

Etiology

## Comparison of a New Soilborne Virus from Cucumber with Tombus-, Diantho-, and Other Similar Viruses

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### ABSTRACT

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A virus isolated from cucumber roots grown in soil from the Jiyeh area south of Beirut produced local lesions on a number of plant species, but no systemic infections. Its particles with a diameter of ~31 nm had a sedimentation coefficient of ~120S and a buoyant density in CsCl of 1.343 g/cm<sup>3</sup>, indicating an RNA content of ~18%. Its UV absorption spectrum had a minimum at 245 nm and a maximum at 260 nm. In immunoelectrophoresis at pH 7.0, the virus migrated towards the anode. The viral coat protein and the single genomic RNA species had molecular weights of  $4.14 \times 10^4$  and  $1.5 \times 10^6$ , respectively. Some preparations also

contained a minor RNA species with a molecular weight of  $0.16-0.19 \times 10^6$ , which was not necessary for infection. The base composition of the RNA was C22, A23, G32, and U23. Virus particles were found scattered or in small aggregates in the cytoplasm, but not in nuclei or other organelles. The virus did not induce cytopathic effects typical of tombusviruses or dianthoviruses, nor did it react with antisera to known tombusviruses, dianthoviruses, or 43 other isometric viruses. Cucumber soilborne virus is the name proposed for this apparently newly described virus.

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In the coastal area of Lebanon, vegetables are grown intensively all year round. This continuous cropping enhances the buildup of soilborne pathogens, including viruses. In a previous survey (17) tomato mosaic virus was found to be the most common virus

recovered from soils of the littoral zone. In this paper, we report using cucumber seedlings as bait and recovering from these soils another virus that is described and provisionally named cucumber soilborne virus (CSBV).

### MATERIALS AND METHODS

**Isolation of the virus.** Soil samples were collected during 1977 from five different localities along the coastal region of Lebanon,

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15 samples per locality. Each sample was placed in a steam-sterilized clay pot in a greenhouse. Five cucumber (*Cucumis sativus* L. 'Chicago Pickling') seeds were sown in each soil sample as bait. Four weeks after sowing, roots were washed thoroughly with water and triturated with phosphate buffer and Celite. The sap was rubbed onto the leaves of a number of indicator plants. Isolates recovered from three soil samples coming from the Jiyeh area south of Beirut produced red necrotic local lesions on *Vigna unguiculata* Wolp., 'California Black Eye No. 5.' One of the isolates was passed three times through local lesions on cowpea, and was used for further studies.

**Host range and symptomatology.** Twenty-four plant species belonging to five plant families were inoculated and observed for symptom development. To detect latent infections, the new growth of previously inoculated plants was homogenized and rubbed onto cowpea primary leaves.

**Purification.** The virus was purified by homogenizing 100 g of locally infected cowpea leaves with 200 ml of a solution containing 5 g Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10 H<sub>2</sub>O, 5.38 g H<sub>3</sub>BO<sub>4</sub>, and 0.2% each of ascorbic acid and sodium sulfite at pH 7.8. After centrifugation at 3,500 g for 30 min, the supernatant was stirred for 15 min with one-fourth volume of chloroform and the virus was sedimented from the aqueous phase by centrifugation at 65,000 g for 2 hr. After centrifugation at 3,500 g for 30 min the virus was further purified by centrifugation at 8 C for 4 hr in a Beckman SW 27 rotor on 10–40% linear sucrose density gradients made up in 0.05 M phosphate buffer, pH 7.0. The gradients were analyzed on an Isco density gradient fractionator with UA-5 analyzer. After sedimentation overnight at 65,000 g, the virus was suspended in 0.05 M tris-HCl buffer, pH 8.0, containing 10% glycerol.

