Electron Microscopy of Cucumber Mosaic Virus-Infected Tobacco Leaves Showing Mosaic Symptoms

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ABSTRACT

Thin-sectioning, and a method which allowed easy detection of virus particles, were used to compare the intracellular virus distribution and fine structure of green and yellow tissues of cucumber mosaic virus-infected tobacco (*Nicotiana tabacum* 'Bright Yellow') leaves showing clear mosaic symptoms. Intracellular profiles of green tissues were similar to those of healthy ones, and no abnormalities were found in them. About half the cells of these tissues contained a few virus particles which were distributed randomly throughout the cytoplasm, although they were difficult to differentiate from the ribosomes. The cells of yellow tissues, however, were smaller than those of green tissues, and the cell arrangement was disordered. Intracellular profiles were different from those of green tissues. Degeneration of grana and lamellae and the occurrence of myelin-like structures was prominent in the chloroplasts. The virus particles were numerous in the ground cytoplasm of most cells and they were less frequently aggregated. A considerable number of the nuclei contained virus particles in the nucleoplasm. No virus particles were found in the chloroplasts or mitochondria.

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Although there is considerable information about the intracellular distribution of TMV particles, little is known about intracellular CMV particles (2, 3, 5, 9). This is largely due to the fact that it is difficult to differentiate CMV particles from the ribosomes unless they are in characteristic arrangement, because the size of CMV particles is close to that of the ribosomes. The recent finding that intracellular ribosomes are destroyed and CMV particles are preserved as aggregates in leaves which are incubated on phosphate buffer (6), make it possible to determine whether the cells contain CMV particles by viewing thin-sections under an electron microscope. Electron microscopy accompanied by usual tissue preparation, thin-sectioning, and a procedure which allows easy detection of virus particles (6), should provide more detailed information on CMV-infected leaf cells.

This paper deals with distribution of CMV particles in diseased tobacco leaves which show clear mosaic symptoms and intracellular modifications associated with the mosaic symptoms.

MATERIALS AND METHODS.—*Usual tissue preparation.*—Lower leaves of *Nicotiana tabacum* L. 'Bright Yellow' were inoculated with the ordinary strain of CMV. Ten days after inoculation, newly developed young upper leaves showing clear mosaic symptoms were divided into yellow tissues and green tissues. Both tissues were fixed with 4% paraformaldehyde and 5% glutaraldehyde in Millonig's phosphate buffer at pH 7.3 (8) for 2 h, and were postfixed with 2% osmium tetroxide in Millonig's phosphate buffer for 5 h. Thin-sections stained with uranyl acetate and lead citrate were examined in an electron microscope. Healthy young leaves were also examined for comparison.

Easy detection procedure for CMV by electron microscopy.—Disks 1 cm in diam were punched from the same diseased leaves. The leaf disks were floated on 0.1 M Millonig's phosphate buffer at pH 7.0, and were incubated at 25 C under continuous illumination for 24 h. The leaf disks were then divided into yellow tissues and green tissues. They were prepared and examined as described above. Healthy leaf disks floated on phosphate buffer were also examined for comparison. The leaf disks floated on buffer were designated as leaves on buffer in the present study.

