Visualization of Tobacco Mosaic Virus Within Infected Cells. (17597)

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One of the most interesting problems arising within our present ability to see elementary virus particles deals with their development from the living cells they infect. Much knowledge concerning this has already been gained through the electron microscopic examination of bacteriophages and their bacterial hosts which, growing as separate entities, are accessible to thorough electron microscopic inspection. A broadened attack on this problem of virus proliferation is, however, now being made possible by the development of suitable ways of cutting tissue sections thin enough for study under the electron microscope. We have been using these methods to investigate how certain viruses develop within diseased plants; this note records preliminary observations and interpretations on about 200 photographs made on the occurrence of tobacco mosaic virus (*Marmor tabaci* H.)(1) in leaf cells of diseased Turkish tobacco (*Nicotiana tabacum* L.).

For the electron micrographs shown here, small pieces were cut from leaves showing marked symptoms of systemic infection with tobacco mosaic virus. These bits, most of them taken from leaf margins, and similar pieces cut from healthy Turkish tobacco plants of corresponding age were immediately fixed by immersion in the conventional formalin-aceto-alcohol mixture. When ready for sectioning they were removed from this fixative, washed thoroughly, passed through the usual alcohols, embedded in methacrylate, cut and prepared for electron microscopy by the procedures recently described by Neuman, Borysko and Swerdlow.(2) The finished sections after removal of methacrylate were shadowed with gold-manganin and photographed in RCA electron microscopes. This shadowing contributes little to the visibility of fine structure in such sections but serves other useful purposes. It prevents the accumulation of charge on the thicker portions of the section, with the disturbing effects such charges have on the quality of the electron image, it permits ready distinction between detail lying deep in the section and on its surface and it prevents the serious distortion of the section that otherwise commonly occurs when an electron beam strikes it.

Typical sections through a healthy Turkish tobacco leaf show chloroplasts lining more or less completely the well-defined walls of its cells (Fig. 1). As this figure indicates, most chloroplasts have one or more large opaque inclusions, the starch grains. However, holes are usually seen where these objects have

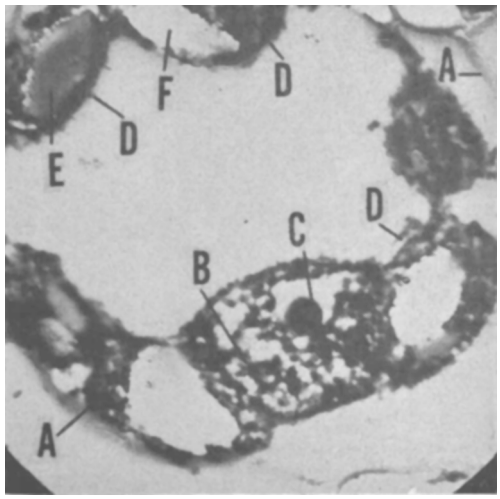

Fig. 1.

A cross section through a cell of a healthy leaf of Turkish tobacco showing at (A) cell wall; (B) interior of a nucleus; (C) nucleolus; (D) chloroplasts; (E) starch grain; (F) cavity from which starch grain was knocked out. Magnification = 8800 ×.

1. Holmes, F. O., Order Virales. The Filterable Viruses, p. 1164, Supplement 2, *Bergey's Manual of Determinative Bacteriology*, 6th edition, Williams & Wilkins Co., Baltimore, 1948.

