# URANYL ACETATE INDUCES GEL PHASE FORMATION IN MODEL LIPID AND BIOLOGICAL MEMBRANES

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ABSTRACT The effect of uranyl acetate on the mesomorphic phase state of lipids in model membranes as well as in isolated biological membranes has been examined. As little as 0.8 mM (0.03% [wt/vol]) uranyl acetate induces a liquid crystal-to-gel phase transformation in egg phosphatidic acid, bovine brain phosphatidylserine, and in lysed chromaffin granule membranes. These results along with others in the literature indicate that the uranyl acetate used in samples for electron microscopy could alter membrane morphology.

Uranyl acetate has long been used as a stain in electron microscopy to preserve and to reveal the morphology of model lipid and biological membranes (Shah, 1969). The manner in which this heavy atom stain interacts with and labels membranes has been the subject of extensive investigation (Bangham et al., 1974; Barton, 1968; Chapman et al., 1974; Huang et al., 1983; Glauert, 1965; McLaughlin et al., 1971; Inoko et al., 1975; Furuya et al., 1976). Recently, the possibility of using the favorable photon-scattering properties of the uranyl ion in x-ray diffraction structural investigations of model and reconstituted membranes has been considered (Stamatoff et al., 1979, 1980). However, in the case of dipalmitoylphosphatidylcholine, uranyl acetate perturbs the lipid by altering lipid acyl chain tilt (Parsegian et al., 1981).

Since most biological membranes contain a variety of negatively charged lipids, the possibility also exists for tight binding of the uranyl cation, $UO_2^{2+}$, which could conceivably induce a gel phase separation. This is indeed what we have found in our examination of how uranyl acetate interacts with phosphatidic acid (PA),[1] phosphatidylserine (PS), and with chromaffin granule lysed membranes (CGLM) derived from bovine adrenal medulla. The interaction was monitored by wide-angle x-ray diffraction which is a sensitive method for detecting a liquid crystal-to-gel phase transformation. We report that uranyl acetate, at concentrations 10- to 20-fold below those used in conventional electron microscopy staining solutions, is an effective inducer of lipid phase separations in both model lipid and biological membranes. Our results highlight the shortcomings of this staining material but also suggest a way in which this problem might be obviated.

The influence of uranyl acetate on the mesomorphic phase properties of model lipid and isolated biological membranes as monitored by x-ray diffraction is illustrated in Fig. 1. Egg PA in the absence of uranyl ion at pH 6 and 23°C displays a typical diffuse wide-angle diffraction pattern centered at $(4.6\ \text{Å})^{-1}$ characteristic of a lipid with disordered acyl chains. The addition of uranyl acetate under the same conditions apparently effects complete liquid crystal-to-gel phase transformation in that the diffraction pattern now reveals a single sharp reflection at $(4.2\ \text{Å})^{-1}$ (Fig. 1 *A*). Similar results were obtained with bovine brain PS (Fig. 1 *C*). These same experiments were repeated with PA and PS at pH 7.0 (0.01 M Hepes, 0.05 M glucose, 2 mM uranyl acetate) with identical results (data now shown).

CGLM prepared from bovine adrenal medulla suspended in buffer at pH 6 and −2°C display a diffuse peak centered at $(4.6\ \text{Å})^{-1}$ in the x-ray diffraction pattern, suggesting that the lipid component of these membranes is in the liquid crystalline phase (Fig. 1 *E*). Under the same conditions, the addition of uranyl acetate at 2 mM induces partial liquid crystal-to-gel phase transformation as evidenced by the coexistence of a sharp line at $(4.2\ \text{Å})^{-1}$ and a diffuse peak centered at $(4.6\ \text{Å})^{-1}$ (Fig. 1 *D*). Upon raising pH to 7.2, no significant difference was observed in the response of CGLM to added uranyl ion (Fig. 1 *G*). However, comparing the diffraction patterns in Fig. 1 (cf., Fig. 1, *F* and *G*), the relative amount of gel phase lipid is reduced somewhat when the uranyl acetate concentration is halved to 1 mM.

[1] *Abbreviations used in this paper:* CG, chromaffin granules: CGLM, chromaffin granule lysed membranes; PA, phosphatidic acid; PS, phosphatidylserine.

