Electron Microscopy of Petunia Vein-Clearing Virus, an Isometric Plant Virus Associated with Specific Inclusions in Petunia Cells

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ABSTRACT

Ultrathin sections of petunia cells infected by petunia vein-clearing virus revealed rounded inclusion bodies a few μm thick. They showed a heavily contrasted, finely granular matrix containing lightly contrasted vacuole-like regions. No membranes were associated with the inclusion body structures. Virus particles occurred in distinct regions of the infected cells, often near the inclusion bodies, but have not been found within the matrix of the inclusion bodies. Conspicuous elements of endoplasmic reticulum were associated with the virus-containing regions and were sometimes arranged in several concentric layers. The diameters of the virus particles were 43 nm in negatively stained crude extracts and about 46 nm in ultrathin sections.

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Additional key words: 43-nm (diam) virus particles, proliferated endoplasmic reticulum.

In petunias with stunted young shoots and vein-clearing symptoms on deformed leaves, virus-like particles and inclusion bodies were found in ultrathin sections. The symptoms had developed six weeks after the petunias had been used in heteroplastic grafting experiments with witches'-broom-diseased *Opuntia tuna* (L.) Mill. (4). The virus-like particles could not be found in ultrathin tissue sections of *O. tuna* and additional transmission experiments from diseased *Opuntia* were unsuccessful. However, the particles and inclusion bodies were later found in petunia plants which had not been used in the transmission experiments, but had been raised from the same seed sample. This could point to seed transmission or perhaps transmission by an unknown vector in the greenhouse. All tests for seed transmission and mechanical transmission were negative. So far, the origin of the particles is not clear.

The virus-like particles were graft-transmissible from petunia to petunia and resembled typical virus particles. Since their properties do not agree with properties of

other viruses of similar structure described in the literature the tentative name "petunia vein-clearing virus" (PVCV) has been proposed (5). In the present study the structure of the isometric virus particles and the fine structure of infected petunia cells are described and discussed.

MATERIALS AND METHODS.—Petunia plants (*Petunia Juss.* × *hybrida hort.* 'Himmelsrõschen') used in this investigation were vegetatively propagated from infected petunias used in another study (4). Virus particles were studied in negatively stained crude extracts obtained by crushing the plant parts in "stain" or in water. Normally, 2% aqueous NaOH-neutralized phosphotungstic acid (PTA) was used, to which one drop per 0.5 ml of 0.1% bovine serum albumin in water had been added. The suspension was sprayed on carbon-stabilized, Formvar-coated, copper grids. For comparison, some preparations were negatively stained with aqueous solutions of 2% PTA at pH 5.0, 2% ammonium molybdate at pH 5.0, 7.0, or 8.5, with saturated aqueous uranyl acetate, or with uranyl formate, the latter prepared according to (1). With the latter two stains, the particle-containing suspension was dropped on a grid, removed after one minute, then stain was added and removed after one additional minute. Only negative staining with neutral PTA and neutral ammonium molybdate allowed observation of the particles. All other stains led to obviously damaged particles or dissolved particle groups. Fixation with 2% glutaraldehyde, or with buffered OsO_4, did not improve the preservation of the particles. TMV particles (300 nm) or unfixed crystals of catalase (23) served as standards for measurements of particle dimensions.

Small pieces were cut from leaf veins or stems of PVCV-diseased and healthy petunia plants. They were fixed for 2 hr or longer in a mixture of 3% acrolein and 3% glutaraldehyde in 0.02 M phosphate buffer, pH 7.3. After being rinsed with buffer, they were postfixed in 2% buffered OsO_4 for 2 hr, dehydrated in ethanol followed by propylene oxide, and embedded in Epon 812 (14). Ultrathin sections were cut with glass knives on an Ultrotome III (LKB), poststained with uranyl acetate and lead citrate (19), and examined with a Siemens-Elmiskop 1 A electron microscope.

For comparison, crude extracts and ultrathin sections from cauliflower mosaic virus-infected turnip plants were used.

