Intracellular Morphology of Two Tobacco Mosaic Virus Strains in, and Cytological Responses of, Systemically Susceptible Potato Plants

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ABSTRACT

Comparative studies were made to investigate the intracellular behavior of two strains of tobacco mosaic virus which cause distinctly different symptoms in systemically infected potato plants. In cells of these plants, which were derived as clonal material from selected susceptible seedlings, both U-1 and a yellow strain formed hexagonal crystals in infected cells. The yellow strain caused degradation of chloroplasts which was correlated with yellowing of leaves and the presence of many osmiophilic globules in the cytoplasm. In cells infected with the U-1 strain, filamentous tubes and microcrystals were found. The phenomenon that different strains of a virus cause distinct cytological responses, which correlate to the external symptoms, yet form almost similar intracellular viral crystals and aggregates, would be explained by the two strains having different genes governing cytological effects and identical genes controlling synthesis of coat protein. The evidence that microcrystals were found only in cells infected with the U-1 strain but not in cells infected with the yellow strain implies that, apparently, different host-virus interactions operate in genetically identical cells of the host.

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The behavior of tobacco mosaic virus (TMV) in plant cells and the cellular response to virus infection at the ultrastructural level have recently received considerable study (6, 8, 10, 12, 16, 17, 18, 19, 20, 21). Details of many aspects of change in plant cells of *Nicotiana tabacum* L. caused by TMV infection are well illustrated by Esau (5). However, few systematic studies on the behavior of different strains of TMV in clonal lines of the same plant species have been made. A comparative study on the behavior of three strains of TMV in tobacco plants lead Granett & Shalla (10) to conclude that closely related virus strains could differ in intracellular behavior. They explained the phenomenon by a hypothesis involving the virus-citron which Kado et al. (14) had postulated.

Although TMV has a wide host range, potato (*Solanum tuberosum* L.) has been considered to be highly resistant to it, since the virus has not been found to occur in field plantings of the crop and experimental inoculations have resulted only in local lesion formation in some cultivars (2, 7, 13) or restricted systemic infection.

Recently, Boyle (3) successfully inoculated potato seedlings with TMV and obtained systemic infections which resulted in the transmission of virus through the tubers. Both the type strain, U-1, and a yellow strain, which was isolated by repeated single lesion transfer from a yellow spot which appeared on a tobacco leaf systemically infected with an isolate from tomato, are infectious to these particular potato cultivars. The leaves of plants infected with the U-1 strain exhibit a green mosaic with considerable leaflet distortion, whereas the foliage of plants infected with the yellow strain exhibit bright yellow mosaic with leaflet distortion.

These host-virus combinations provided an opportunity for observation of the intracellular morphology of a virus in identical clonal material of an unusual host and especially for comparison of the cytological response of the host plant to virus strains which incited distinctly different systemic symptoms. Therefore, the present study compares the U-1 and a yellow strain of TMV with respect to their intracellular morphology and cytological responses in systemically infected potato.

MATERIALS AND METHODS.—Plants of a susceptible, seedling-derived cultivar of *Solanum tuberosum* L. were used. Sprouted tubers were planted in a steam-sterilized mixture of soil, peat, and perlite (1:1:1) contained in 6-inch clay pots. When the plants were about 6 inches high, we inoculated them with TMV U-1 or the yellow strain by rubbing Carborundum-dusted leaves with the virus suspensions. Samples for electron microscopy were taken from the systemically infected apical leaves after they had fully expanded. Small leaf pieces were fixed 3 hr in 5% glutaraldehyde in 0.1 M potassium phosphate buffer in an ice bath. Postfixation was carried out in 1% OsO_4 in the same buffer at 4 C for 2 hr. After a staining overnight in 1% aqueous solution of uranyl acetate at room temperature, the material was dehydrated by passage through a graded series of acetone up to 100%. The pieces were kept for 30 min in each of a 1:1 and then a 1:3 mixture of acetone and a low viscosity embedding medium (22) before they were placed in the medium at room temperature overnight. The pieces were then transferred into fresh medium and cured at 70 C for 16 hr. Sections were cut with a glass or diamond knife on a Porter-Blum MT-2 ultramicrotome and stained with lead citrate.

Observations were made with a RCA-EMG-3G electron microscope with a 50 μ objective aperture, operating at 50 kv or with a Hitachi HU-11E electron microscope with an adjustable objective aperture from 20 to 70 μ, operating at 75 kv.

