RELATION OF TOBACCO MOSAIC VIRUS TO THE HOST CELLS

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ABSTRACT

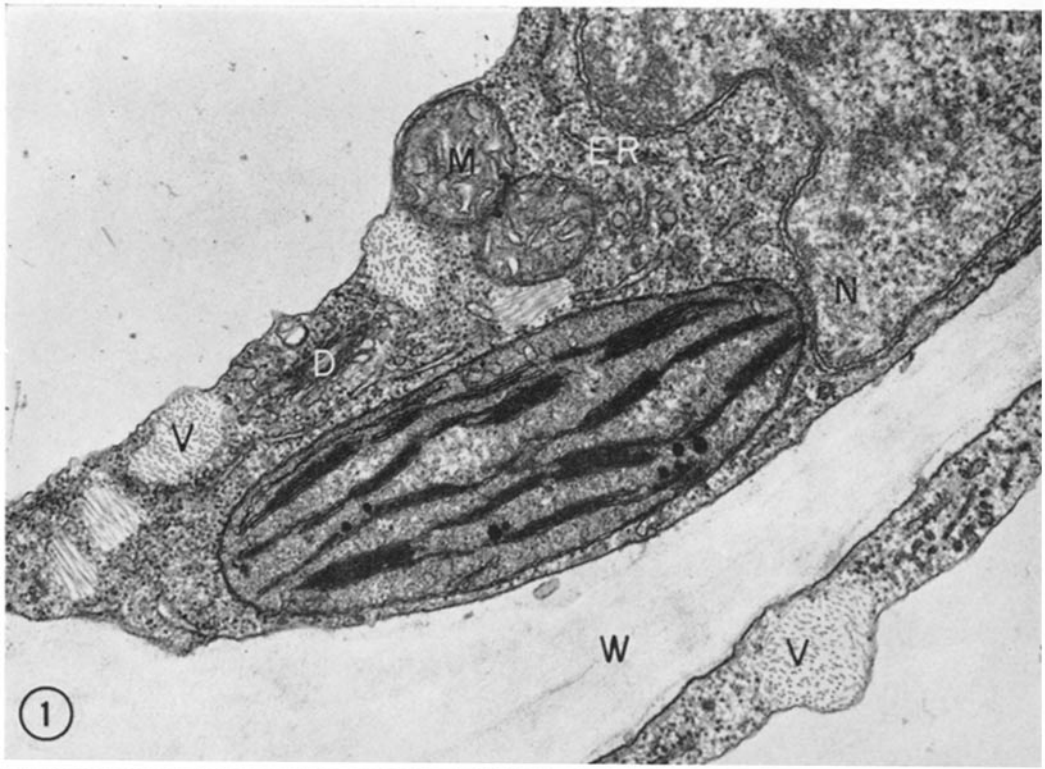
The relation of tobacco mosaic virus (TMV) to host cells was studied in leaves of *Nicotiana tabacum* L. systemically infected with the virus. The typical TMV inclusions, striate or crystalline material and ameboid or X-bodies, which are discernible with the light microscope, and/or particles of virus, which are identifiable with the electron microscope, were observed in epidermal cells, mesophyll cells, parenchyma cells of the vascular bundles, differentiating and mature tracheary elements, and immature and mature sieve elements. Virus particles were observed in the nuclei and the chloroplasts of parenchyma cells as well as in the ground cytoplasm, the vacuole, and between the plasma membrane and the cell wall. The nature of the conformations of the particle aggregates in the chloroplasts was compatible with the concept that some virus particles may be assembled in these organelles. The virus particles in the nuclei appeared to be complete particles. Under the electron microscope the X-body constitutes a membraneless assemblage of endoplasmic reticulum, ribosomes, virus particles, and of virus-related material in the form of wide filaments indistinctly resolvable as bundles of tubules. Some parenchyma cells contained aggregates of discrete tubules in parallel arrangement. These groups of tubules were relatively free from components of host protoplasts.

As part of a study on the relation of viruses to the phloem tissue, we have reported extensively on the distribution and some effects of the beet yellows virus in the leaf tissues of the sugar beet (5, 7, 8). The particles of that virus were identified in the parenchyma cells and the sieve elements of the phloem and in mesophyll cells. Their occurrence in the mesophyll agrees with the interpretation that the beet yellows virus is not as strictly limited to the phloem as some other yellows viruses. At the same time, the first post-inoculation appearance of the beet yellows inclusions in phloem cells conforms with other data that the virus depends on the phloem tissue for its introduction into and its initial spread through the plant. Significantly, virus particles were found in the lumina of mature sieve elements and also in sieve plate pores and various plasmodesmata. This observation was interpreted as indicating the passage of virus from cell to cell in the form of complete particles and its mass movement in the sieve elements.

The study of the tobacco mosaic virus (TMV) was undertaken for the purpose of comparing the relation to host tissue of a virus having apparently only an incidental association with the phloem (TMV is transported in the phloem if it enters that tissue but can become systemic by spreading through parenchyma tissue) with the beet yellows virus that has a closer relation to the phloem.

MATERIAL AND METHODS

Nicotiana tabacum L. plants systemically infected with TMV were kindly supplied by Dr. W. N. Takahashi of the University of California, Berkeley, and by Dr. S. G. Wildman of the University of California, Los Angeles. Control plants were grown locally. Fragments of young stems and medium-size leaves,



