SOME CONDITIONS AFFECTING THE INTRACELLULAR ARRANGEMENT AND CONCENTRATION OF TOBACCO MOSAIC VIRUS PARTICLES IN LOCAL LESIONS

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ABSTRACT

Tobacco mosaic virus particles were found in small packets and in small numbers, with the electron microscope, in necrotic leaf cells of *Nicotiana glutinosa* when the samples were fixed in glutaraldehyde and postfixed in OsO_4, and the sections were stained with heavy metals. The numbers and size of the virus packets were increased greatly when the leaves were detached from the plant after inoculation. Assay of concentration showed that detachment resulted in a 30-fold increase of virus. A similar increase in the number of virus particles detected by electron microscopy was produced by keeping inoculated plants at an air temperature of 26°C. A still greater increase in concentration was effected by incubating detached inoculated leaves at 26°C. Moreover the arrangement of virus particles in these cells resembled that of a systemic virus infection. Cells in local lesions of *Chenopodium amaranticolor* contained large numbers of virus particles both as packets and in the loose arrangement characteristic of systemic infection. Neither the number of particles nor their arrangement was affected in this host by detaching the leaf or by changing the air temperature. It is suggested that there may be two types of localized virus infections, one of which produces virus in low concentration and is amenable to changes in virus concentration and arrangement as a result of environmental manipulation.

INTRODUCTION

There has been, in recent years, controversy over the visualization of virus particles within the cells of necrotic lesions produced by tobacco mosaic virus (TMV) in a variety of hosts. The first detailed study of the cells in a localized infection by TMV on leaves of *Nicotiana glutinosa* (26) failed to reveal the presence of virus particles at any stage from 0 to 78 hr after infection, but Carroll and Shalla (2) subsequently demonstrated their presence unequivocally and in large numbers in local lesions on *Datura stramonium*.

More recently, Milne also demonstrated their presence in *N. glutinosa* (11) and, with considerably less difficulty, in *Chenopodium amaranticolor* (10). Milne attributed part of his success in finding the particles in *N. glutinosa* to improved preparative techniques. The number of virus particles per cell in a local lesion of *N. glutinosa* is not in dispute, since Milne calculated (11) a virus content substantially in agreement with our estimate of about 10^8 particles (25).

However, in our studies on virus multiplication in localized infections, several factors were found to influence the amount of virus present in the infected cells which, together with the improved preparative methods now in general use, have

TABLE I

TMV Concentration

Microgram per milliliter of *N. glutinosa* Leaf Extract*

After inoculation	Attached leaves	Detached leaves
hr		
0	0	0
12	0	0
24	0	11.2
36	0	179.2
40	2.8	—
48	11.2	358.4
60	11.2	358.4
72	0‡	179.2

* The figures given are calculated from the highest dilution of the antigen, i.e. the leaf extract, that produced an identifiable precipitation line in the agar-gel after immuno-osmophoresis. This dilution end point was equivalent to 0.28 μg purified TMV.
‡ Failure to detect virus serologically at 72 hr is probably a result of too low a concentration in the extract, although TMV can be seen at this time with the electron microscope.

disclosed conditions responsible for ease in visualizing the virus particles with the electron microscope. These conditions are reported here.

MATERIALS AND METHODS

Serological Assay of Virus Content

Leaves on plants of *N. glutinosa* were inoculated with a purified TMV preparation so concentrated that the leaves became covered with lesions. Some of the inoculated leaves were left attached to the plants, while others were removed and floated on distilled water, or placed on wet filter paper in Petri dishes. All were placed under 500 footcandles of light for 18 hr per day, at a room temperature of about 21°C.

Samples of attached and detached leaves were removed every 12 hr from 0 to 72 hr, with additional samples at 40 hr from attached leaves only. The samples were ground with a mortar and pestle, and strained through cheesecloth. The extracts were then assayed for virus concentration by the immuno-osmophoretic method (13), using 0.1 ml of the extract in each test. Preliminary determinations made with mixtures of purified TMV, the concentration of which had been determined spectrophotometrically (19), and healthy *N. glutinosa* leaf extracts showed that the end point for production of a precipitation line in these experiments was 0.28 μg TMV. Calculations of TMV concentrations in the infected leaf extracts were therefore based on this end point, and the results of the assay were expressed as microgram TMV per milliliter of leaf extract. In cases where no precipitation line was produced at the highest concentration of leaf extract (Table I), additional checks were included by concentrating the leaf extract 10 times after high-speed centrifugation.

Electron Microscopy

Leaves on plants of *N. glutinosa* and *C. amaranticolor* were inoculated with TMV as described. Both the leaves attached to the plants and 22-mm discs cut out of other leaves and floated on distilled water were kept in a chamber with 500 footcandles of light for 16 hr per day, at 21°C, until sampled. This was repeated at 26°C for both hosts, and again at 15°C for *C. amaranticolor* alone.

