As part of a study of the way in which tobacco rattle viruses multiply, we have examined the intracellular distribution of the CAM isolate (Harrison & Woods, 1966) by means of electron microscopy of thin sections of leaves. The micrographs show a remarkable association between virus particles and mitochondria. Nothing comparable seems to have been reported with other viruses.

Tip leaves, about 2 cm. long, were taken from plants of Nicotiana clevelandii Gray 5 days after their lower leaves were inoculated with the CAM isolate. Narrow strips of leaf blade were fixed in 5 % (v/v) glutaraldehyde, post-fixed in 0.1 % OsO₄, stained during dehydration for 16 hr in a saturated solution of uranyl acetate in 70 % (v/v) acetone, and embedded in methyl methacrylate + styrene (6:1, v/v). Sections, mostly about 600 Å thick, were cut with a Huxley ultramicrotome, using a glass knife.

In sections through the palisade and spongy mesophyll a high proportion (often more than 80 %) of the mitochondria in any given cell were associated with numerous virus particles (Pl. 1, fig. 1). No particles were associated with either chloroplasts or nuclei. The particles radiated from the outer surface of the mitochondrion, and often 40 or more could be seen around a single mitochondrion (Pl. 2, fig. 2). By examining serial sections, 100 to 500 particles could be counted on each mitochondrion. The particles were not randomly distributed on the mitochondrial surface, but where they occurred they were usually numerous; they were often found packed along one side but not along the opposite side of a mitochondrion. In some sections, groups of particles in close-packed arrays were cut transversely, but examination of serial sections showed that such arrays were invariably associated with mitochondria (Pl. 2, fig. 2, 3). Although some particles could be discerned in the cytoplasm, these were not in aggregates and seemed randomly oriented. Mitochondria associated with very many particles tended to have a distorted outline, as though the packing of the particles along a side had made it abnormally straight (Pl. 2, fig. 4). A similar association of particles with mitochondria was found when the tissue was fixed with 1 % potassium permanganate (Pl. 3, fig. 5) instead of with glutaraldehyde and OsO₄. There is no evidence that it is an artifact.

Although the CAM isolate of tobacco rattle virus has particles of two distinct length categories (520 and 1950 Å, Harrison & Woods, 1966), and these two kinds of particle interact during infection in a manner analogous to symbiosis (Lister, 1966; Frost, Harrison & Woods, 1967), only the longer type was associated with the mitochondria. Electron microscopy of extracts of leaves similar to those sectioned showed that the leaves contained both types of particle and that in some samples the shorter outnumbered the longer ones. We think the short particles came from the cytoplasm, where they would be less readily discernible in sections than particles associated with mitochondria.

The association of the long tobacco rattle virus particles with mitochondria can be interpreted in two main ways: (1) mitochondria are sites of assembly of the particles;
(2) the particles become adsorbed to mitochondria after assembly elsewhere. The first possibility is attractive because in tissues infected with a defective isolate of tobacco rattle virus, which produces virus RNA but not nucleoprotein particles, the virus nucleic acid is associated with, and partially protected from inactivation by, readily sedimentable cell structures (Cadman, 1962). These could be mitochondria. Against this possibility is the observation that nearly all the particles around the mitochondria are close to the standard length (1800 to 2000 Å); few if any seem to be in process of assembly. The second possibility is more compatible with this finding, and the apparent difference in surface charge of long and short particles of isolate CAM (J. I. Cooper, unpublished results) could explain the association of only the long particles with the mitochondria. However, none of these arguments is conclusive.

Close inspection of the region of contact between tobacco rattle virus particles and mitochondria (Pl. 3, figs. 6, 7) gave no evidence that the particles penetrated the outer mitochondrial membrane, though their ends seemed closely appressed to it. There was little evidence that tobacco rattle virus particles formed bridges linking mitochondria, suggesting that if surface charge is important, one end of a particle may differ in surface charge from the other, as it does morphologically (Harrison & Woods, 1966). The sides of the particles would also carry a different charge from the adsorbed end.

De Zoeten (1966) examined sections of Nicotiana tabacum L. leaves infected with a Californian isolate of tobacco rattle virus and did not note any association between virus particles and mitochondria. Hence other isolates may not behave like isolate CAM.

Scottish Horticultural Research Institute  
Invergowrie, Dundee, Scotland  

REFERENCES


(Received 12 December 1967)
EXPLANATION OF PLATES
(See overleaf)
EXPLANATION OF PLATES

Fig. 1 to 7. Electron micrographs of sections of palisade cells of *Nicotiana clevelandii* leaves infected with the CAM isolate of tobacco rattle virus. Fixed in glutaraldehyde and post-fixed in OsO₄ unless otherwise stated.

PLATE 1

Fig. 1. General view of cells, showing numerous virus particles cut in longitudinal (L) or transverse (T) section and associated with mitochondria (M).

PLATE 2

Fig. 2. Section showing mitochondrion cut transversely, with fringe of virus particles. Part of chloroplast at left.

Fig. 3. Same mitochondrion as in fig. 2 after removing three intervening sections. Note array of virus particles cut transversely.

Fig. 4. Area including angular mitochondrion associated with numerous virus particles.

PLATE 3

Fig. 5. Section from tissue fixed with potassium permanganate, showing mitochondrion with fringe of virus particles. Second mitochondrion at bottom left. Parts of chloroplasts at left and right.

Fig. 6, 7. Enlarged views of mitochondrial membranes, showing virus particles appressed to but not penetrating the outer membrane (arrows).
Plate 3

B. D. HARRISON AND I. M. ROBERTS