RESULTS.—*Negatively stained virus particles.*—Virus particles could be detected in crude extracts only when the plants showed distinct symptoms. Usually the particle concentration was low, and single particles could not be clearly distinguished from contaminating cell components. However, the presence of particles could often be detected, since they showed a tendency to lie in loose groups in the negatively stained drops (Fig. 1).

Although most particles showed rounded outlines, a few were slightly hexagonal (Fig. 1, arrows). They were penetrated by PTA to different degrees. The shell of apparently completely penetrated particles (Fig. 1-a) was approximately 8-nm thick. Some micrographs seemed to indicate the substructure of the shells (Fig. 1-b), but the resolution was not sufficient for an analysis.

Measurements of approximately 300 particles revealed particle diam between 35 nm and 51 nm. Seventy-five percent were between 40 nm and 45 nm in diam, with the mean value of these at 43 nm.

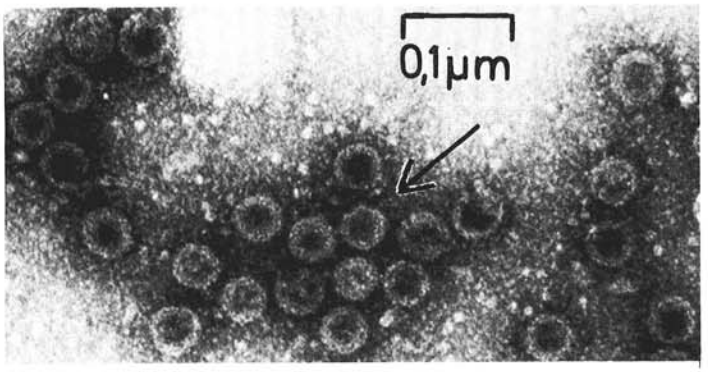
Fine structure of infected plant cells.—Many cells of infected petunia contained one or more densely stained inclusion bodies (Fig. 2, 6). Most had diam of 1-2 μm, but occasionally diam up to 10 μm were observed. The inclusion bodies normally were rounded, but in some instances appeared irregular in shape. The matrix of the bodies appeared finely granular but showed no defined substructure. Within the bodies many lightly contrasted, rounded, vacuole-like regions occurred which were often large in the body center and small at the periphery (Fig. 2, 6). In some cases, the large central vacuole-like region seemed to be continuous with the cytoplasmic region outside the inclusion body. The vacuole-like regions were not delimited from the matrix by a membrane, likewise the inclusion bodies had no surrounding membrane. In a few cases, endoplasmic reticulum (ER) was adjacent to portions of the inclusion body surface (Fig. 2). Inclusion bodies were not present in healthy petunias.

Virus particles within infected cells consisted of electron opaque rings, 32 nm in diam, with translucent centers 19 nm in diam (Fig. 3). The rings were surrounded by an area of intermediate contrast with an outer diam in the range of 46 nm. These outer areas were sometimes faint, but were consistently seen in good quality ultrathin sections, so it is probable that they are parts of the virus particles. If included in the particle diam, the total diam would be about 46 nm in sections, corresponding closely to the diam of about 43 nm in negatively stained crude extracts. The particle diam of approximately 30 nm given in the previous report (4) has to be corrected. It was mainly based on measurements from thin sections before the outer portion of the particles was found. This value thus represented the inner parts but not the entire particle.

Virus particles have not been found within the matrix, but sometimes close to the inclusion bodies and only occasionally within the vacuole-like regions (Fig. 2). Single particles were seen scattered in the cytoplasm of infected cells. In one instance virus particles occurred within a nucleus (Fig. 5). However, most virus particles found in infected cells were concentrated in distinct cytoplasmic regions (Fig. 4). The virus-containing regions generally appeared more electron dense than the other parts of the cytoplasms, because they contained irregular clusters of virus particles mixed with proliferated, often branched rough-surfaced ER (Fig. 2, 4). Sometimes lipid droplets were visible in these regions (Fig. 4) but no dictyosomes, mitochondria, or plastids.