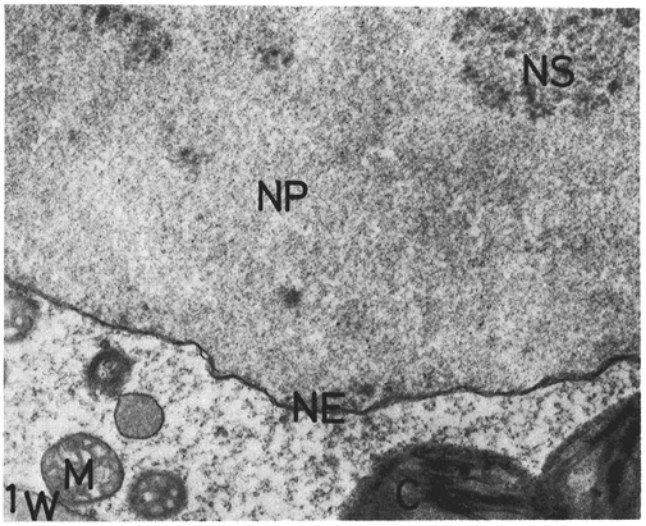
RESULTS.—*Intracellular effects induced by incubation on phosphate buffer.*—Intracellular profiles of healthy leaves on buffer (Figs. 1, 2) were different from those of healthy leaves without incubation on buffer (Fig. 3). The former somewhat resembled those fixed with potassium permanganate. The ribosomes in the cytoplasm no longer retained their normal appearance, but were changed into fine amorphous particles (Fig. 2). The nucleolus and heterochromatin were indiscernible, or heterochromatin was omnipresent around the nucleus. Although the nuclei, chloroplasts, and mitochondria were somewhat swollen, no disintegration of these organelles was observed.

Green tissues of diseased leaves.—The intracellular fine-structural details of spongy, palisade, and epidermal cells of green diseased tissues were similar to those of healthy leaves (Fig. 3). No abnormalities were evident in the nucleus, chloroplasts, or mitochondria. No CMV or CMV-like particles were detectable in any portions of the cells. Although 500 cells were examined in the present study, only two cells contained aggregates of CMV particles in the central vacuole. Thus, it was not clear whether the cells in green tissues actually contained CMV particles when the usual tissue preparation procedures were followed.

Fig. 1-5. 1-3) Healthy tobacco leaves on buffer: **1)** General view of cell (× 11,000). **2)** Details of cytoplasm. Note disappearance of ribosomes (× 75,000). **3)** Details of healthy tobacco leaf. The cytoplasm is filled with ribosomes; note dense heterochromatin (× 75,000). **4)** CMV aggregates in the cytoplasm and nucleus of green tissue cell of diseased tobacco leaf on buffer (× 68,000). **5)** Heavily affected cell from yellow tissue of diseased tobacco leaf (× 5,000).