**Physical properties.** Ultraviolet absorption spectra were measured in a PYE UNICAM SP 8-100 spectrophotometer. Absorption values ranged from 220 to 400 nm and were corrected for light scattering (7). The sedimentation velocity ( $S_{rel}$ ) relative to that of tobacco mosaic virus (194S), top (53S) and bottom components (116S) of turnip yellow mosaic virus, and middle (95S) and bottom components (115S) of cowpea yellow mosaic virus was estimated by sucrose density gradient centrifugation for 90 min at 35,000 rpm in a Beckman SW 40.1 rotor. To determine the buoyant density of the virus, it was suspended in a 36% (w/w) CsCl solution and centrifuged for 27 hr at 35 rpm in the same rotor. The gradients were separated into 0.3-ml fractions and the density of each fraction was calculated by using the relationship  $\rho_{CsCl} = \eta \times 10.2402 - 12.6483$  (4). The molecular weight of the protein subunits was determined by SDS polyacrylamide gel electrophoresis as described previously (16). The nucleic acid was extracted by the two-phase phenol method (21). The molecular weights were estimated after electrophoresis under nondenaturing conditions in 2.5% polyacrylamide gels prepared in 0.036 M tris-acetic acid buffer, pH 7.2. RNAs of brome mosaic virus served as markers. For

further purification, the nucleic acid of CSBV was layered on linear log sucrose density gradient columns designed for a density of 1.5 g/cm<sup>3</sup> (13) and made in 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0. The gradients were centrifuged for 5 hr at 6 C in a Beckman SW 55 Ti rotor. The base composition of the RNA was determined after hydrolysis overnight in 0.3 N KOH at 37 C. Potassium ions were removed by passage through a column of Dowex 50. After concentration with an evaporator the nucleotides were dissolved in distilled water and separated by thin-layer electrophoresis using cellulose MN 300<sub>uv</sub> (Macherey and Nagel, Düren, W. Germany) in 0.05 M triethanolamine-acetic acid buffer, pH 3.6. The nucleotides were eluted from the cellulose with 0.1 N HCl and their absorption was measured at 260 nm. The molar concentrations of the nucleotides were calculated (1).

**Serology.** A rabbit was immunized by two intramuscular injections, spaced 1 wk apart, of purified virus suspensions emulsified in an equal volume of Freund's complete and incomplete adjuvant, respectively. Bleedings were taken at 2-wk intervals. Agar gel double diffusion tests were done with 0.85% Difco Noble agar containing 0.85% sodium chloride, 0.25% sodium azide, and 0.01 M tris-HCl buffer, pH 8.0. The reactant wells, 4 mm in diameter, were spaced 2 mm apart. For immunoelectrophoresis, 1% agarose in 0.025 M phosphate buffer (pH 7.0) was used. ELISA was done as previously described (5).

**Electron microscopy.** Virus particles were detected in crude sap of infected leaves and in purified preparations after floating carbon-pioloform coated copper grids on the samples for a few min, washing the grids with 40 drops of distilled water, and staining them with 2% aqueous uranyl acetate. Negative staining with phosphotungstate (NaPT) was done by using spray droplet preparations (15). Local lesions from *Gomphrena globosa* L., *Nicotiana clevelandii* Gray, and *Vigna unguiculata* were embedded, thin sectioned, and stained as previously described (25).

