

results of enzyme studies summarized in Table 1 indicate that activity of pancreatic carboxypeptidase A of zinc-deficient rats was consistently lower than that of rats that had been fed zinc. However, zinc deficiency did not alter the activities of pancreatic carboxypeptidase B and liver alcohol dehydrogenase.

The different responses in the observed enzymic activities by zinc-deficient animals are of particular interest. It is known that zinc is a functional component of carboxypeptidase. The nature of the binding of zinc to carboxypeptidase A appears to be different from that of binding to carboxypeptidase B (9, 10). According to Vallee and Coombs (11), zinc is bound strongly in alcohol dehydrogenase. Thus it is possible that in dietary deprivation of zinc the activities of each of the zinc metalloenzymes may be affected differently.

Nevertheless, the reduction of the specific enzyme, carboxypeptidase A, in zinc-deficient rats suggests that zinc deficiency may decrease proteolysis within the intestinal tract and result in poor utilization of feed. This possibility is supported by the observation of Stirn *et al.* (12) which indicates that the zinc-deficient animals require 52 percent more ration to gain 1 g of body weight.

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#### References and Notes

1. E. Hove, C. A. Elvehjem, E. B. Hart, *J. Biol. Chem.* **134**, 425 (1940).
2. H. G. Day and E. V. McCollum, *Proc. Soc. Exp. Biol. Med.* **45**, 282 (1940).
3. M. J. Millar, M. I. Fischer, P. V. Elcoate, C. A. Mawson, *Can. J. Biochem. Physiol.* **36**, 557 (1958).
4. J. E. Folk and E. W. Schirmer, *J. Biol. Chem.* **238**, 3884 (1963).
5. J. E. Folk, K. A. Piez, W. R. Carroll, J. A. Gladner, *ibid.* **235**, 2272 (1962).
6. E. Racker, *ibid.* **184**, 313 (1950).
7. B. L. Vallee and F. L. Hoch, *ibid.* **225**, 185 (1957).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *ibid.* **193**, 265 (1951).
9. B. L. Vallee, J. A. Rupley, T. L. Coombs, H. J. Neurath, *ibid.* **235**, 64 (1960).
10. J. E. Folk and J. A. Gladner, *Biochim. Biophys. Acta* **48**, 139 (1961).
11. B. L. Vallee and T. L. Coombs, *J. Biol. Chem.* **234**, 2615 (1959).
12. F. E. Stirn, C. A. Elvehjem, E. B. Hart, *ibid.* **109**, 347 (1935).
13. Supported in part by Air Force contract No. AF 49(638)-1338.

12 May 1966

19 AUGUST 1966

## Cylindrical Inclusions in the Cytoplasm of Leaf Cells Infected with Tobacco Etch Virus

**Abstract.** Combined tracings from electron micrographs of serial sections of leaf tissue infected with tobacco etch virus show that one type of cytoplasmic inclusion, when sectioned in different planes, can produce configurations which have been interpreted as being two distinct types of inclusion bodies.

Electron micrographs of sections of plant tissue infected with certain flexuous rod viruses characteristically exhibit cytoplasmic inclusions of the pinwheel and bundle types, as shown in a portion of a parenchyma cell of tobacco leaf infected with tobacco etch virus (Fig. 1). Inclusions very similar to these have been described in various host tissues infected with tulip mosaic (1), turnip mosaic (2), tobacco etch (3, 4, 5), wheat streak mosaic (6), bean yellow mosaic (5, 7), watermelon mosaic, lettuce mosaic, bean common mosaic, potato Y, or sugarcane mosaic viruses (5). These inclusions have been variously interpreted as distinct types of inclusion bodies (2, 3, 6) or the same structure viewed from different angles (1, 4, 8).

Recently (5) these pinwheels and bundles have been considered as different aspects of a single type of inclusion, which was assumed to be cylindrical in shape and composed of curved plates with their inner edges converging around the central axis of the cylinder. Outer edges of the plates were assumed to diverge to form the boundary of the cylinder. Pinwheels would arise from cross sections of the hypothetical cylindrical inclusion, and longitudinal sections of the inclusion would show bundles.

Electron micrographs used in this study were obtained from portions of tobacco leaf that had been fixed in 6.5 percent phosphate-buffered glutaraldehyde for 17 hours, fixed again in 1 percent OsO<sub>4</sub> for 3 hours, and embedded