Symbol Legend

Abbreviations used in Fig. 1-16: C = chloroplast; CP = cytoplasmic protrusion; CY = cytoplasm; ER = endoplasmic reticulum; G = Golgi apparatus; HC = heterochromatin; I = crystalline inclusion; M = mitochondrion; N = nucleus; NE = nuclear envelope; NP = nucleoplasm; NS = nucleolus; V = vacuole; and W = cell wall.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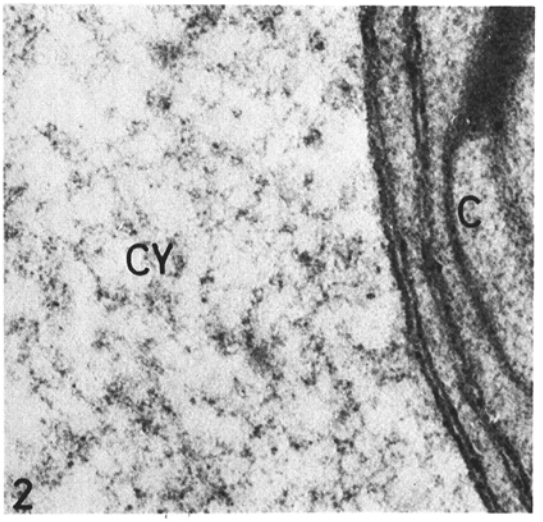
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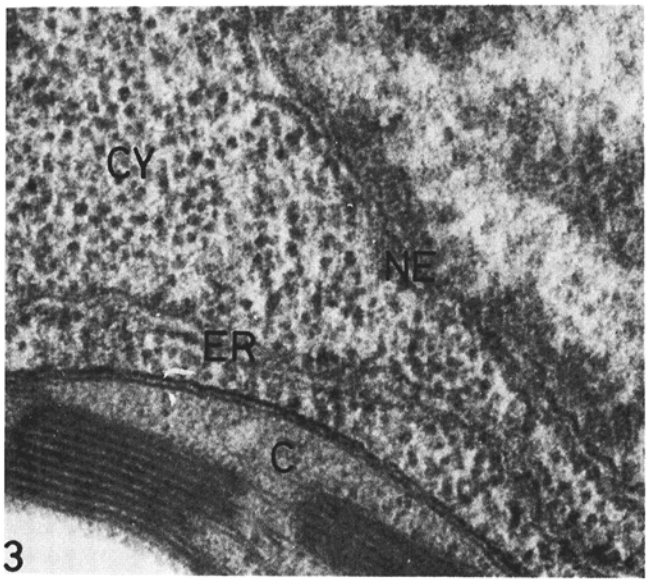
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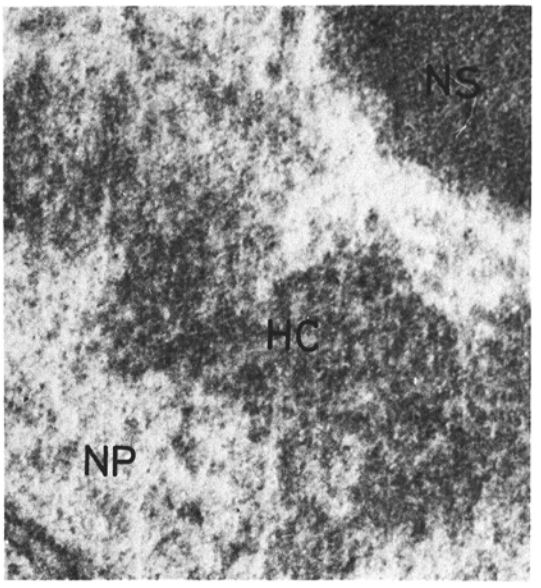
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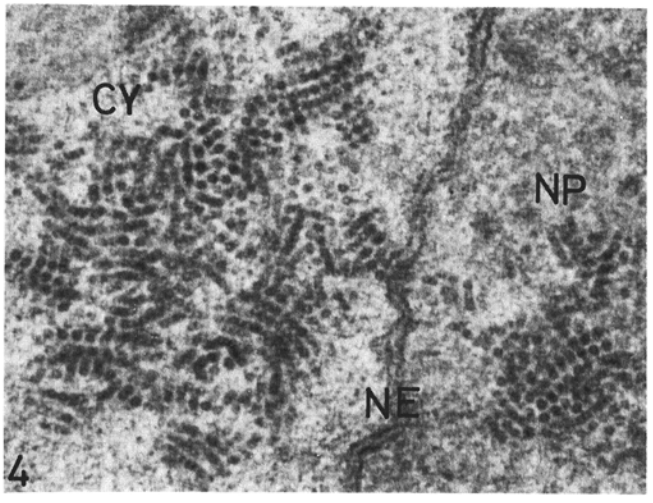
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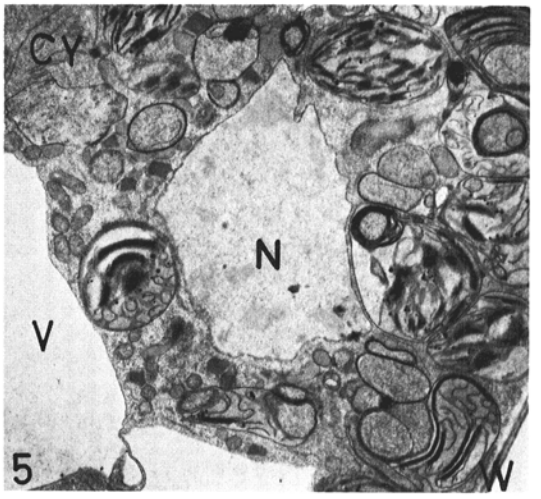
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On the other hand, in green tissues floated on buffer, the ribosomes were completely destroyed (Fig. 4), and it was easy to detect CMV particles. Aggregates of CMV particles were detected in the cytoplasm of about 40% of the examined cells (Fig. 4). The CMV aggregates usually appeared in some portion of the cytoplasm, and not throughout the cytoplasm. The amount of CMV aggregates varied in individual cells. At any rate, intracytoplasmic CMV aggregates in green tissues were far smaller in numbers than those in yellow tissues. Intranuclear aggregates of CMV particles were found in about 5% of the examined nuclei (Fig. 4). No CMV particles were found in the chloroplasts or mitochondria.