The *N. glutinosa* leaves and discs were sampled 40 and 72 hr after inoculation, while the *C. amaranticolor* were sampled at 64 and 136 hr, at 19 days in the 21°C chamber, and at 19 days only in the 26°C and 15°C chambers. The *C. amaranticolor* leaves were not entirely covered with lesions, as in the case of *N. glutinosa,* and samples were taken from the necrotic areas, from the yellow areas bordering the lesions, and from green areas between the lesions, as well as from green areas in the upper, uninoculated portions of the plants. All the *C. amaranticolor* leaves and discs were also sampled in these areas for infectivity tests on *N. glutinosa.*

The samples for electron microscopy were leaf pieces 2–3 mm², which were fixed in 5% glutaraldehyde in phosphate buffer, pH 7.2, at room temperature for 1½ hr. After rinsing for 30 min with distilled water, they were postfixed for 1 hr in 1% Palade's OsO_4 solution, pH 7.2. They were rinsed three or four times in distilled water and were dehydrated in graded dilutions of ethanol, followed by treatment with propylene oxide, and finally embedded in Epon 812 (8). The thin sections were cut with glass knives and were stained with uranyl acetate and lead citrate.

RESULTS

Serological Assay of Virus Content

The average results of five separate experiments with attached leaves and of two experiments with detached leaves are given in Table I. In attached leaves, the concentration of TMV increased from 2.8 μg/ml, when the virus was first detected at 40 hr, to a maximum of 11.2 μg/ml at 48–60 hr, after which it was no longer detectable at 72 hr. Further samples of attached leaves at 84, 98, and 119 hr after inoculation, in

separate experiments, did not yield detectable precipitation lines.

In detached leaves, TMV was detectable at 11.2 μg/ml 24 hr after inoculation. A line appeared in the 12 hr sample, but it was vague and diffuse, and we are not certain that it was really produced by a low concentration of less than 2.8 μg TMV per milliliter. There was a sharp rise in TMV concentration to a maximum of 358.4 μg/ml between 48 and 60 hr, followed by a decline to 179.2 μg/ml at 72 hr. Thus the maximum TMV concentration attained in the detached leaves was about 30 times as great as the maximum found in the attached leaves.

At this point we became concerned about the possibility that such a large discrepancy might have been caused by the differing amounts of virus extractable from attached and detached leaves. A difference was possible because the detached leaves were turgid and fleshy, while the attached leaves were wilted, especially at the end of the sampling periods, when the infected tissues were dehydrated and hardened and the cells presumably were filled with polymerized polyphenolic compounds (12, 26).

To determine the effect of this wilting and hardening of the tissues on the recovery of TMV, two leaves on each of two *N. glutinosa* plants were inoculated with TMV. The leaves on one of the plants were covered with plastic bags containing a small amount of water in a pool that did not touch the leaves, while the leaves on the other plant were left uncovered. After 72 hr, the covered leaves were turgid, and the lesions were clearly seen but they were not so necrotic as those in the uncovered leaves which were wilted and dehydrated. When the inoculated leaves were detached and weighed, the covered leaves weighed almost twice as much as the uncovered ones, 1.56 and 0.85 g, respectively. Each leaf was then ground separately with an equal volume of water, and the extracts were tested for infectivity and dilution end point, on *N. glutinosa*.

At higher concentrations, the extracts from the covered leaves produced nearly twice as many lesions as those from uncovered leaves, although the dilution end points were identical. It was clear that not much more than one-half the virus would be lost as a result of hardening and wilting of the attached leaves. The difficulties of extraction could not, therefore, account for the observed 30-fold increase in virus concentration in detached leaves.

Electron Microscopy

N. glutinosa, ATTACHED, 21°C

The necrotic cells in the leaves left attached to the plants and kept at 21°C were examined extensively, because of our previous inability (26) to find virus particles in such cells. In the present experiments, packets of particles about 285 mμ long that resembled TMV particles were found, but only in five cells out of 96 examined in 15 sections. These sections were from three different lesions, and each lesion was cut at several levels. The virus packets were very small, consisting of about 25 particles in a single row (Fig. 1), or occasionally in a double row. Only rarely was a larger accumulation found.

Another loose arrangement of particles was also seen, always close to a chloroplast or to a starch grain left after the disintegration of the chloroplast that had contained it, and similar to that shown in Fig. 3. There was no clear difference between the number of particles seen in the 40- and 72-hr samples.

N. glutinosa, DETACHED, 21°C

At least one-half of the cells examined contained TMV particles, usually in long single or double rows, or in triple rows (Fig. 2), as well as the loose, random arrangement of particles close to starch grains (Fig. 3). There was no apparent difference between the 40- and 72-hr samples. The number of particles and packets seen clearly substantiated the increase in TMV concentration established by serology.

N. glutinosa, ATTACHED, 26°C

A considerable difference was seen between the 40- and 72-hr samples. The 40-hr samples contained many cells in which pathological damage was extensive but was not at the final stages of necrosis (Fig. 4). No virus particles were found in these damaged cells, but small packets were found in those cells that had become totally necrotic.