2. Neuman, S. B., Borysko, E., and Swerdlow, M., *J. Research Nat. Bur. Standards*, 1949, v43, 183.

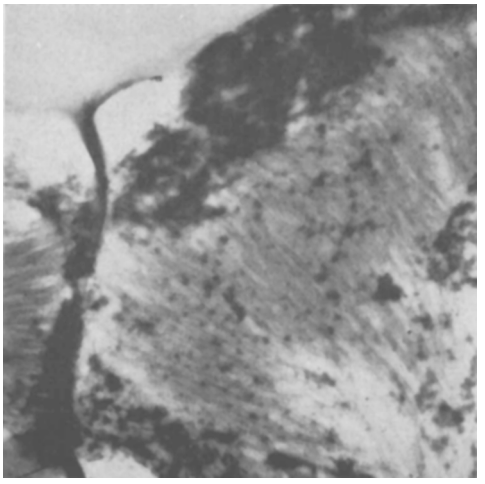

FIG. 2.
Section through parts of two cells of a tobacco leaf diseased with tobacco mosaic virus. Some of the finer filaments in the right hand cell (which occupies most of the figure) have the thickness to be expected of single tobacco mosaic rods, though their lengths are many times 2800A. 8800 ×.

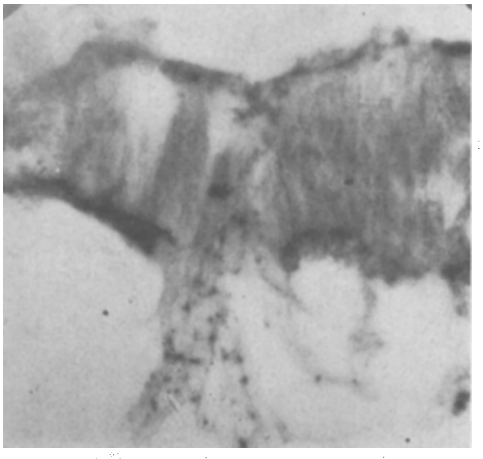

FIG. 3.
A mass of virus with filaments and strands extending into the region of the cell vacuole. 8000 ×.

been punched out during the sectioning. The cell nuclei which occasionally are sectioned appear as coarse, especially opaque networks having a well marked membrane and often a very opaque nucleolus.

Sections through diseased leaves show some cells of normal appearance, but most cells contain visible amounts of virus in the form of masses of strands or fibers. In Fig. 2 and 3 some of these fibers appear as separate filaments having approximately the 150A diameters typical of tobacco mosaic virus rods. It is important that these separate filaments, where they have been seen clearly, have nearly always been many times longer than the 2800A particles that predominate in suspensions freshly made from diseased tissue.(3) Evidently in the plant these relatively short elementary particles are usually associated

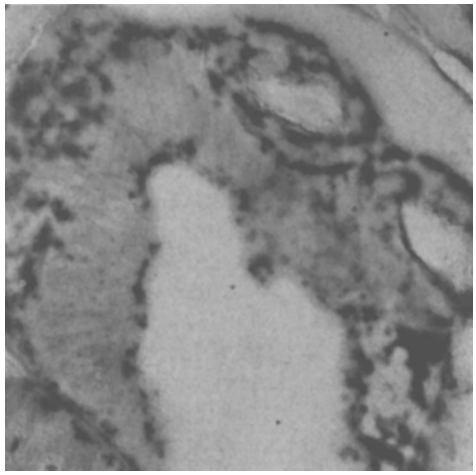

FIG. 4.
Virus filaments radiating inwards from a cell wall and the chloroplasts that line it. 8000 ×.

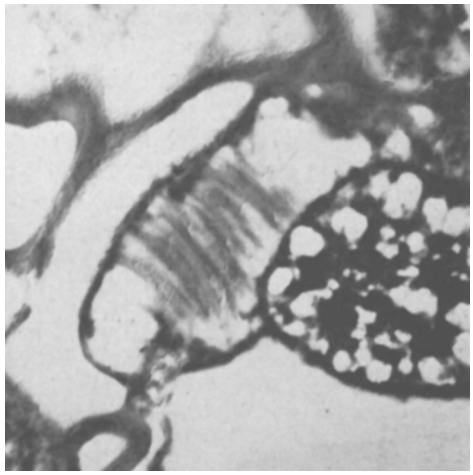

FIG. 5.
Body with well-defined membrane containing virus. The adjacent nucleus appears normal and virus-free. 9300 ×.

