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# LIQUID CRYSTALLINE PHASES OF HYDRATED PHOSPHATIDYLETHANOLAMINE

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SUMMARY

In order to investigate liquid crystalline phases of hydrated phosphatidylethanolamine by electron microscopy, a negative staining technique was used. Since the presence of the different phases is a function of lipid concentration and temperature, the phosphatidylethanolamine–water system was studied at different states of swelling at constant temperature. Two phases were found to be in rather good agreement with X-ray results (lamellar and hexagonal type II), whereas in one case, the striking dimensions of an hexagonal network did not afford a definite correlation.

## INTRODUCTION

Structural analysis of lipid phases is of common interest because of the physiological functions of lipids in biological membranes. Research of lipid polymorphism has been carried out using optical and X-ray diffraction techniques[1–4]. This study is an attempt to correlate X-ray diffraction data with those obtained with electron microscopy using negative staining.

The main objective of the investigation was to examine the nature of the different lipid phases by studying the progressive states of swelling of the lipid. In order to attain a particle dispersion suitable for electron microscopy, the lipid or the lipid–water system was dispersed in an excess of water. Changes in the lipid–water proportions of the slightly hydrated forms was avoided by keeping the preparation time short with respect to the time required for sufficient water uptake into the system.

## MATERIALS AND METHODS

Phosphatidylethanolamine was prepared from rat liver by the method of HANAHAN *et al.*[5]. The extracts of rat liver were dissolved in chloroform and transferred to a column of silic acid (50–100 mesh). The triglycerides, cholesterol and cardiolipin, were eluted with chloroform and chloroform–methanol respectively. Using a solvent system of chloroform–methanol–water (65:30:5, by vol.) phosphatidylethanolamine was then separated from phosphatidylcholine, phosphatidylserine and sphingomyelin. The purity of the sample was checked with one- and twodimensional thin-layer chromatography[6].

The ether or chloroform solutions of phosphatidylethanolamine were dried in $N_2$ and placed under a vacuum. The lipid was then suspended in water, either by shaking or by sonication. Samples were stained for electron microscopy either immediately or after set intervals.

For negative staining a 2 % solution of sodium phosphotungstate was used at pH 7, mixing equal volumes of the stain and the lipid suspension. A carbon-formvar-coated grid was dipped into the mixture, and the excess of liquid was quickly removed using fine-grained filter paper. The experiments were carried out at room temperature.

A Siemens Elmiscop I electron microscope was used at 80 or 100 kV with a double condenser, a 200-$\mu$ condenser aperture and 50-$\mu$ objective aperture. Electron optical magnifications was 40000:1.

## RESULTS AND DISCUSSION

For phosphatidylethanolamine–water mixtures, REISS–HUSSON[4] has described two liquid–crystalline phases: the lamellar phase and the hexagonal phase type II (H II). The latter exists at 55°. At lower temperatures, 25–35°, the lamellar and hexagonal phases are observed simultaneously over a wide concentration range.

The hexagonal phase, type II, was found to be a two-dimensional array of rods whose interior is occupied by the polar groups of the molecules, suggesting that the water cylinders are separated by the lipid phase[7].

In an ultrastructural study either the hexagonal packing of these rods or a linear pattern caused by the inclination of the rods with respect to the beam direction should be observed. Depending on the angle of rotation, different periodic distances should also be observed, as indicated in Fig. 1.

Results showed that samples in a very early state of swelling exhibit a phase whose structural dimensions are in good agreement with X-ray data of the hexagonal phase type II. The micrographs illustrate to a large extent a linear pattern (Figs. 2a and 2b), and in a few areas, the cylinders may be seen from above (Fig. 2b, arrow). Fig. 2c shows the change from the hexogonal arrangement to linear patterns of diffe-

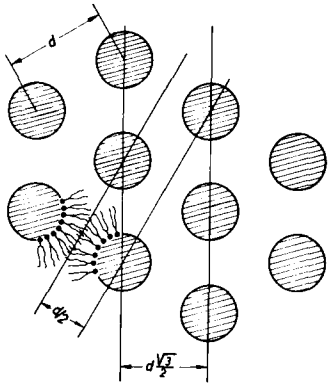


Fig. 1. The hatched areas represent the water cylinders of the hexagonal network viewed head on. The center to center distance is represented by $d$. Inclination of these cylinders with respect to the beam direction would result in a linear pattern. The observed distances of equal width are drawn in.

