trols. Furthermore, several experiments seem to indicate that, at least in the case of polyoma virus in hamsters, the interaction leading to tumor formation occurs within 24 hours after inoculation, and further viral replication, if it occurs, does not play any significant role in tumorigenesis (*9*). Thus, the increased frequency of tumors with irradiated viruses must be related to the properties of the virus population at the time of inoculation.

The possibility that irradiations could have modified those properties of the virus coat, which conceivably would favor adsorption of the virus to the target cells, can be excluded, since there is no enhancement of infectivity or of ability to produce complement-fixing antigen by the irradiated viruses. Furthermore, hemagglutination activity of polyoma virus—a property of the viral coat—was not affected by the maximum amount of ultraviolet radiation used (*10*).

Because ultraviolet and gamma radiations inactivate biological material through different mechanisms of action, the fact that both radiations produced enhanced tumorigenicity in three different viruses suggests nonspecificity of locus of action. At present we can propose two alternative hypotheses for the mechanism of increased oncogenicity of irradiated DNA viruses, both compatible with established experimental evidence.

1) Oncogenicity is a property of "defective" virus particles, and, in irradiated samples, because of inactivation of particles capable of expressing all the genome functions, the proportion of "defective" particles increases. As a further hypothesis it could be suggested that the fragments of viral DNA responsible for oncogenesis may be more easily incorporated into the cell genome if the integrity of the whole viral DNA is altered by radiation.

2) The tumors produced by the irradiated viruses are defective for the viral-induced specific transplantation antigens (*11*) and, consequently, their growth is not hampered by immunological interaction with the host. None of the tumors produced by the irradiated viruses that were examined was defective for the SV40-induced complement-fixing antigen.

Apart from the mechanism involved, these findings suggest the need for cautious use of inactivated tumor viruses for vaccine purposes and suggest that other viruses (for example, reoviruses, herpes, pseudorabies) be tested after suppression of their cytocidal properties by irradiation for possible unmasking of oncogenic potential.

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References and Notes

1. V. Defendi, F. Jensen, G. Sauer, in *Molecular Biology of Viruses*, J. S. Colter, Ed. (Academic Press, New York, 1967).
2. D. Gershon, P. Hausen, L. Sachs, E. Winocour, *Proc. Nat. Acad. Sci. U.S.* **54**, 1584 (1965).
3. R. I. Carp, S. Kit, J. L. Melnick, *Virology* **29**, 503 (1966).
4. C. Basilico and G. diMayorca, *Proc. Nat. Acad. Sci. U.S.* **54**, 125 (1965); T. L. Benjamin, *Proc. Nat. Acad. Sci. U.S.* **54**, 121 (1965).
5. R. Latarjet, R. Cramer, A. Golde, L. Montagnier, in preparation.
6. D. H. Moore in *Tumour Viruses of Murine Origin*, G. E. Wolstenholme and M. O'Conner, Eds. (Little, Brown, Boston, 1962), p. 107.
7. R. Latarjet and L. Chamaillard, *Bull. Cancer* **49**, 382 (1962).
8. A. Golde and R. Latarjet, *Comp. Rend. Ser. D* **262**, 420 (1966).
9. K. Habel and R. J. Silverberg, *Virology* **12**, 463 (1960); V. Defendi and H. Koprowski, *Nature* **184**, 1579 (1959).
10. R. Frey and V. Defendi, unpublished results.
11. V. Defendi, R. I. Carp, R. Gilden, in *Viruses Inducing Cancer*, W. J. Burdette, Ed. (Univ. of Utah Press, Salt Lake City, 1966), p. 269.
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Tubular Structures Associated with Turnip Yellow Mosaic Virus in vivo

Abstract. *Plants infected with a necrotic strain of turnip yellow mosaic virus contain tubes averaging about 80 millimicrons in diameter and attaining 3 microns in length. The main constituent of these tubes is a protein that is related chemically and immunologically to the protein of the virus. The tubes are composed of hexagonally packed hexagonal subunits resembling the hexamer protein subunits of the virus particles and are probably helical in construction.*