3. Oster, G., and Stanley, W. M., *Brit. J. Exp. Path.*, 1946, v27, 261.

together end-to-end as well as side-to-side. In Fig. 2 the virus appears to fill much of the cytoplasmic region of the diseased cell; it has reached deeply into the central vacuole in the cell of Fig. 4. In the fully diseased leaf tissue described here, the virus has usually occurred in thick fibrous masses (Fig. 6 and 8) rather than as separate, individual particles. In all cases the fibrous strands seem to extend inwards from the cell wall (Fig. 2 and 4).

Many of the virus masses are in close association with the chloroplasts and even appear to be internal to them. A cell body containing virus is shown in Fig. 5. This body has a well-defined membrane, but it is not certain whether it is a chloroplast or an inclusion body. The elongated body of Fig. 7 containing a fibrous mass of virus has a hole like that left in chloroplasts when the starch grain is dislodged. Virus masses are often seen extending out from what appear to be damaged chloroplasts (Fig. 2, 4, 6 and 8). Whether such disintegration of the chloroplasts is due to virus action alone or to virus plus the treatment of the material in preparation for electron microscopy is of course not known. It has not been encountered in healthy cells. The inner edges of the virus masses of Fig. 4, 6 and 8 carry lines of dark granules that may well be fragments of broken chloroplasts. Unlike the chloroplasts from some other plants we have examined, the inner detail of those from both healthy and diseased Turkish tobacco has not been sufficiently well defined in section to identify these granules with the grana.

In contrast with the marked changes in the aspect of the chloroplasts, the nuclei appear the same in healthy and diseased cells and show no evidence that they contain strands of virus (Fig. 1, 5 and 8).

This work is being continued with plant

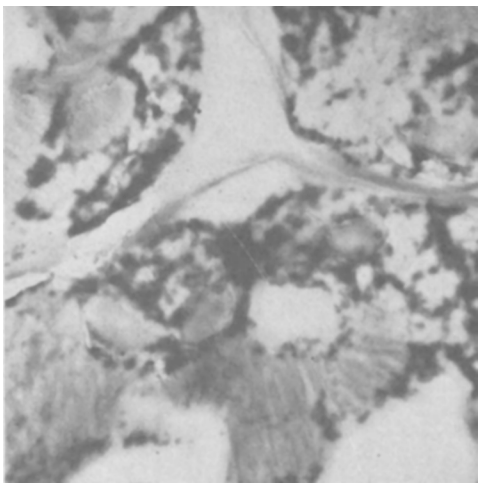

FIG. 6.
Much virus appears in the bottom cell of the three that meet at the center of the photograph; less virus can be seen in the other two. 8000 ×.