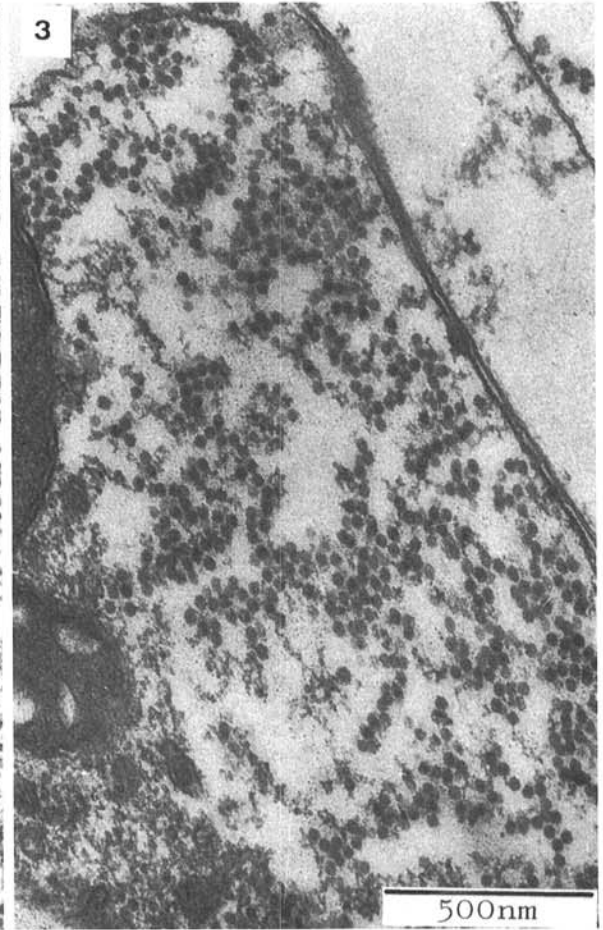
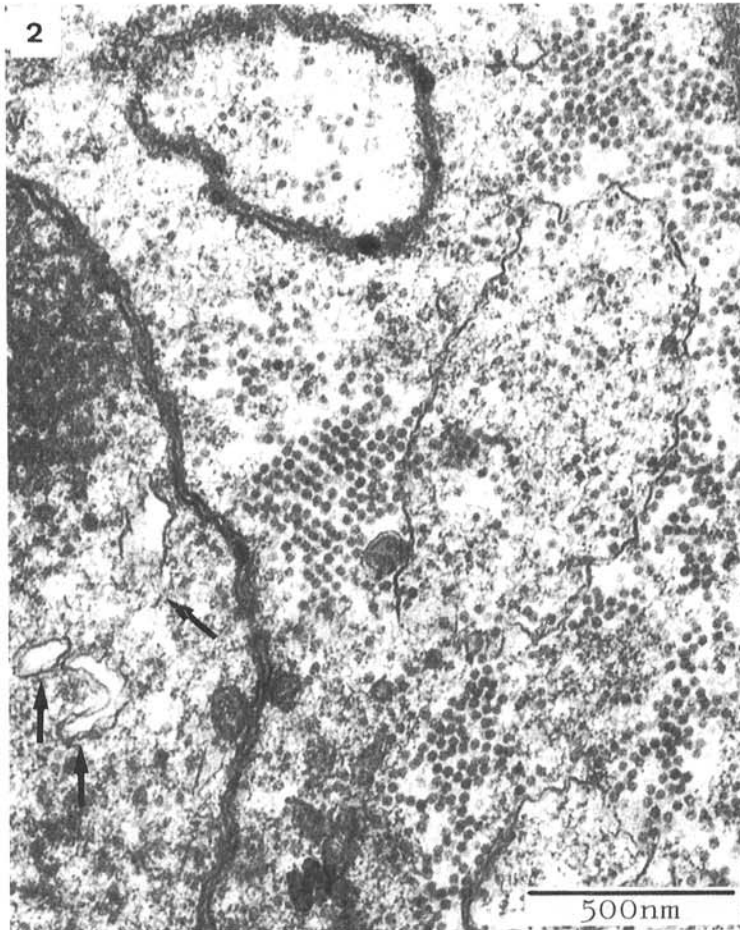