Yellow tissues of diseased leaves.—The cells in yellow tissues were smaller than those in healthy or diseased green tissues. Cell arrangement was disordered. Occasionally, the palisade cells were deformed to round or elliptical shape. Intracellular fine-structural details of spongy and palisade cells (Fig. 5) were different from those of healthy or green tissues. The epidermal cells were rather similar to those of green tissues. The most prominent modifications were degeneration of grana and lamellae, and occurrence of myelinlike structures in the chloroplasts (Figs. 5, 6, 7), though the limiting membranes of the affected chloroplasts were intact. The myelinlike structures in the chloroplasts appeared as bands, semicircles or circles (Figs. 5, 6, 7). The cytoplasmic protrusions containing doughnutlike particles were usually encountered in the affected chloroplasts (Fig. 6). Some chloroplasts contained crystalline inclusions (Fig. 7). No CMV-like particles were detected in the chloroplasts.

In some nuclei, dense heterochromatin [which was prominent in the nucleus of cells in healthy (Fig. 3) or green tissues] became indiscernible (Fig. 5). In these nuclei, small dense particles or doughnutlike particles were present in the nucleoplasm (Fig. 8). About 30% of the examined nuclei contained these particles. No abnormalities were observed in the nucleolus and nuclear envelope.

The cytoplasm was thicker than that of healthy or green tissues, and was filled with doughnutlike particles (Figs. 9, 10). Myelinlike structures were also encountered in the cytoplasm (Fig. 10), and doughnutlike particles were arranged between the layers of some myelinlike structures. The diam of the doughnutlike particles observed in the ground cytoplasm (Figs. 9, 10), cytoplasmic protrusions in the chloroplasts (Fig. 6), intracytoplasmic myelinlike structures and nuclei (Fig. 8) was about 25 nm. These particles were somewhat larger and rounder than the ribosomes (Fig. 3). Although these doughnutlike particles (Fig. 11) resembled CMV particles, it was impossible to determine whether they were CMV particles, using usual preparation procedures.

Five hundred different cells were examined in yellow tissues, and intracytoplasmic aggregates of CMV particles were encountered in only two cells (Fig. 12). On the other hand, aggregates of CMV particles in the central vacuoles were encountered in 30 cells. Some of these aggregates consisted of randomly arranged virus particles, and others consisted of regularly arranged virus particles (Fig. 13). Aggregates of CMV particles attached to the tonoplast or associated with the membranous structures were also encountered in the central vacuoles. It was difficult to determine whether these aggregates of CMV particles in the vacuoles were representative of the general appearance of intracellular CMV particles, because they were encountered in only a small number of cells.

On the contrary, aggregates of CMV particles were easily encountered throughout the cytoplasm of about 90% of the examined cells in yellow tissues on buffer (Fig. 14). They were also encountered in about 70% of the examined nuclei (Fig. 15). No CMV particles were detected in the chloroplasts and mitochondria. Since the profiles of CMV particles in yellow tissues on buffer (Fig. 16) were similar to the doughnutlike particles which were observed in the ground cytoplasm (Figs. 9, 10), cytoplasmic protrusions in the chloroplasts (Fig. 6), some intracytoplasmic myelin-like structures and some nuclei (Fig. 8), it is considered that these doughnutlike particles observed by the usual tissue preparation procedures correspond to randomly distributed CMV particles.