RESULTS.—Results quite similar to those reported by Kolehmainen et al. (16) were obtained. They studied the structure of cells of a *Nicotiana tabacum* cross (Samsun × White Burley) systemically infected with a flavum strain of TMV, and observed hexagonal crystals, filamentous tubes, microcrystals, osmiophilic globules in the cytoplasm, chloroplast degradation, and single layers of oriented virus particles. In the present study, both the U-1 and the yellow strain formed hexagonal crystals and small virus aggregates. However, only in cells infected with U-1 were filamentous tubes and microcrystals observed. The yellow strain incited chloroplast degradation and osmiophilic globules in the cytoplasm. No monolayers of virus particles were observed in cells infected either with U-1 or the yellow strain.

Crystalline inclusions.—Both U-1 and the yellow strain formed hexagonal crystals which lay free in the ribosome-rich cytoplasm without any type of membrane or other visible boundary. Individual crystals consisted only of virus particles, except for some cytoplasmic elements such as ribosomes and mitochondria in a few localized areas (Fig. 1, 2). Virus crystals in cells infected with U-1 contained more of these cytoplasmic elements, including mitochondria, than those in cells infected with the yellow strain. The basic orientation of particles within individual layers in a viral crystal was parallel. Adjacent layers seemed either to be oriented parallel to each other or at a characteristic angle suggesting a herringbone pattern (23). In many places, loosely aggregated and small, poorly oriented groups of particles, identical in structure and dimensions with those in the virus crystals, were found within clear areas of the cytoplasm. Individual virus particles were seen in vacuoles. The location of crystals in cells was not specific in relation to cell components. They occurred next to mitochondria, the nucleus, or the chloroplasts (Fig. 3), and sometimes in the vacuole. The virus crystals varied in size, but sometimes they occupied all the available space in the cell (Fig. 3). No crystalline inclusions or any other forms of virus aggregates were observed in the specimen obtained from noninfected potato plants.

Filamentous tubes and microcrystals.—In some cells infected with U-1, certain areas of the cytoplasm were filled with long tubes. They were dispersed in small, randomly oriented groups between parts of the endoplasmic reticulum, or they were well oriented and evenly spaced to form a large, regular mass (Fig. 4, 5). No such tubes were observed in cells infected with the yellow strain or in cells of noninfected plants. The U-1 infected cells under study also contained many small rhomboidal crystals with a lattice spacing of 100 Å (Fig. 6). The crystals were contained in a single membrane-bound organelle. None of these microcrystals was found in cells infected with the yellow strain or in cells of noninfected plants.

Osmiophilic globules.—In yellow-TMV-infected cells, relatively large areas of the cytoplasm were filled with strongly electron-scattering globules of varying sizes which tended to fuse into relatively large masses (Fig. 7, 8). Esau (5) has suggested that the origin of osmiophilic globules in the cytoplasm of cells infected with some viruses was due to metabolic changes that increase the deposition of insoluble lipid materials. However, an increase of the yellow pigments in cells, following a virus infection, has also been suggested to be the progenitor of the osmiophilic globules observed in TMV-infected cells (4, 11). According to our unpublished data, there was no increase in the amounts of carotenes or xanthophylls in the yellow leaf areas from plants infected with the yellow strain when compared with green leaf areas from the same plant or with noninfected plants. In fact, the amounts of these pigments were decreased in the yellow areas of the infected plants.

Disintegration of chloroplasts.—In cells infected with the yellow strain, chloroplasts were almost always observed in various stages of degradation (Fig. 7, 8, 9). The lamellar system was frequently deranged, swollen, or completely eliminated. An increase in number and size of osmiophilic globules in the chloroplasts compared to those in cells of noninfected plants or in cells infected with TMV U-1 was observed. A similar condition has been reported to occur in the senescence of plant leaves (24). In U-1 infections, despite massive accumulation of virus in cytoplasm, the chloroplasts appeared normal(Fig. 3).

No virus particles or aggregates were found in the nucleus or mitochondria. These organelles appeared essentially normal even though aggregates of virus occurred next to them (Fig. 3).

DISCUSSION.—In the present studies, both strains, even though symptomatologically distinct, were similar in crystal formation. Both strains formed hexagonal crystals in potato plant cells with minor differences. However, the specific cytological response of the host plant to these strains was distinct. The disintegration of chloroplasts in cells infected with the yellow strain was correlated with the external symptom of leaf chlorosis. Filamentous tubes and microcrystals occurred in cells infected with U-1, whereas osmiophilic globules in the cytoplasm were seen only in cells infected with the yellow strain.

