Appearance of Tobacco Mosaic Virus in Thin Section
Using Different Staining Procedures

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Tomato fruit infected with the *vulgare* strain of tobacco mosaic virus were used for the isolation of protoplasts infected with tobacco mosaic virus (Gregory & Cocking, 1965). Protoplasts were fixed in glutaraldehyde, post-fixed in osmium tetroxide and embedded in a butyl methacrylate+styrene mixture (70:30, v/v) as previously described (Mohr & Cocking, 1968). Two different staining procedures were employed. The first (method A) involved incubation of protoplasts during dehydration in a freshly prepared, Millipore-filtered 1% solution of uranyl acetate in absolute ethanol for 1 hr at 18°C. In the second procedure (method B) incubation was for 15 hr at 25°C in a freshly prepared, Millipore-filtered 1% solution of uranyl acetate in 70% ethanol. With both staining procedures sections were post-stained for ½ hr with lead citrate (Reynolds, 1963). Electron micrographs were made with an A.E.I. EM6B at 60 kv using a 25 μ objective aperture.

When method A was employed the particles of tobacco mosaic virus appeared in cross-section with an electron transparent central hole of about 30 Å diameter and an overall cross-sectional diameter of about 80 Å (Pl. 1, fig. 1). This means that probably only the RNA core of the virus was being detected using this staining procedure. With the longer staining procedure (method B) the particles possessed a dense core of about 60 Å diameter with a more lightly stained periphery giving an overall cross-sectional diameter to the virus particle of about 150 Å (Pl. 1, fig. 2). The dense core of the virus was also clearly visible in median longitudinal section as was also the lighter space between it and the more densely stained periphery of the virus particle. Clearly with this staining procedure the protein coat of the virus was being detected. The dense core of the tobacco mosaic virus which was observed corresponds to the RNA core together with the central hole of the virus. This central hole was electron opaque and largely observed as a result of greater infiltration of uranyl acetate during the prolonged soaking.

The chief differences in the appearance of tobacco mosaic virus in thin section from the appearances previously reported appear to fall into two major categories. First, that corresponding to the appearance given by staining Method A in which mainly only the RNA core of the virus is detected (Shalla, 1964; Kolehmainen, Zech & Von Wettstein, 1965; Esau & Cronshaw, 1967); and second, that corresponding to method B in which the complete virus particle is observed (Milne, 1966). Less pronounced differences have, however, been reported (Milne & De Zoeten, 1967).

Staining method A has been employed routinely in electron microscopic studies of protoplasts infected with tobacco mosaic virus. A typical partially-membrane-bounded aggregate of virus is shown in Pl. 1, fig. 3. Often in cross-section the hollow centre of the virus is visible, facilitating the identification of the virus and avoiding any possible confusion of virus in cross-section with ribosomes. Both staining procedures are currently being employed to elucidate more fully the fate of tobacco mosaic virus
pinocytosed by isolated protoplasts (Cocking, 1966), since comparative studies using these two different staining procedures should facilitate the detection of any removal of the protein coat of the virus particle within the pinocytic vesicle.

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REFERENCES


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EXPLANATION OF PLATE

Fig. 1. One hr staining with uranyl acetate during dehydration (method A). In cross-section the hollow centre of the virus is clearly seen.

Fig. 2. Fifteen hr staining with uranyl acetate during dehydration (method B). Usually a dense central core is clearly seen. Occasionally in cross-section a very small hollow centre is discernible.

Fig. 3. A partially-membrane-bound virus aggregate in the cytoplasm of an isolated protoplast (method A). Tobacco mosaic virus particles, many in cross-section and showing the characteristic hollow centre of the virus, are to be seen aggregating in a protrusion into a small vesicle in the cytoplasm.