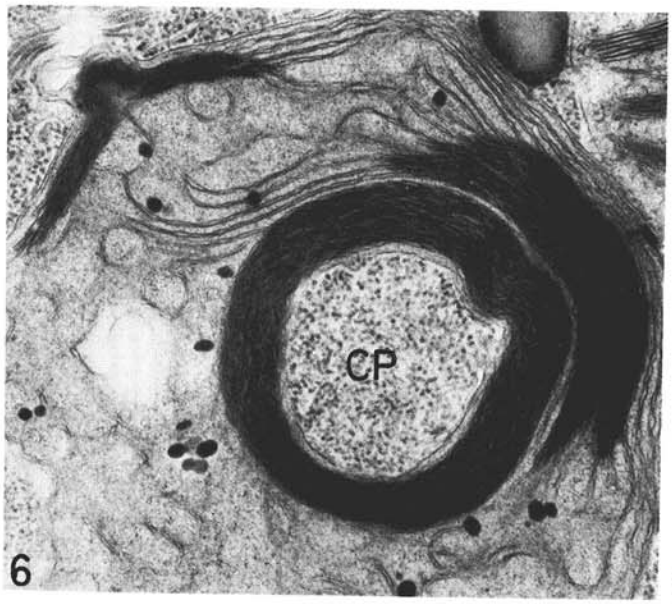
According to the present observation, it is concluded that about 90% of the cells in yellow tissues of CMV-infected leaves contained large numbers of CMV particles, and that the intracellular CMV particles were scattered throughout the ground cytoplasm. Furthermore, a considerable number of the nuclei contained CMV particles in their nucleoplasm. The chloroplasts and mitochondria contained no CMV particles.

DISCUSSION.—The modifications in the chloroplasts induced by virus infection have been extensively investigated in various plant-virus systems (4, 7). Nevertheless, no generalized interpretation of the responses of chloroplasts to virus infection has been established. The crystalline inclusions in the chloroplasts of yellow tissues resembled those observed in tobacco mesophyll protoplasts (10). It was considered that the crystalline inclusions in the chloroplasts of tobacco mesophyll protoplasts corresponded to polymerized fraction I protein (10).

Occurrence of CMV particles in the nuclei was reported by Doi and Yora (1). However, there is no evidence whether intranuclear CMV particles were formed in situ, or whether they were transmitted from the cytoplasm. If these many intranuclear CMV particles correponded to the transmitted ones from the cytoplasm to the nucleus through the nuclear pores, CMV particles passing through the nuclear pores should be encountered. No CMV particles were detected in the nuclear pores in the present study.

Presumably, small doughnutlike particles observed in CMV-infected leaves on buffer are CMV particles, because no ribosomes were observed in healthy leaves on