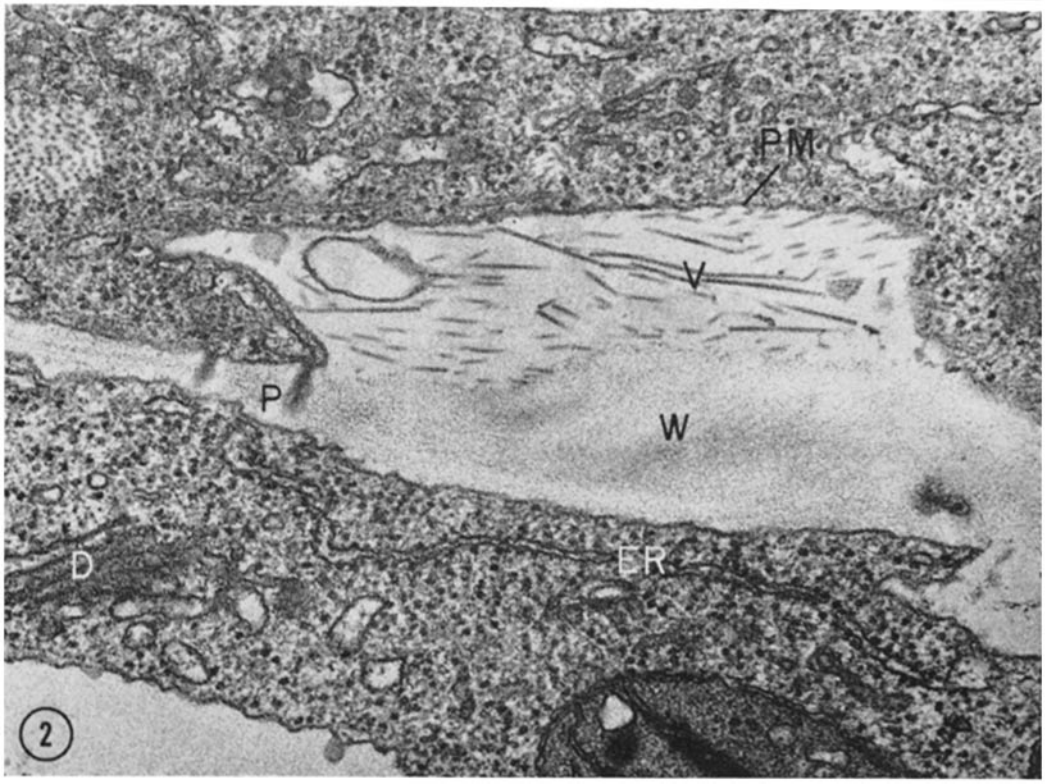


FIGURE 1 *Nicotiana tabacum.* Parenchyma cells from a vascular bundle with small aggregates of TMV particles. Cell components show no evidence of disorganization. The chloroplast contains grana and osmiophilic globules. Details: *D*, dictyosome; *ER*, endoplasmic reticulum; *M*, mitochondrion; *N*, nucleus; *V*, virus particles; *W*, cell wall. × 26,000.

FIGURE 2 *Nicotiana tabacum.* Parenchyma cells from a vascular bundle with virus particles in the cytoplasm (left, above) and between the plasma membrane and the cell wall. Details: *D*, dictyosome; *ER*, endoplasmic reticulum; *P*, plasmodesma; *PM*, plasma membrane; *V*, virus particles; *W*, cell wall. × 52,500

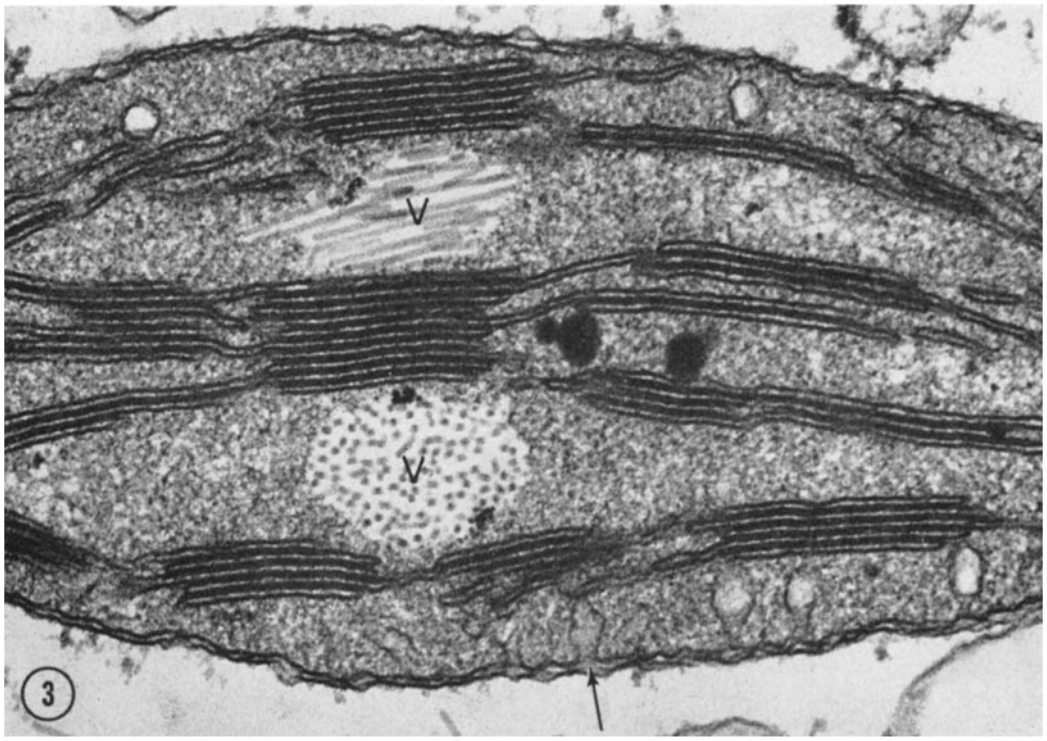


FIGURE 3 *Nicotiana tabacum.* Chloroplast containing virus particles (*V*) in two aggregations. In one of these the particles are seen in their longitudinal extent, in the other they were sectioned transversely. One of the vesicles near the periphery of the chloroplast is seen as a continuation of the inner membrane of the chloroplast envelope (arrow). × 72,000.

including vascular bundles, were fixed for light and electron microscopy. For the former, a common combination of chromic acid, acetic acid, and formalin served as fixative. Paraplast (Scientific Products, Inc., Detroit, Mich.) was used for embedding, and hematoxylin according to Heidenhain for staining. For electron microscopy the material was fixed in glutaraldehyde-formaldehyde according to the method of Karnovsky (11). Postfixation was with phosphate-buffered 2% osmium tetroxide. The material was dehydrated in acetone solutions and embedded in Epon epoxy resin. Sections were cut with a diamond knife on a Porter-Blum MT 2 ultramicrotome and stained with uranyl acetate and lead (7). Observations were made with a Siemens Elmiskop I.

RESULTS

Distribution of Virus in Host Tissues

The microscopically visible inclusions typical of tobacco mosaic virus infections and the rodlike virus particles revealed by the electron microscope served for the identification of infected cells in the survey of cell types that may harbor the virus. Two kinds of inclusions seen with the light microscope occurred in the fixed material from diseased plants, the so-called striate material, that is, aggregates of virus rods representing the crystalline inclusions modified by fixation, and the amorphous X-body containing virus-related material (2). (Both kinds of inclusions are depicted in Fig. 11.)

The microscopic inclusions (combinations of striate material and X-bodies, or striate material alone) occurred in all types of protoplast-containing cells but showed an uneven distribution. They were abundant in some regions of leaf and stem, sparse in others. They occurred in the epidermis, mesophyll, and parenchyma cells of the xylem and phloem. Striate material and X-bodies were recognized in differentiating tracheary elements