The virus-containing regions showed a tendency to be separated from the remainder of infected cells cytoplasms by rough-surfaced ER (Fig. 2, 4). It has not been shown in serial sections that the virus-containing regions are surrounded by ER, but a close association of ER with the periphery of these regions can be seen. The ER elements often appeared as a single obviously interrupted "envelope" (Fig. 2, 4), or they consisted of many (up to 13) very conspicuous cisternae arranged in parallel. Arrangements were concentric or more often on one side of the virus containing regions (Fig. 6). Virus particles were also found in the cytoplasmic area between the parallel cisternae (Fig. 6).

Infected cells occurred in apparently all tissues of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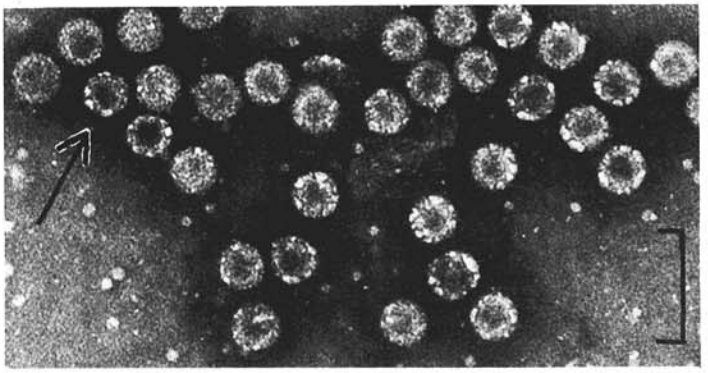

