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Virus Particles in Apparently Healthy *Peregrinus maidis*

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Virus-like particles were found in apparently healthy *Peregrinus maidis* (Ashm.) in Venezuela. The particles were observed in the salivary gland, intestine, mycetome, adipose tissue, ovary, and hemolymph. In the cells, the particles occurred in the cytoplasm singly, in groups, free or within vesicles, and in hexagonally arranged crystals. In *P. maidis* from Hawaii such particles were not found. However, in these insects, single particles or crystals were observed after injecting suspensions of intestines from *P. maidis* from Venezuela. The particles were not observed in insects feeding on plants soiled with excretions from particle-containing *P. maidis*. Particles in organs of insects or in pellets of intestines were polygonal and showed a weakly contrasted envelope and a highly contrasted core. The particles had a diameter of 54 ± 9 mμ. They are believed to represent a *Peregrinus* virus causing latent infection.

During an investigation (8) of the rod-shaped maize mosaic virus I (Smith) in different organs of *Peregrinus maidis* (Ashm.), spherical virus-like particles were also found. The present paper reveals information on the occurrence, localization, transmission, and structure of these particles.

MATERIALS AND METHODS

Materials. Most investigations were carried out with *P. maidis* of a maize mosaic free colony maintained for 6 years in a greenhouse on corn plants (*Zea mays* L.), variety Sicarigua. This strain of insects originated from corn fields in Maracay, Venezuela, and for identification purposes was designated as the Maracay strain. Other *P. maidis* were collected from plants in Maracay just before use. A third group of *P. maidis* insects originated from corn plants in Hawaii and was reared in a greenhouse in rooms separated from the Maracay strain on corn grown from seeds from Hawaii (variety Hawaiian Sugar). It was designated as the Hawaii strain.

Some *P. maidis* insects from the Maracay and the Hawaii strain were infected with maize mosaic virus I by feeding on infected corn plants. After an incubation period of 10 to 12 days, these insects were checked on healthy corn plants for presence of maize mosaic virus I.

Purification. Suspensions of at least 80 intestines from *P. maidis* insects (for tissue culture and mice inoculation purposes, 160 intestines) were prepared in the following manner. The insects were anesthetized by cooling (0 to 4 C), and the intestines were dissected in a drop of 0.01 M phosphate buffer (*p*H 8) containing 250 units of penicillin and 250 μg of streptomycin per ml. Immediately afterwards, the intestines were frozen in dry ice in a 0.5 ml TenBroeck microgrinder and stored for about 20 hr at −55 C. The intestines were then thawed rapidly, ground in 0.5 ml (1 ml if 160 intestines) of phosphate buffer, and centrifuged for 15 min at 3,000 × *g*. The supernatant fluid was called suspension A. This was concentrated for 2 hr at 33,000 × *g* in a Spinco model L ultracentrifuge. The resulting sediment was resuspended in phosphate buffer and divided into three equal parts, and each was centrifuged for 1 hr at 74,000 × *g*. Two of the resulting pellets were fixed for embedding and the third one was resuspended in 0.1 ml of distilled water (suspension B).

Transmission of the virus-like particles. (i) *P. maidis.* Adults or nymphs in the last instar of *P. maidis* Hawaii strain were anesthetized by cooling and then received a lateral injection in the abdomen with 0.2 μliter of suspension A by use of a microliter syringe (Hamilton Co., Inc., Whittier, Calif.). Afterwards, the insects were maintained on corn grown from Hawaiian seeds. In other experiments, adult males of the Maracay strain were placed for 10 days on Hawaiian Sugar corn plants (seven insects per plant). When taking off the males, nymphs of the last instar of the Hawaii strain were placed on the plants. This was done to determine whether the insects might become infected by the excretions of the Maracay insects.

(ii) *Tissue cultures.* Cell lines were supplied by Microbiological Associates, Bethesda, Md. MA 111 rabbit kidney, MA 134 green monkey kidney, MA 104 Rhesus monkey kidney, and baby hamster kidney (BHK/21) (*see* reference 10), were used. Monolayers of cells in Falcon plastic 30-ml flasks were inoculated with 0.1 ml of suspension A and were maintained in a serum-free medium (Bergold and Mazzali, J. Gen. Virol., *in press*). The cultures were observed up to 4

weeks, with the exception of BHK/21 cultures which were observed up to 10 days.

(iii) *Suckling mice.* Mice 2 days old were injected intracerebrally with 0.02 ml of suspension A and were observed until the adult stage.

(iv) *Plants.* Insects of *P. maidis* Maracay strain were transferred to corn variety Laguna, to six other species of *Gramineae*, six of *Solanaceae*, three of *Leguminosae*, and one species each of *Amaranthaceae*, *Compositae*, *Malvaceae*, *Sterulaceae*, *Tropeolaceae*, and *Plantaginaceae*. On Laguna corn and some other *Gramineae*, the insects were killed after 4 days with a systemic insecticide (Systox, 0.1%; Bayer, Germany). On the other plants they died after several days.