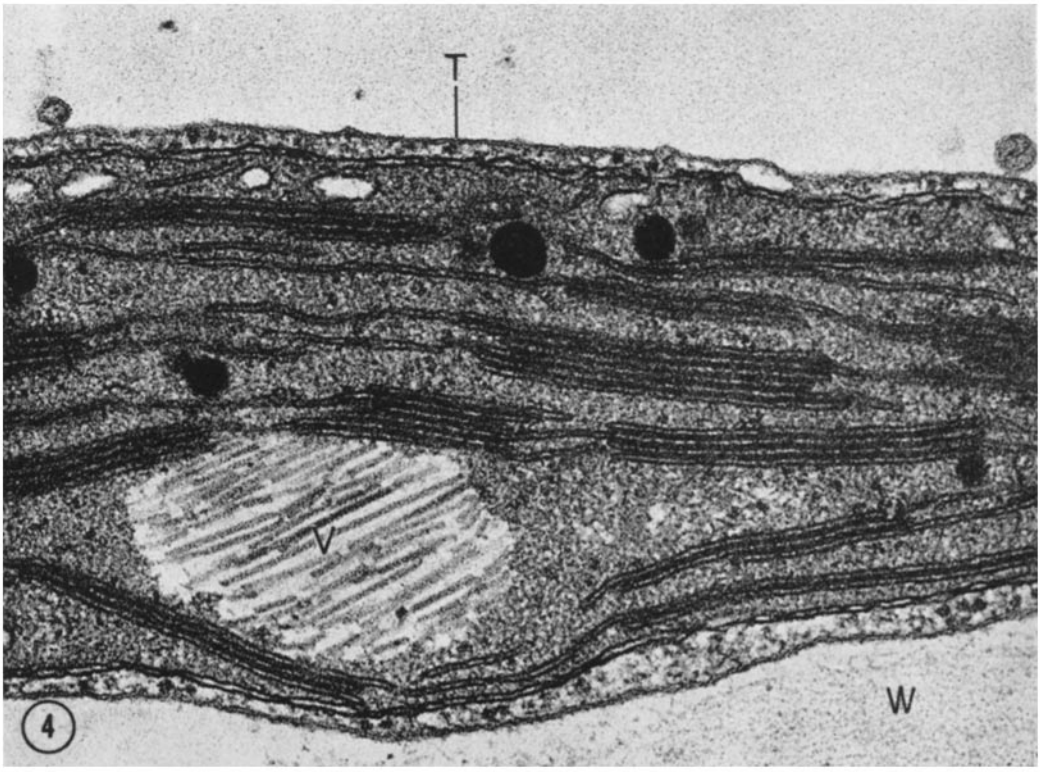


FIGURE 4 *Nicotiana tabacum.* Chloroplast containing virus particles in an aggregate that apparently has caused the displacement of grana. Osmiophilic globules and vesicles are present. Details: *T*, tonoplast; *V*, virus particles; *W*, cell wall. × 80,000.

and companion cells in the phloem, only striate material in immature sieve elements.

With the electron microscope, typical TMV particles (see references 13, 15–17, 21) were identified in all cell types that were found to contain the microscopic inclusions (Figs. 1–9). In addition virus particles were detected in mature tracheary elements and mature sieve elements (Fig. 10).

Relation of Virus Particles to Cell Protoplasts

In parenchyma cells, the virus particles were seen within the ground cytoplasm (Fig. 1), the vacuole (Fig. 10), between the plasma membrane and the cell wall (Fig. 2), and within the chloroplasts (Figs. 3–5) and the nucleus (Fig. 7). The aggregates of virus particles in the cytoplasm of parenchyma cells and other cell types were apparently free from components of host protoplasts and were not delimited by membranes (Fig. 1). In the vacuoles, the virus particles were either thinly dispersed or appeared in aggregates in which the tendency toward an alignment typical of the crystalline aggregates was unmistakable (Fig. 10, lower left).

The aggregates in the chloroplasts were similar to those in the cytoplasm and were lacking a bounding membrane (Figs. 3–5). The evident displacement of chloroplast grana near the enclaves containing the virus (especially in Fig. 4) probably indicates that the virus aggregates were increasing in volume within the plastids. The chloroplasts containing virus showed no signs of degeneration. The small peripheral vesicles, so prominently displayed in Figs. 3 and 4, were present in noninfected controls also and were recognized as invaginations of the inner membrane of the chloroplast envelope (Fig. 3, arrow). Shalla (21) assumed that these vesicles were associated with the infection.

The particle aggregates encountered in the nuclei were rather small and occurred in the matrix of the nucleus (Fig. 7), sometimes next to

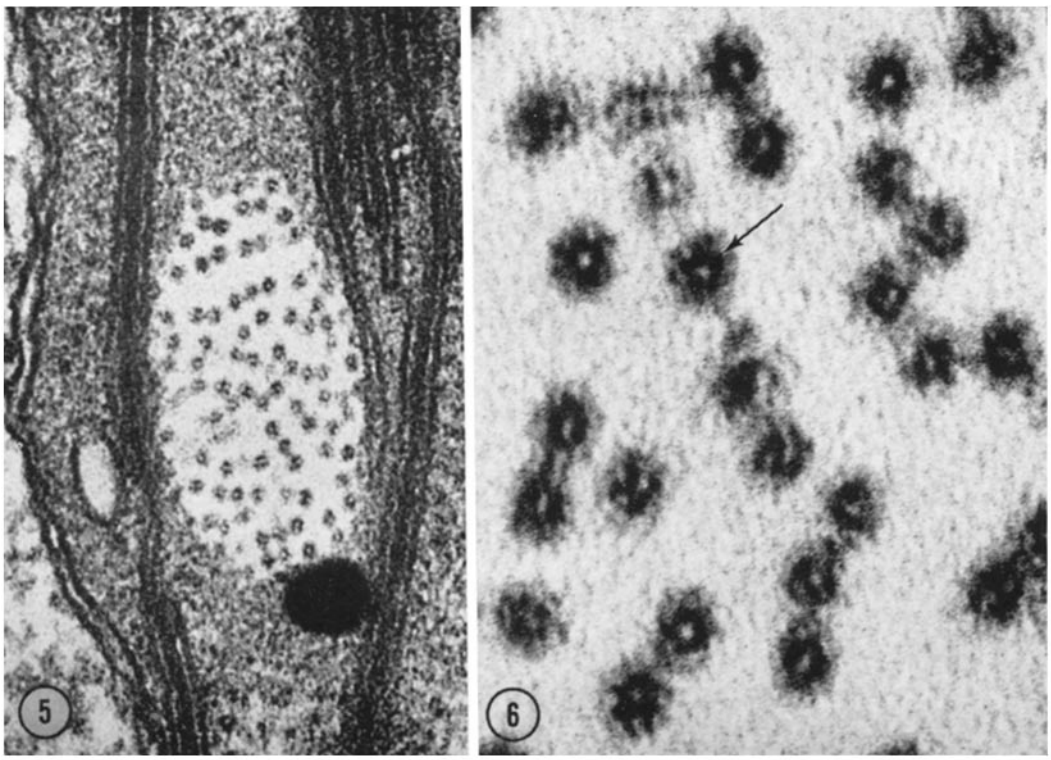


FIGURE 5 *Nicotiana tabacum.* Chloroplast with an aggregate of virus particles seen in transection. In the particles, a dark hollow core may be distinguished from a lighter peripheral region. × 158,000.

FIGURE 6 *Nicotiana tabacum.* Virus particles from an aggregate in the cytoplasm seen in transection. A dark hollow core, possibly RNA, may be distinguished from a lighter peripheral region, especially at arrow. × 640,000.

the nucleolus. The aggregates usually appeared to consist of particles only, without obvious admixture of nuclear material. No membrane delimited the aggregates. In some sections of material obtained from Dr. Wildman, spherical bodies, 450 A in diameter, occurred in the nucleus together with occasional virus rods (Fig. 8). The identity of these bodies was not determined.

In immature sieve elements and xylem elements the virus particles occurred in the cytoplasm in the same form as in parenchyma cells. They were dispersed in mature tracheary elements. In sieve elements lacking tonoplasts the particles were widely dispersed or occurred in aggregates partially or completely delimited by degenerated material (Fig. 10). The dispersed particles were frequently mixed with components of the slime but could be distinguished from the latter by their straight form (Fig. 10).