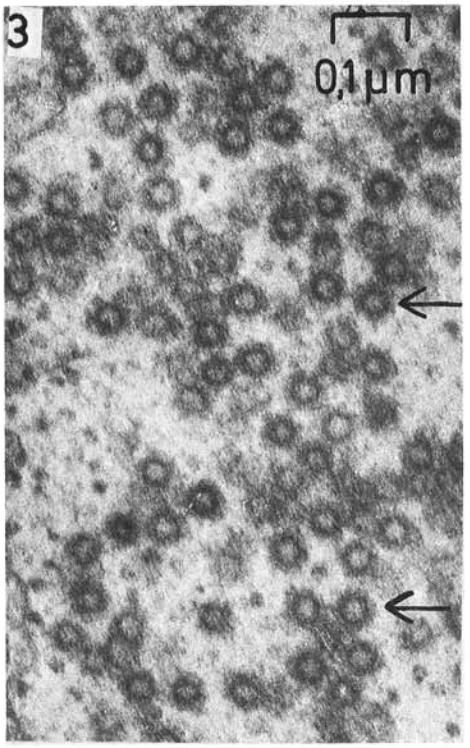
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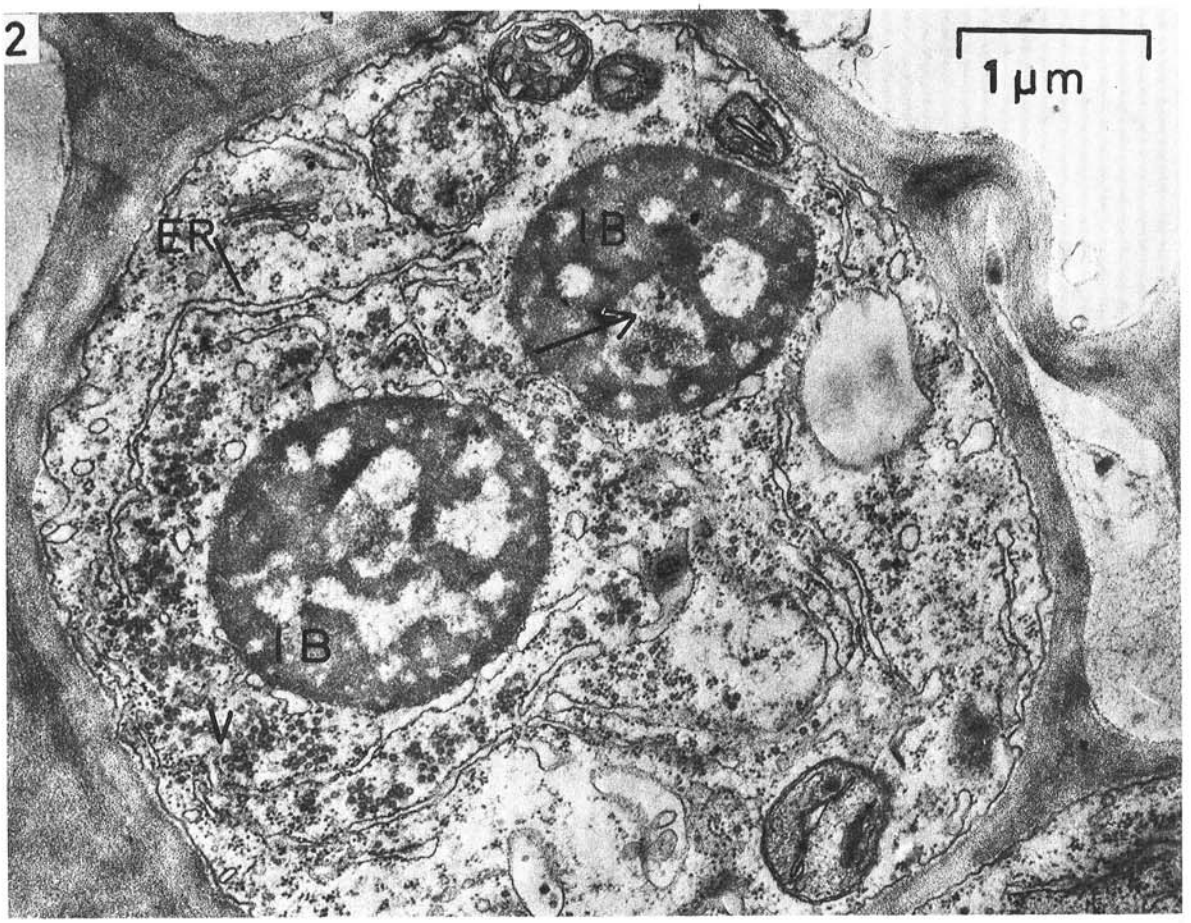
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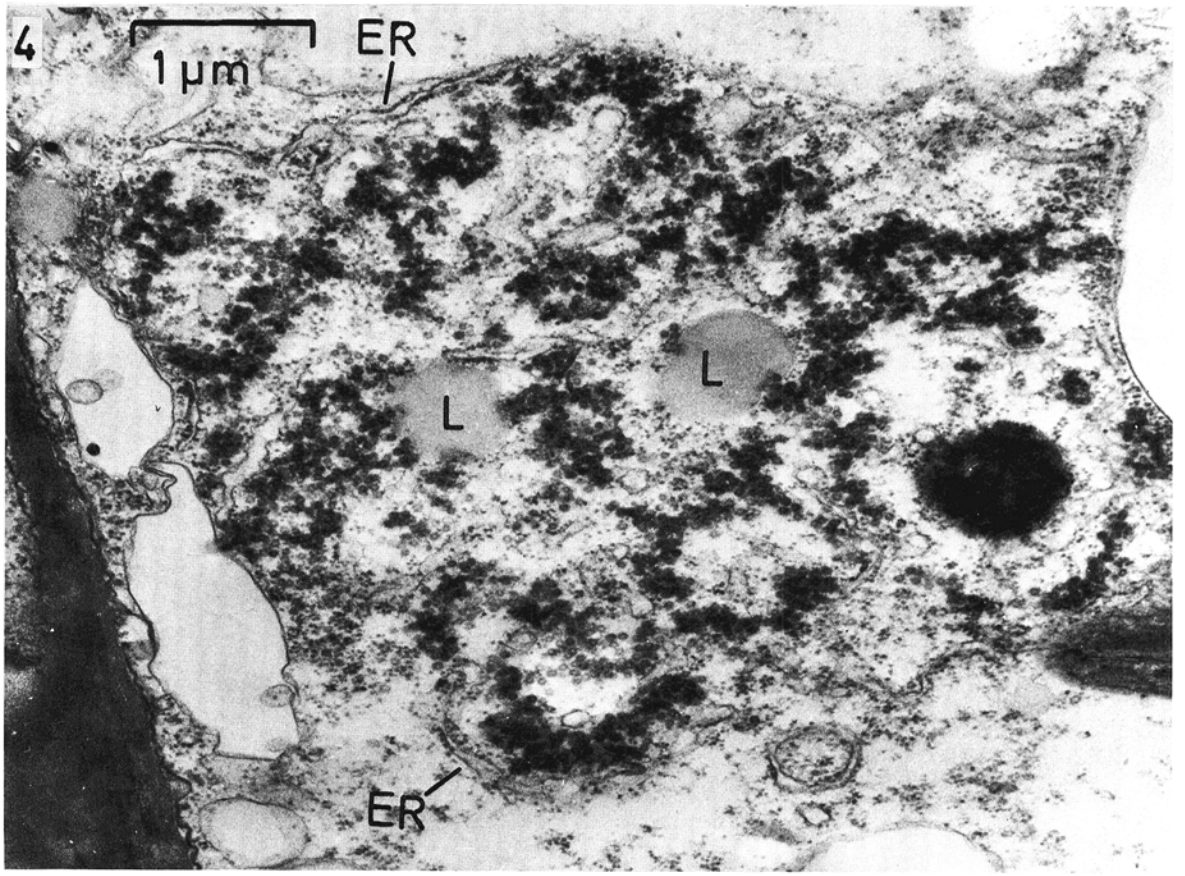