Electron microscopy. Different organs of adult *P. maidis* insects were dissected in a drop of 1% Veronal-buffered osmic acid (*p*H 7.4), fixed for 30 min, dehydrated in graded ethyl alcohol, and embedded in butyl-methyl methacrylate, 7:3. Pellets of purified suspensions were fixed and embedded by the same procedure. Small pieces of corn leaves (1 × 1 mm) were fixed for 3 hr and then processed as described above. Sections cut with a Porter-Blum MT-2 microtome equipped with a diamond knife were stained for 30 min in 4% aqueous uranyl acetate or for 2 min in lead citrate at 60 C (14).

Hemolymph of *P. maidis* was obtained from cut legs; the protruding drop was sucked off with a silicon-coated micropipette and was mixed on carbon-stabilized Formvar grids with approximately the same volume of 0.2% aqueous phosphotungstic acid adjusted to *p*H 6.5 (PTA). Suspensions A and B from purification experiments were mixed 1:1 with 2% PTA and sprayed with a nebulizer (no. 4659, Vaponefrin Co., Edison, N.J.) onto carbon-coated grids or mixed 1:1 with aqueous uranyl acetate on Formvar grids.

All preparations were examined in a Siemens Elmiskop I electron microscope.

TABLE 1. *Occurrence of Peregrinus virus in P. maidis from Venezuela and from Hawaii*

Treatment of the insects	Material investigated	*P. maidis* Venezuela[a]	*P. maidis* Hawaii[a]
None	Organs	25/47	0/27
None	Suspension A of at least 80 intestines	13/13[b]	0/7[b]
Injected with suspension A, strain Hawaii	Organs		0/13
Injected with suspension A, strain Maracay	Organs		16/30

[a] Number of insects with virus/total number of insects.

[b] Figures refer to number of lots.

RESULTS

Natural occurrence of the particles. Virus-like particles were found in 25 of 47 embedded intestines of *P. maidis* insects from Venezuela and in suspensions A and B of all 13 lots of *P. maidis* from Venezuela. No such particles were found in 27 embedded intestines (7 of these from individuals grown in Hawaii) or in 7 lots of suspension A of intestines of *P. maidis* Hawaiian strain (Table 1).

In *P. maidis* from Venezuela, the particles were found: (i) in individuals from the Maracay strain reared on corn free of maize mosaic virus; (ii) in individuals from the Maracay strain which had fed on plants with maize mosaic virus and did or did not transmit maize mosaic (Table 2); and (iii) in two of eight insects freshly collected from corn fields in Maracay. No obvious difference in proportion of males to females was noticed in insects containing the virus-like particles (14 ♂, 11 ♀) and not containing them (12 ♂, 10 ♀).

The virus-like particles were not found in leaf sections of about 50 corn plants on which *P. maidis* Maracay strain had fed.

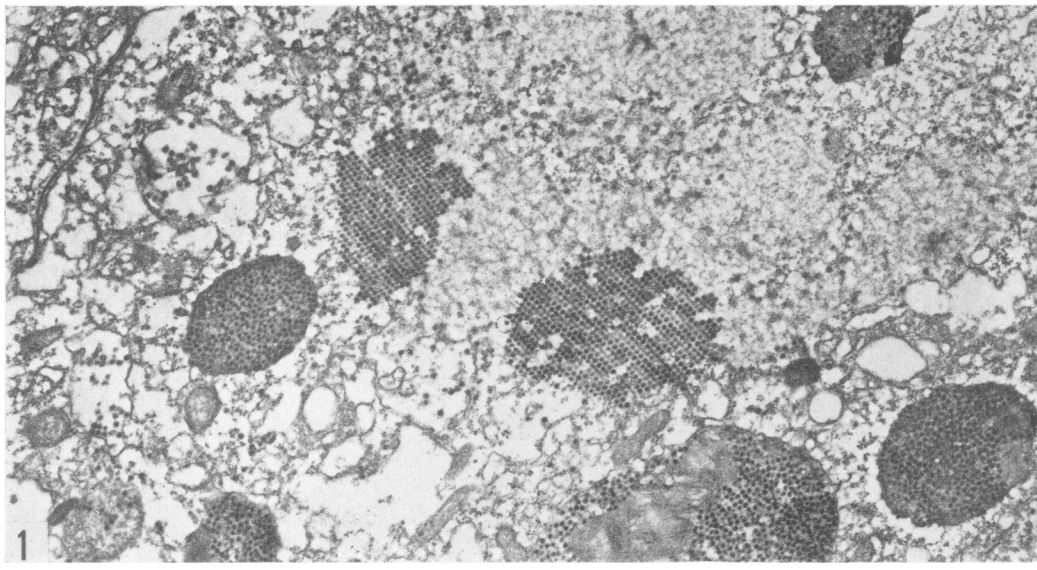
Localization and ultrastructure of the particles. The virus-like particles were located in cells of the intestine (Fig. 2–5, 9–11), Malpighian tubules, ovary (Fig. 8), salivary gland (Fig. 1, 6), mycetome (Fig. 7), adipose tissue adhering to the intestine, and in the hemolymph.