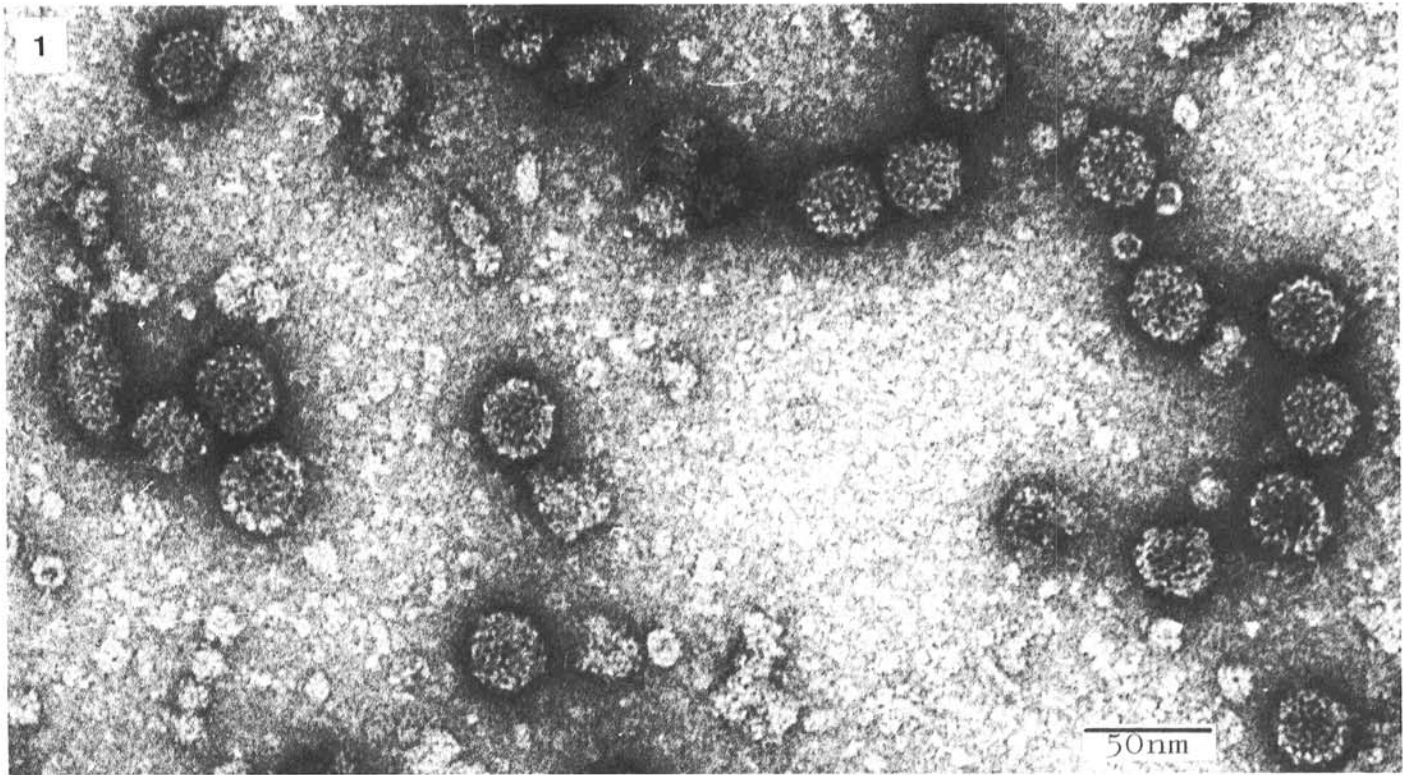
## RESULTS