The phenomenon observed in the present study, i.e., distinct differences in symptoms and cytological response of the same host plant to the different strains but with similar viral crystal formation, can be explained by the hypothesis of Kado et al. (14) that two stains of a virus can have identical genes controlling coat protein synthesis but differ with respect to genes that are somehow involved in symptom expression and cytological changes. Presumably, similarity in formation of crystals and aggregates indicates similarity of coat proteins and thus similarity in genes coding for coat proteins. Intracellular changes and symptoms are presumably determined by the interaction of other viral genes and the host's

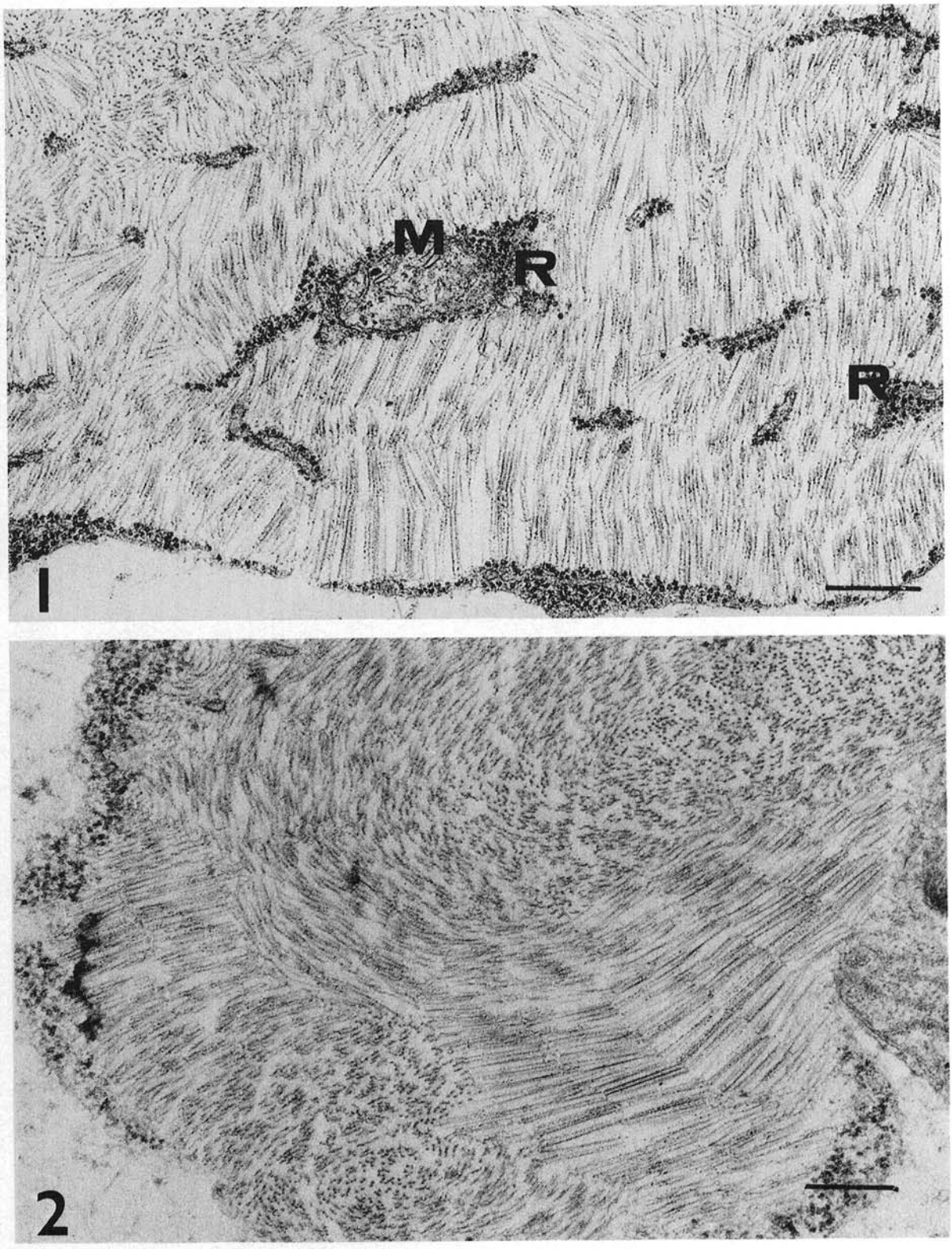

Fig. 1-2. Electron micrographs of tobacco mosaic virus crystals found in the infected mesophyll cells of potato plants. **1)** Portion of a hexagonal crystal found in cells infected with U-1. Ribosomes and a mitochondrion are seen in the crystal. **2)** Portion of a crystal found in cells infected with the yellow strain. Similar in constitution to the crystal in U-1 infected cells, but rather free from cytoplasmic organelles. M = mitochondrion; R = ribosomes. Bars equal 0.3μ.