The particles were found in the cytoplasm but never in the nucleus. They formed big crystals (Fig. 1–3, 7–9, 11), were distributed in smaller or bigger groups (Fig. 5, 10, 11), lay scattered individually in the cytoplasm (Fig. 1, 6, 11), or appeared enclosed in vesicles (Fig. 1, 4, 6). In the crystals the particles were arranged hexagonally. Often dislocations (Fig. 2) and other irregularities (Fig. 1–3, 7–9, 11, 12) in the crystalline array were observed. In the vesicles, the particles were distributed at random. In some vesicles, relatively few particles and much lamellated material (Fig.

TABLE 2. *Occurrence of Peregrinus virus in Venezuelan P. maidis after feeding on corn with maize mosaic virus*

Virus	Intermediate feeding on plants		
	With maize mosaic virus[a]		Without maize mosaic virus[a]
Maize mosaic virus....	20/20	0/13	0/6
Peregrinus virus.....	12/20	9/13	2/6

[a] Number of insects with virus/total number of insects.



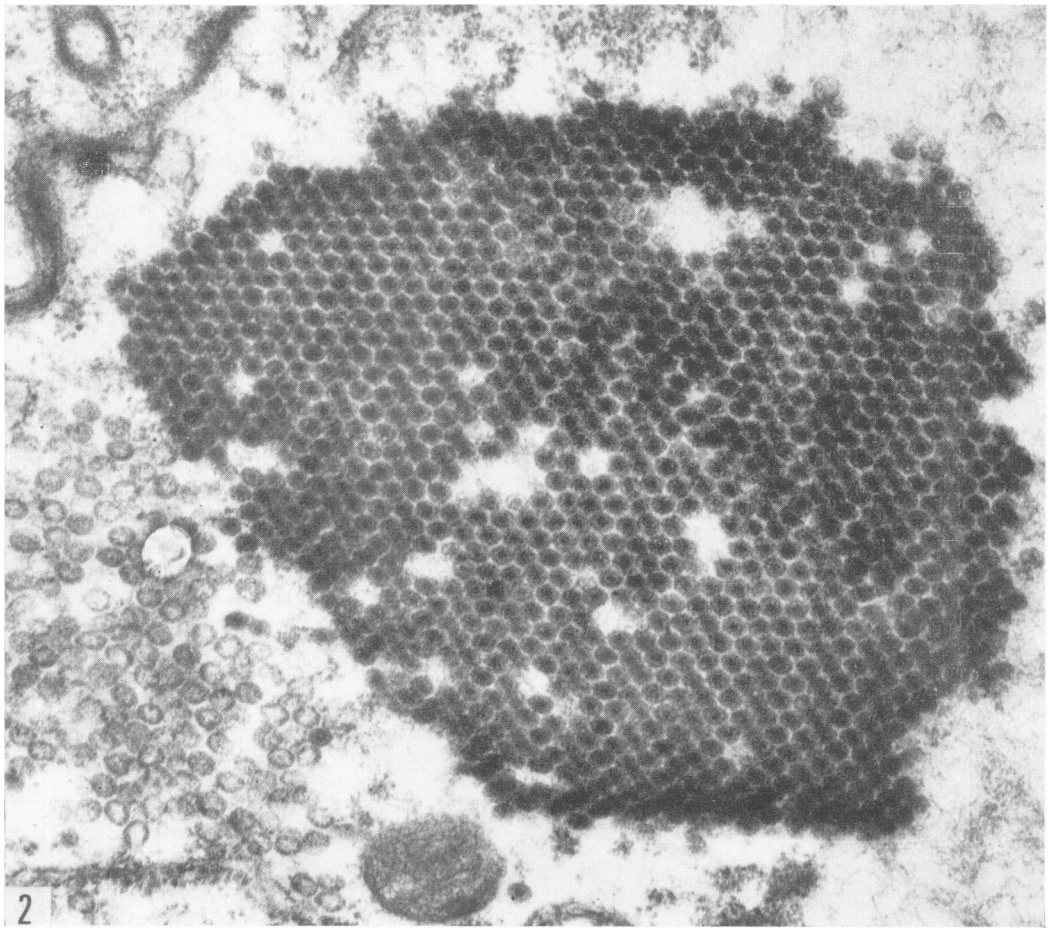
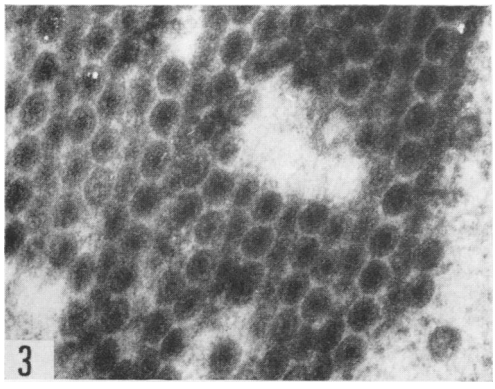
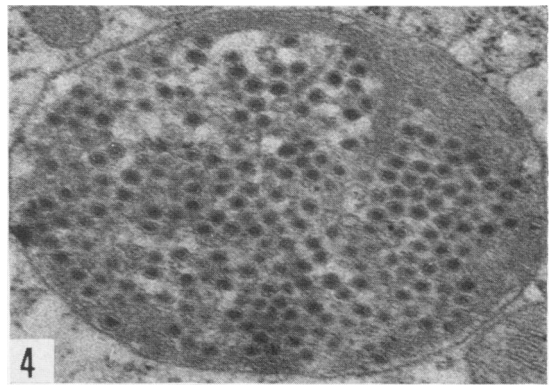
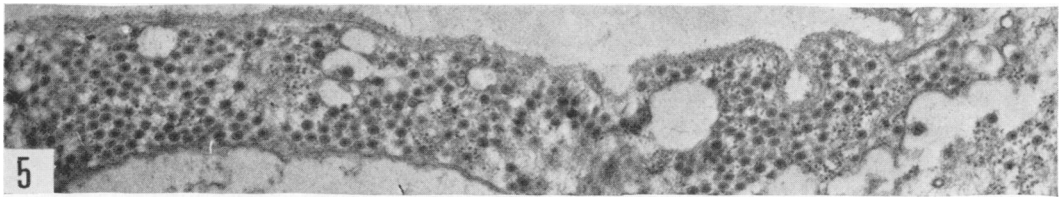