**Host range.** The virus caused local lesions without systemic infection in *Beta vulgaris* L.; *Chenopodium amaranticolor* Coste & Reyn.; *C. quinoa* Willd.; *Cucumis sativus*; *Cucurbita pepo* L.; *Datura stramonium* L.; *Gomphrena globosa*; *Lycopersicon esculentum* Mill. 'Marmande'; *N. clevelandii*; *N. glutinosa* L.; *N. tabacum* L. 'Havana 423,' 'Xanthi,' and 'White Burley'; *Peunia hybrida* Vilm.; *Phaseolus vulgaris* L. 'Romano'; and *V. unguiculata* 'California Blackeye No. 5.' *V. unguiculata* was the best source for virus purification. The following plants were not infected either locally or systemically: *Capsicum annum* L. 'Yolo Wonder'; *Physalis floridana* Rydb.; *Solanum melongena* L. 'Black Beauty'; *Spinacea oleracea* L. 'Virofle'; and *Vicia faba* L. ELISA yielded no evidence of transmission of virus to cucumber roots in sterilized soil.

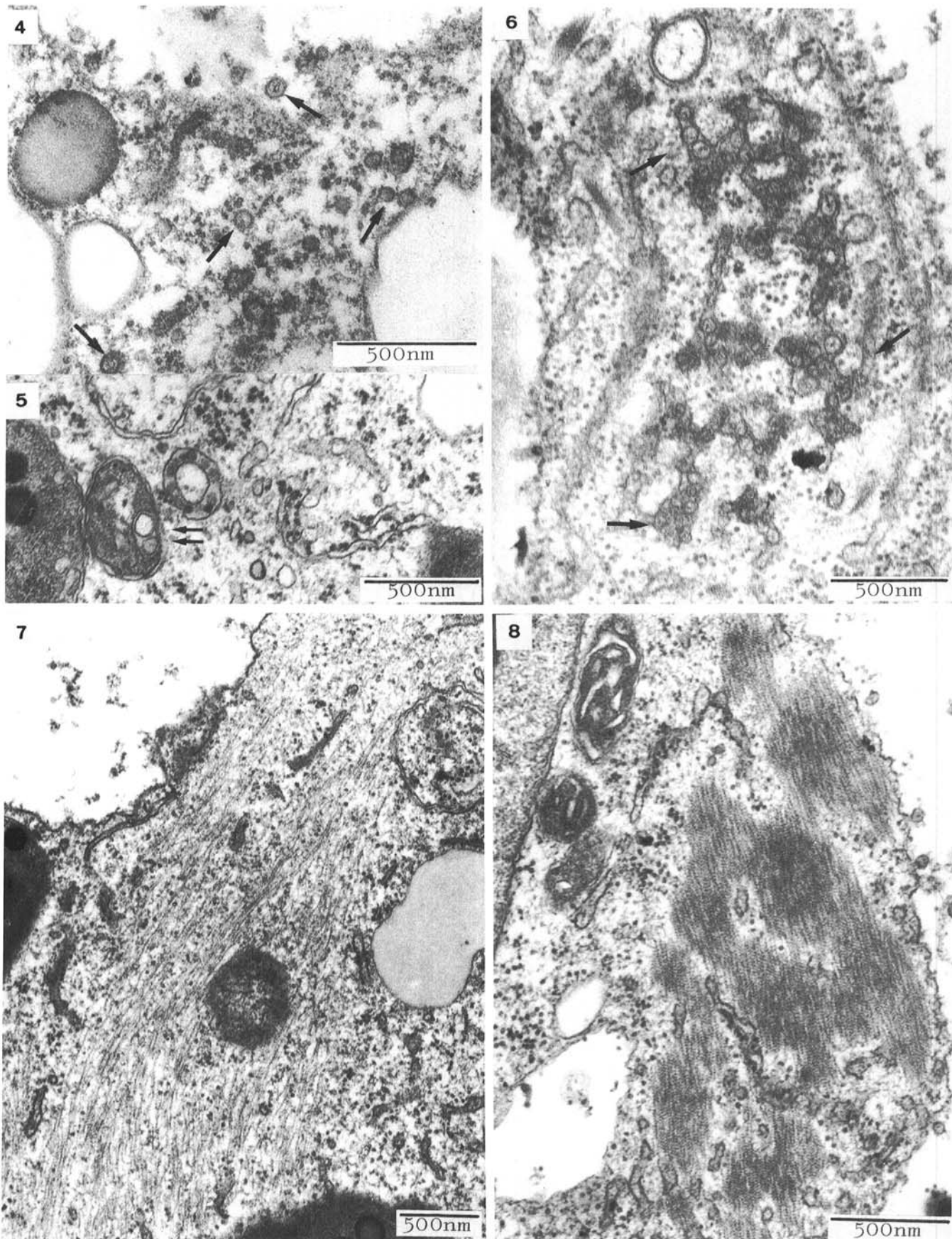
TABLE 1. Comparison between cucumber soilborne virus and some other serologically unrelated viruses with similar properties