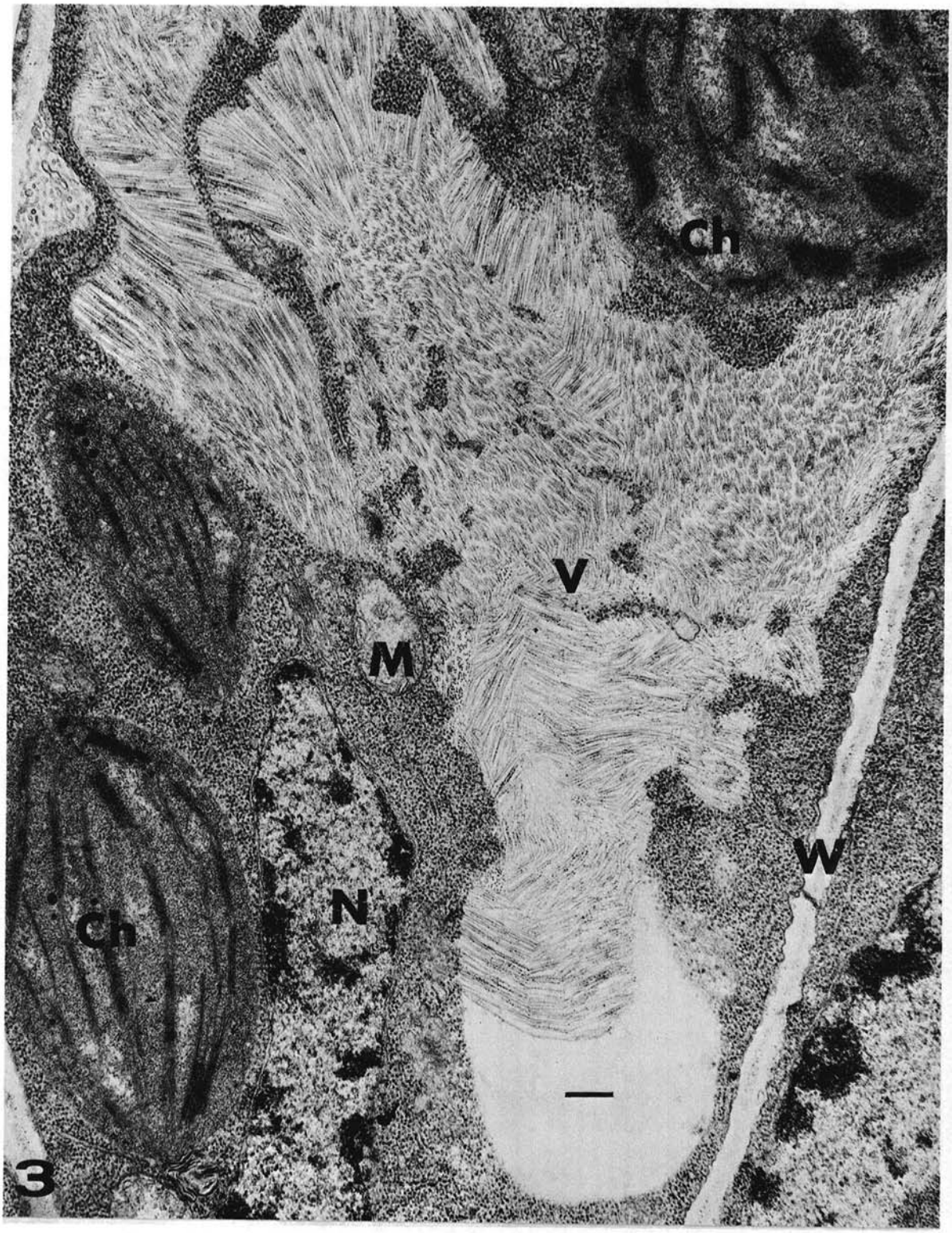

Fig. 3. Portion of a mesophyll cell infected with U-1. Virus occurs in the cytoplasm with rods nonoriented. Cellular organelles clearly demarcated in spite of heavy accumulation of virus particles. Ch = chloroplast; N = nucleus; M = mitochondrion; V = virus; W = cell wall. Bar equals 0.3μ.