FIG. 1. *Virus particles forming crystals, enclosed in vesicles, lying in groups or singly in the cytoplasm of a salivary gland.* × *15,000.*

FIG. 2. *A big crystal in an intestine cell. Note the hexagonal arrangement of the particles and a dislocation in the upper right side of the crystal. Next to the crystal many electron-transparent, round structures are seen.* × *50,000.*







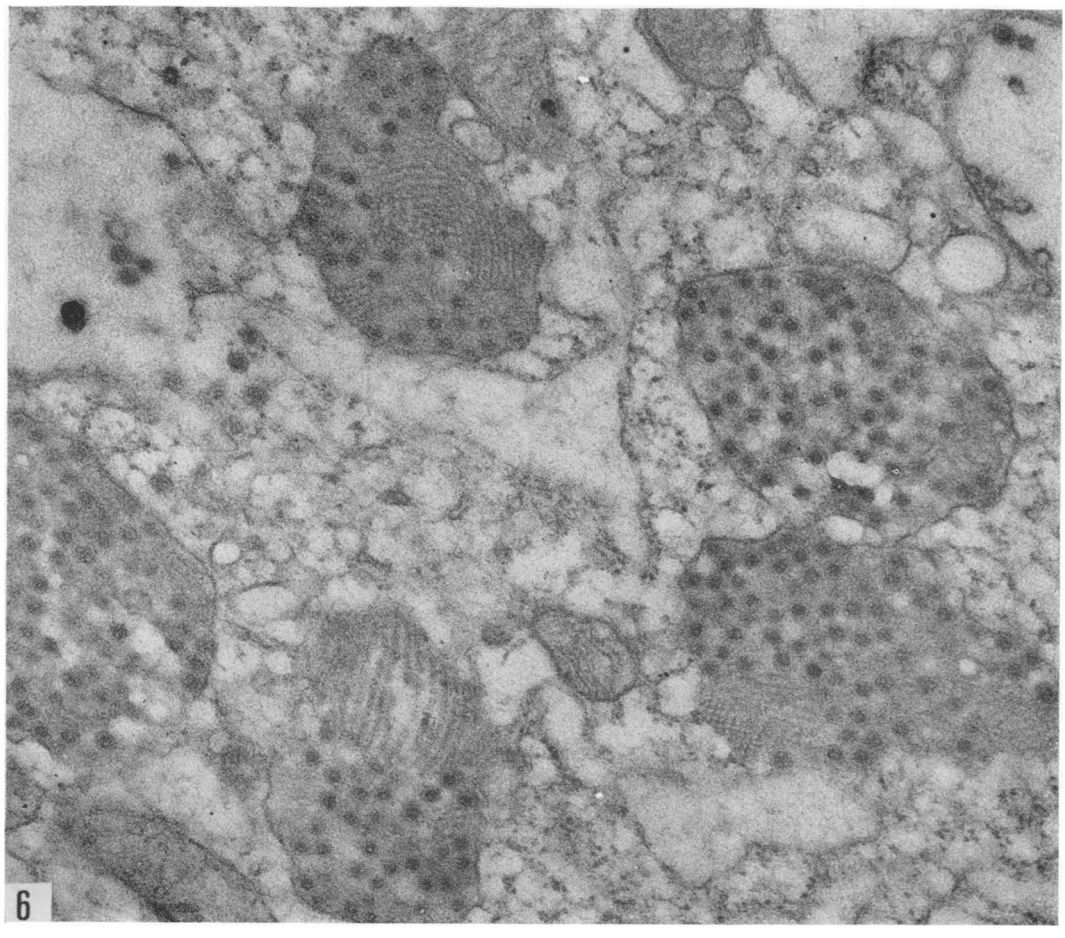
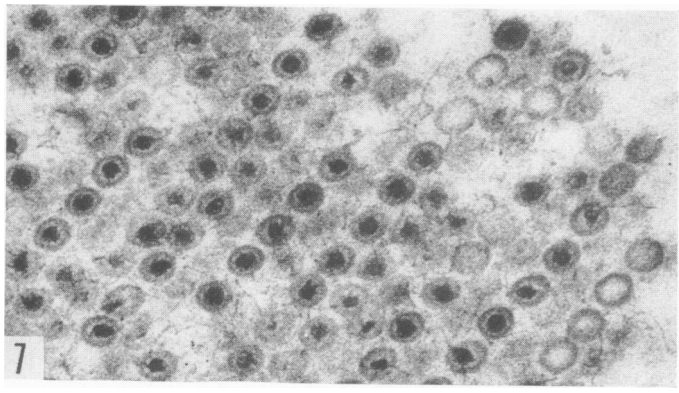


