TECHNICAL PAPERS

Electron Micrographic Observations of Tobacco Mosaic Virus in Crude, Undiluted Plant Juice[1]

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Several reports have been published (*2, 3, 4, 5, 6*) of electron microscopic studies of the tobacco mosaic virus in various degrees of impurity. In no study, however, has the infected juice been used in its crude, undiluted form. There has always been some degree of treatment, if only with distilled water, prior to observation in the electron microscope. The published micrographs of the impure material are qualitatively similar to those obtained from freshly purified material.

We have recently obtained electron micrographs of a wild strain of tobacco mosaic[2] grown in White Burley tobacco plants. The specimens for the microscopic examination were prepared in this way: A small portion of the tobacco leaf was crushed by a round-end glass rod in a small glass tube. A drop of expressed juice was picked up in a micropipette, and a bubble was blown over the surface of the specimen screen. The bubble was quickly wiped across the screen, and a very thin film of material was left behind. This film dried almost instantly, and the dried specimen was then shadowed with uranium. The total elapsed time between expressing the juice and drying the film was about 15 sec.

A typical micrograph from a 14-day-old infection is shown in Fig. 1. As can be readily seen, the virus particles appear in the form of large bundles, or sheaves, whose length is roughly 1 to 3 μ, and whose diameter, or width, varies greatly but might average 150 mμ. The background appears quite coarse, since it consists of all of the nonvolatile plant material. Uninfected plants are found to exhibit none of these bundle-like forms. The size and frequency of the typical forms shown in Fig. 1 increase with the age of the infection, being barely visible at 36 hrs after inoculation.

Fig. 2 illustrates the probable reason why previous work with "crude" juice has not exhibited the sheaves of virus particles. The specimen shown in this micrograph was prepared like that in Fig. 2, up to the shadow-casting, but was then washed with two drops of distilled water and allowed to dry. It was then shadowed with uranium. Even such a light and brief treatment with distilled water is evidently sufficient to bring about extensive disintegration of the bundles and to cause many typical, single particles to become visibly detached from the larger groups.

It is well known from observations with the optical microscope (*1*) that needle-like forms are found to exist *in vivo* in the hair-cells of an infected plant, particularly

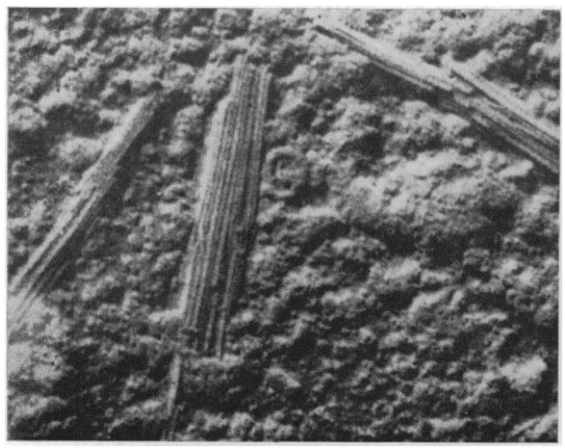

FIG. 1. Electron micrograph of expressed, crude juice of White Burley tobacco leaf infected with a wild strain of tobacco mosaic virus. Note the bundle-like forms in which the virus is seen. Magnification is 30,000 ×.

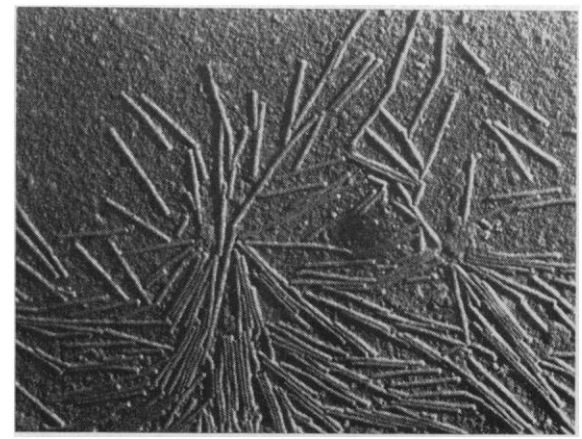

FIG. 2. Electron micrograph of the same infected material as is shown in Fig. 1, except that the dried preparation was washed briefly with distilled water. Note that the virus particles are now partially dispersed in individual rods. Magnification is 30,000 ×.

[1] These results have been orally reported at the recent meeting in Toronto of the Electron Microscope Society of America and at the Autumn Conference on Electron Microscopy in Cambridge, England. The work is part of a program supported by a grant-in-aid from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.

[2] The initial virus used for propagation in our laboratory was kindly furnished by Gerald Oster.

when the cell has been slightly injured or treated with mild acid. It is entirely possible that what is seen in detail in the electron micrographs is identical with the needle-forms recognized in the optical microscope.