Electron-microscopic examination of negatively stained crude extracts (*1*) of plants infected with a necrotic strain (*2*) of turnip yellow mosaic virus showed that infection led to the production of tubular structures, in addition to the near-spherical particles of the virus. The tubes, which flatten when dried for electron microscopy, varied in diameter between 30 and 130 mμ and were up to 3 μ long. Amino acid analysis of purified preparations of the tubes provided strong evidence that they are composed of viral protein. This conclusion was supported by demonstration of a serological relation between the virus and the tubes. In electron micrographs these tubes showed a somewhat confused appearance, although the presence across them of sets of evenly spaced lines suggested that regular fine detail had been resolved. The clear optical transforms of the tubes, formed with an optical diffractometer described elsewhere (*3*), confirmed this and indicated that the construction of the tubes was based on a hexagonal lattice with a periodicity of about 42Å. A typical transform is shown in Fig. 1. Doubling of the spots and their disposition indicate that the transform was produced by two superimposed identical lattices intersecting at a small angle and set symmetrically on either side of the long axis of the tube. This is a situation that would arise if the tube were helical in construction and, as can occur with negatively stained objects (*4*), had both upper and lower surfaces resolved in the electron micrograph. Since the lattices on the two sides of the tube are not in register, mutual interference between structural detail on each side would be expected and would explain the disordered appearance of the tube. The high degree

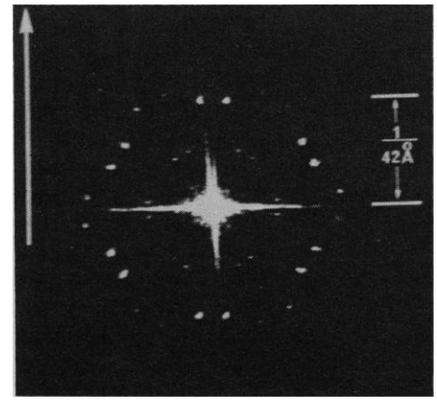

Fig. 1. Optical transform of the turnip yellow mosaic virus shown in Fig. 2. An arrow indicates the corresponding orientation of the long axis of the tube.

of structural order revealed by the transform suggested that it might be appropriate to use the technique of linear integration (*5*) on the electron micrograph to reinforce the structural regularities on one surface of the tube while at the same time suppressing those on the other surface. The effect of carrying out this procedure can be seen in Fig. 2, the pattern of hexagonally packed hexagons obtained on integrating part of the tube shown being contained within the rectangular insert. The integration interval employed was equivalent to twice the lattice spacing deduced from the optical transforms, and the axis of the tube had first been rotated clockwise through an angle of 7° relative to the direction in which integration was carried out. If the tube was first rotated through about 7° anticlockwise, a similar pattern was obtained, representing structure on the opposite surface. Theoretically, the angle of inclination necessary to allow integration to take place along the parallel rows of subunits on one surface of the tube could be measured on an optical transform. In practice, however, it was difficult to locate exactly the direction of the tube axis on the highly magnified portion of tube that was used. This meant that a few trial integrations had to be made before the inclination giving a clear overall pattern was found. The pattern shown was actually obtained by a process of double integration (reintegration of an integrated picture at 60° across it), which further accentuated repeating detail. To the same end the technique of rotation (*6*) was applied to integrated pictures. Both double integration and rotation suggested (i) that the hexagonal subunits were placed slightly skew relative to the direction in which the rows ran and (ii) that each was composed of six smaller units, presumably individual peptides, located at the vertices (Fig. 3).