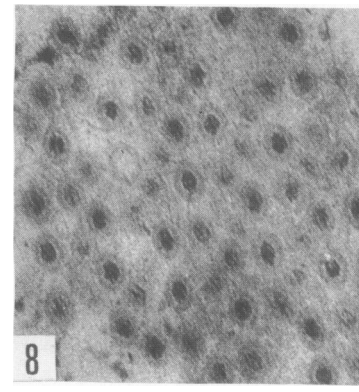
FIG. 3. *Part of a crystal with irregularities in an intestine cell.* × *75,000.*

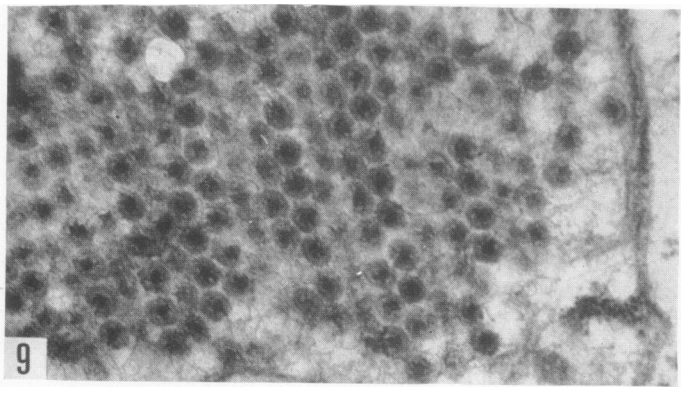
FIG. 4. *Many particles enclosed in a vesicle in an intestine cell. Note the lamellated material in the vesicle.* × *50,000.*

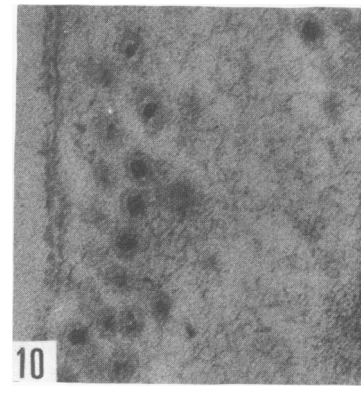
FIG. 5. *Group of particles scattered in the cytoplasm of an intestine cell.* × *25,000.*

FIG. 6. *Particles enclosed in vesicles in a salivary gland. Some of the vesicles contain relatively few particles and much lamellated material.* × *50,000.*









