c* direction, with the polarizer making an angle of 45° with the field. Because the crystals have a weak optical anisotropy in the *ab* plane with the optical axis in the *a* direction, the rotation became manifest as a disappearance of the platelets for a very short time immediately after the field application. Therefore, a thin platelet of Pam<sub>2</sub>PtdCho is oriented with its *c* direction (parallel to hydrocarbon chains) perpendicular to the magnetic field and at the same time with its *a* direction (parallel to phosphorylcholine groups) also perpendicular to the magnetic field. Under a polarizing microscope, polyethylene crystals suspended in xylene showed a magneto-orientation effect similar to that of Pam<sub>2</sub>PtdCho crystals; however, they were observed only to be oriented with their hydrocarbon chains perpendicular to the field. We could not observe any evidence of orientation in a direction perpendicular to the hydrocarbon chains.

To demonstrate the magneto-orientation effect of Pam<sub>2</sub>PtdCho and polyethylene crystals, we measured the intensity of polarized light transmitted through suspensions by a simple setup. It was constructed by crossed Nicol prisms and a microscope whose eyepiece was replaced by a photoelectric cell of a phototransistor. The intensity of transmitted light detected by the cell was amplified and recorded on a recording chart. Xylene suspension of single microcrystals contained in a glass tube for NMR measurement was placed between the pole pieces of an electromagnet. The diameter of the pole piece was 30 mm and the gap between the pole pieces was 6 mm. At 9 kG, 90% rise time of the magnetic field strength was about 150 msec. Before each run of measurements the suspension was mixed thoroughly by shaking the tube. We used about 0.5 cm<sup>3</sup>

of sample suspension. In the observed volume there were several tens of microcrystals.

Typical time dependencies of light intensity after turning on and off a magnetic field are shown in Fig. 2. In higher fields we could observe little change in the intensity of transmitted light in xylene without crystals. The field-induced change, however, was about 2 orders of magnitude smaller than in crystal-suspended xylene, and it was not clear whether the change might be a significant manifestation of the Cotton-Mouton effect of xylene or not. Nevertheless, the observed intensity change must be mainly caused by the crystals in xylene.

Taking into account the visually observed orientation behavior of both Pam<sub>2</sub>PtdCho and polyethylene platelets, we can explain the behavior of the transmitted-light intensity from the orientation of platelets, whose optical axes with the largest refractive index are in the *c* direction—the hydrocarbon-chain direction. When crystals were randomly oriented in xylene suspension without the magnetic field, each crystal's optical axis with the largest refractive index, whose direction coincides with that of the thickness of the crystal (i.e., with the direction of hydrocarbon chains), was also oriented at random. Therefore, considerable transmitted-light intensity was detected mainly because of crystals whose optical axes were in the diagonal

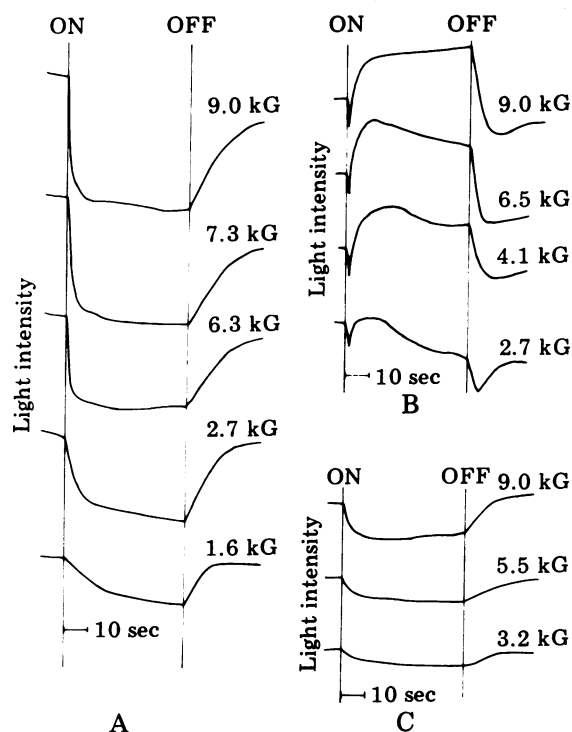


FIG. 2. Typical results of variation of transmitted-light intensity in the magnetic field when the field has been turned on or off. The field strength is shown beside each curve. The intensity (arbitrary units) was recorded by use of a simple setup, which was constructed by crossed Nicols and a microscope whose eyepiece was replaced by a photoelectric cell of a phototransistor. Measurements were made at 22°C. (A) Pam<sub>2</sub>PtdCho when the polarizer was oriented parallel to the magnetic field. (B) Pam<sub>2</sub>PtdCho when the polarizer was oriented to make an angle of 45° with the field. (C) Polyethylene crystals suspended in xylene; the polarizer was oriented parallel to the field direction. On application of a magnetic field parallel to the polarizer (A and C), crystals rapidly orient so as to direct their optical axes perpendicular to the magnetic field (i.e., they are in the extinction position under crossed Nicols), hence the transmitted-light intensity is rapidly decreased. On application of a magnetic field with the polarizer at the 45° position (B), the variation of intensity of transmitted light is almost reversed.