The stability of the uranyl acetate-induced gel phase lipid in CGLM was shown to be temperature sensitive in the range of 2°–10°C (Fig. 1, *G–J*). As sample temperature was increased in this range, the intensity of the gel phase signal at $(4.2\ \text{Å})^{-1}$ gradually decreased, and at 10°C had completely disappeared. This result illustrates the broad nature of the chain "melting" transition characteristic of biological membranes of mixed lipid composition (Winkler and Carmichael, 1982) and shows that the transformation to the liquid crystal phase is complete by 10°C indicating that the upper bound to phase coexistence in uranyl acetate treated CGLM lies between 2° and 10°C.

We also attempted to investigate the sensitivity of intact CG to uranyl acetate. Unfortunately the residual water content in these samples was so high, and the water

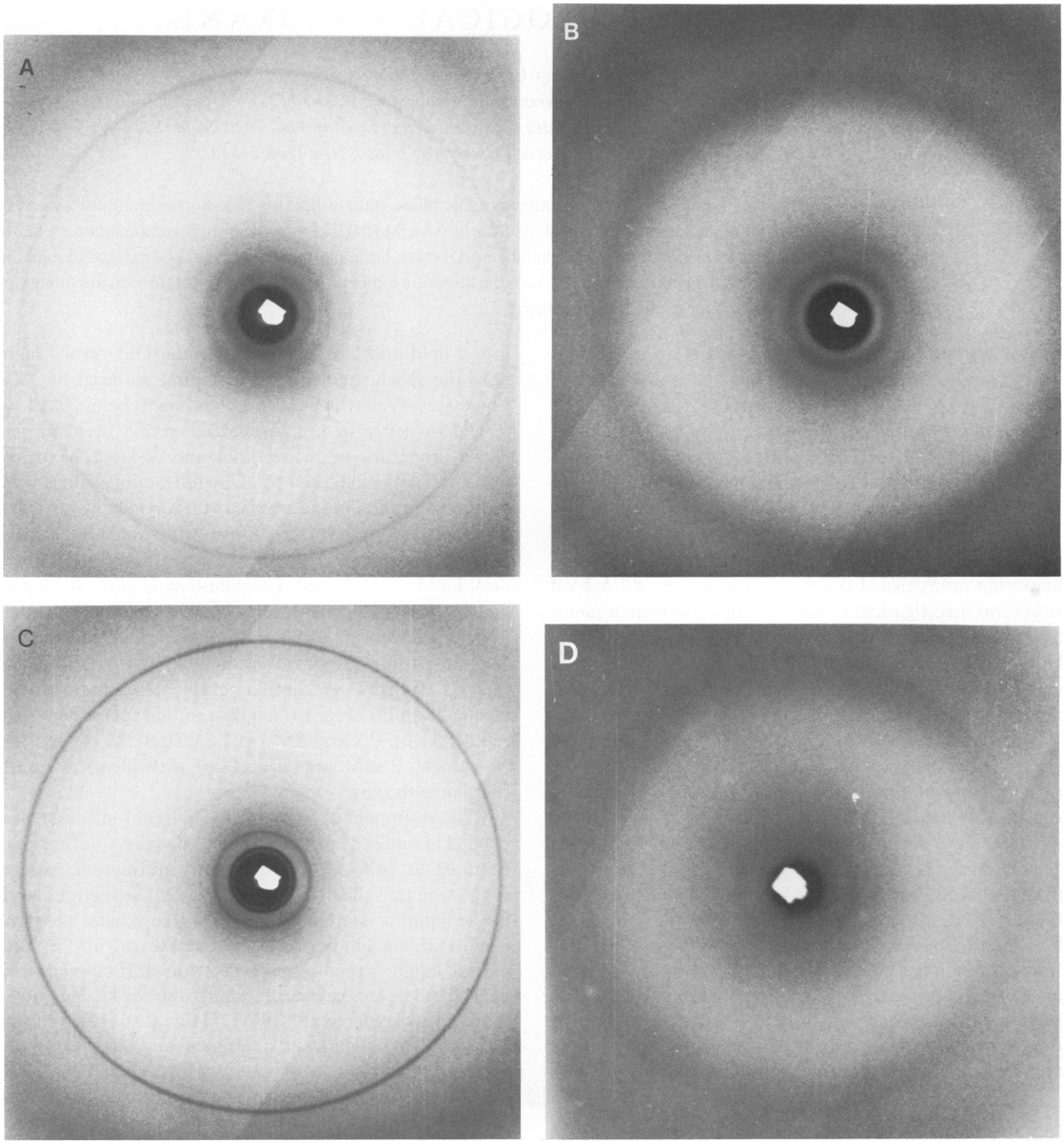


FIGURE 1 See caption on page 504.