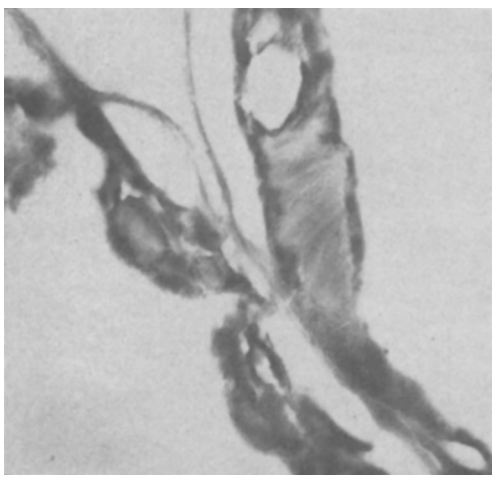

FIG. 7.
A mass of virus within a body that may have been a chloroplast. 8800 ×

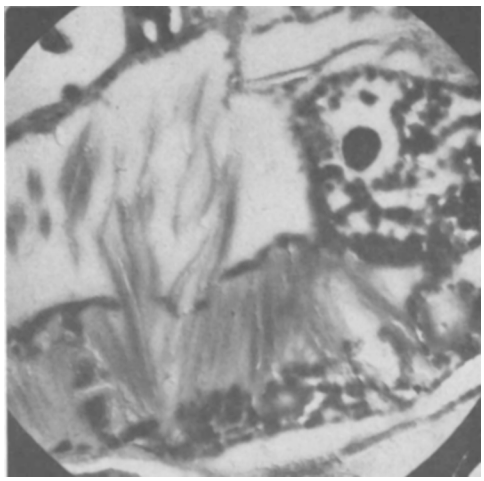

FIG. 8.
Virus dispersed into the region of the central vacuole of a cell from the mass at the bottom. The apparently normal nucleus is at the right. 8800 ×.

tissues diseased with tobacco mosaic and with other viruses to see what further information can thus be gained concerning the focus and mechanism of virus proliferation.

Summary. Electron micrographs of sections cut through tobacco leaves infected with tobacco mosaic indicate that the virus is often present in fibrous masses associated with chloroplasts which are severely affected. Virus can clearly be seen in the cytoplasm of the diseased cells sometimes as single filaments. The nuclei seem unaltered by the infective process and have appeared free of virus. The great length of these filaments, much in excess of 2800A, indicates an end-to-end arrangement of the rods *in situ*.

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Increased *in vitro* Tuberculin Leucocyte Cytolysis Following a Tuberculin Skin Test.* (17598)

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It has recently been shown that leucocyte suspensions prepared from the blood of animals showing tuberculin hypersensitivity are injured when exposed to tuberculin.(1,2) Further analysis of this cell-tuberculin-system has shown that a serum factor, present in the euglobulin portion of serum from tuberculous subjects,(3,4) is capable of destroying portions of the white cells of the tuberculous as well as the non-tuberculous guinea pig and man, if first these cells are exposed to tuberculin.(5) A convenient way of demonstrating this serum factor consists in following the white blood counts in samples of heparinized whole blood to which appropriate amounts of tuberculin have been added.(6)

The purpose of this report is to describe an increase in *in vitro* tuberculin-leucocyte cytolysis in blood of tuberculin sensitive guinea pigs following an intracutaneous tuberculin reaction. This study was prompted by earlier findings that a positive tuberculin test in man may be followed by a similar increase in *in vitro* tuberculin cytolysis.(6)

Materials and Method.

Materials. Guinea Pigs. Four hundred to 900 g animals were inoculated one to 3 times at 2-month intervals in both groins and pectoral regions with 10 mg of heat-killed tubercle bacilli (H37Rv) suspended in 1 ml of Bayol F (0.25 ml at each site). Animals were selected for study who showed hard masses 1 to 2 cm in diameter in the above areas and who also demonstrated cutaneous induration greater than 1 cm in diameter at 48 hours from a second strength PPD (5 γ in 0.1 cc) skin test.(7) Normal tuberculin-negative guinea pigs were used as controls. No skin tests were done more recently than 3 weeks before the experiment began.

Leucocytes. Two ml blood samples were taken by cardiac puncture into a syringe containing 0.2 ml heparin (Liquaemin), mixed,

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1. Favour, C. B., PROC. SOC. EXP. BIOL. AND MED., 1947, v65, 269.
2. Fremont-Smith, P., and Favour, C. B., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 502.
3. Miller, J. M., Favour, C. B., Wilson, Barbara A., and Umbarger, Merle A., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 738.
4. Miller, J. M., Favour, C. B., Wilson, Barbara A., and Umbarger, Merle A., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 287.
5. Favour, C. B., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 369.
6. Favour, C. B., Fremont-Smith, R., and Miller, J. M., *Am. Rev. Tuberc.*, 1949, v60, 212.
7. Seibert, F. B., and Glenn, J. T., *Am. Rev. Tuberc.*, 1949, v44, 9.