Morphology of the Virus Particles

The appearance of the TMV particles in our preparations agrees with that illustrated in the most recent papers dealing with the ultrastructure of this virus within the host cells (13, 15–17). In Fig. 10 the rodlike particles have a length of approximately 3000 A. The strongly electron-scattering rod seen in longitudinal views possibly represents only the core of ribonucleic acid, for the protein coat, or the capsid, may fail to stain (13). Some of our preparations, stained on the grids with uranyl acetate and then with lead, showed a differentiation between a core and a coat (Figs. 5, 6; see also reference 15). The core was hollow and resolvable into strongly electron opaque units (Fig. 6). The diameter of the core was approximately 76 A and that of the inner space 40 A. The mean width of the entire particle,

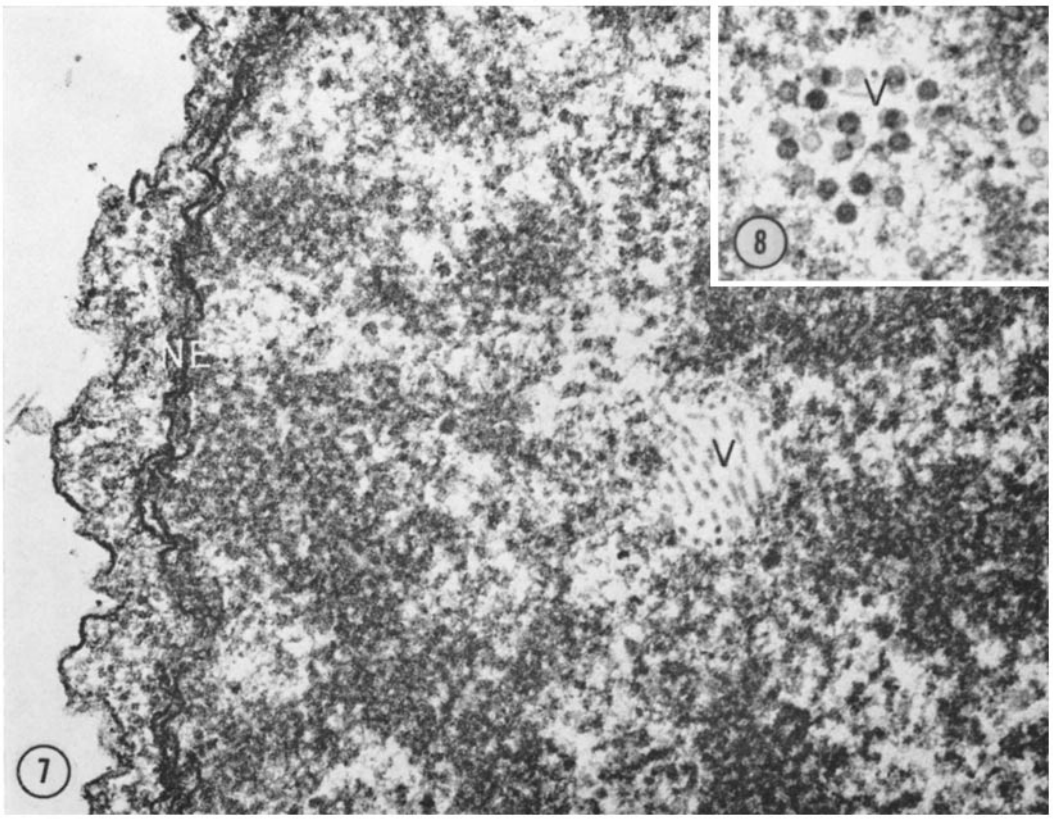


FIGURE 7 *Nicotiana tabacum.* Portion of a nucleus from a parenchyma cell with an aggregate of virus particles (*V*) in its matrix. Nuclear envelope at *NE*. × 66,000.

FIGURE 8 *Nicotiana tabacum.* Portion of a nucleus from a parenchyma cell showing one TMV particle (*V*) and a group of unidentified spherical particles. × 66,000.

as seen in Fig. 6, was 144 A. The dark core probably indicates the position of the RNA helix and the lighter coat would appear to be the protein capsid.

The X-Body

A special effort was made to determine the ultrastructural features of the ameboid body. Reviews in the literature (e.g. reference 24, pp. 12–13) indicate that the composition of the X-bodies observed in material infected with different plant viruses may vary but that the bodies are in some manner related to the crystalline inclusions, that is, to the aggregates of virus particles. An adequate correlation between the structures identified as X-bodies with the light microscope and their counterparts seen with the electron microscope has not been made for TMV (13, 15, 21) or any other virus (cf. reference 24).

In our light microscope study the ameboid bodies (Figs. 11, 13) proved to be most conspicuous in the epidermis and the subepidermal layers in vein ribs and stems. In similar material embedded in Epon and sectioned at 0.5 μ ameboid bodies were identified by the use of phase contrast optics after which sectioning of the same block was continued for electron microscopy. The X-bodies recognized with the light microscope could thus be positively identified at the ultrastructual level.

Partial sections of X-bodies are shown in Figs. 12 and 14–16. The bodies are aggregations of virus-related material in the form of broad filaments and of components of the host cell, chiefly

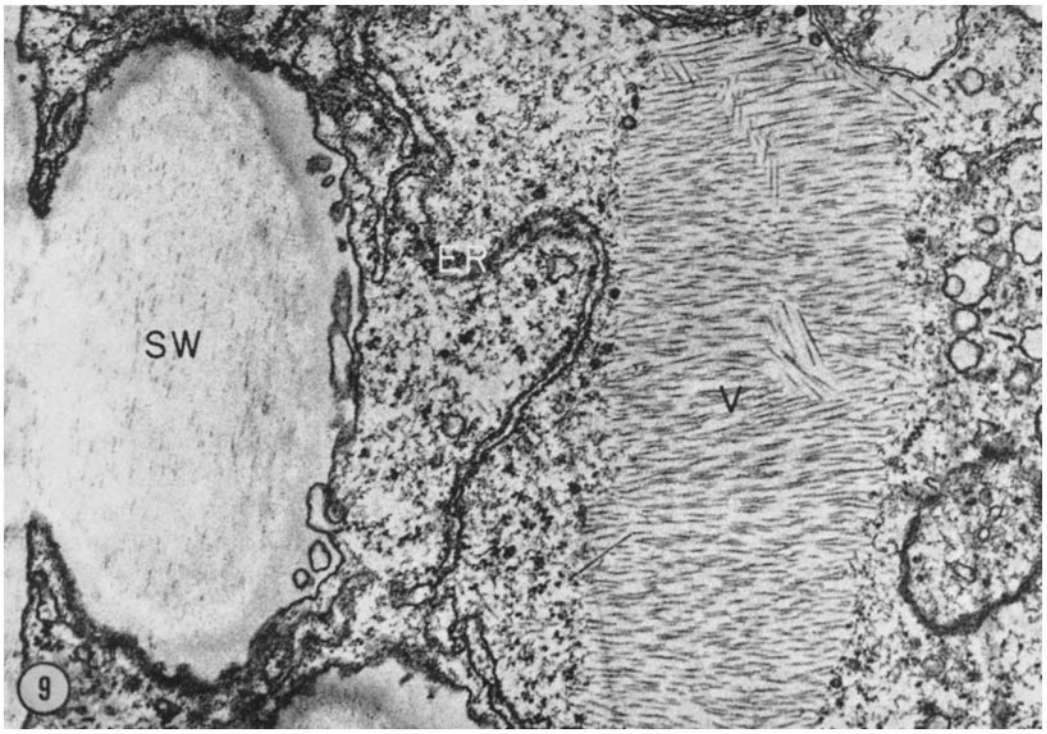


FIGURE 9 *Nicotiana tabacum.* Section of differentiating vessel member with a rather large aggregate of virus particles. Details: *ER*, endoplasmic reticulum; *V*, virus particles; SW, secondary wall. × 33,000.

