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Electron Micrographs of Lecithin Films

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Monolayers of a synthetic lecithin transferred from a water surface to electron-microscope supports show a pronounced porous structure at low and intermediate surface pressures. The small "holes" and the irregular structure of the film may be related to a microporosity of cell membranes.

Although lecithins are one of the principal lipids in the membranes of cells that control important biological processes, relatively little is known in detail about the structure of lecithin films. [1,2] Electron micrographs now show a remarkable microporosity in the ultimate thin film or monolayer of a dipalmitoyl lecithin transferred from a water surface. In many respects, the water corresponds to the aqueous phases that bound cell membranes.

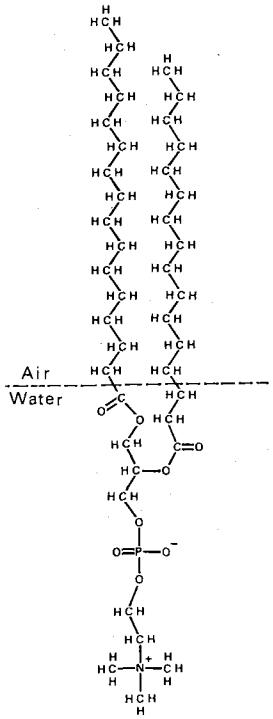

Fig. 1. Schematic drawing of lecithin molecule.

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A high purity synthetic lecithin, β-γ-dipalmitoyl-DL-α-glycerylphosphorylcholine, dissolved in chloroform was spread on a substrate of twice-distilled water. The Wilhelmy balance technique with continuous recording provided the surface pressure data.[1] A modified Langmuir-Blodgett method was used to transfer the monolayers to electron-microscope screens sandwiched between formvar and a glass plate.[3] Variable speed