Fig. 4. Virus-containing region from a cortical parenchyma cell of a petunia stem. Cisternae of endoplasmic reticulum loosely envelop the region, branched cisternae and lipid droplets(L) occur within it.

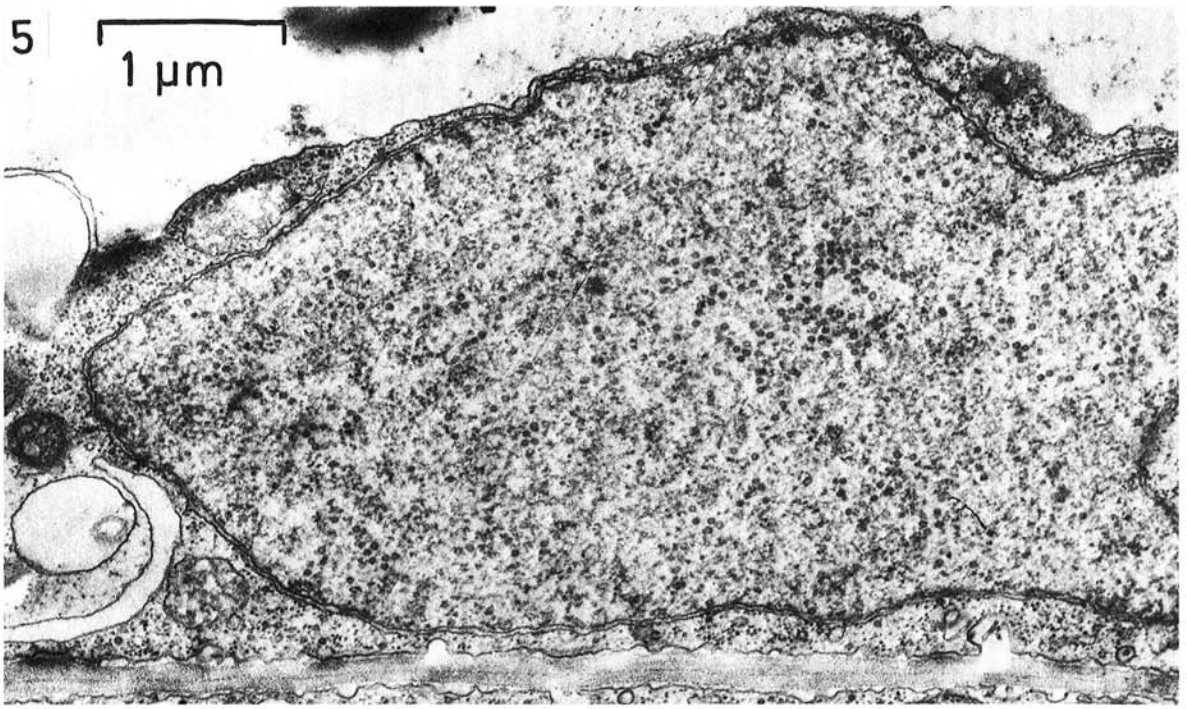
symptom-bearing plant parts, although a smaller number was found in leaf tissues than in stems. Almost every cell seemed to be infected in small side shoots that had severe symptoms and sometimes necrotic apices. No inclusion bodies were found in epidermal cells and sieve elements, but virus particles were present (Fig. 7). Mature sieve elements contained scattered virus particles or ordered arrays of particles mixed with filaments, presumably P-protein (Fig. 7-b, c). Sometimes virus particles adhered to irregular membranes in mature sieve tubes (Fig. 7-c).

