Uranyl Acetate Useful as a Specimen Supporting Membrane on Electron Microscope Grids

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ABSTRACT

Uranyl acetate is useful for its staining properties and also produces an instant, specimen-supporting membrane. Virus particles are embedded in a film of uranyl acetate that is allowed to dry on an uncoated copper grid. This reduces the need for prefilming grids before use.

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Additional key words: tobacco mosaic virus, satellite virus of tobacco necrosis virus.

Specimen grids for electron microscopy are often overlaid with a film of Formvar, collodion, or carbon for supporting the specimen over the viewing areas of the grid. Regardless of the ease with which precoated grids are prepared, the coating materials are not always readily available. We find that uranyl acetate mixed in equal volume with a virus preparation will form a relatively stable membrane showing good staining qualities on uncoated screens. Our results with two virus preparations are reported in this communication.

Tobacco mosaic virus (TMV) and the defective satellite (SV) of the tobacco necrosis virus complex were partially purified by the precedures of Gooding & Hebert (2) and Uyemoto et al. (5), respectively. Negative stains tested included 2% solutions of phosphotungstate (PTA, pH 4 and 6.5, values adjusted with 1N NaOH) and uranyl acetate (UA, pH 4.1). Samples were prepared for the electron microscope (Jeolco 100B) by mixing a drop each of stain and virus suspension on a 400-mesh grid. Excess stain (1% final conc.) was removed with tissue paper and the grids dried in a desiccator.

The relative ease with which uranyl acetate forms

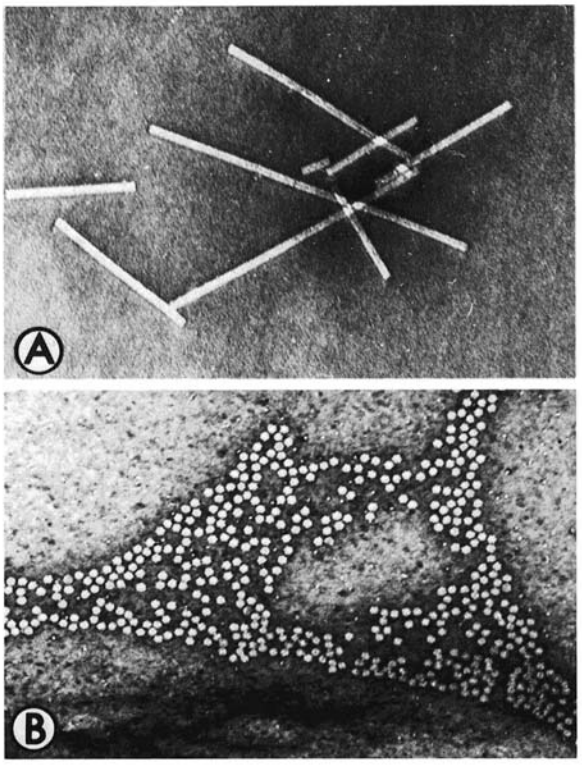

Fig. 1. Electron microscopy of viral samples prepared with uranyl acetate as a stain and specimen support. **A)** tobacco mosaic virus and **B)** satellite virus of tobacco necrosis virus. (× 100,000).

a supporting substrate is illustrated in Fig. 1. Virus particles of TMV and SV are clearly stained and found embedded in the uranyl acetate film. In contrast, PTA produced unsatisfactory support under similar preparative conditions.

Huxley & Zubay (4) and Finch & Klug (1) reported that PTA and UA produced a limited membrane over holey carbon substrate. The results herein are similar to their findings except that film-covered screens were not required. Our attempts to produce holey collodion film (3) overlaid with a thin sheet of carbon were unsuccessful. Although pseudoholes were abundant, actual holes were infrequent.

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