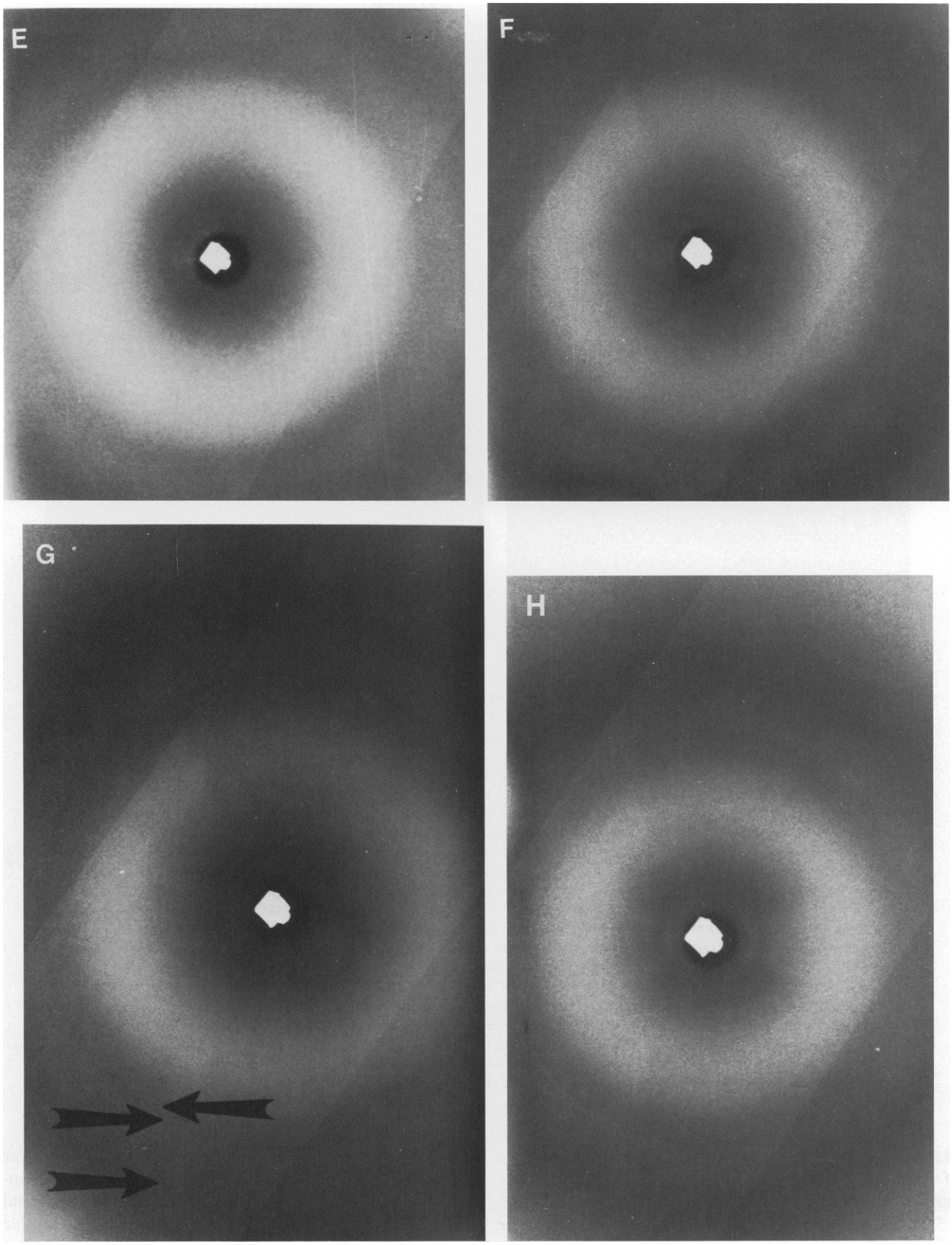


FIGURE 1 (continued). See caption on page 504.