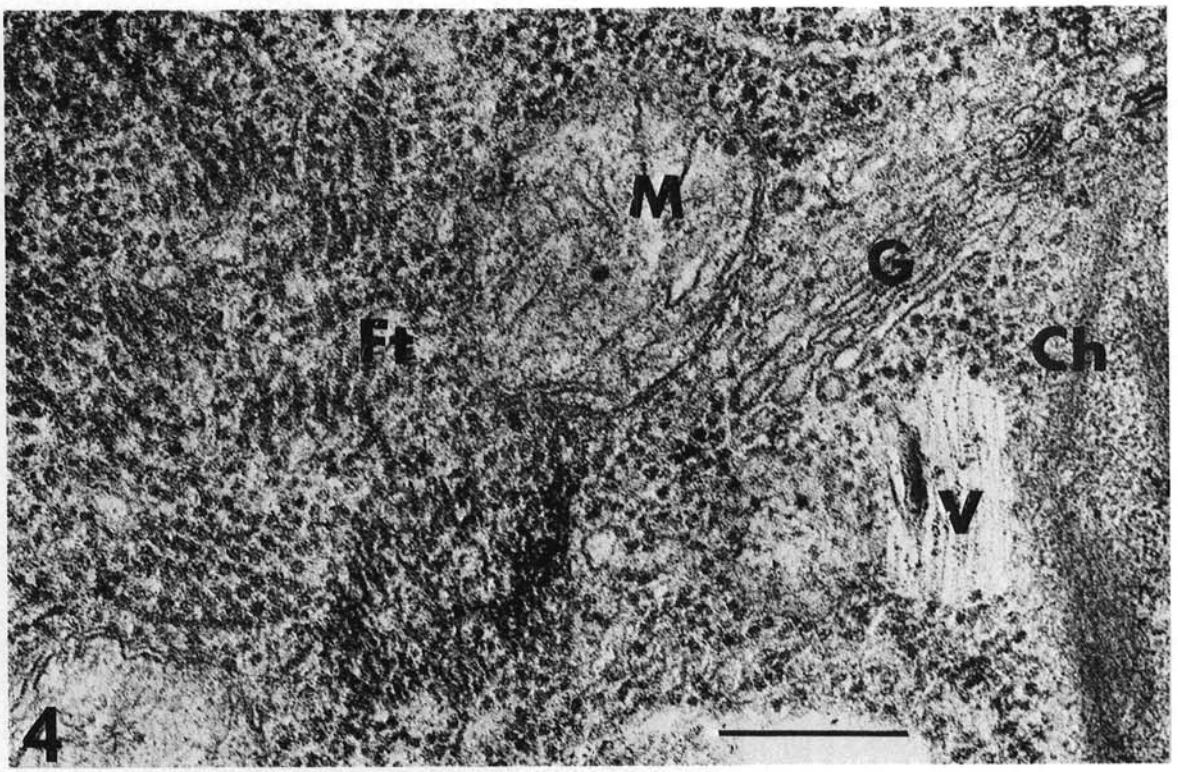

Fig. 4-5. Filamentous tubes found in U-1 infected cells. **4)** Clusters of filamentous tubes in the cytoplasm cut obliquely to their long axes in association with some cellular organelles and a small aggregate of virus. **5)** Clusters of filamentous tubes in the cytoplasm near the nucleus cut longitudinally. The single arrowhead indicates cross section of a cluster of tubes. Ch = chloroplast; Ft = filamentous tube; G = Golgi apparatus; M = mitochondrion; V = virus. Bars equal 0.3μ.