Fig. 6-10. Yellow tissue cells of diseased tobacco leaves: **6)** Myelin-like structures in the chloroplast (× 26,000). **7)** Crystalline inclusions in the chloroplast (× 22,000). **8)** Small spherical or doughnutlike particles distributed in the nucleoplasm (× 66,000). **9)** The cytoplasm is filled with small spherical or doughnutlike particles (× 27,000). **10)** Myelinlike structures in the cytoplasm filled with small spherical or doughnutlike particles (× 32,000).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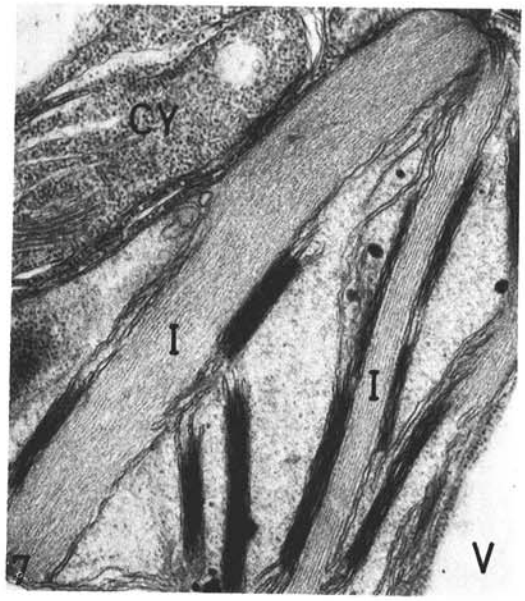
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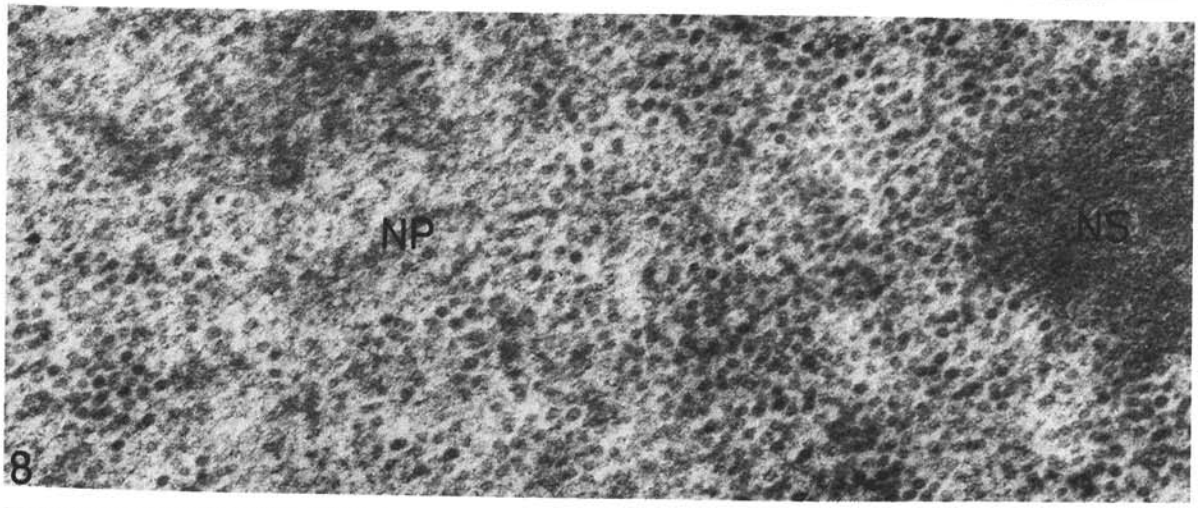
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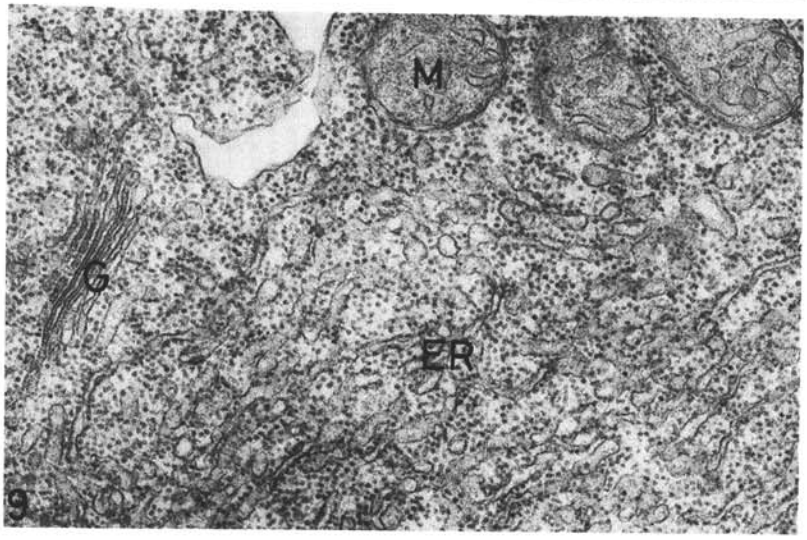
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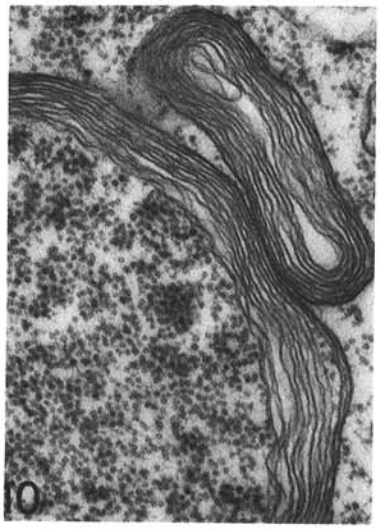
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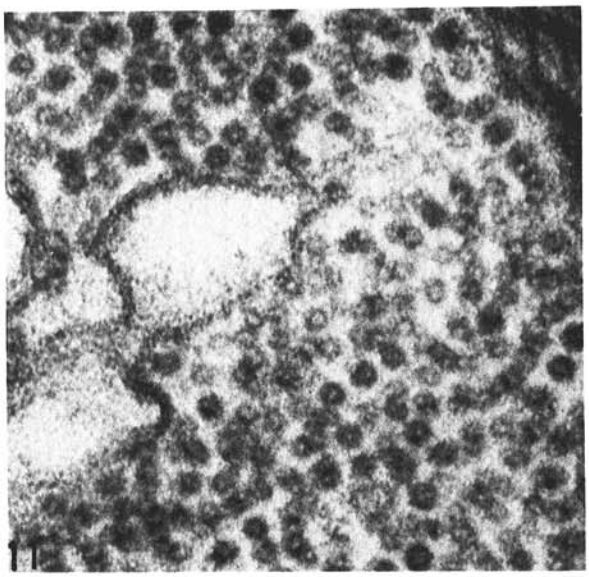