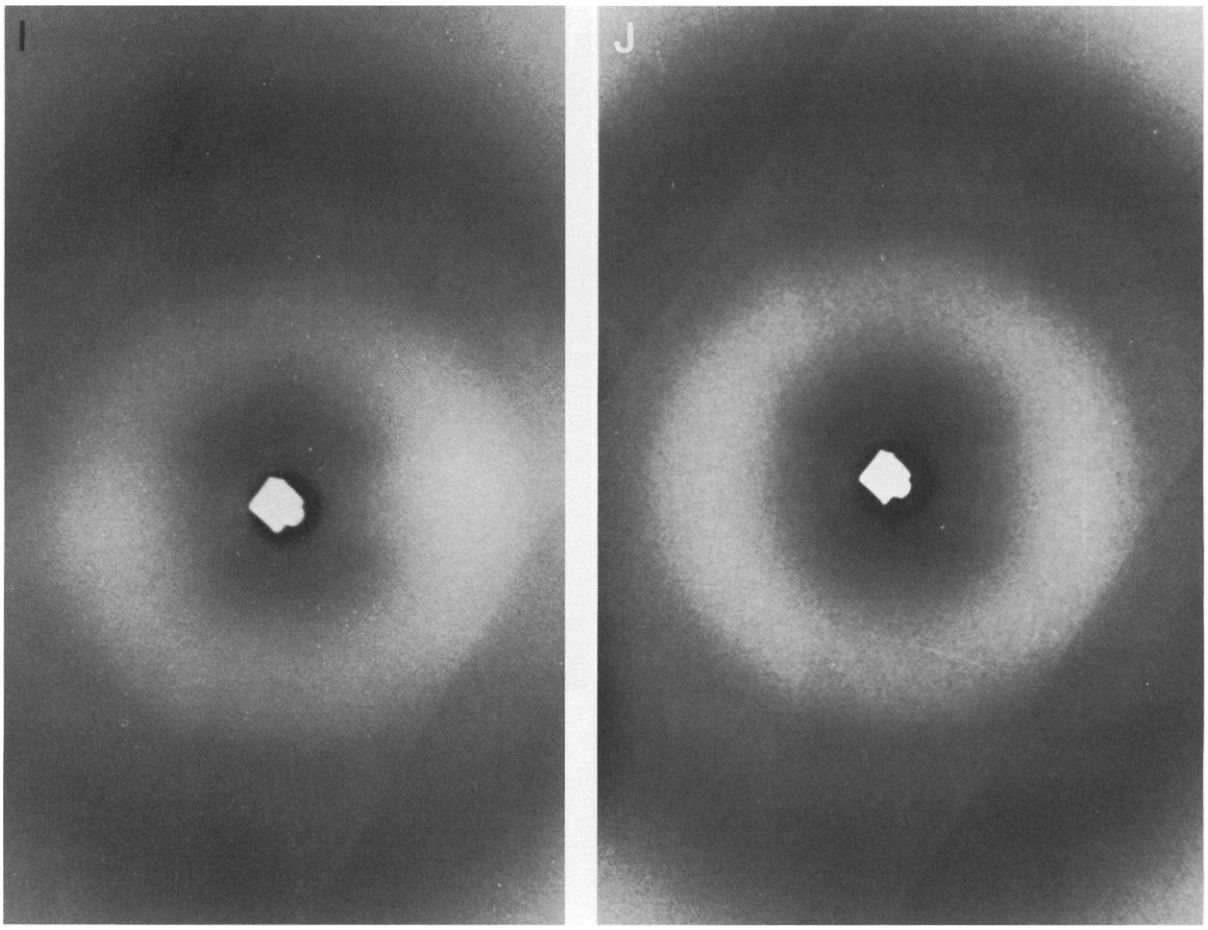


FIGURE 1 Mesomorphic phase sensitivity of egg PA and of CGLM to uranyl acetate as revealed by x-ray diffraction. (*A* and *B*) Egg PA (2 mM), 0.1 M Hepes, pH 6, with and without 0.8 mM uranyl acetate, 23°C; (*C*) bovine brain PS (2 mM), 0.1 M Hepes, pH 5.7, 0.8 mM uranyl acetate, 23°C; (*D* and *E*) CGLM, pH 6, with and without 2 mM uranyl acetate, −2°C; (*F*) CGLM, pH 7.2, 1 mM uranly acetate, −2°C; (*G–J*) CGLM, pH 7.2, 2 mM uranyl acetate at −2°C, 2°C, 10°C, and 14°C, respectively. The arrowed reflections in *G* correspond to the water peak at ~$(3.3\ \text{Å})^{-1}$, to the gel phase line at $(4.2\ \text{Å})^{-1}$ and to the liquid-crystalline peak at $(4.6\ \text{Å})^{-1}$ in order of decreasing scattering angle. CG were isolated from bovine adrenal medulla and stored on ice (max. 3 d) in 0.3 M sucrose, 10 mM Na-Hepes, pH 7.4 before x-ray diffraction (Cahill and Morris, 1979). CGLM ghosts were prepared from CG by the method of Cahill and Morris (1979) except that lysis was in 10 mM Na-Hepes, 10 mM KCl, pH 7.4 and instead of overnight dialysis, the ghosts were pelleted, supernatant removed, and the moist pellet stored on ice until used. CG or CGLM were resuspended at a final protein concentration of 1.0 mg/ml (Bradford, 1976) in the appropriate buffer, diluted with an equal volume of buffer containing a two-fold excess of uranyl acetate, and treated for 30 min at 0–4°C with solutions of the following composition: (*A*) 1.0 mM uranyl actate, 0.1 M Na-Hepes, pH 7.2 (final $[Na^+]$ ~20 mM); (*B*) 2.0 mM uranyl acetate, 0.1 M Na-Hepes, pH 7.2 (final $[Na^+]$ ~20 mM), and (*C*) 2.0 mM uranyl acetate, 80 mM K-MES, pH 6.0. The samples were then pelleted at 100,000 rpm for 20 min at ~4°C in an airfuge (Beckman Instruments, Inc., Palo Alto, CA) and the supernatant removed from each pellet. Lipids (Avanti Polar Lipids, Inc., Birmingham, AL) were shown to be ≥98% pure by thin-layer chromatography as previously described (Caffrey and Feigenson, 1984). Aqueous dispersions of PA and PS were prepared by vortexing the dry lipid with the suspending medium and were treated with uranyl acetate as described before (Caffrey and Feigenson, 1984). X-ray diffraction measurements on samples contained in thin-walled (10 μm) glass capillaries (1-mm internal diam, Charles Supper