endoplasmic reticulum and ribosomes. Dictyosomes also may be seen in some sections (Fig. 16). Enclaves of virus particles, resembling those in the cytoplasm, are typically present. No bounding membrane is detectable around the X-body. Ameboid bodies are known to give the reactions of the protein characteristic of the virus (2). The presence of ribosomes, endoplasmic reticulum, and dictyosomes indicates that the machinery necessary for protein synthesis is contained within the X-body. The occurrence of aggregates of virus particles, on the other hand, would explain the infectivity of the ameboid bodies as determined by Sheffield (22) for the aucuba strain of TMV.

The material illustrated in Figs. 12 and 16 resembles that shown by Kolehmainen et al. (13) in their figures 11 and 12. The authors interpret the filaments as groups of long flexuous rods. Transectional views of the filaments reveal clusters of circles each with an electron transparent core, indicating possible tubular nature of the rods. Similar images are discernible at arrows in our Figs. 12 and 14. Occasional longitudinal sections of the broad filaments also faintly suggest their compound nature (Fig. 16, arrows). Thus, the broad filaments appear to be aggregates of tubules.

As Figs. 13 and 15 indicate, "vacuolate body" is an appropriate alternate term for the X-bodies. At the ultrastructural level the vacuoles appear to be regions within the body where cytoplasmic components have undergone degeneration (Fig. 15). The globular granular aggregations in these vacuoles resemble those depicted by Kolehmainen et al. (13) and interpreted by them as possibly components of the X-body.

Inclusion with Discrete Tubular Elements

In addition to the X-body another type of inclusion was recognized with the electron microscope. It consisted of tubular units that were not in bundles as those of the X-body and were arranged in parallel arrays (Fig. 17). Their tubular nature was revealed in transectional

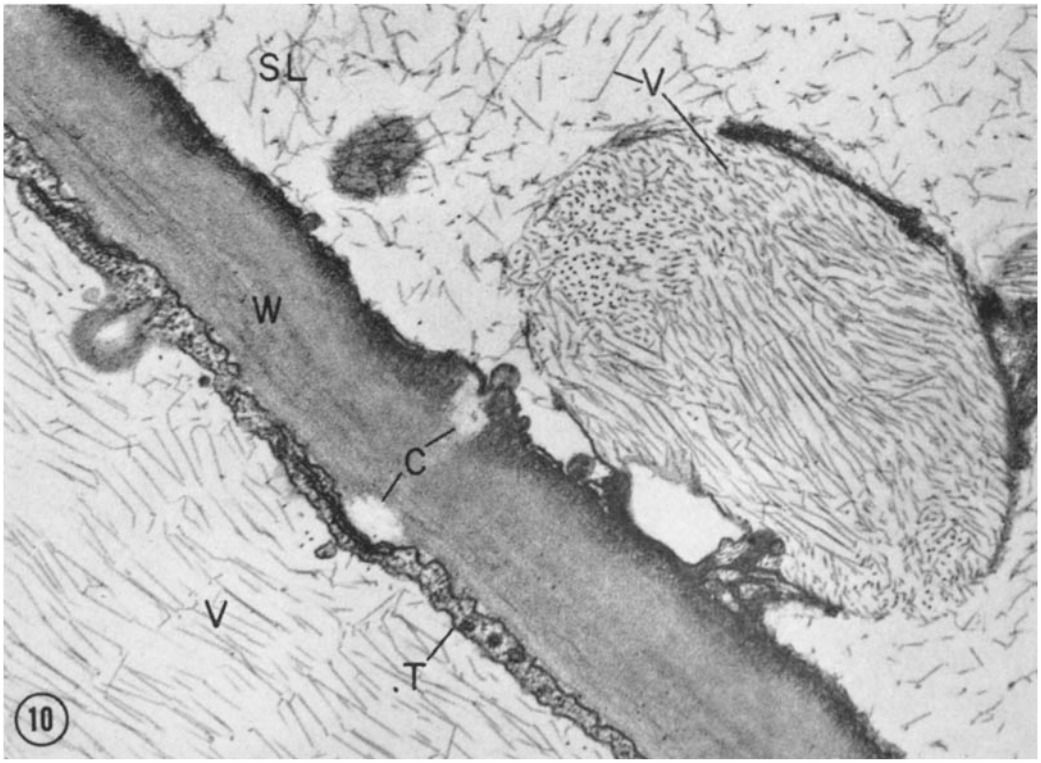


FIGURE 10 *Nicotiana tabacum.* Section of mature sieve element, right above, and parenchyma cell, left below. The sieve element is devoid of a tonoplast. It contains an aggregate of virus particles surrounded by degenerated material, some scattered particles, and scattered elements of slime. The virus particles in the parenchyma cell are approaching a layered arrangement characteristic of TMV crystals. Details: *C*, callose; *SL*, slime; *T*, tonoplast; *V*, virus particles; *W*, cell wall. × 28,000.

views, in which the particles exhibited an electron transparent center (Fig. 18). The tubules showed some similarity to the microtubules (Figs. 14 and 17, *MT*) normally present in the peripheral cytoplasm of plant cells with growing walls, but their electron-transparent central canal was narrower than that of the microtubules.

DISCUSSION

The tobacco mosaic virus is the best known example of a plant virus that shows no selective relation to a specific tissue of the host, and the occurrence of inclusions and particles of this virus in representatives of all types of cells is one of the expressions of this lack of specificity. Furthermore, studies on the multiplication of this virus in the infected cells at various times after inoculation (e.g. references 15, 21) show that the virus particles first appear in the mesophyll with no relation to transport in conducting tissues. Some viruses multiply first in the phloem tissue and egress into the mesophyll later (e.g. beet yellows virus) or largely remain restricted to the conducting tissue (e.g. curly-top virus).