Nearly every cell in the 72 hr sample was necrotic, and virus was found in at least as many cells as in the detached leaves at 21°C. The virus aggregates were either linear or in large masses (Fig. 5) of the kind that are found in systemic

virus infections (9). We are not certain whether this difference between the 40- and 72-hr samples was the result of the conditions of this experiment, but it appeared that large deposits of virus were not readily detectable until most of the cells were totally necrotic.

N. glutinosa, DETACHED, 26°C

Both the 40- and 72-hr samples showed the presence of large amounts of TMV particles. Nearly every cell contained some virus, arranged in single or double, long aggregates, or in large masses which showed a crystalline array in cross-section (Fig. 6). Virus particles, either loosely arranged or closely packed, were often closely apposed to starch grains, or they filled the vacuole in which the starch grain lay. Frequently virus particles were found at the edges of former vacuoles, within the cytoplasmic membrane which bulged out to form a vesicle, similar to those found in *C. amaranticolor* and seen in Fig. 9.

There were frequently found regular crystalline inclusions within membrane-bounded granular organelles (Fig. 7) which were distinct from the virus particles in the cells (Fig. 7, arrow). Crystals of similar appearance have been observed in healthy *N. glutinosa* leaves exposed to high temperatures (5, 21).

C. amaranticolor

There was no essential difference among any of the samples taken from the lesion areas of TMV-infected *C. amaranticolor* leaves and discs. The degree of necrosis, with the accompanying electron-opaque material, and complete cell collapse was lower in the 64- and 136-hr samples than in *N. glutinosa* cells after 72 hr. TMV particles were seen in large concentrations in most of the cells and were arranged in two ways. The first arrangement was in compact, multilinear aggregates, in herringbone, and in straight end-to-end patterns (Fig. 8). This arrangement was the usual formation in necrotic cells of *C. amaranticolor*, and resembled that in necrotic cells of *N. glutinosa*. In the second arrangement, where the cells were relatively undamaged, as in the yellow areas bordering the lesions, large accumulations of virus particles were found, usually in loose, and occasionally in compact, aggregates (Fig. 9). In some cells both arrangements were seen in different parts of the cell. Outpouchings of the cytoplasmic membrane to form vesicles filled with TMV particles were frequently found, sometimes ruptured (Fig. 9, arrow), probably having ruptured during preparation of the samples.

In Fig. 8 there can also be seen elongated bodies (*DB*) that probably correspond to the "filaments" observed by Milne (10) and Shalla (16) with TMV infections, and to the "dense bands" formed by bean yellow mosaic virus in broad bean (27), and by other rod-shaped viruses (4).

Similar observations were made in the cells of attached leaves and detached discs, at all three

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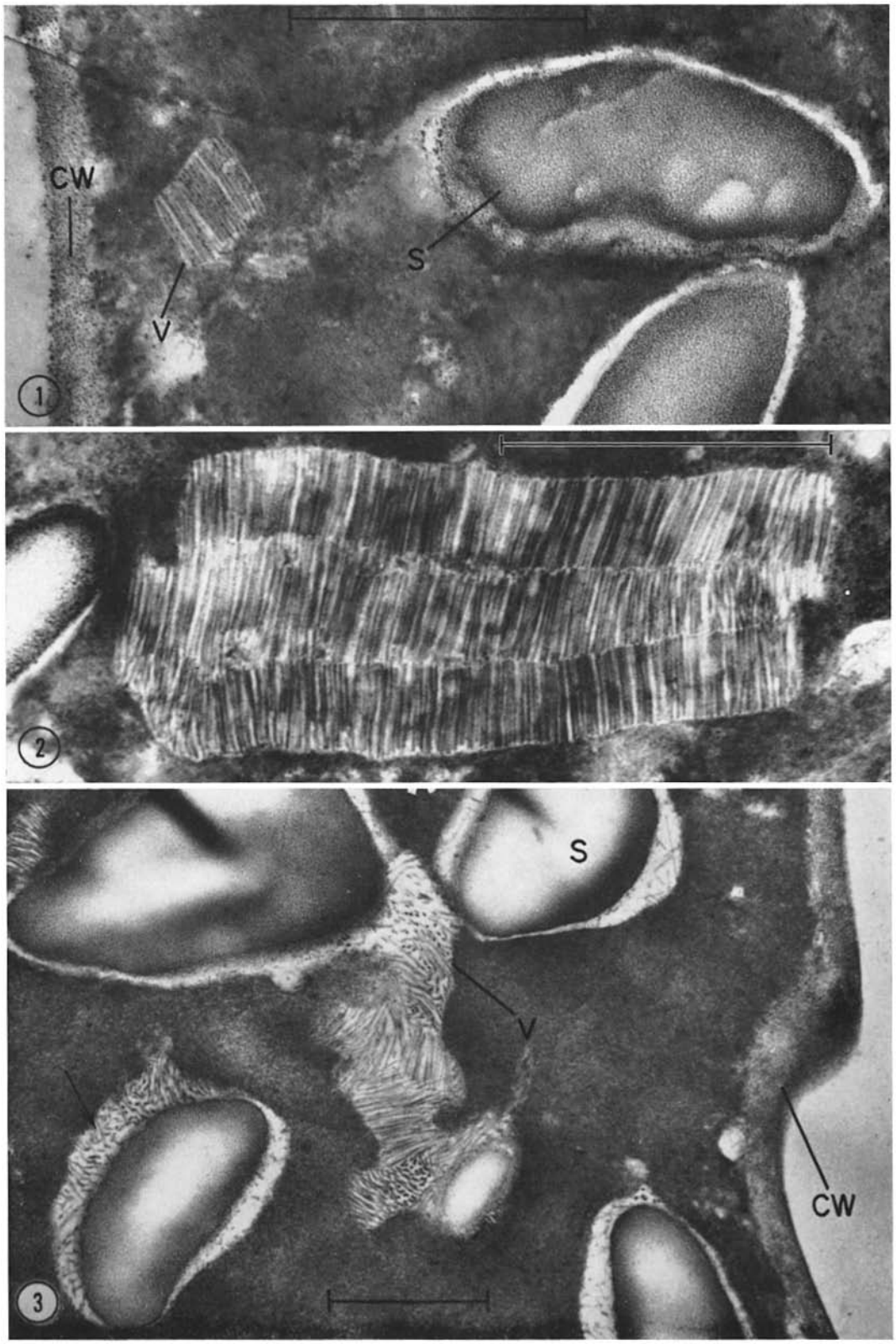
Abbreviations