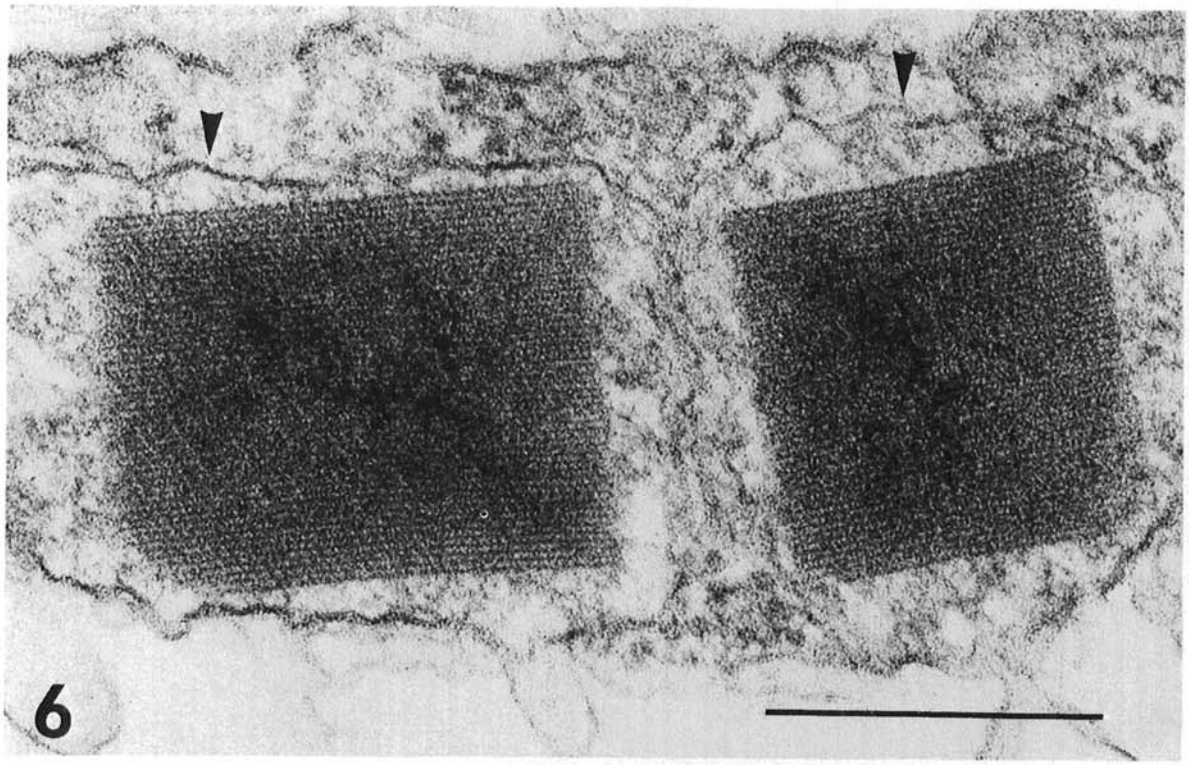

Fig. 6. Microcrystals found in the cytoplasm of a cell infected with U-1 are contained in single membrane-bound organelles. Arrowheads indicate single membranes. Bar equals 0.3μ.

metabolic system. Since use of a single clone provided the same cellular metabolic system for the two virus strains, differences in the viral genes conditioning host response are indicated.

Crystalloids similar to microcrystals observed in the present study have been reported in cells surrounding TMV lesions in tobacco (12), in plant cells infected by a mycoplasmalike organism (9), in insects infected with a virus (1), in phagocytes of the ovary of sea urchin, *Arbacia puntulata* (15), and in midgut glands of a wood-eating marine isopod, *Limoria lignorum* (Rathke) (25). Concerning the function and origin of these crystals, it has been postulated that since they are an iron-containing protein, a synthesizing mechanism similar to that involved in the aggregation of ferritin might be involved in their formation (15). In their study on the fine structure of local lesions induced by TMV in tobacco, Israel & Ross (12) found microcrystals in the encircling zone of resistant and synthetically active cells, and they suggested that formation of microcrystals reflects some aspect of the resistant mechanism. In the present study, microcrystals were found only in the cells infected with TMV-U-1 and not in the cells from noninfected plants or cells infected with the yellow strain. The significance of this difference is not clear at this time, since it appears that the potato cultivar is equally susceptible to both strains of the virus.