DISCUSSION.—Virus particles with dimensions similar to those described here from PVCV are uncommon among plant viruses. Carrot mottle virus has particles of a similar diam, 50 nm, but they appear to be enveloped by a membrane derivative (16). More similarity seems to exist to cauliflower mosaic virus (CIMV) (6, 7, 9, 13, 15, 18, 21, 22), dahlia mosaic virus (DMV) (2, 3, 12, 17), carnation etched ring virus (CERV) (8, 10, 20), and two other viruses cited by (3). These viruses have particle diam of about 50 nm.

The diam of negatively stained particles of PVCV was 43 nm; whereas, in parallel measurements in this laboratory, particles of CIMV from negatively stained crude extracts had diam of 51 nm. The diam of the latter was reported to be near to 50 nm by most authors (7, 11, 18, 22) and only in one case to be 40-45 nm (8). So a difference between particle diam of the CIMV group viruses and PVCV seems to be established.

Also the virus particle structure of the CIMV group as revealed from thin sections seems to be different from PVCV. The former are visible in sections as electron-dense rings with transparent or sometimes electron-dense

Fig. 1-3. 1) Groups of particles of petunia vein-clearing virus, negatively stained with neutral PTA in crude extracts from petunia. Arrows point to particles with hexagonal outlines. **a)** Particles heavily penetrated by PTA, **b)** particles less penetrated, some showing substructure of the shells. **2)** Ultrathin section from petunia vein-clearing virus-infected parenchyma cell in the vascular cylinder of petunia. Note inclusion bodies (IB) and virus particles (V), virus-containing region loosely enveloped by single cisternae of endoplasmic reticulum (ER), and virus particles within the vacuole-like regions of the inclusion bodies (arrow). **3)** Details of petunia vein-clearing virus particles from ultrathin section of a parenchyma cell of petunia. Note lightly stained centers within electron-opaque rings that are surrounded by an area of intermediate contrast (arrows).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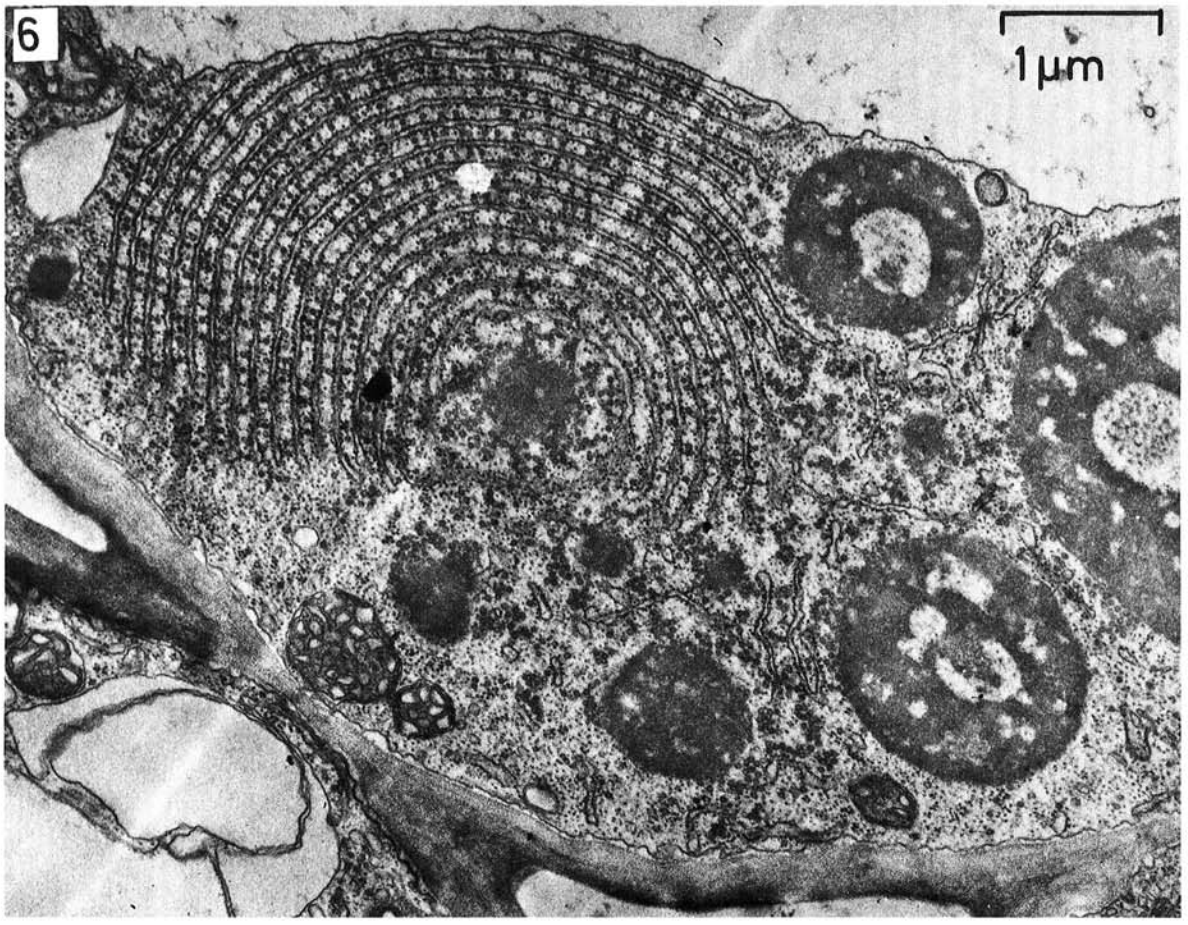
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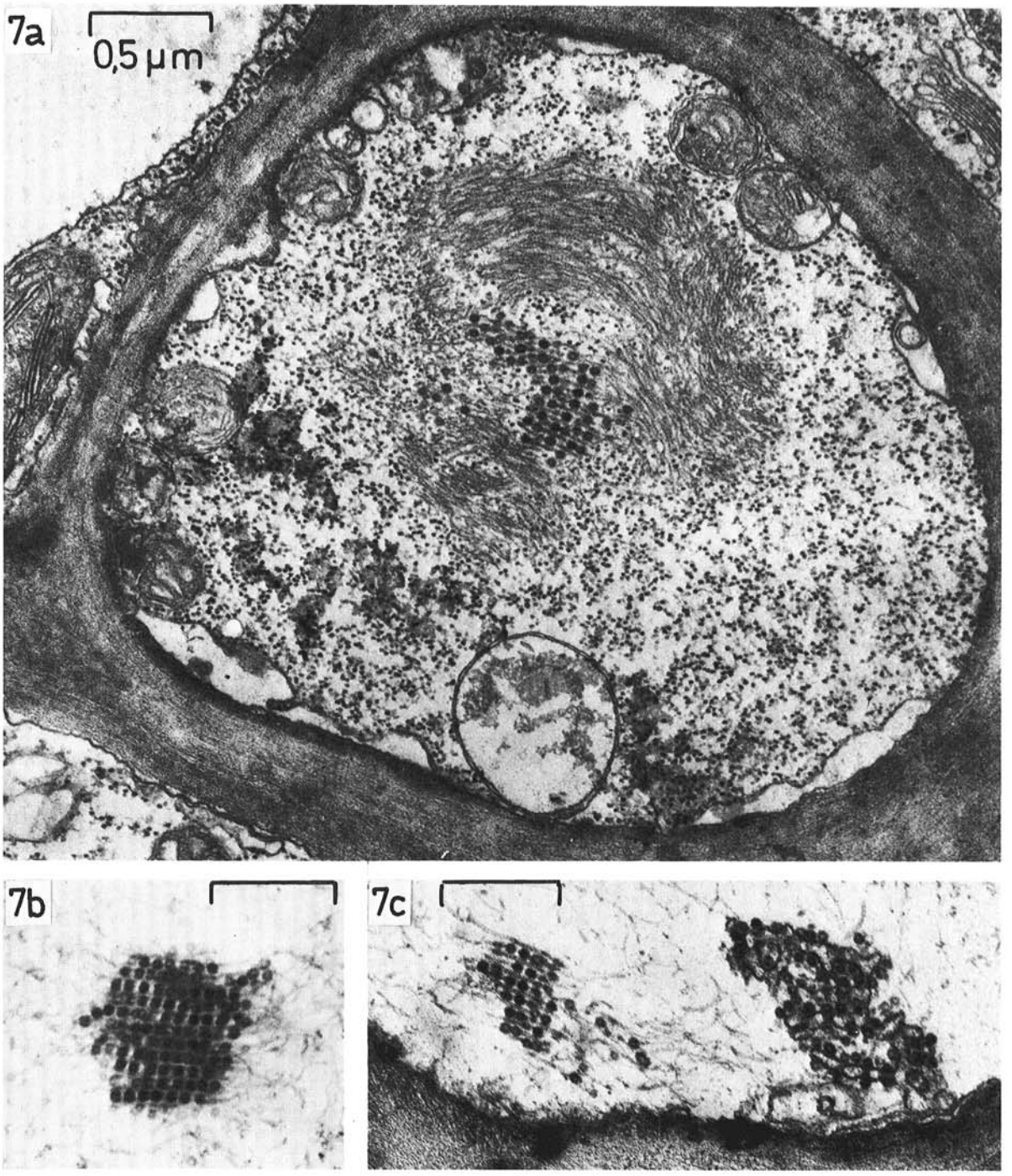