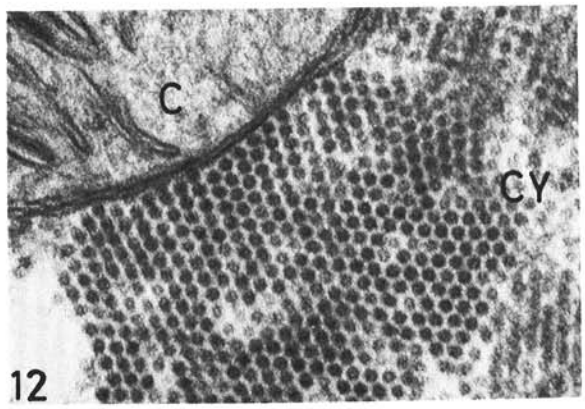

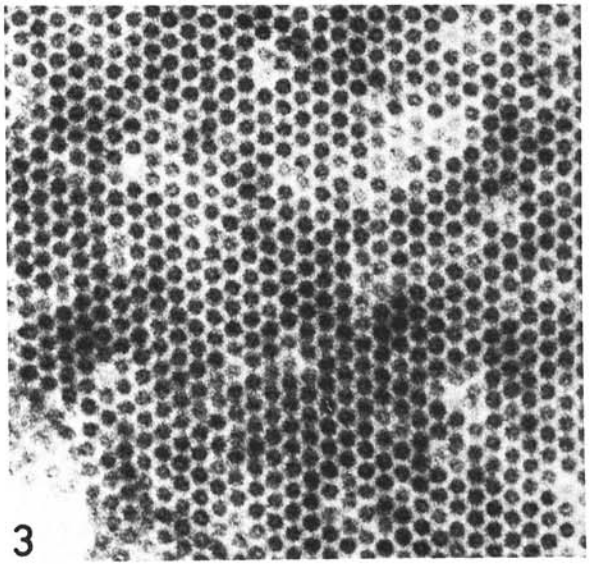

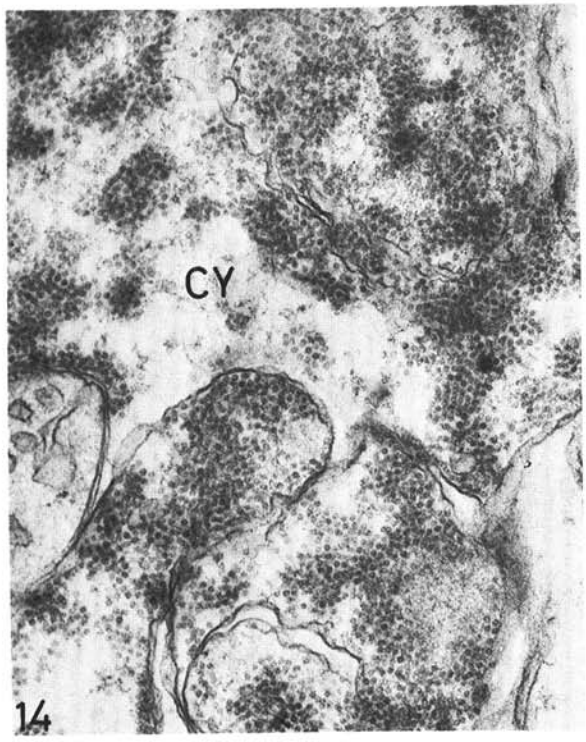

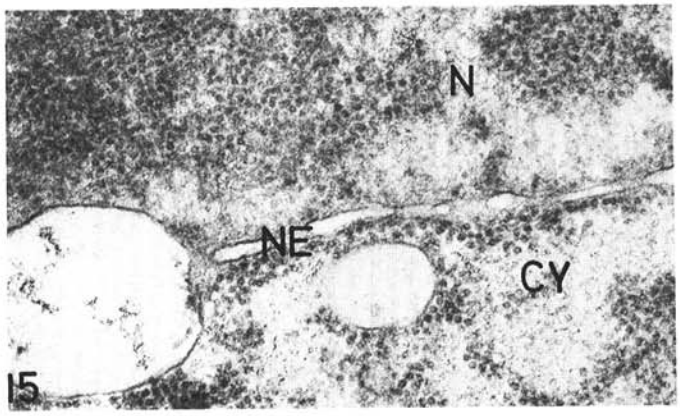

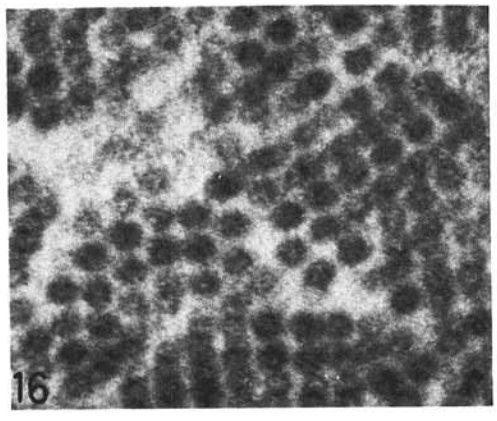

Fig. 11-16. 11-13) Yellow tissue cells of diseased tobacco leaves: **11)** Details of doughnutlike particles in the cytoplasm (× 160,000). **12)** CMV aggregates in the cytoplasm (× 80,000). **13)** CMV aggregates in the vacuole (× 84,000). **14-16)** Yellow tissue cells of diseased tobacco leaves on buffer: **14)** The cytoplasm is filled with CMV aggregates (× 37,000). **15)** CMV aggregates in the nucleus and cytoplasm (× 36,000). **16)** Details of CMV aggregates in the cytoplasm (× 170,000).

buffer and the cowpea primary leaves which were inoculated with crude juice extracted in buffer from CMV-infected leaves produced local lesions. It seems that the incubation on phosphate buffer used in the present study is useful for easy detection of intracellular small spherical virus particles which are scattered randomly throughout the cytoplasm and are difficult to differentiate from the ribosomes, because of degradation of the ribosomes and preservation of virus particles in normal form.

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