The ubiquity in the distribution of TMV inclusions in systemically infected host plants has been previously reported in the literature (6, 10) and virus particles have been seen in mature tracheary elements (9). The present study shows that TMV particles occur also in the sieve elements, including the mature ones. The presence of the virus in the sieve elements is in harmony with the concept that the long-distance movement of this virus occurs in the phloem; but the question must be raised as to whether the occurrence of particles in mature tracheary elements indicates that the virus is transported with the transpiration stream as well. If it is so transported, the further question

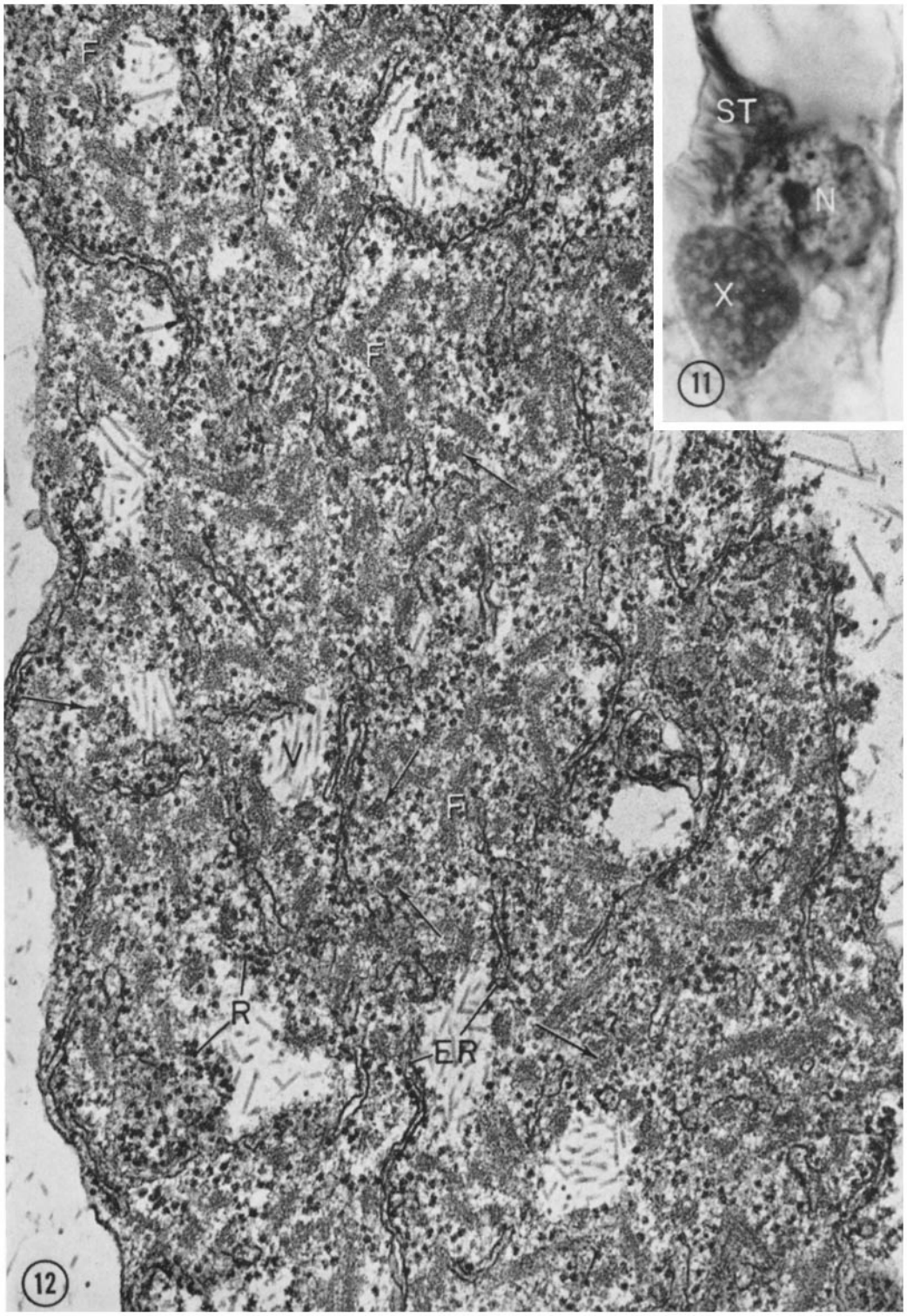


FIGURE 11 *Nicotiana tabacum.* Light-microscope view of nucleus (*N*), striate material (*ST*), and X-body (*X*) from cortical parenchyma cell in young stem. × 1500.

FIGURE 12 *Nicotiana tabacum.* Portion of an X-body from a ground parenchyma cell of a midvein. The body has no delimiting membrane and contains abundant endoplasmic reticulum (*ER*), ribosomes (*R*), enclaves with virus particles (*V*), and filaments (*F*). In transections (arrows), the filaments appear as groups of circles, an indication that the filaments are composed of tubules. × 53,000.

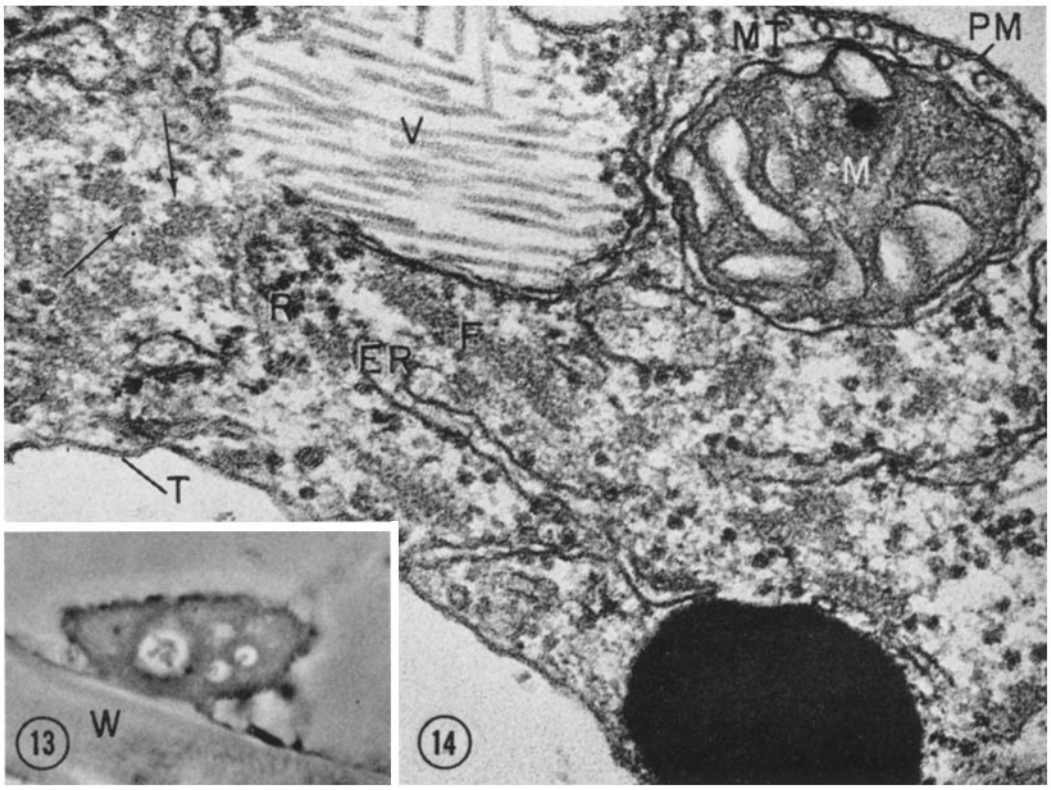


FIGURE 13 *Nicotiana tabacum.* X-body fixed in glutaraldehyde-osmium tetroxide and photographed with phase optics. The vacuoles are prominently displayed. Cell wall at *W*. × 1500.