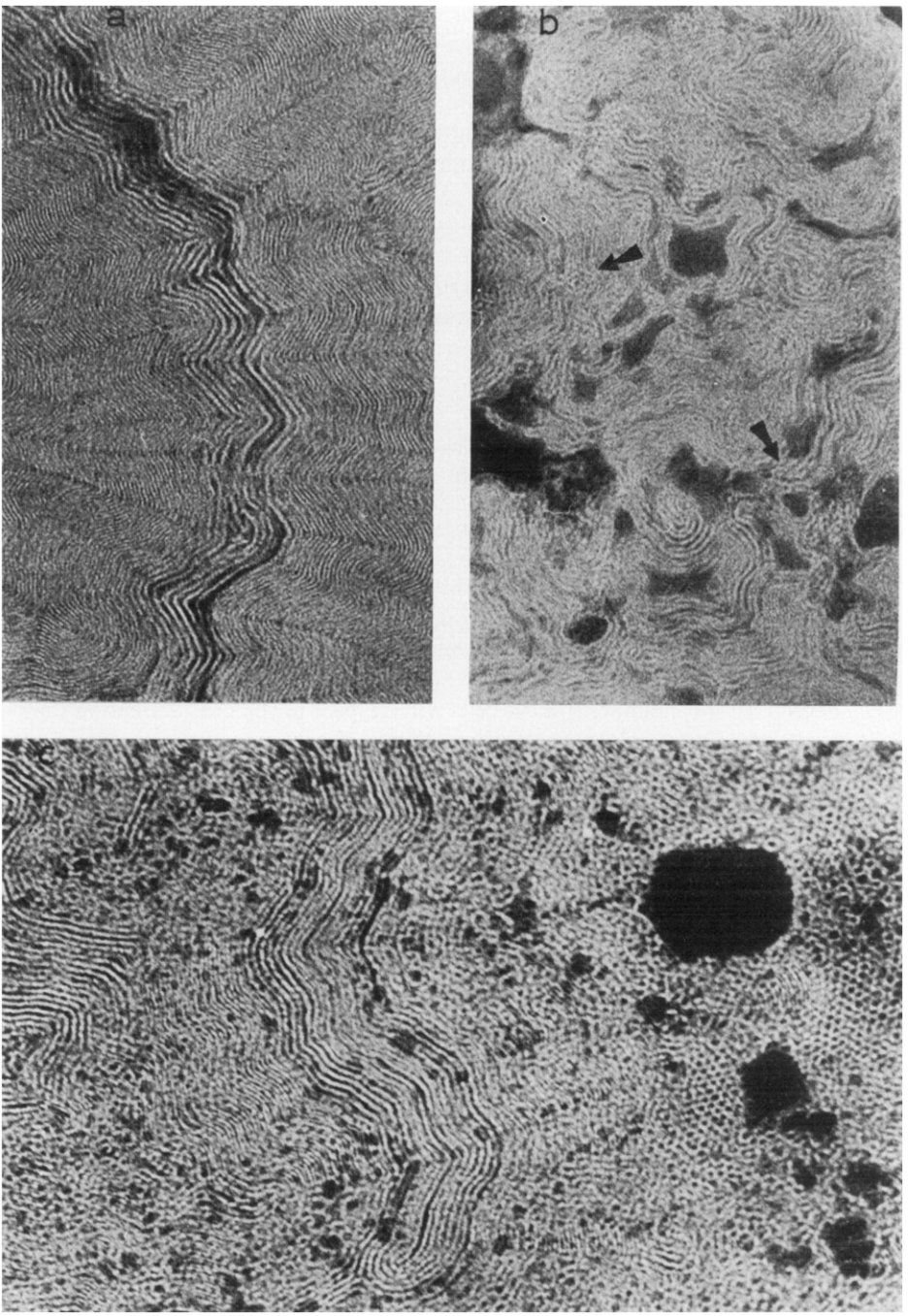


Fig. 2. a–c. Phosphatidylethanolamine in very early states of swelling, 10–30 min. ( × 160 000)