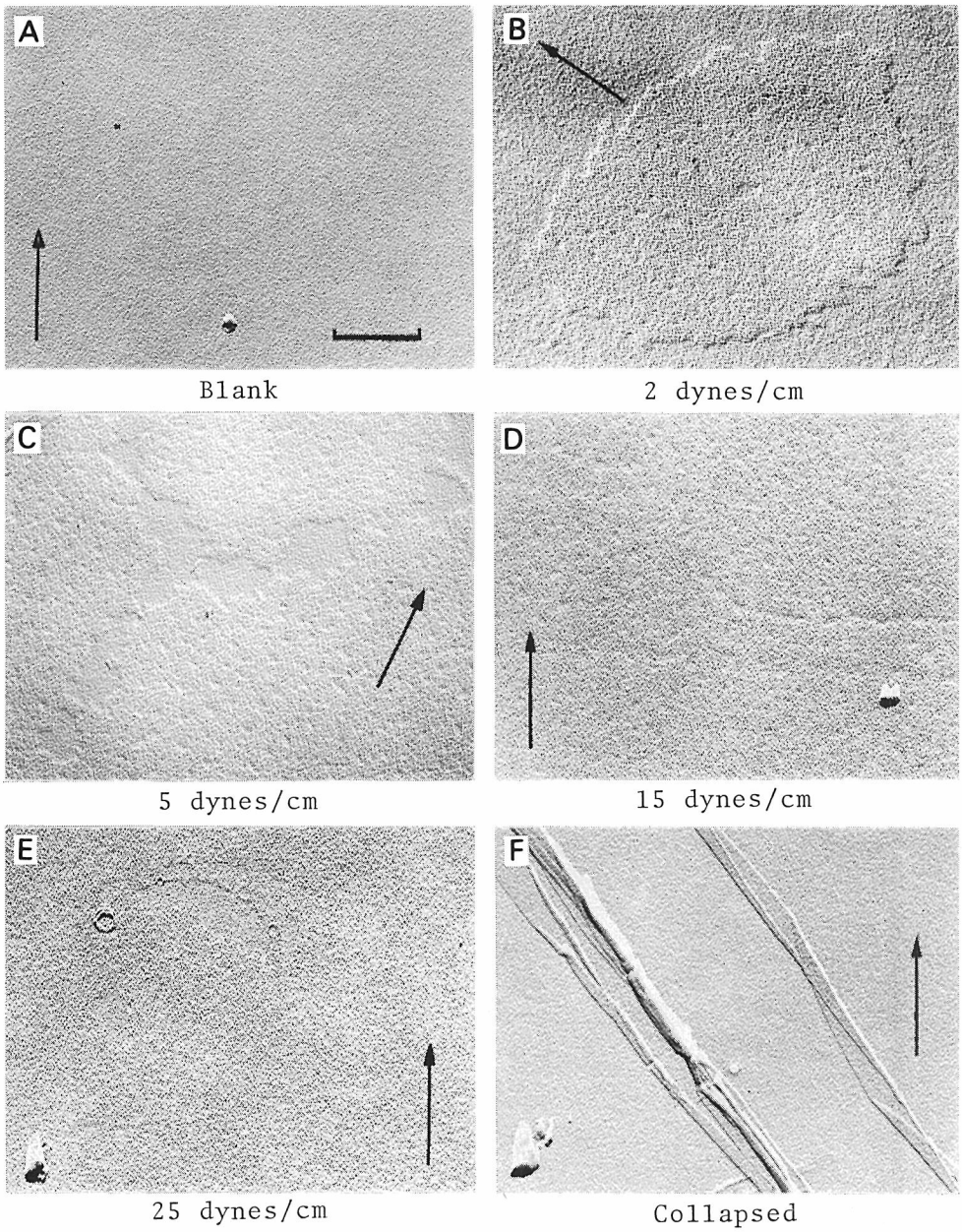

Fig. 2. Electron micrographs of lecithin films transferred at various surface pressures. The scale shows 1 μ and the arrows indicate the direction of shadowcasting. All micrographs are at the same magnification.

motor drives raised the plate slowly through the water-air interface and moved the compressing barrier at such a rate that the surface pressure remained constant during transfer. The entire apparatus was mounted on a heavy concrete base remarkably free from vibration--essentially no movement of the water-air interface was shown by a long optical beam reflected from the water surface.

After transfer, the film samples were placed in high vacuum and shadowcast with germanium at an angle of approximately 10°. They were then examined at a direct magnification of 5,000 times in a modified JEM-7 electron microscope. Many samples were transferred at low surface pressures and large areas, about 2 dynes/cm and 50 to 60 $Å^2$/molecule, respectively, because the lipids in membrane layers are undoubtedly subjected to relatively small "horizontal" or surface pressures.

A schematic drawing of an oriented dipalmitoyl lecithin molecule at the water-air interface is shown in Fig. 1. Representative electron micrographs are presented in Fig. 2; arrows indicate the direction of shadowcasting. A one-to-one correspondence between the film structures on the water surface and those observed in the microscope is not claimed, but the sequence of changes on the water surface undoubtedly parallels the sequence of changes observed in the microscope.

The blank sample shown in Fig. 2A was obtained by moving the formvar-covered screens through a clean water-air interface before the film was spread. This electron micrograph establishes both the flat smooth surface of the formvar and the fine texture of the vapor-deposited germanium.

Figure 2B shows a typical island structure for the lecithin film transferred at a low surface pressure, 2 dynes/cm. The islands contain many more small "holes" and have edge contours that are considerably more irregular than those of n-hexatriacontanoic acid (C-36) studied earlier.[3] At 5 dynes/cm, as shown in Fig. 2C, the micrographs indicate large areas of a two-dimensional continuous phase with the so-called uncovered areas discontinuous. At 15 dynes/cm (2D), the ratio of the covered to the uncovered area increases, but the micropores persist. At 25 dynes/cm (2E), much of the uncovered area has disappeared and large areas are covered homogeneously. After collapse (2F), long flat ribbon-like structures, apparently two molecules thick, appear. These collapse fragments are much less regular in structure than the collapsed material formed by the C-36 acid.

There are thus three principal differences between the film structures observed for lecithin and those for the simple C-36 acid: (a) lecithin islands have many more small holes and less regular edges, (b) the small holes in lecithin films persist at higher surface pressures, and (c) the collapse fragments formed by lecithin are less structured. Such differences are, of course, related to molecular geometry and polarity. The hydrocarbon chains of the lecithin are much shorter than those of the C-36 acid and the forces of cohesion are thus smaller. The polar extremity of lecithin is much larger and stronger (high dipole moment) than the carboxy group of the acid and may therefore interfere with close packing.

An elongated structure of the lecithin molecule with the polar groups extending well into the aqueous phase (Fig. 1) is also suggested by the thickness of the film (ca. 40Å) as observed in the electron microscope. Furthermore there may well be staggered packing of the vertically oriented molecules, partly to satisfy the electrical forces of the polar

groups. Relatively tight packing of the molecules in the islands combined with a much less dense packing in the "uncovered" areas may of course account for area values in the neighborhood of 60 Å^2/molecule at low surface pressures. Areas approach 40 Å^2/molecule at the higher pressures.

The "holes" or small uncovered areas observed throughout the micrograph series may be related to a microporosity of cell membranes as well as to the possibility of contiguous protein molecules penetrating into the lipid layer.

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It was indeed a special privilege for one of us (H. E. R., Jr.) to spend two years (1972-74) in Professor Suito's laboratory. A happier or more stimulating atmosphere for research would be difficult to find. Throughout his long and distinguished career at Kyoto University, Professor Suito has demonstrated rare qualities of leadership. We trust that for many years to come, the entire scientific community will continue to benefit from his advice and guidance. This paper is dedicated to Emeritus Proferror E. Suito on the commemoration of his retirement.

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