position under crossed Nicols. On application of a magnetic field parallel to the polarizer (Fig. 2 A and C), crystals rapidly oriented so as to direct their optical axes perpendicular to the magnetic field (i.e., they were in the extinction position under crossed Nicols), hence the transmitted-light intensity rapidly decreased. On application of a magnetic field with the polarizer at the 45° position (Fig. 2B), however, the variation of intensity of transmitted light was almost reversed. Except for a small dip just after the application of the magnetic field, the transmitted-light intensity of the Pam<sub>2</sub>PtdCho suspension was increased by the application of the field, suggesting that crystals oriented into the diagonal position under the crossed Nicols. We could not observe any dip for polyethylene suspension with the 45° polarizer.

Thus, the dip of Pam<sub>2</sub>PtdCho could be considered to be a contribution of the polar head. The "rotational motion" of Pam<sub>2</sub>PtdCho with the 45° polarizer mentioned before might be a cause of the phenomena causing the dip. In fact, the dip disappeared when we observed the transmitted-light intensity at about 32°C, and it was reversibly recovered when observed again at about 22°C. The weak optical anisotropy in the plane of the platelet at the same time, was observed to disappear beyond about 30°C, and the change was reversible. Therefore, the *b*-direction orientation of Pam<sub>2</sub>PtdCho should arise from a contribution of the polar head components. The polar head with phosphorylcholine groups nearly parallel to the *a* direction should give a magnetic anisotropy in the *ab* plane, which is favorable for the *a* direction to be oriented perpendicular to the field.

After removal of the magnetic field, the orientation of crystals turned again into a disordered state, and the transmitted-light intensity reversibly returned toward the original intensity level. The magnitude of the magnetic field-induced change in the transmitted-light intensity was nearly linear to the squared field strength, and for Pam<sub>2</sub>PtdCho the effect of the magnetic field seemed near saturation around 7 kG. The behavior of the transmitted-light intensity can thus be summarized to suggest that the optical axis can be oriented parallel or perpendicular to the magnetic field, hence being in the extinction (Fig. 2 A and C) or the diagonal (Fig. 2B) positions depending upon the orientation of the polarizer. Taking into account results of visual observations, we know that the optical axis is oriented perpendicular to the field. The polar head of Pam<sub>2</sub>PtdCho gives an additional contribution to the orientation of the crystal, as suggested by the *b*-direction orientation of the crystal and the dip of the transmitted-light intensity.

From the orientation behavior of crystals in the magnetic field, we tried to obtain values of volume diamagnetic susceptibility anisotropy,  $\Delta\chi$  (i.e., the difference between susceptibilities parallel and perpendicular to the hydrocarbon-chain direction), of both Pam<sub>2</sub>PtdCho and polyethylene. As densities of both crystals are higher than xylene, a slow sedimentation effect was superimposed on the intensity change, resulting in a slow change in the number of crystals in the observed volume. The effect manifested itself as the lack of reproducibility of the magnitude of the field-induced intensity change and of the transient behavior of intensity change after the field was turned off. The transient change in light intensity after the field was turned on, however, showed a good reproducibility, at least within the first 10 sec or so. Therefore, we used this behavior to evaluate  $\Delta\chi$ .

The transient behavior was recorded in the initial 5 or 6 sec by putting the polarizer parallel to the field and by using a digital memory of 12 bits resolution in magnitude and 20 msec resolution in time. We used data taken from about 0.2 sec to 2 sec after application of the field to minimize the sedimentation