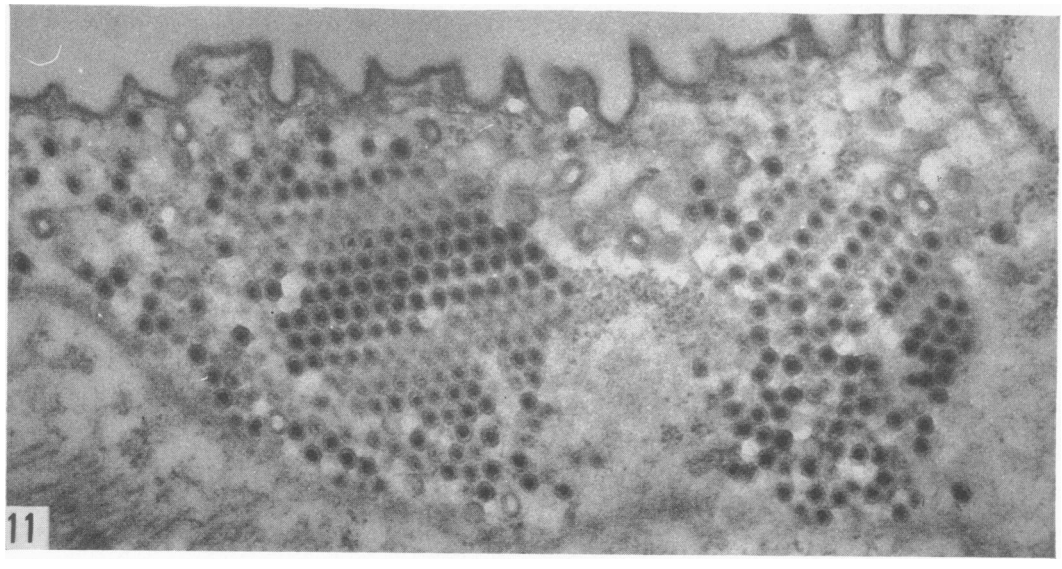


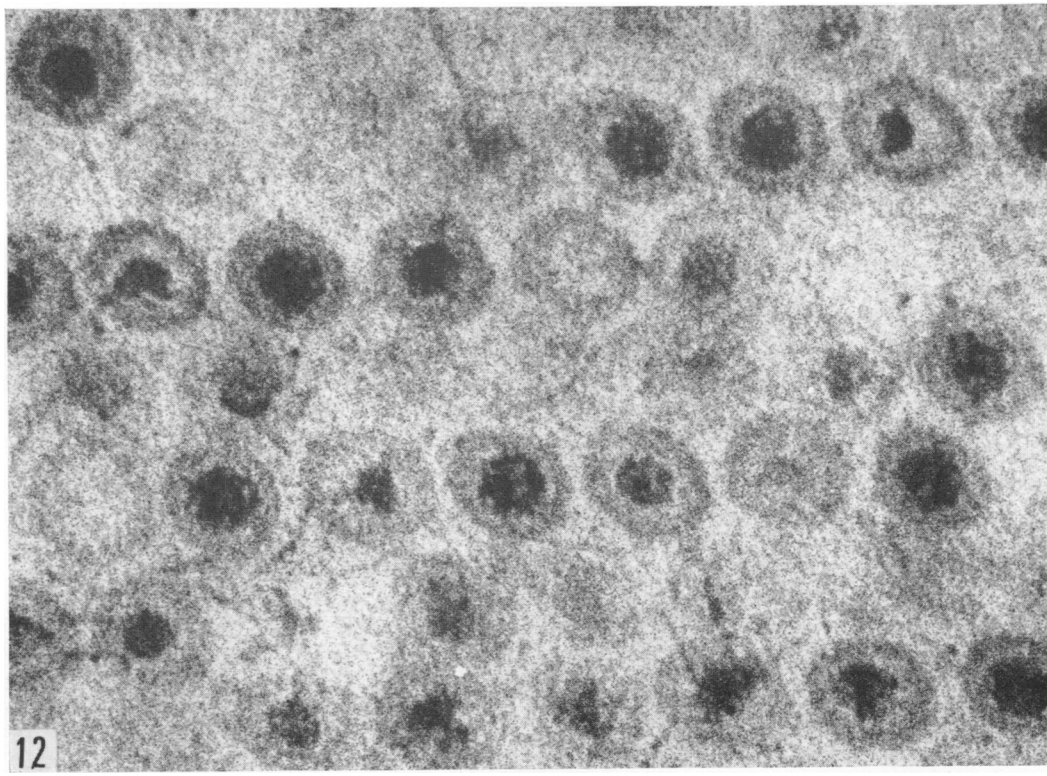
FIG. 7. *Part of a crystal in a mycetome.* × *75,000.*

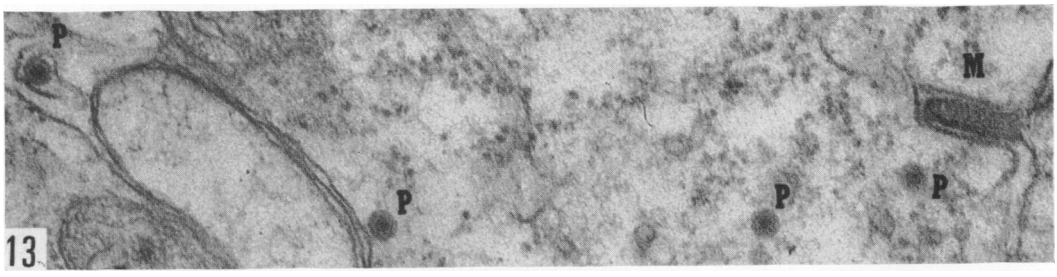
FIG. 8. *Part of a crystal in an ovary.* × *75,000.*