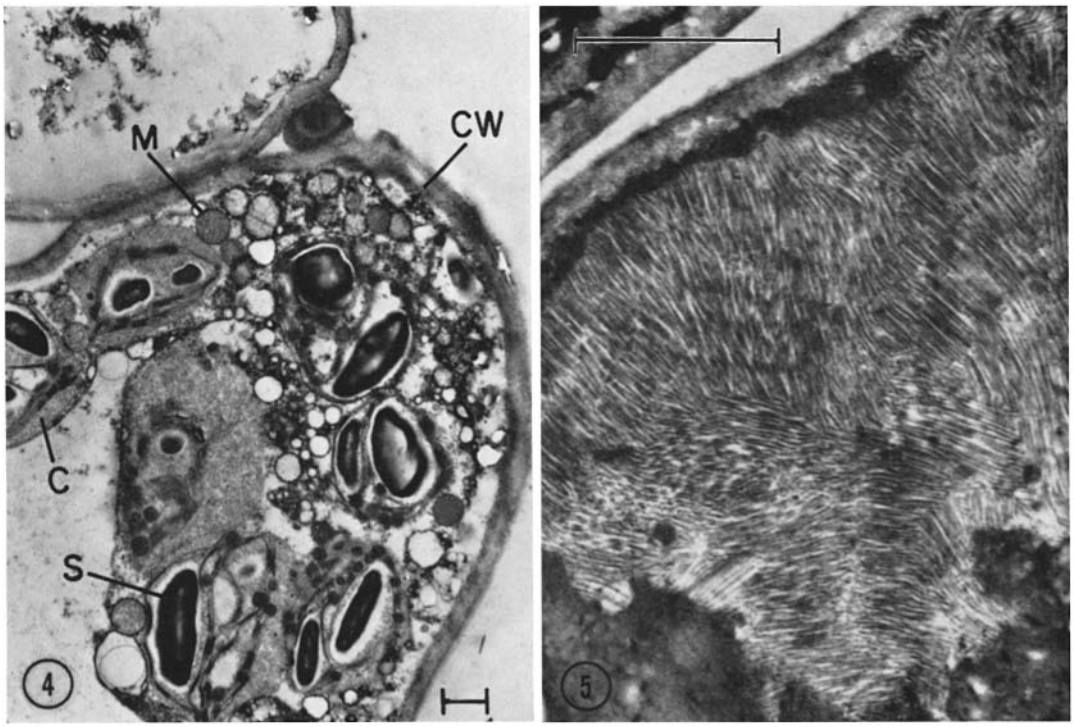
C, chloroplast	*CyM* cytoplasmic membrane
CI, crystalline inclusion body	*DB*, dense band
CL, chloroplast lamellae	*M*, mitochondrion
CV, cell vacuole	*S*, starch grain
CW, cell wall	*V*, virus (TMV) particles
Cy, cytoplasm	

The scale on each electron micrograph indicates 1 μ.

FIGURE 1 Portion of a necrotic cell of *N. glutinosa* leaf left attached to plant, at 21°C; 40 hr after inoculation with TMV. The virus particles are arranged as a small compact aggregate. × 46,000.

