ELECTRON MICROSCOPY OF POTATO YELLOW-DWARF VIRUS

by

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None of the group of plant viruses transmitted by leafhoppers has been obtained in purified form. This is probably due, in part at least, to their instability in vitro and to the difficulty or impossibility of transmitting them from plant to plant by present juice-inoculation techniques. Nevertheless, the many indications that they differ fundamentally from the more easily transmitted plant viruses make it important to continue efforts at purification and recognition of their infectious particles.

Previous studies[1] of the rates at which infectiousness is sedimented in the centrifuge have demonstrated that the elementary particles of two of these viruses [potato yellow-dwarf virus (*Aureogenus vastans* H. var. *vulgare* Black) and aster yellows virus (*Chlorogenus callistephi* var. *vulgaris* H.)] are much heavier than those of the plant viruses hitherto investigated in purified form. Electron micrographs made of infectious centrifugates of these viruses, however, failed to demonstrate particles that might represent the virus.

We have recently carried out a new series of ultracentrifugal fractionations of juices from plants infected with a number of different leafhopper transmitted viruses. Electron micrographs were made of metal-shadowed preparations taken at the various steps of these fractionations. Results of this preliminary survey suggested that a careful study of the juice from *Nicotiana rustica* L. infected with potato yellow-dwarf virus was most likely to reveal the elementary particles of a leafhopper virus.

Accordingly, in further experiments, leaves of *N. rustica* thoroughly infected with potato yellow-dwarf virus were cooled to O° C and the virus kept as near this low temperature as possible throughout the following treatment. The leaves were ground in the presence of an alkaline buffer which brought the expressed juice to a p_H of ca 6.5. This was achieved by adding ca ½ ml of a ¼ molar Na_2HPO_4 solution to each gram of leaf. Juice, expressed from the resulting pulp by squeezing through several layers of cheese cloth, was cleared of intact chloroplasts and large fragments of leaf tissue by 10 minutes' run in an angle centrifuge at 2 000 r.p.m. Smaller fragments were then eliminated by sedimentation at 7 000 r.p.m. for 20 minutes in the ultracentrifuge. The clarified supernatant prepared in this way was run at 18 000 r.p.m. for an hour to sediment the virus activity and yield large nearly transparent but deeply green pellets. These were dissolved in water and put through a second series of low and high speed centrifugations. It has been considered important not to sediment the virus at a higher speed or for a longer time than necessary to precipitate the activity; the foregoing regime of 18 000 r.p.m. (ca 25 000 times gravity) for an hour is probably more than the optimal.

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As controls equal amounts of healthy *N. rustica* leaves were ground and subjected to the same ultracentrifugal treatment, the amount of Na_2HPO_4 added being such as to yield a juice of p_H 6.5. Large pellets were thrown down from this juice as well as from the juice of diseased leaves. Solutions of the final pellets from the second ultracentrifugation, whether from infected or healthy plants, have gradually precipitated a dark material on standing. Since this obviously, in part at least, results from decomposition of macromolecular material other than the virus the solutions have been examined after standing in the ice box, as well as immediately after being prepared.