Fig. 7-a, b, c. Ultrathin sections showing virus particles in sieve elements of a petunia stem. **a)** Immature sieve element with an ordered array of virus particles intermixed with protein filaments. **b)** Mature sieve element with a similar particle arrangement. **c)** Mature sieve element that, in addition, contains irregular membranes associated with virus particles.

Fig. 5-6. 5) Ultrathin section showing nucleus of an infected cell that contains particles of petunia vein-clearing virus. **6)** Ultrathin section of a virus-containing region in a petunia parenchyma cell, showing anamolous concentric cisternae. Note virus particles between the cisternae.

centers. Strongly contrasting material is always seen at the particle peripheries. PVCV-particle structure differs in that the strongly contrasting material of its particles is surrounded by a lightly stained envelope.

Unlike PVCV, all of the observed ClMV-group virus particles are, with only a few exceptions, located within the matrix and the vacuole-like regions of the inclusion bodies. This holds true for all of the different plant species infected by viruses of the ClMV group, and was confirmed by comparing thin sections of ClMV-infected turnip leaves in our laboratory. PVCV further differs from viruses of the ClMV group in the association of ER-elements with the virus-containing regions. DMV-induced inclusions show an association with dictyosome systems, for ClMV, only association with ribosomes is reported.

Additionally, in preliminary serological tests, no reaction could be found between antisera against ClMV or DMV and PVCV in crude extracts from petunia (A. A. Brunt, *personal communication*).

On the whole, the conclusion seems to be justified, that PVCV is different from the known members of the CIMV group. However, the similarity in structure of the inclusion bodies is striking. So, it remains an open question whether there is no relation at all between PVCV and the CIMV group.

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