FIGURE 14 *Nicotiana tabacum.* Portion of an X-body from a ground-parenchyma cell of a midvein. The body lies between the plasma membrane (*PM*) and tonoplast (*T*). At arrows are transectional views of the filaments showing the electron transparent centers in the component units. The diameters of these units are smaller than those of the microtubules (*MT*) near the plasma membrane. Details: *ER*, endoplasmic reticulum; *F*, filament; *M*, mitochondrion; *MT*, microtubule; *PM*, plasma membrane; *R*, ribosomes; *T*, tonoplast; *V*, virus particles. × 94,000.

requiring answer is whether this virus is able to leave the nonliving tracheary elements and cause infection. According to the familiar experiment by Caldwell (4), mosaic virus artificially introduced into the xylem in the lower part of a tomato plant reached the leaves but failed to leave the xylem unless the latter was mechanically injured. Schneider and Worley (see reference 20), on the other hand, obtained evidence that the southern bean mosaic virus is capable of entering and leaving the water conduit.

The two typical TMV inclusions, the striate material and the ameboid or X-bodies have distinctive ultrastructural features. The striate material is composed of virus particles which form crystalline aggregates in fresh material. The ameboid body includes a virus-related product, a protein in the form of tubules, groups of virus particles, and components of the host protoplast known to be concerned with protein synthesis. Kolehmainen et al. (13) speculate that this inclusion is the region of synthesis of virus protein and of the coating of virus nucleic acid with protein. Shalla (21) suggests that the tubular elements in TMV-infected tomato represent a developmental form of the virus. Milne (15) leaves the question regarding the relation between the tubules and the virus particles open. It is possible that the

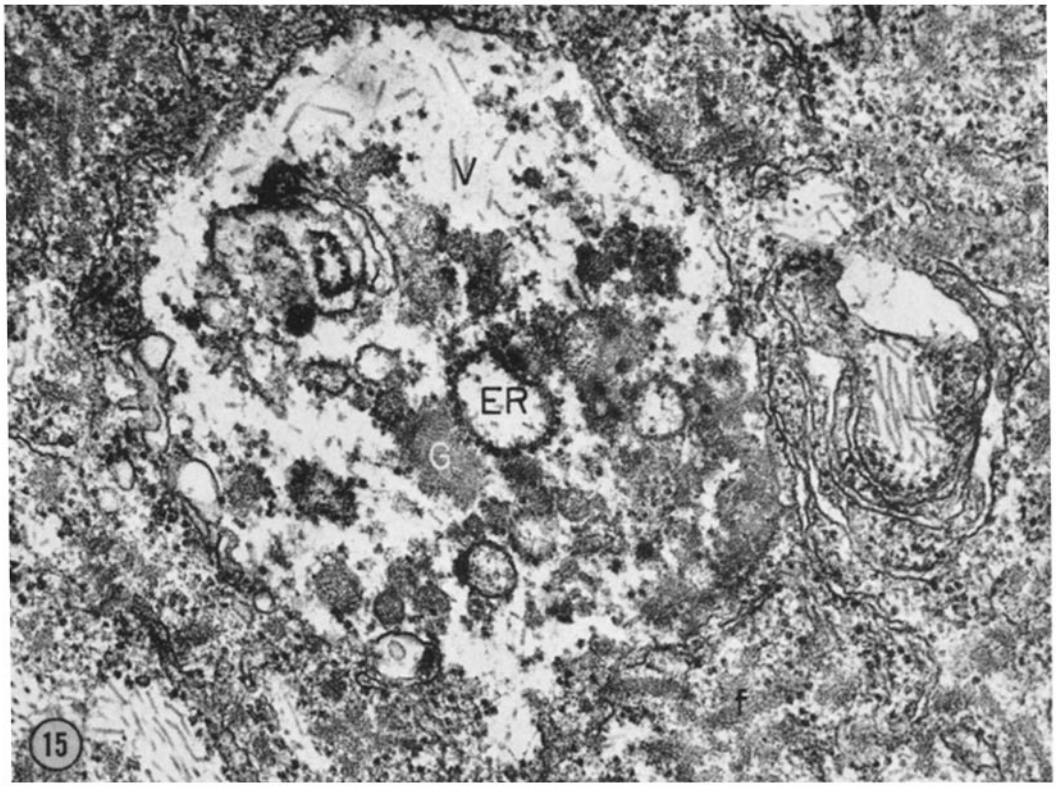


FIGURE 15 *Nicotiana tabacum.* Portion of an X-body with a vacuole containing globules (*G*), virus particles (*V*), rough endoplasmic reticulum (*ER*), and some apparently partly disorganized cell components. The globules possibly represent a disorganized form of the filaments. Details: *G*, globule; *ER*, endoplasmic reticulum; *V*, virus particles. × 52,000.

filaments composed of tubules in the X-bodies constitute excess protein commonly manufactured in TMV-infected cells (cf. reference 12, pp. 118–119).

The inclusions composed of discrete tubules in dense arrays as shown in our Figs. 17 and 18 probably correspond to the material suggested to be X-protein by Kolehmainen et al. (13). It is not unlikely that this inclusion is related to the one we identified as the X-body in that both contain virus-related protein. As Bawden (1) has pointed out, virus infection induces the synthesis of not one specific type of particle but of a range of types related to one another in different ways. It is conceivable that the virus-related protein occurs in different forms and is assembled into aggregates that are more or less free of host cell components or are combined with such components. One might think also of developmental stages among which the most advanced would show the protein in rather homogeneous aggregates, whereas the less advanced would be a combination of host-cell entities concerned with protein synthesis and the newly formed virus-related protein.

The possible role of chloroplasts in virus synthesis continues to be discussed. Zaitlin and Boardman (25) and Boardman and Zaitlin (3), for example, reported the isolation of TMV from the chloroplast fraction of diseased-leaf homogenates. Reddi (18), on the other hand, detected no virus in chloroplasts of TMV infected tobacco. In studies with the electron microscope, Kolehmainen et al. (13) recognized no virus inclusions in the chloroplasts. Shalla (21) and Milne (15) interpreted the enclaves with virus particles which occurred in the chloroplasts in their material as invaginations in the chloroplast continuous with the cytoplasm. In the illustrations reproduced by these authors