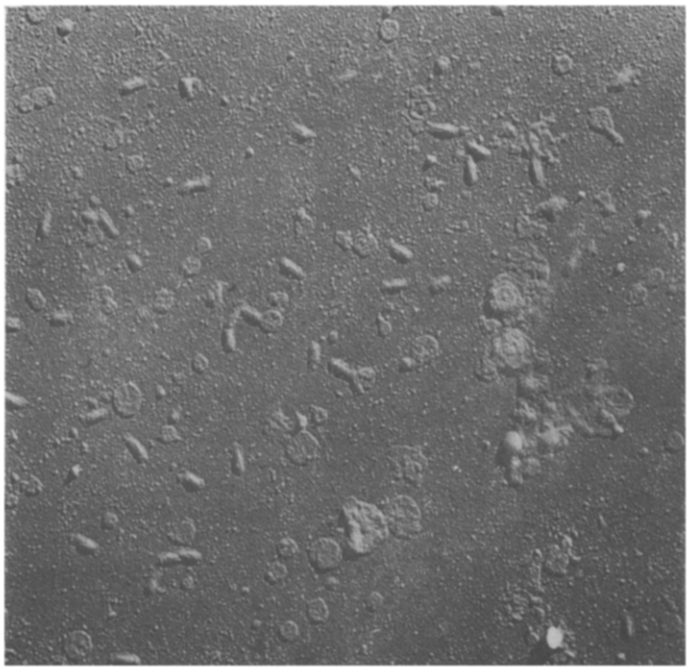

Fig. 1. A typical field in an electron micrograph of ultracentrifugally fractionated juice of leaves of *N. rustica* diseased with the potato yellow-dwarf virus. The short rod-like objects which in the picture are about 3 mm long and 1 mm in diameter have not been found in similar preparations from healthy *N. rustica* leaves. Chromium shadowing. Magnification, ca 19 500 times.

For electron microscopy a drop of one of these solutions was serially diluted with either pure water or with water containing one part in 500 of calcium chloride and 0.1 % formalin. Micro drops of these dilutions were dried on the usual collodion-covered grids, shadowed obliquely with chromium and examined with an RCA type EMU microscope. In each of three separate experiments, the purified solutions from infected material have yielded fields of which Fig. 1 is typical. These contain many short rod-like forms that are ca 0.2 micron long and ca 0.05 micron in diameter; they have never been seen in preparations made from healthy leaves of *N. rustica*. Fields from healthy preparations have been strewn with flat disks, irregularly sized fragments and many very small particles. As can be seen in the figure they appear along with the short rods in the diseased preparations. The irregular fragments are probably membrane and the disks grana from disintegrated chloroplasts; the small particles, of macromolecular dimensions,

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have the same size as the fine detail seen within the grana of intact chloroplasts. After standing for several weeks in the ice box, the rods could no longer be found in ultracentrifuged preparations which initially were rich in them.

Examination of *N. rustica* leaves infected with the milder New Jersey strain of potato yellow-dwarf virus (*A. vastans* H. var. *agalliae* Black) has also revealed the rod-shaped bodies but in greatly reduced numbers. Healthy leaves were again negative.

Because they have always been seen in partially purified solutions from infected leaves and never from healthy material and because they are sedimented under conditions that are known to sediment the virus of potato yellow dwarf, the short rod-like bodies of Fig. 1 may well be the elementary particles of this virus. The work is being continued to develop more evidence bearing on this point.

SUMMARY

Electron micrographs have been prepared of an ultracentrifugally fractionated suspension from the juice of *N. rustica* leaves diseased with the potato yellow-dwarf virus. These show bodies that have not been seen in similarly treated juice from healthy plants and that well may be the elementary particles of this leafhopper propagated virus.

RÉSUMÉ

Des microphotographies électroniques ont été faites, représentant une suspension, fractionnée par ultracentrifugation, de jus de feuilles de *N. rustica* infecté du virus nain jaune de la pomme de terre. On y voit des corpuscules qui n'existent pas dans le jus de plantes saines et qui pourraient bien être les particules élémentaires de ce virus qui se propage par les pucerons.

ZUSAMMENFASSUNG

Elektronenmikrographien einer mit der Ultrazentrifuge fraktionierten Suspension aus dem Saft von *N. rustica* Blättern, die durch den Kartoffel-Gelbzwergvirus erkrankt waren, wurden verfertigt. Diese zeigen Teilchen, die in ähnlich behandeltem Saft aus gesunden Pflanzen nicht wahrgenommen wurden und sehr gut die Elementarteilchen dieses, durch Heuschrecken verbreiteten, Virus sein könnten.

REFERENCES

[1] L. M. BLACK, Partial purification of potato yellow-dwarf virus, *Phytopath.*, 31 (1941) 3; Some properties of aster yellows virus, *ibid.*, 33 (1943) 2; also unpublished observations with W. M. STANLEY.

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