The interesting question in connection with these preliminary results is whether the groups of virus particles exist as such in the intact plant cells, or whether they form subsequent to the crushing of the cells. If the latter is true, they can be considered aggregates of the individual virus particles. But if further work should show that they exist in the intact cells, then the interesting question will arise as to whether or not the virus bundles represent the geometrical growth pattern of the virus.

References

1. BAWDEN, F. C. *Plant viruses and virus diseases.* Waltham, Mass: Chronica Botanica, 1943.
2. CROOK, E. M., and SHEFFIELD, F. M. L. *Brit. J. exp. Path.*, 1946, **27**, 328.
3. OSTER, GERALD, and STANLEY, W. M. *Brit. J. exp. Path.*, 1946, **27**, 261.
4. SIGURGEIRSSON, T., and STANLEY, W. M. *Phytopath.*, 1947, **37**, 26.
5. STANLEY, W. M. *Chronica Botanica*, 1943, **7**, 291.
6. TAKAHASHI, WILLIAM N., and RAWLINS, T. E. *Phytopath.*, 1948, **38**, 279.

Infrared Spectra and the Amide Linkage in a Native Globular Protein

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The presence of absorption characteristic of amides in the 6-μ region of the spectrum has been observed in proteins by several investigators (*1*, *6*, *8*) and has frequently been cited as evidence against the "cyclol 6" structure postulated by Wrinch (*9*) for native protein molecules. The results reported, however, have been for insoluble, fibrous proteins or for samples in the form of a dried film. In view of the pronounced sensitivity of proteins to drying, as well as to deposition in a film, it has always been possible to cast doubt on this evidence for the amide linkage, at least insofar as native globular proteins are concerned, on the ground that the molecule had been denatured during the experiment.

A protein which is relatively stable to the drying process is serum albumin. It has also been observed recently that native serum albumin produces a modification in the visible spectra of many compounds (*3*) and that this ability is lost on denaturation (*5*). It has seemed appropriate, therefore, to examine the infrared absorption of a dried film of crystallized bovine serum albumin and then to test the sample for possible denaturation by seeing whether it is still capable of causing characteristic spectrophotometric shifts.

The infrared absorption spectrum of bovine serum albumin in the 6-μ region is illustrated in Fig. 1, together with the synthetic, water-soluble, polypeptide, polylysine

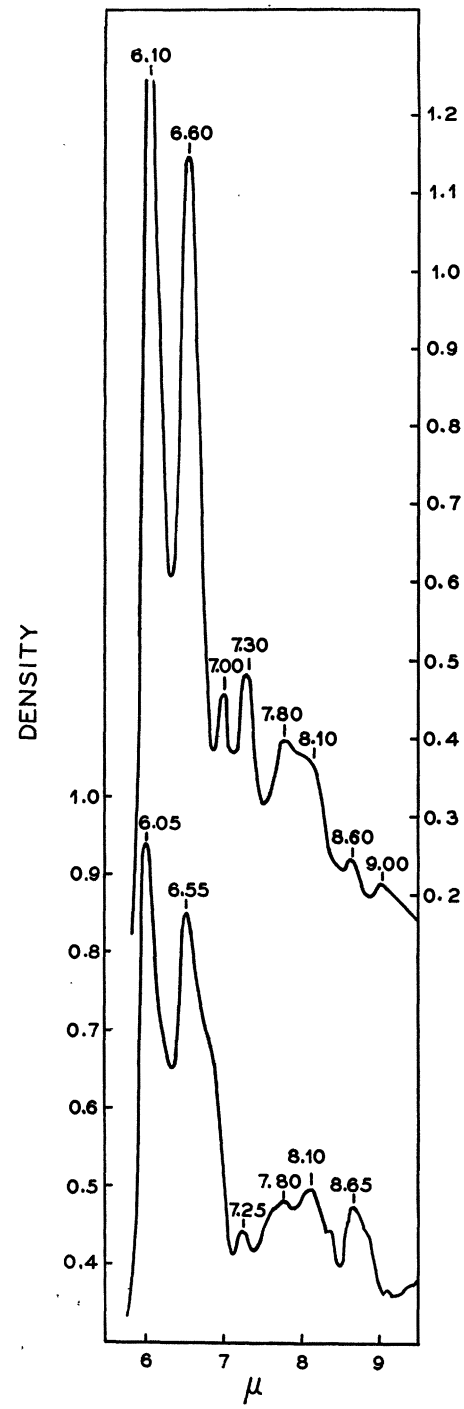

FIG. 1. Infrared absorption spectra of films of bovine serum albumin (top) and polylysine (bottom).

(*2*)[1] for comparison. The C=O peaks at 6.05 and 6.10 μ are essentially identical in position and characteristic of the simple amides in the solid state (*4*, *7*). Thus there can be no doubt that amide carbonyl groups are present

[1] We are very much indebted to E. Katchalski for his generous gift of a substantial sample of polylysine hydroiodide.