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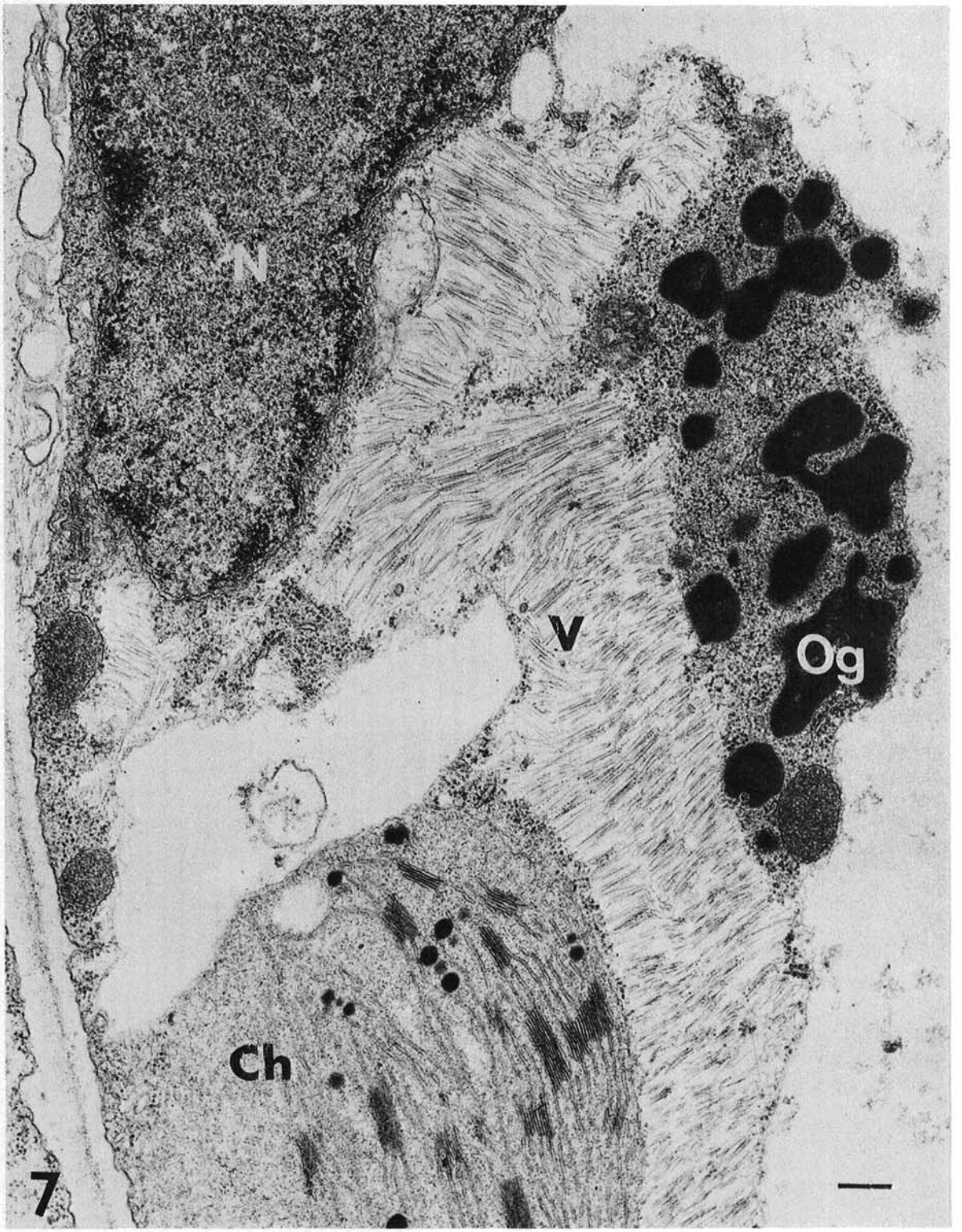
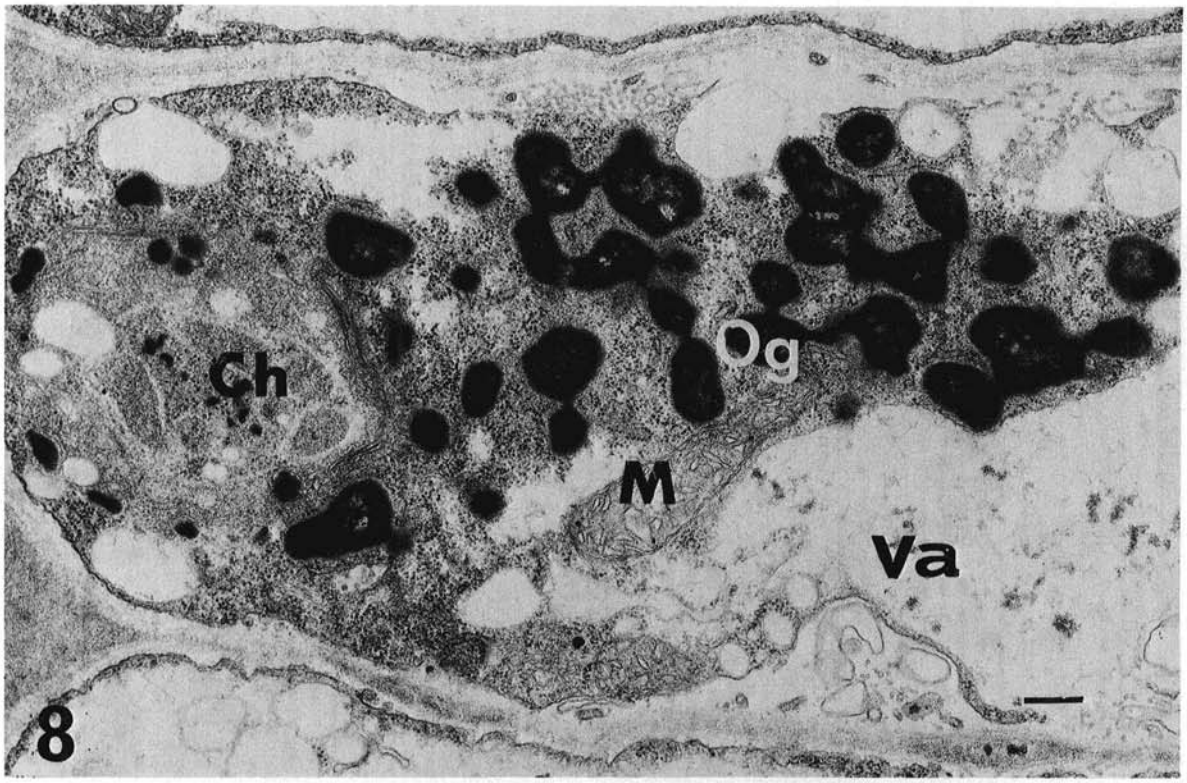