Co., Natick, MA) were performed using the focused, monochromatic x-ray source at the Cornell High Energy Synchrotron Source (CHESS) as previously described (Caffrey and Feigenson, 1984; Caffrey, 1985). Sample temperature as regulated by securing the glass capillary in a brass sample holder through which water from a thermostated water bath was circulated. Diffraction patterns were recorded on CEA Reflex 25 film (CEA American Corp., Greenwich, CT) at sample-to-film distances of 4.5 and 5.5 cm and exposure times of 5–30 min with the storage ring operating at 5 GeV and 5–10 mA of electrons.

scattering peak so strong, that it precluded determination of lipid short-range order in these samples that were not further examined in this connection.

The induction of a gel phase in CGLM by uranyl acetate suggests a cautious approach to the use of this stain in electron microscopy. We used 0.8–2 mM uranyl acetate for these experiments, a concentration 10- to 20-fold lowe than that used in conventional electron microscopy stain ing solutions. The fact that the gel phase lipid disappeare above 10°C at 2 mM uranyl acetate suggests that sampl preparation and manipulation above this temperatur might eliminate this problem for studies of the CG system

We expect such concentration and temperature effects to be dependent on the nature of the particular membrane examined.

We have previously studied the multivalent cation-induced phase separations in biological membranes (Caffrey and Lew, 1986) and redistribution of polypeptides (Feigenson, 1983), or of intramembrane particles (Morris et al., 1982) in membrane vesicles. The detection of gel phase in these chromaffin granule membranes further suggests that a cation-induced gel phase is, at least in principle, a plausible driving force for such redistribution.

In conclusion, the results presented above demonstrate explicitly that uranyl acetate causes a gel phase separation to occur in both model and biological membranes, specifically egg PA, bovine brain PS, and CGLM. These findings have implications for electron microscopy techniques employing uranyl acetate as a staining material since uranyl-induced phase separations may induce artifactual changes in protein distribution and other features of membrane morphology.

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