FIG. 9. *Part of a crystal in an intestine cell of Peregrinus maidis Hawaii strain, 35 days after injection with a suspension of intestines of the Maracay strain.* × *75,000.*

FIG. 10. *Group of particles in an intestine cell of P. maidis Hawaii strain, 16 days after injection with a suspension of the Maracay strain.* × *75,000.*

FIG. 11. *Crystals and groups of particles in an intestine cell of P. maidis Hawaii strain, 35 days after injection with a suspension of intestines of the Maracay strain.* × *50,000.*





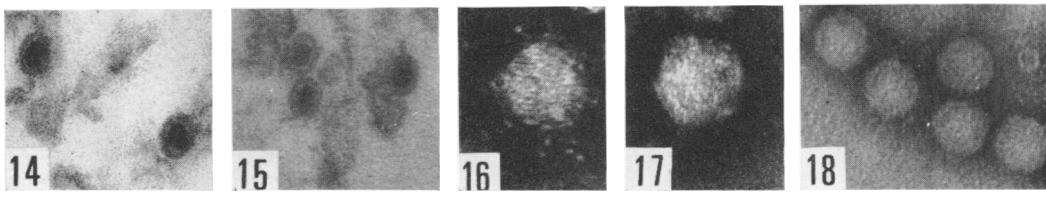


FIG. 12. *Part of a crystal at higher magnification demonstrating the weakly contrasted envelope and the highly osmiophilic core.* × *200,000.*

FIG. 13. *Part of an intestine cell showing some particles of the Peregrinus virus (P) in the vicinity of a rod-shaped particle of maize mosaic virus 1 (M).* × *75,000.*

FIG. 14 and 15. *Particles in a pellet of a purified suspension of intestines.* × *100,000.*

FIG. 16 and 17. *Bigger particles with damaged envelopes from a purified suspension of intestines.* × *200,000.*

FIG. 18. *Smaller particles from a purified suspension of intestines.* × *250,000.*

6) were seen; in other vesicles many densely packed particles and little lamellated material (Fig. 4) were observed. Electron-transparent, round structures with a diameter similar to that of the particles were recognized next to (Fig. 2) and in the crystals (Fig. 7, 8, 12), in the proximity of groups of particles (Fig. 11), and within the vesicles (Fig. 4, 6).

In several cases, the virus-like particles were found together in the same cell with the elongated particles of maize mosaic virus I (Fig. 13).

The particles appeared to have a polygonal form. In particles from embedded insect organs as well as from pellets, a weakly contrasted envelope and a highly osmiophilic core were recognized (Fig. 2–12, 14, 15). Often a clear space was observed between the envelope and the core. The particles measured 54 ± 9 mμ (Fig. 19) and the dense cores 27 ± 8 mμ. In suspensions A and B, some particles (Fig. 16, 17) had a diameter of 60 to 65 mμ, and many particles (Fig. 18) had a diameter of approximately 33 mμ.

Transmission of the particles. No particles could be found in nine intestines from insects of *P. maidis* Hawaiian strain 8 and 12 days after injection of suspension A from the Maracay strain. However, few scattered particles (Fig. 10) were observed in 3 of 14 intestines 16 to 22 days after injection. Many scattered particles (Fig. 9, 11) and many crystals (up to 15 in the cutting plane of one cell) were observed in 14 of 18 intestines 22 to 38 days after injection. Altogether, intestines of 16 of 30 injected insects contained virus particles (Table 1).

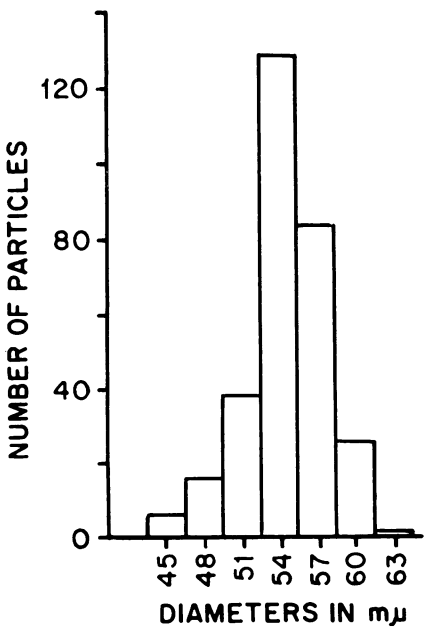


FIG. 19. *Distribution of diameters of particles in crystalline arrangement.*

In 13 *P. maidis* insects of the Hawaii strain, injected with suspension A of the Hawaiian strain, no particles were found 18 to 43 days after injection.