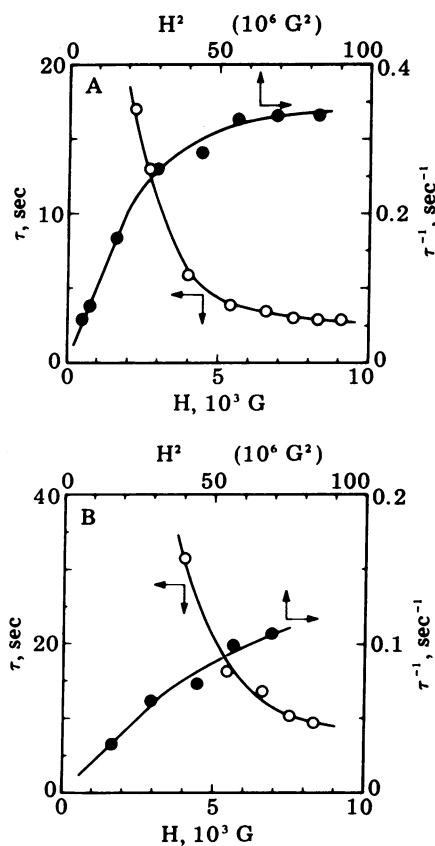


FIG. 3. Magnetic field,  $H$ , dependence of orientational relaxation time,  $\tau$ , which is obtained from a plot of logarithms of transmitted-light intensity against time after the magnetic field turned on. (A)  $\tau$  plotted against  $H$  and  $\tau^{-1}$  plotted against  $H^2$  for Pam<sub>2</sub>PtdCho. (B)  $\tau$  plotted against  $H$  and  $\tau^{-1}$  plotted against  $H^2$  for polyethylene.  $\circ$ ,  $\tau$ ;  $\bullet$ ,  $\tau^{-1}$ .

effect. The transient time was described by a simple exponential law giving an orientational relaxation time,  $\tau$ , shown in Fig. 3. In the linear approximation, the orientational relaxation time in the magnetic field is given as  $\tau = \zeta / \Delta\chi \cdot V \cdot H^2$ , where  $\zeta$  is the rotational friction coefficient,  $V$  is the volume of the platelet, and  $H$  is the field strength (18). In the low-field region,  $\tau^{-1}$  varied almost linearly with  $H^2$ , and  $\Delta\chi$  could be evaluated from the slope. In making a crude estimation of  $\Delta\chi$ , we assumed the thin platelet to be an oblate ellipsoid of revolution with the semiaxis of revolution half the thickness and the equatorial axis half the average length of two dimensions of the plane of the platelet, and we evaluated  $\zeta$  as described by Perrin (19). The volume susceptibility anisotropy,  $\Delta\chi$ , was  $-9 \times 10^{-8}$  cgs (i.e., the molar anisotropy of about  $-68 \times 10^{-6}$  EMU/mol, with the density  $\approx 1$  g/cm<sup>3</sup>) and  $-1 \times 10^{-8}$  cgs for Pam<sub>2</sub>PtdCho and polyethylene, respectively.

$\Delta\chi$  obtained for Pam<sub>2</sub>PtdCho is larger than that for polyethylene. This does not necessarily indicate that a large contribution to the anisotropy should come from the polar head, because in a comparison between the two, the molar anisotropy must be used. Moreover, because a crystal of polyethylene contains amorphous regions such as chain foldings (17),  $\Delta\chi$  obtained for polyethylene is a rather smaller value than is the  $\Delta\chi$  of the crystalline hydrocarbon chains. However, the magneto-orientation of polyethylene crystals shows at least qualitatively that the well-organized hydrocarbon chains give enough magnetic anisotropy to the crystal to be oriented in a magnetic field. The molar anisotropy for crystalline stearic acid, on the other hand, is reported to be about  $-26 \times 10^{-6}$  EMU/

mol by Lonsdale (8), which accounts for most of the  $-68 \times 10^{-6}$  EMU/mol of Pam<sub>2</sub>PtdCho having two hydrocarbon chains. The balance of the anisotropy probably comes from the polar head components including ester bonds. The value of  $\Delta\chi$  reported for egg yolk lecithin in  $L\alpha$  phase is  $-0.27 \times 10^{-8}$  cgs (about  $-2 \times 10^{-6}$  EMU/mol) (21) and is an order of magnitude smaller than the present value for Pam<sub>2</sub>PtdCho. We think that the difference arises mainly from the different degree-of-ordering of hydrocarbon chains, which are homogeneous and ordered in crystalline Pam<sub>2</sub>PtdCho and inhomogeneous and disordered in the  $L\alpha$  phase of egg yolk lecithin; additionally the difference arises from the contribution of polar head components, which are in an ordered state in the crystalline phase and in a less-ordered state in  $L\alpha$  phase.