FIGURES 2 and 3 Portions of necrotic cells of *N. glutinosa* leaves detached from plant, at 21°C; 40 hr after inoculation. Fig. 2, the TMV particles are arranged here in a compact aggregate of three rows, side-by-side. Fig. 3, TMV particles close to starch grains and in the vacuole surrounding the grains (arrow). Fig. 2, × 51,000; Fig. 3, × 25,000.





FIGURES 4 and 5 Portions of cells of *N. glutinosa* leaves attached to plant, at 26°C. Fig. 4, a cell that is damaged, 40 hr after inoculation with TMV, but is not yet necrotic. TMV particles were not found in such cells. Fig. 5, characteristic TMV aggregate, 72 hr after inoculation, with many particles in each large group. Fig. × 4, 5,500; Fig. 5, × 27,000.

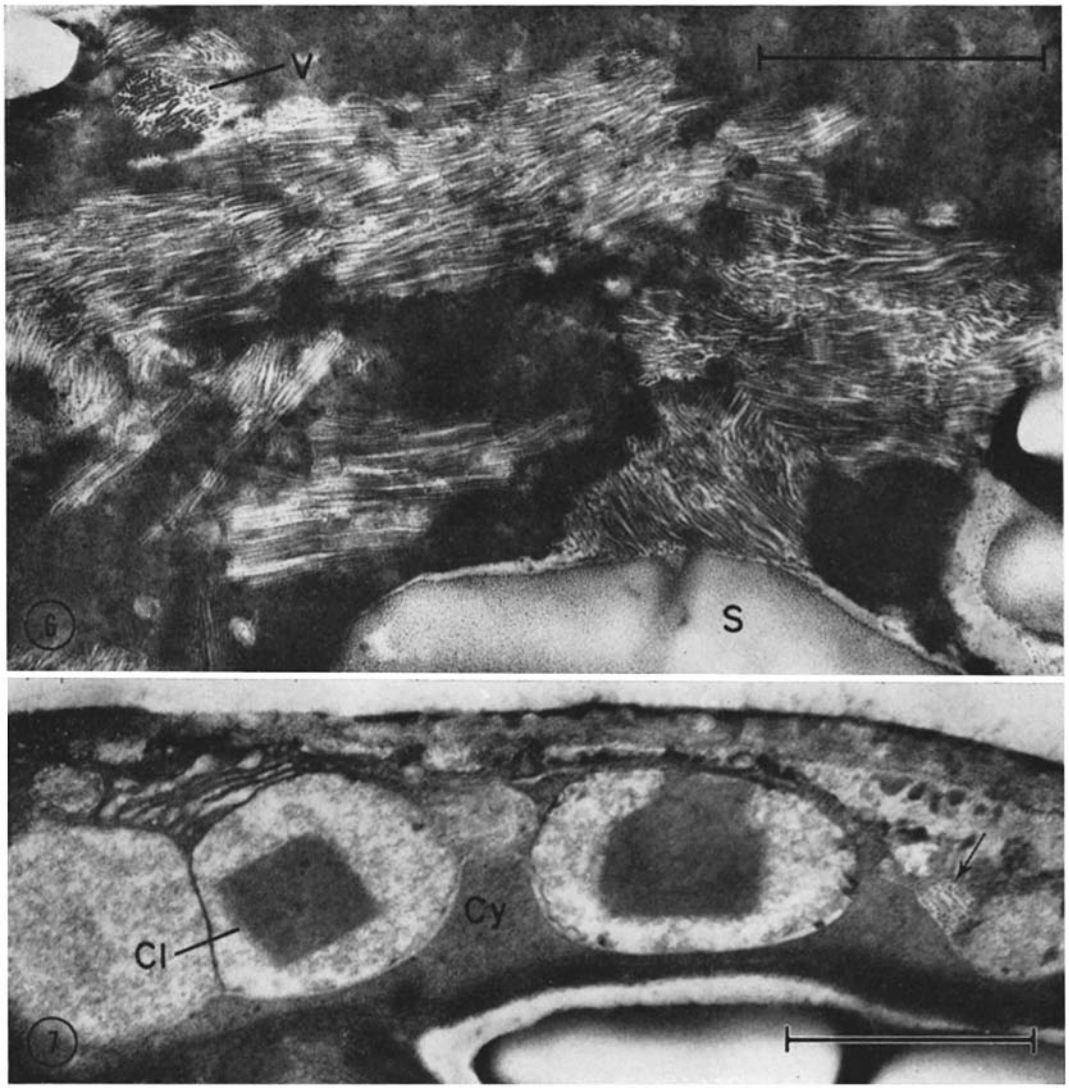
temperatures, and at all times of sampling, so that very large numbers of particles in the several arrangements described were seen. However, virus particles were not found in the green areas between lesions, or in the upper, uninoculated leaves. These areas were not infectious when tested on *N. glutinosa*, whereas samples from the yellow and necrotic areas were.