Virus	Soil-borne	Particle diameter (nm)	Protein mol. wt. ( $\times 10^4$ )	RNA					Sedimentation coefficient	References	
				%	mol. wt. ( $\times 10^6$ )	Base composition					
						G	A	C			U
Cucumber soilborne	+	31	4.1	18	(0.17), 1.5	32	23	22	23	120	
Tombusviruses	+	28–30	~4.1	15–18	1.5–1.7	~28	25	21	26	132–140	(18)
Dianthoviruses (carnation ringspot)	+	31–34	~4.0	20	0.45, 1.4	26	27	23	24	135	(19)
Blackgram mottle		28	3.8	20	1.4	25	26	25	24	122	(23)
Carnation mottle virus		28	4.1 <sup>a</sup>	20		27	30	19	24	122	(9)
Cowpea mottle		30	4.4	20	1.4					122	(3)
Cucumber necrosis	+	31	~4.0	16	1.4					133	(6)
Elderberry latent		30(+17)	4.0	23	1.55					112(+48)	(14)
Narcissus tip necrosis		30	4.2	18	1.6					123	(20)
Pelargonium flower break		27–34	4.1	17						125	(11)
Saguaro cactus		32	3.9	17	1.4	29	24	21	27	118	(22)
Tephrosia symptomless	–	30	4.2		1.5					127	(2)
Turnip crinkle		30	3.8(+2.8)	17	1.4	28	26	24	22	129	(10)

<sup>a</sup>R. Koening (unpublished).



**Figs. 1-3.** Electron microscope images of particles of cucumber soilborne virus: **1**, from a partially purified preparation negatively stained with uranyl acetate; **2** and **3**, in ultrathin sections of palisade cells of cowpea. Note membranous structures in the nucleus in Fig. 2 (arrows).



**Figs. 4-8.** Cytological alterations in leaf cells of cowpea infected by cucumber soilborne virus: **4** and **5**, small vesicles free in the cytoplasm or in a mitochondrion, respectively (arrows); **6**, vesicles containing fibrillous material enclosed in cisternae of the endoplasmic reticulum (arrows); **7** and **8**, fibrous strands in the cytoplasm in loose arrangement, or in densely aggregated masses, respectively. Note the regular substructure of the aggregates in Fig. 8.

**Physical properties.** In sucrose density gradient centrifugation the virus sedimented as a single component with  $S_{rel}$  of S120. The buoyant density in CsCl was 1.343 g/cm<sup>3</sup> (average of six determinations), suggesting an RNA content of ~18% (24). The ultraviolet absorption spectrum showed a minimum at 245 nm and a maximum at 260 nm. The  $A_{280/260}$  ratio was 0.66. In SDS polyacrylamide gel electrophoresis the coat protein migrated as a single band for which a molecular weight of  $4.14 \pm 0.02 \times 10^4$  (average of six separate determinations) was calculated. The viral nucleic acid migrated in polyacrylamide gel electrophoresis as one major band with a molecular weight of  $1.5 \times 10^6$ . In some preparations, an additional minor band with a molecular weight of  $0.16-0.19 \times 10^6$  was observed. In comparative runs the nucleic acid of a tombusvirus (petunia asteroid mosaic virus) migrated as a single band with a molecular weight of  $1.65 \times 10^6$ , whereas the nucleic acid of a dianthovirus (carnation ringspot virus) migrated as two bands with molecular weights of  $0.45 \times 10^6$  and  $1.4 \times 10^6$ . The major CSBV nucleic acid isolated by sucrose density gradient centrifugation produced numerous local lesions on *V. unguiculata*. Therefore, the minor CSBV nucleic acid is probably a subgenomic fragment. The base composition of the CSBV RNA was C22, A23, G32, and U23.

**Serology.** In agar gel double diffusion tests the virus yielded a single precipitin line with its homologous antiserum that had a titer of 1:256. It did not react with antisera to tombusviruses (cymbidium ringspot, eggplant mottled crinkle, pelargonium leaf curl, petunia asteroid mosaic viruses, or the BS-3 strain of tomato bushy stunt virus), to dianthoviruses (carnation ringspot and red clover necrotic mottle) and 10 other viruses that resemble CSBV in protein molecular weight and a number of other properties (Table 1). Also, no reactions were observed with antisera to tobacco necrosis virus, sowbane mosaic virus, southern bean mosaic virus, 17 tymoviruses, three nepoviruses, three comoviruses, and seven unclassified viruses. In immunoelectrophoresis at pH 7.0, CSBV migrated towards the anode.