The contribution from polar head components manifesting itself in the  $b$ -direction orientation of Pam<sub>2</sub>PtdCho in the magnetic field, can be attributed, at least in part, to the ester groups of a lecithin molecule, which connect the hydrocarbon chains to the glyceryl group. The ester group has been reported to have rather large diamagnetic anisotropy (8), by which the plane of the ester group is oriented parallel to the magnetic field. Therefore, if we assume a locally similar crystal structure of dilauroyl-DL- $\alpha$ -phosphatidylethanolamine reported by Hitchcock *et al.* (20) or of Myr<sub>2</sub>PtdCho reported by Pearson and Pasher (13) to the polar head of Pam<sub>2</sub>PtdCho, in which the ester groups have the right orientation for putting the mean axis of the phosphorylcholine groups nearly perpendicular to the field, we can qualitatively account for our present results. Recently, magnetic field effects have been observed with dipalmitoyl-DL- $\alpha$ -phosphatidylcholine, which will be reported in detail elsewhere.

In conclusion, hydrocarbon chains give enough magnetic anisotropy to thin crystalline platelets of Pam<sub>2</sub>PtdCho and polyethylene to align them with their hydrocarbon chains perpendicular to the field. This is consistent with the reported  $\Delta\chi$  for long-chain alkyl molecules (8) and for egg yolk lecithin vesicles (21). In addition, there is a contribution from the ordered polar head components to the anisotropy of the magnetic susceptibility of Pam<sub>2</sub>PtdCho. If we assume that the planes of the ester bonds in the polar head of Pam<sub>2</sub>PtdCho are oriented in a similar manner to those of Myr<sub>2</sub>PtdCho (13) or dilauroyl-DL- $\alpha$ -phosphatidylethanolamine (20), the diamagnetic susceptibility anisotropy of ester groups (8) will give an additional contribution to the magneto-orientation of Pam<sub>2</sub>PtdCho.

The authors would like to thank Professor K. Okano of Tokyo University and Dr. T. Sakurai of the Institute of Physical and Chemical Research for their valuable suggestions on the present study. Thanks are also due to Mr. S. Sakurai of Japan Electron Optics Laboratory Co., Ltd., for his collaboration in diffraction measurements.

1. Chalazonitis, N., Chagneux, R. & Arvanitaki, A. (1970) *C. R. Acad. Sci. Ser. D* **271**, 130-133.
2. Chagneux, R. & Chalazonitis, N. (1972) *C. R. Acad. Sci. Ser. D* **274**, 317-320.
3. Geacintov, N. E., Van Nostrand, F., Pope, M. & Tinkel, J. B. (1971) *Biochim. Biophys. Acta* **226**, 486-491.
4. Geacintov, N. E., Van Nostrand, F., Becker, J. F. & Tinkel, J. B. (1972) *Biochim. Biophys. Acta* **267**, 65-79.
5. Breton, J., Michel-Villaz, M. & Paillontin, G. (1973) *Biochim. Biophys. Acta* **314**, 42-56.
6. Becker, J. F., Geacintov, N. E., Van Nostrand, F. & Van Metter, R. (1973) *Biochem. Biophys. Res. Commun.* **51**, 597-602.
7. Neugebauer, D.C., Blaurock, A. E. & Worcester, D. L. (1977) *FEBS Lett.* **78**, 31-35.
8. Lonsdale, K. (1939) *Proc. Soc. London, Ser. A* **171**, 541-568.
9. Worcester, D. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 5475-5477.

10. Pauling, L. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2293-2294.
11. Hong, F. T. (1980) *Biophys. J.* **29**, 343-346.
12. Sakurai, I., Sakurai, S., Sakurai, T., Ikegami, A., Seto, T. & Iwayanagi, S. (1980) *Chem. Phys. Lipids* **26**, 41-48.
13. Pearson, R. H. & Pasher, I. (1979) *Nature (London)* **281**, 499-501.
14. Sakurai, I., Iwayanagi, S., Sakurai, T. & Seto, T. (1977) *J. Mol. Biol.* **117**, 285-291.
15. Franks, N. P. (1976) *J. Mol. Biol.* **100**, 345-358.
16. Worcester, D. L. & Franks, N. P. (1976) *J. Mol. Biol.* **100**, 359-378.
17. Geil, P. H. (1963) *Polymer Single Crystals* (Interscience, New York).
18. Hong, F. T. (1977) *J. Colloid Interface Sci.* **58**, 471-497.
19. Perrin, F. (1934) *J. Phys. Radium Ser. 7* **5**, 499-511.
20. Hitchcock, P. B., Mason, R., Thomas, K. M. & Shipley, G. G. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3036-3040.
21. Boroske, E. & Helfrich, W. (1978) *Biophys. J.* **24**, 863-868.