DISCUSSION

Preparative techniques are undoubtedly a factor in detecting virus particles, as Milne correctly surmised (11). Our earlier study on local lesions in TMV-infected *N. glutinosa* (26), which was completed early in 1961, depended upon OsO_4 fixation alone and sections that were either unstained or stained only with uranyl acetate. Thus, until fixation and staining techniques were available to enhance contrast (1, 6, 14, 15, 17, 20), it was difficult to locate virus particles in the electron-opaque necrotic cells.

Serological data (Table I) and electron microscopy show clearly that in *N. glutinosa* the amount of TMV present and observable is governed by at least two factors. The first factor, the detaching of the infected leaf from the plant, results in an increase of virus concentration of up to 30 times that in attached leaves. The second factor, the increasing of the air temperature, also brings about an increase in virus concentration which, as judged from the ease of finding particles in the cells and from the number of particles seen in each cell, is at least comparable to the effect of detachment at room temperature. Data based on the respiration of TMV-infected *N. glutinosa* leaves at high temperatures (22) have indicated that an increase in virus concentration must occur.

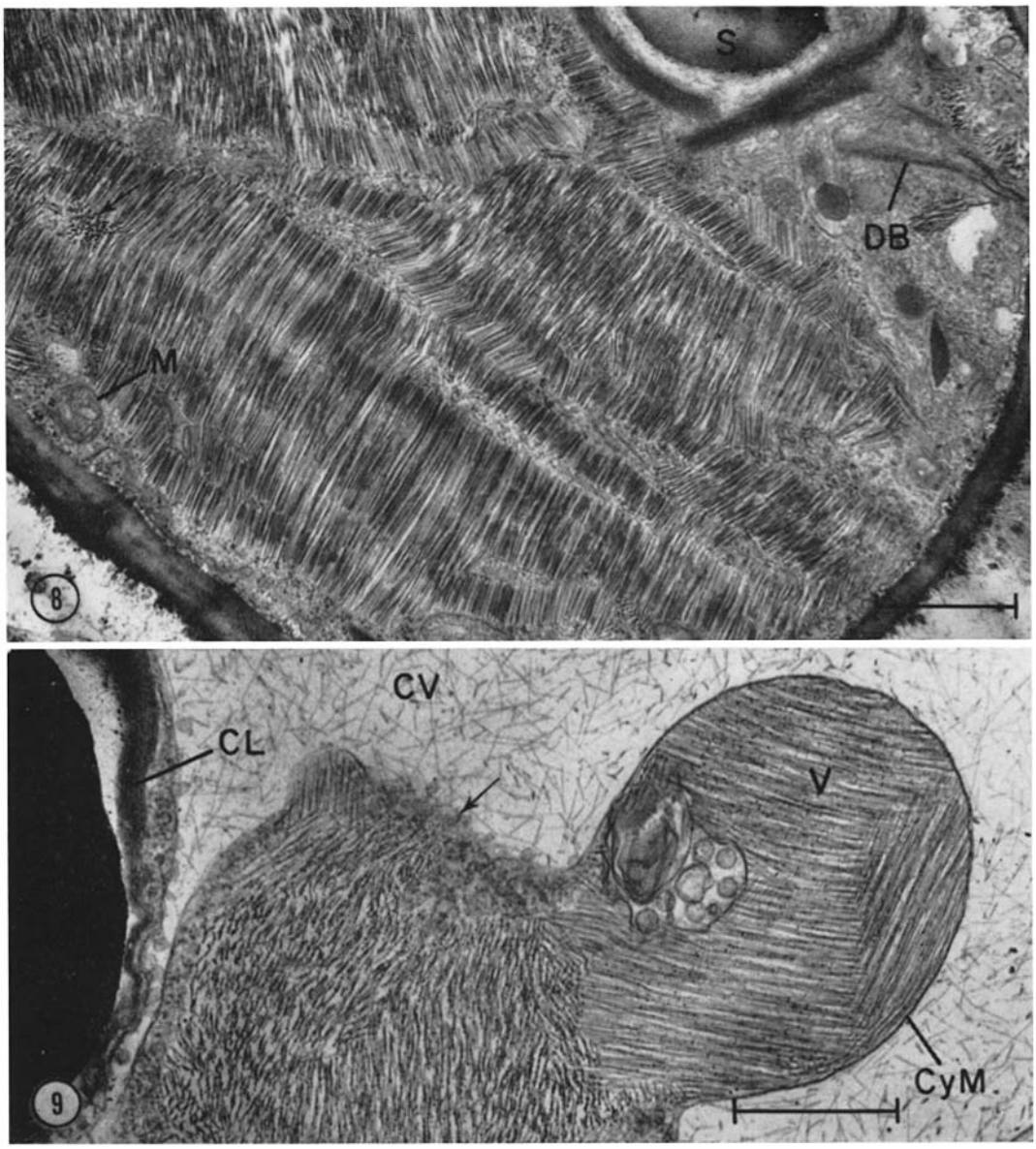
When the two factors are applied together, as in detached leaves at 26°C, the infection takes on a new character, since the amount of virus present and the arrangement of the particles in the cells are now no longer characteristic of a localized infection but are similar to those of virus in systemic TMV infections (7, 9, 16). We have already