Fig. 7. Cytological change seen in a cell infected with the yellow strain. Large osmiophilic globules occur in the cytoplasm. The chloroplast shows some degree of deterioration. Numbers of osmiophilic globules in the organelle increase. The nucleus appears rather normal. Ch = chloroplast; N = nucleus; Og = osmiophilic globules; V = virus aggregates.



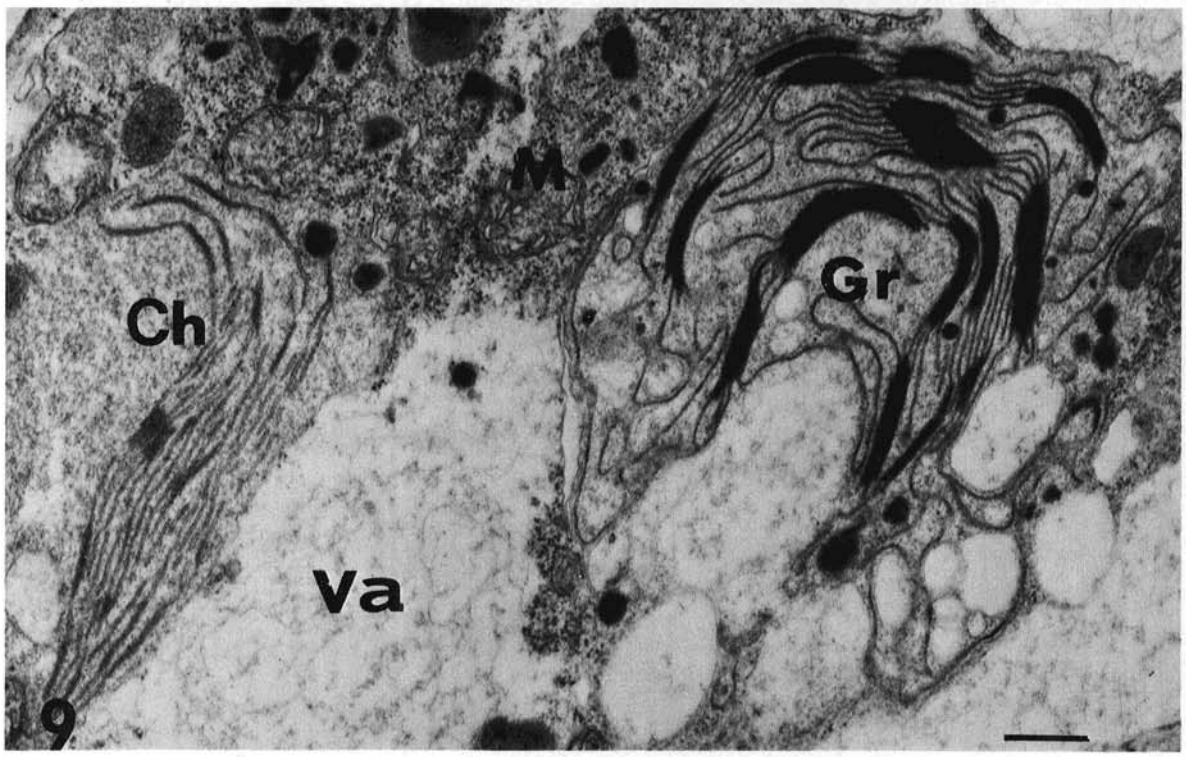


Fig. 8-9. 8) Cytological change seen in a guard cell infected with the yellow strain. Osmiophilic globules fill the cytoplasm. The chloroplast shows an extensive deterioration. Only scattered individual virus particles seen in the vacuole. **9)** Distorted chloroplasts seen in a cell infected with the yellow strain. Fused grana in the right hand chloroplast become more electron-opaque. Numerous virus particles seen in the vacuole. Ch = chloroplast; M = mitochondrion; Va = vacuole; Gr = granum. Bars equal 0.3μ.

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