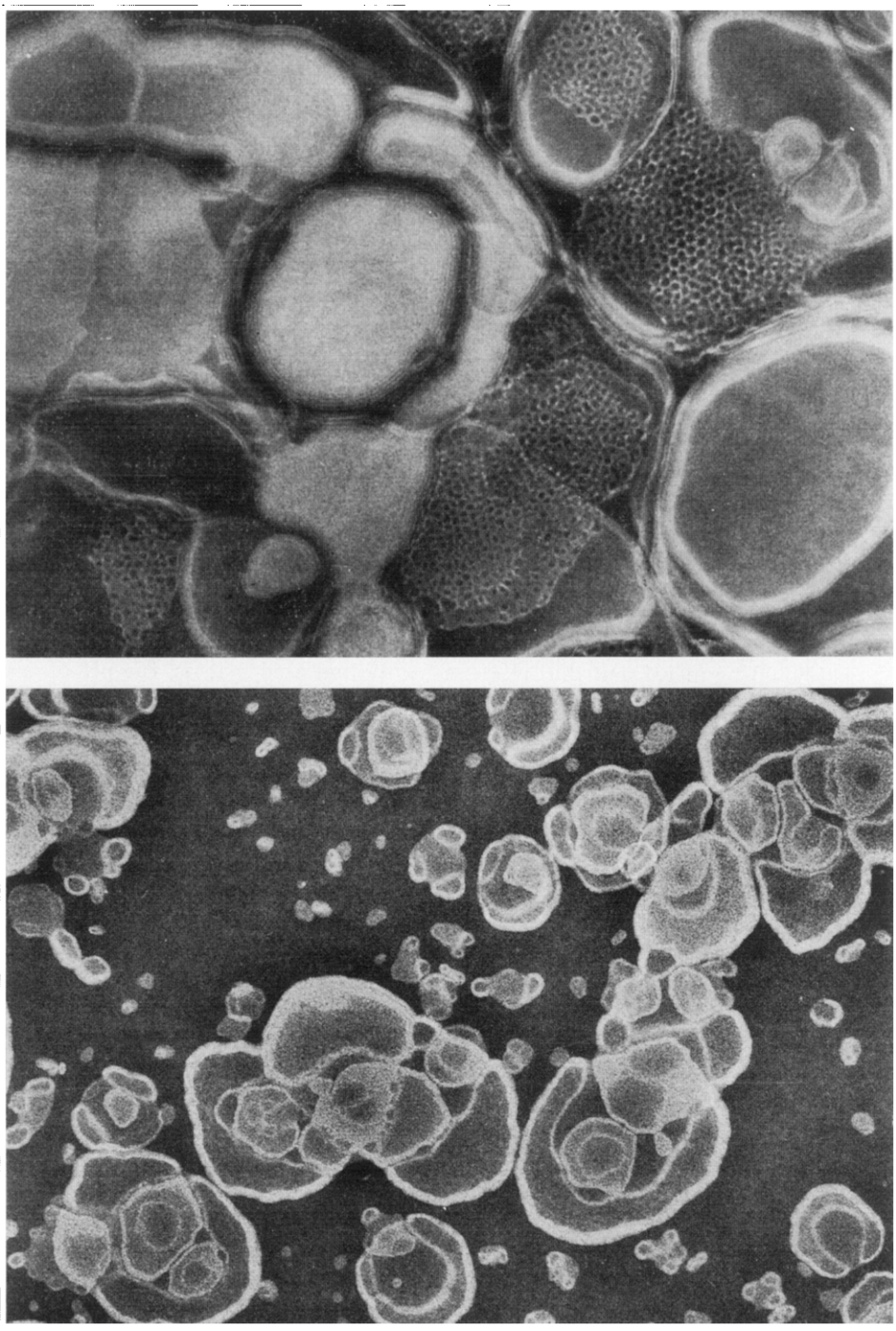

Fig. 3. Phosphatidylethanolamine has been suspended in water for several h. (× 100 000)

Fig. 4. Phosphatidylethanolamine had been suspended in water for several days. (× 100 000)

rent periodicity. The values of the center to center distance $d$, either measured in the hexogonal array or calculated from the linear structures, usually varied from 60 to 80 Å. This difference may be due to the specific water content of the lipid particles. In the hexagonal areas of Fig. 1c, the center to center distance $d$ is 80 Å. The values for the linear distances are 40 Å, corresponding to $d/2$, and 70 Å corresponding to $d\sqrt{3/2}$.

Samples which have been in water for several h show the well-established lamellar phase together with hexagonal patterns (Fig. 3). The center to center distances, $d$, are strikingly large (115 Å) compared with X-ray data of the hexagonal phase type II. However, electron micrographs suggest that this phase cannot consist of indefinite rods. Perhaps this phase is related to one of the intermediate phases, similar to phase R, characterized by limited rods linked three by three to form an hexagonal two-dimensional network. Although LUZZATI *et al.*[8,9] described phase R in almost dry lipid preparations, the existence of a phase like R is thought to be possible in more extensively hydrated systems.

In the lamellar phase, the form of the negatively stained particles essentially depends on their state of hydration. The capacity of phosphatidylethanolamine to take up water over a long period of time is demonstrated in Figs. 3 and 4. Fig. 3 shows a preparation made at an early state of swelling (several h). The lamellae are piled up, showing a repeating distance of about 50 Å, while in more highly hydrated systems, which have been suspended in water for several days, single leaflets are frequently observed. These leaflets are usually folded, producing a disorderly assembly of lamellae (Fig. 4).

It should be mentioned that in these highly hydrated sytems, the pH of the stain solution significantly influences the findings. In addition, the investigation of highly hydrated phosphatidylethanolamine examined after swelling for several days is further complicated by the decomposition of the lipid.

In spite of the special experimental conditions created by the distribution of lipid–water particles in water and treatment with negative stain, the electron microscopic results were found to be in rather good agreement with those of X-ray studies.

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