FIGURES 6 and 7 Portions of cells of *N. glutinosa* leaves detached from the plant, at 26°C; 40 hr after inoculation with TMV. Fig. 6, large masses of TMV particles found in nearly every cell. A crystalline array of the particles can be seen where the aggregate appears in cross-section. Fig. 7, crystalline inclusions within a membrane-bounded granular body. A few TMV particles in cross-section are also seen (arrow). Fig. 6, × 37,000; Fig. 7, × 32,000.

suggested (23, 24) that conditions tending to increase virus concentration in local lesions will convert this type of infection to a systemic one. This suggestion is supported by the results reported here.

The characteristic arrangement of TMV particles in compact packets is of interest since we do not know whether the virus is assembled in this pattern prior to the cells' becoming totally necrotic. Since TMV particles have not been found in cells still relatively undamaged, it is possible, as Milne suggests (11), that the shrinkage in cell volume during necrosis is responsible for the formation of these packets. Certainly, the virus particles appear to be quite impervious to the crushing forces that must be present in col-



FIGURES 8 and 9 Portions of cells of *C. amaranticolor* leaf discs, floated on water at 21°C; 136 hr after inoculation with TMV. Fig. 8, portion of cell from yellow area surrounding lesion. Large, compact TMV aggregate is seen in both longitudinal section and cross-section (arrow). Fig. 9, loosely arranged mass of TMV particles. A vesicle extruded into the cell vacuole also contains TMV particles. The virus particles lying freely in the cell vacuole may have broken through ruptures in the cytoplasmic membrane (arrow) caused by sample preparation. Fig. 8, × 19,000; Fig. 9, × 22,000.

lapsing necrotic cells, since finally the only recognizable objects are the starch grains, cell walls, and packets of virus.

The close association of the TMV particles with starch grains cannot be taken as evidence that chloroplasts are the sites of virus synthesis (28), or that the TMV is located within them, which appears to be the case with sugar beet

yellows virus in *Beta* leaves (3). TMV particles were seen, in cells with advanced necrosis, closely apposed to the chloroplast but never inside it (Fig. 3), so that it is likely that the close association with starch grains resulted merely from the destruction of the rest of the chloroplast and the subsequent pressing together of the TMV particles and the starch grains. It would be interesting to know the origin of the electron-transparent vacuoles around the starch grains which resemble vacuoles formed during chloroplast degeneration in yellowing tobacco (18), since TMV particles tend to accumulate loosely in these spaces.

In contrast to *N. glutinosa* with respect to response to environmental factors in the synthesis of TMV, *C. amaranticolor* was found to be comparatively insensitive. Lowering the air temperature to 15°C reduced the number of lesions formed and slowed their development, but it still resulted in numbers of TMV particles per cell that were as great, as estimated by electron microscopy, as in the cells of leaves kept at 21° and 26°C, both attached and detached. The general appearance of the virus particles in the cells of *C. amaranticolor* was similar to that of virus particles in *N. glutinosa*, with the highest concentration of TMV resulting from detachment and 26°C, and differed in no way from the appearance of TMV in systemically infected cells of other hosts such as tobacco and tomato (7, 9, 16). Nevertheless, the infection was still clearly localized since, even after 19 days at 26°C, the TMV did not move into the interlesion areas or uninoculated parts of the plant.

We suggest that there may be two types of localized infections. The first, as in *N. glutinosa*, which under normal conditions produces little virus in the necrotic cells, is responsive to environmental changes, so that, in the extreme, it produces an internal arrangement of virus particles and a virus concentration per cell similar to those produced by the usual systemic infection in other hosts. The second type, as in *C. amaranticolor*, is unresponsive at least to the two environmental factors studied here, and is already similar to a systemic infection in terms of virus particle arrangement and concentration of virus per cell.

As judged by the electron micrographs published by Carroll and Shalla (2) which show TMV particles in lesions, *Datura stramonium* would also appear to fall into the second type of localized infection, although the effects of the environmental changes on this host have not yet been tested. The factors that determine whether a virus–host system is of one type or the other could be very subtle: for instance, the rate of spread of virus into adjacent cells and the rapidity with which the invaded cells collapse and die. Whatever these factors may be, it seems reasonable to conclude that the term "local lesion host," as typified by TMV on *N. glutinosa*, is a rather arbitrary description of a condition dictated to a large extent by environmental factors, and that manipulation of environment can result in an internal cellular response of a local lesion host that is equivalent to the response of individual cells in a normally systemic host, and finally (23, 24) in conversion of the infection to a systemic conditon.