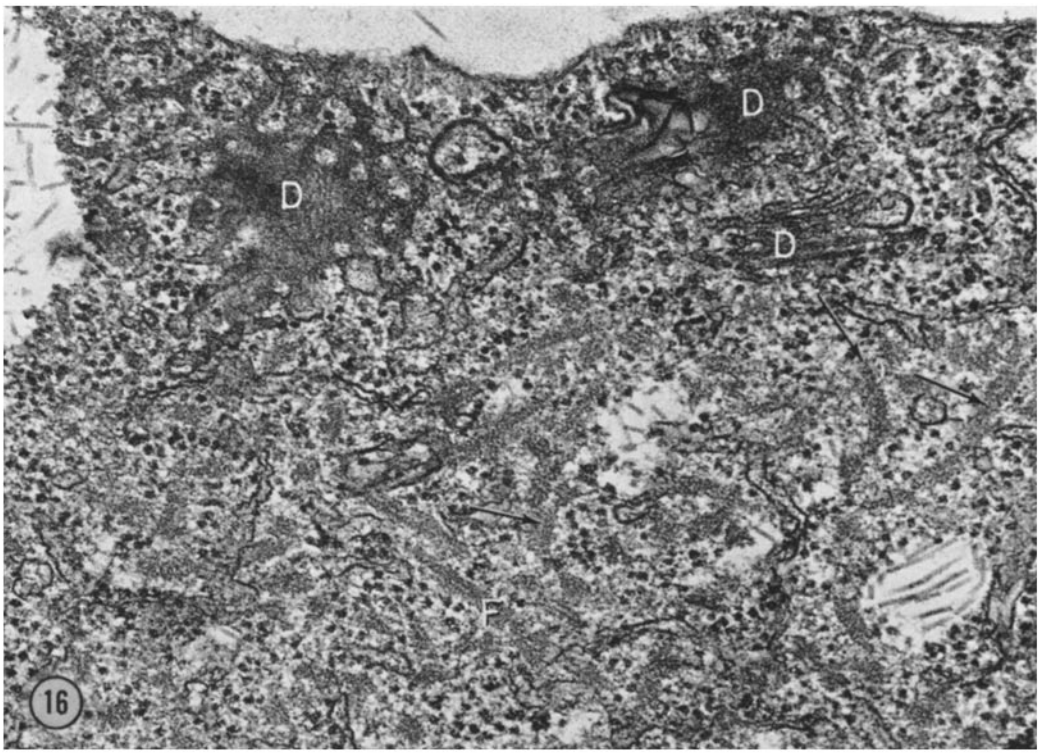


FIGURE 16 *Nicotiana tabacum.* Fragment of an X-body associated with three dictyosomes (*D*), two of which are seen in surface view and show netlike structure along the periphery. At arrows the tubules composing the filaments (*F*) may be discerned as separate entities. × 57,000.

the enclaves appear to be surrounded by a membrane, sometimes obviously double, and some include ribosomes or mitochondria. Our Figs. 3–5 show a distinctly different relation between the virus particles and the chloroplasts. The aggregates of virus particles resemble those in the cytoplasm, that is, they are free of host-cell components and are not surrounded by membranes. The aggregates have the appearance of having been produced in the plastids, for the grana in their neighborhood are more or less displaced as they often are near a developing starch grain. The possibility of TMV synthesis in the chloroplast cannot be rejected at this time.

In *Beta* leaves infected with the beet yellows virus, particles resembling the virus particles in the cytoplasm were observed in the chloroplasts. For several reasons the identity of those particles is not as certain as is that of the TMV particles in the chloroplasts of *Nicotiana*.

More than one investigator has interpreted the nucleus as the site of virus reproduction (see reference 20), Reddi (18) with particular emphasis. With the light microscope, inclusions were recognized in the nuclei or nucleoli in plants infected with certain viruses, including a strain of TMV (see references 14, 23, and 24). With the electron microscope, unequivocal demonstrations of virus particles in nuclei in sectioned material were made by Rubio-Huertos and Hidalgo (19) for severe etch virus, by Shikata and Maramorosch (23) for pea enation mosaic virus, and by us for beet yellows virus (5) and for TMV (present study). Thus, as judged by the present studies, both nuclei and chloroplasts may be involved in one or more aspects of virus multiplication.

The occurrence of virus particles in the vacuoles is considered to be an artifact by Shalla (21) and Milne (15), possibly a result of rupture of the tonoplast during the preparation of material. When the virus particles in the vacuoles assume an orderly arrangement, as was revealed by some

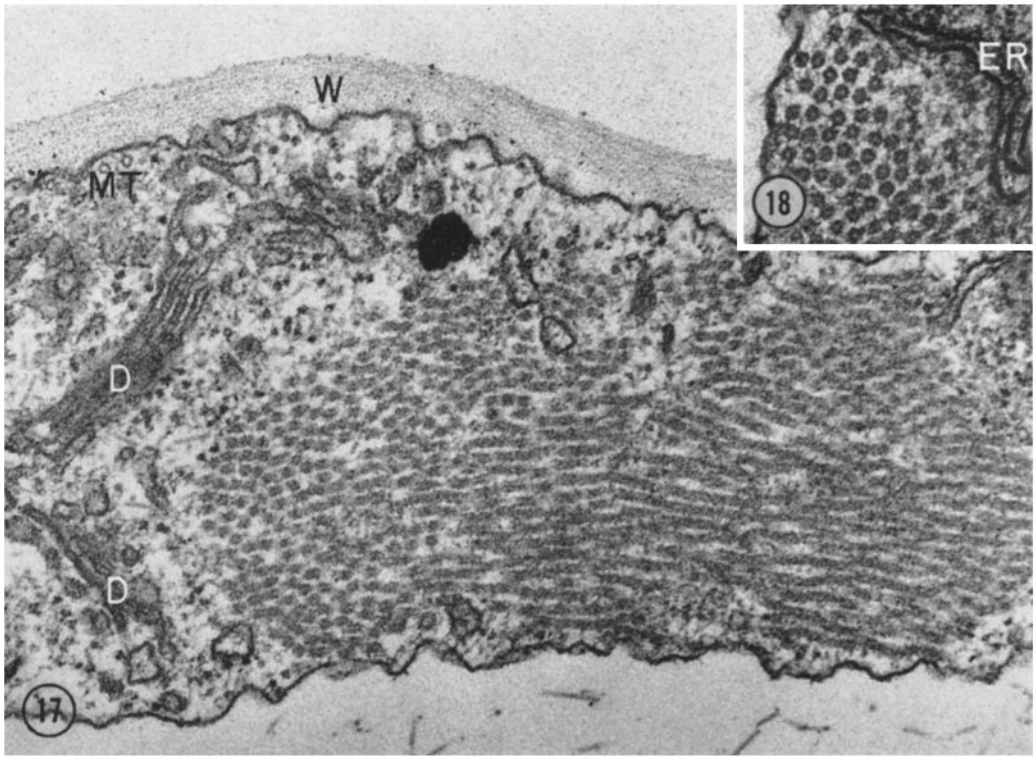


FIGURE 17 *Nicotiana tabacum.* Aggregate of virus-associated discrete tubules in parallel arrangement, from a parenchyma cell. Microtubules are visible at *MT*. Details: *D*, dictyosome; *MT*, microtubule; *W*, wall; an intercellular space above *W*. × 67,000.

FIGURE 18 *Nicotiana tabacum.* Aggregate of virus-associated tubules in transverse section. The hollow center is discernible. Endoplasmic reticulum at *ER*. × 80,000.

of our preparations (Fig. 10), the egress of the virus into the vacuole does not seem to be accidental. It is conceivable that the virus can affect cytoplasmic membranes, the tonoplast and the plasma membrane, and pass beyond their limits. The presence of virus particles between the plasma membrane and the cell wall, which was seen occasionally in our material, may have a bearing on the movement of virus between cells.

The study was supported in part by National Science Foundation grant GB-1523 and in part by faculty grant 308 from the University of California. The authors also acknowledge the assistance of Mr. R. H. Gill and Mrs. B. Osterhoff.

Received for publication 16 November 1966.

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