Intestines of 14 insects of *P. maidis* Hawaii fed 35 to 55 days on corn plants, which previously served as food for males of the Maracay strain, did not contain the virus-like particles.

In tissue cultures, no differences were observed between monolayers inoculated with suspension A from Maracay strain and suspension A from Hawaii strain. Mice inoculated with suspension A of both strains also remained without symptoms.

Corn variety Laguna and the other plants on which *P. maidis* Maracay had fed revealed no abnormality during 3 months of observation.

DISCUSSION

It is concluded that the described particles in the insect organs are virus particles, because (i) their structure and crystallization pattern are very similar to those of known viruses, and (ii) they can be trasmitted to and multiply in virus-free insects. The occurrence of the particles in injected *P. maidis* is an actual transmission and not an effect of stress, since insects injected with suspensions of virus-free intestines did not reveal the particles. Moreover, the development of virus in the Maracay strain is not a consequence of the conditions in the greenhouse because the virus was also found in *P. maidis* collected in the field.

In the intestines of injected insects, the incubation period seems to be at least 12 days. At incubation times of 31 to 38 days, 85% of the insects contained the virus-like particles. This is a higher percentage than in naturally infected *P. maidis* (53%).

The clear space between the envelope and the core in most embedded particles may be due to loss of material or a shrinkage effect or both. The smaller particles in suspensions seem to be damaged forms of the virus, because (i) they occur only in suspensions of *P. maidis* Maracay strain, (ii) they are found in the suspensions of which the pelletted part showed the bigger particles, and (iii) they are never seen in sectioned intestines. The envelope of the particles may be highly susceptible to PTA and uranyl acetate. Similarly, in *Tipula* iridescent virus (TIV) from *Bibio marci* L., rupture of membranes occurred when neutralized PTA was added (17). Furthermore, in *Sericesthis* iridescent virus (SIV), damage of the membrane after PTA contrasting was observed (3).

The polygonal outline of the particles with a weakly contrasted envelope and an osmiophilic core are similar to the appearance of sectioned particles of maize rough dwarf virus (6, 18), SIV (1, 3), and TIV (16, 17, 19). However, all these viruses have larger diameters. A further similarity of the virus from *Peregrinus* with maize rough dwarf virus (18), SIV (1), and TIV (16, 17) is indicated by the electron-transparent, round structures in the vicinity of complete particles. At present, it cannot be decided whether these structures are incomplete forms of particles lacking the dense core. Another similarity of the newly discovered virus with earlier described ones is the occurrence of particles in vesicles (5) and of lamellated material (15). No effort was made to interpret the origin of the vesicles nor the role of the lamellated material.

The virus from *P. maidis* could be a plant-insect virus, systemically invading the insect, but causing no detectable disease as in the case of wound tumor virus (15), rice dwarf virus (4), and maize rough dwarf virus (7, 18). The cicadellid insect vector of the latter virus and *P. maidis* share the same food plant (6, 18). However, *Laguna* corn, which is highly susceptible to maize rough dwarf (Harpaz, *personal communication*), did not become infected with the virus of *P. maidis*.

Virus-like particles have been found earlier in organs of the aphids *Myzus persicae* (Sulz.) (11, 13) and *Rhopalosiphum maidis* (Fitch) (12) and the leafhopper *Endria inimica* Say (9). In some instances, similar particles were detected in symptomless food plants of the insects (13), whereas in other instances no particles were found in the plants (9, 11, 12). In our experiments, the particles were not observed in corn, which is the main food plant of *P. maidis*. No other plants, exposed to *P. maidis*, showed signs of disease. These plants were not checked for presence of the particles, because it seems very improbable that the virus would be transmitted to plants on which the insect would not likely breed or would die after few days. Consequently, as no plant host of the virus infecting *P. maidis* is known and as none has been found among 21 plants of nine families tested, the virus is considered at present as an insect virus causing latent infection. It is not believed to belong to the arboviruses because of the absence of cytopathic effects in tissue cultures of cell lines of vertebrates and because of its noninfectivity to suckling mice. Experiments are being carried out regarding the possible transovarial infection and other ways of natural transmission.

The described virus occurs in *P. maidis* independently of the presence of maize mosaic virus I. It seems that there is not even a distant connection to maize mosaic virus, because on the one hand maize mosaic virus occurs also in Hawaii (2) and on the other hand in our experiments *P. maidis* from Hawaii transmitted maize mosaic virus. Investigations are under way to find out whether there is any influence of the virus of *Peregrinus* on the transmission of maize mosaic virus.

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