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REFERENCES

1. DALTON, A. J., and R. F. ZEIGEL. 1960. A simplified method of staining thin sections of biological material with lead hydroxide for electron microscopy. *J. Biophys. Biochem. Cytol.* **7**:409.
2. CARROLL, T. W., and T. A. SHALLA. 1965. Visualization of tobacco mosaic virus in local lesions of *Datura stramonium*. *Phytopathology*. **55**: 928.
3. CRONSHAW, J., L. HOEFERT, and K. ESAU. 1966. Ultrastructural features of *Beta* leaves infected with beet yellows virus. *J. Cell Biol.* **31**:429.
4. EDWARDSON, J. R. 1966. Electron microscopy of cytoplasmic inclusions in cells infected with rod-shaped viruses. *Am. J. Botan.* **53**:359.
5. JOHN, V. T., and M. WEINTRAUB. 1966. Symptoms resembling virus infection induced by high temperature in *Nicotiana glutinosa*. *Phytopathology*. **56**:502.
6. KARNOVSKY, M. J. 1961. Simple methods for "staining with lead" at high pH in electron microscopy. *J. Biophys. Biochem. Cytol.* **11**:729.
7. KOLEHMAINEN, L., H. ZECH, and D. VON WETTSTEIN. 1965. The structure of cells during tobacco mosaic virus reproduction. *J. Cell Biol.* **25**:77.
8. LUFT, J. H. 1961. Improvements in epoxy resin

embedding methods. *J. Biophys. Biochem. Cytol.* **9**:409.

9. MILNE, R. G. 1966. Multiplication of tobacco mosaic virus in tobacco leaf palisade cells. *Virology*. **28**:79.
10. MILNE, R. G. 1966. Electron microscopy of tobacco mosaic virus in leaves of *Chenopodium amaranticolor*. *Virology*. **28**:520.
11. MILNE, R. G. 1966. Electron microscopy of tobacco mosaic virus in leaves of *Nicotiana glutinosa*. *Virology*. **28**:527.
12. OWEN, P. C. 1958. Some effects of virus infection on leaf water contents of *Nicotiana* species. *Ann. Appl. Biol.* **46**:205.
13. RAGETLI, H. W. J., and M. WEINTRAUB. 1964. Immuno-osmophoresis, a rapid and sensitive method for evaluating viruses. *Science*. **144**: 1023.
14. REYNOLDS, E. S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**:208.
15. SABATINI, D. D., K. BENSCH, and R. J. BARRNETT. 1963. Cytochemistry and electron microscopy. The preservation of cellular ultra structure and enzymatic activity by aldehyde fixation. *J. Cell Biol.* **17**:19.
16. SHALLA, T. A. 1964. Assembly and aggregation of tobacco mosaic virus in tomato leaflets. *J. Cell Biol.* **21**:253.
17. STEMPAK, J. G., and R. T. WARD. 1964. An improved staining method for electron microscopy. *J. Cell Biol.* **22**:697.
18. STRUNK, C., and H. WARTENBERG. 1960. Licht- und elektronenmikroskopische Untersuchungen der Chloroplasten chlorotischer Maispflanzen. *Phytopathol. Z.* **38**:8.
19. TAKAHASHI, W. N. 1951. Ultraviolet absorption as a measure of tobacco-mosaic virus nucleoprotein. *Phytopathology*. **41**:142.
20. WATSON, M. L. 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophysic. Biochem. Cytol.* **4**:475.
21. WEINTRAUB, M., and V. T. JOHN. 1966. Cytological abnormalities induced by high temperature in *Nicotiana glutinosa*. *Phytopathology*. **56**:705.
22. WEINTRAUB, M., W. G. KEMP, and H. W. J. RAGETLI. 1960. Studies on the metabolism of leaves with localized virus infections. I. Oxygen uptake. *Can. J. Microbiol.* **6**:407.
23. WEINTRAUB, M., W. G. KEMP, and H. W. J. RAGETLI. 1961. Some observations on hypersensitivity to plant viruses. *Phytopathology*. **51**: 290.
24. WEINTRAUB, M., W. G. KEMP, and H. W. J. RAGETLI. 1963. Conditions for systemic invasion by virus of a hypersensitive host. *Phytopathology*. **53**:618.
25. WEINTRAUB, M., and H. W. J. RAGETLI. 1964. Studies on the metabolism of leaves with localized virus infections. Particulate fractions and substrates in TMV-infected *Nicotiana glutinosa* L. *Can. J. Botan.* **42**:533.
26. WEINTRAUB, M., and H. W. J. RAGETLI. 1964. An electron microscope study of tobacco mosaic virus lesions in *Nicotiana glutinosa* L. *J. Cell Biol.* **23**:499.
27. WEINTRAUB, M., and H. W. J. RAGETLI. 1966. Fine structure of inclusions and organelles in *Vicia faba* infected with bean yellow mosaic virus. *Virology*. **28**:290.
28. ZAITLIN, M., and N. K. BOARDMAN. 1960. The association of tobacco mosaic virus with plastids. I. Isolation of virus from the chloroplast fraction of diseased-leaf homogenates. *Virology*. **11**:499.