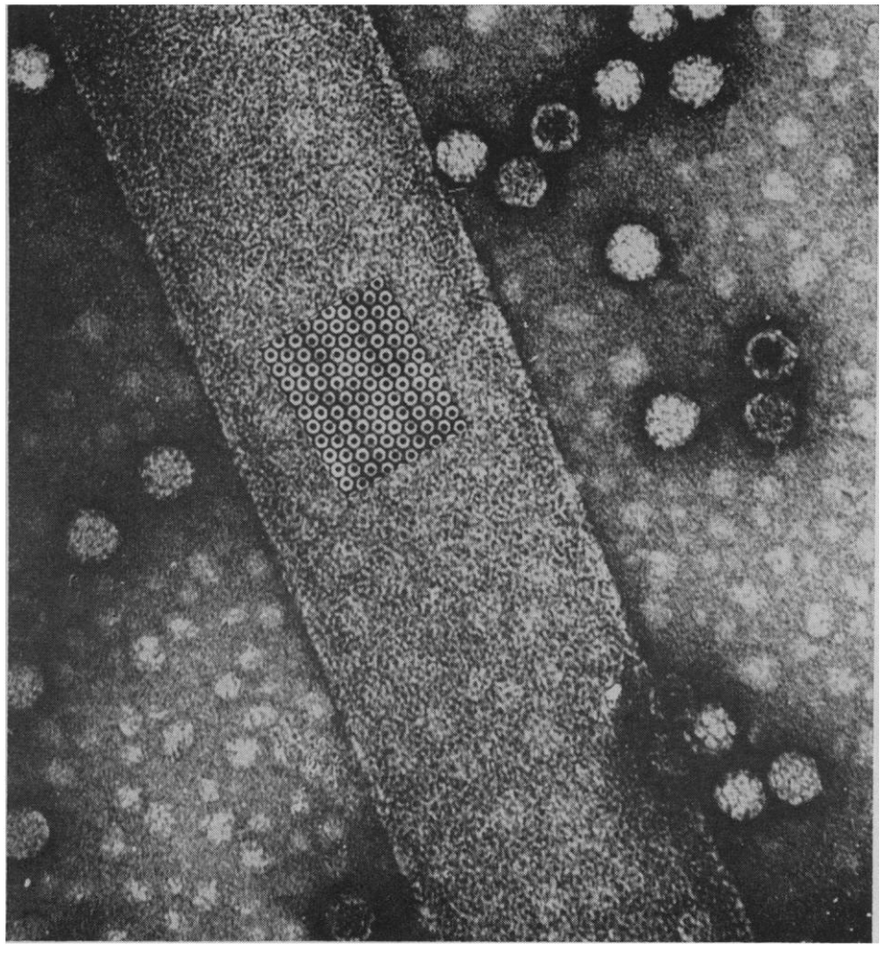

Fig. 2. Electron micrograph of a tube of the type present in plants infected with a necrotic strain of turnip yellow mosaic virus. Imposed on the picture is a pattern, obtained by a process of linear integration, showing the packing arrangement and orientation of the hexagonal subunits that make up the tube; also shown are "full" and "empty" virus particles. (× 268,000)

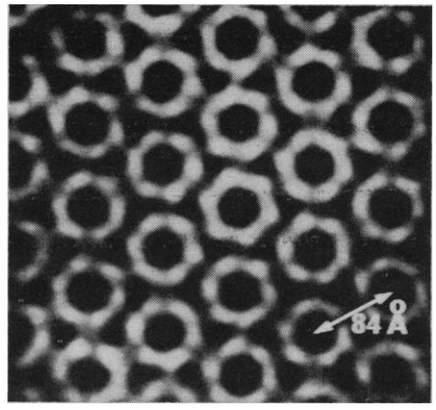

Fig. 3. Pattern obtained by using the rotation technique on an integrated picture of a tube of turnip yellow mosaic virus. Rotation was through an angle of 60° between photographic exposures. There is a clear indication that matter is concentrated at the vertices of the hexagons and also that the latter are slightly skew. (× 1,350,000)

Our results show that tubes of the turnip yellow mosaic virus are constructed of subunits resembling the hexamer morphological subunits of the virus particles. It is also clear that the manner in which they are assembled is subject to some variation for, not only are the tubes not constant in diameter, but optical transforms revealed that the angle between the lattices on the upper and lower tube surfaces could vary. If, as seems likely, the tubes are helices, it must follow that they are of variable pitch, a situation analogous to that which occurs among the T4 bacteriophage "polyhead" tubes (*7*).

A detailed account of this work is being prepared and will be published elsewhere.

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References and Notes

1. J. H. Hitchborn and G. J. Hills, *Virology* **27**, 528 (1965).
2. D. B. Dunn and J. H. Hitchborn, *ibid.* **30**, 598 (1966).
3. J. B. Bancroft, G. J. Hills, R. Markham, *ibid.* **31**, 354 (1967).
4. A. Klug and J. E. Berger, *J. Mol. Biol.* **10**, 565 (1964).
5. R. Markham, J. H. Hitchborn, G. J. Hills, S. Frey, *Virology* **22**, 342 (1964).
6. R. Markham, S. Frey, G. J. Hills, *ibid.* **20**, 88 (1963).
7. R. Favre, E. Boy de la Tour, N. Segrè, E. Kellenberger, *J. Ultrastruct. Res.* **13**, 318 (1965); E. Kellenberger and E. Boy de la Tour, *ibid.*, p. 343.
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