**Electron microscopy.** Isometric particles about 31 nm in diameter were present in crude sap from lesions of infected plants and in purified preparations. Particles were stable in NaPT. They were usually rounded, but occasionally showed an angular outline. Often, particles were penetrated by the stains. The particle surface stained in uranyl acetate showed distinct morphological subunits (Fig. 1).

In non-necrotic epidermal, parenchyma, and vascular parenchyma cells of local lesions distinct viruslike particles, ~28 nm in diameter, were found scattered or in small aggregates in the cytoplasm (Fig. 2). They were considered to be CSBV particles. Clusters of these particles occurred in the cytoplasm of living (Fig. 3) and of necrotic cells. Sometimes particles accumulated between the cytoplasm and cell wall or were found in the central vacuoles of affected cells with a damaged tonoplast. Virus particles were not found in nuclei or other organelles.

Endoplasmic reticulum proliferation was observed in infected tissues. Small vesicles, delimited by a single membrane and containing fibrillar material resembling nucleic acid, were often seen (Figs. 4 and 6). These were mostly free in the cytoplasm (Fig. 4), but sometimes were enclosed within endoplasmic reticulum cisternae (Fig. 6). Similar vesicles were also found occasionally within mitochondria between the outer and inner membrane (Fig. 5).

Infected cells of all three host plants studied also contained thin fibrils ~9 nm thick, which occurred either in loose parallel aggregates (Fig. 7) or in denser elongated fibrous masses (Fig. 8). Especially in the dense aggregates there was an indication of a regular substructure of the fibrils (Fig. 8).

No alterations were observed in nuclei except for membranous structures occurring sometimes at their periphery (Fig. 2). Chloroplasts of affected tissues often had an altered structure showing accumulations of starch and/or phytoferritin, or a reduction of their membrane system.

Cucumber soilborne virus has a number of properties in common with viruses in the tombusvirus and the newly established dianthovirus groups (19), i.e., protein subunits with a rather high molecular weight ( $\sim 4.0 \times 10^4$ ), isometric particles with a diameter of ~31 nm and transmission through soil. The molecular weight of its single genomic RNA is similar to that of the single RNA of tombusviruses and that of the heavier of the two RNAs of dianthoviruses. Host range and the lack of serological relationships, however, clearly differentiate CSBV from all known tombus- and dianthoviruses and in infected cells it induces fibrillar cytoplasmic inclusions, which are not found with tombus- or dianthoviruses. It lacks the ability to induce the cytoplasmic multivesicular inclusions that are typical for tombusviruses (18), and its cytopathic effects also differ from those produced by a dianthovirus (27). From dianthoviruses it is also differentiated by the lack of a second major RNA species with a molecular weight of  $\sim 0.5 \times 10^6$ .

CSBV also shares some properties with a number of other viruses (Table 1). Some of these viruses (i.e., carnation mottle, pelargonium flower break, elderberry latent, and narcissus tip necrosis viruses) have tentatively been assigned to a carnation mottle virus group (12), which so far has not been approved by the International Committee on Taxonomy of Viruses. CSBV is differentiated from all viruses in Table 1 by the lack of serological relationships and differences in host range. If future studies justify the establishment of a carnation mottle virus group, CSBV may be a candidate for this group.

The fact that the plant species commonly grown in the area where the CSBV was isolated were not invaded systemically after leaf inoculation might indicate that the virus has no economic importance at the present time. As a soilborne pathogen, however, it may resemble beet necrotic yellow vein (26) and tobacco rattle (8) viruses, which predominantly affect roots or other subterranean parts of the host plants, and thus may cause damage without moving systemically to the foliage.

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