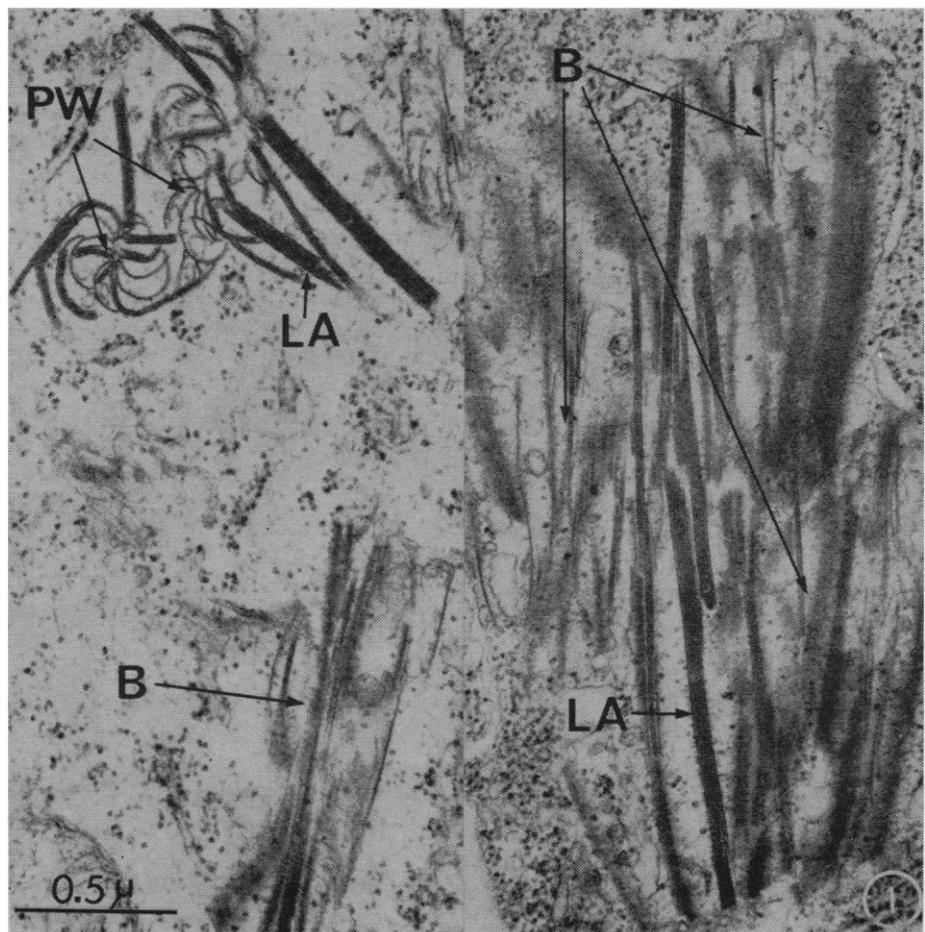
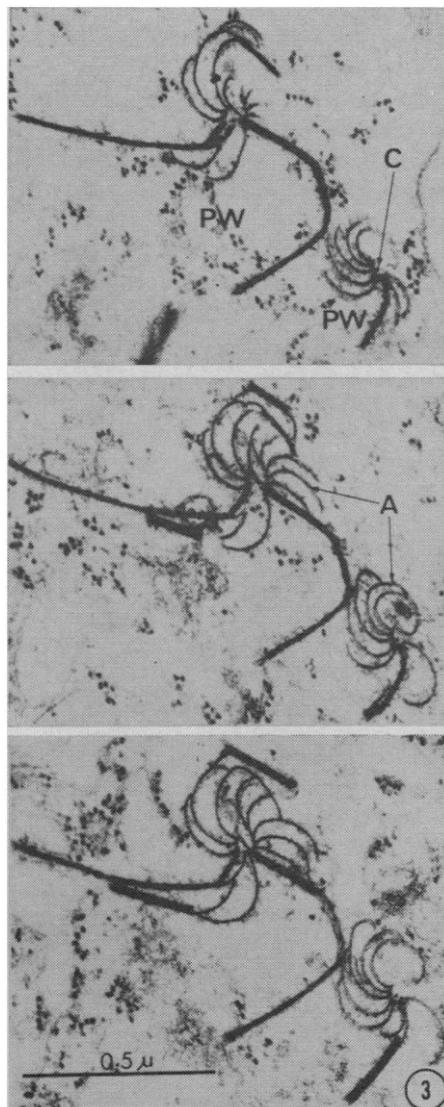
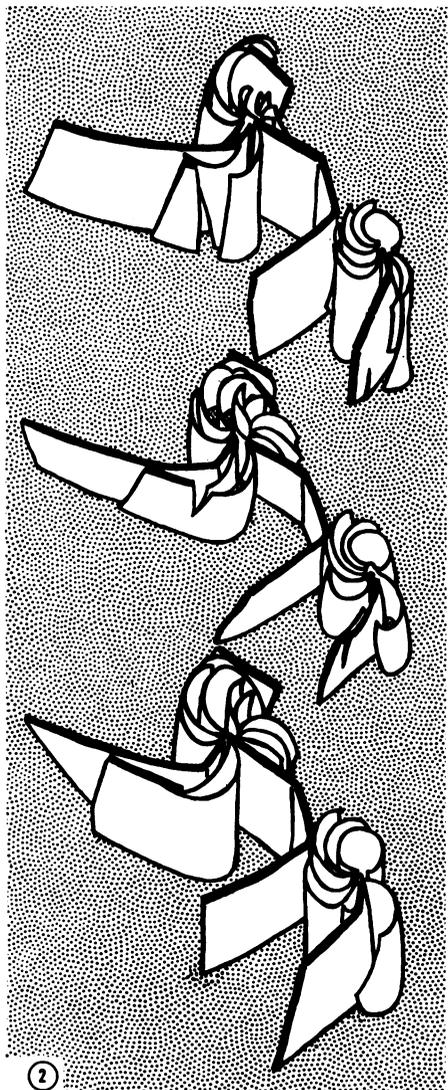


Fig. 1. Portions of leaf cells from tobacco infected with tobacco etch virus, which contain pinwheel (PW), bundle (B) inclusions, and laminated aggregates (LA).



Figs. 2 and 3. Fig. 2 (left). Representation of three-dimensional cylindrical inclusion from which pinwheels and bundles are derived by sectioning in cross or longitudinal planes. Fig. 3 (right). Pinwheels in selected sections from the series of serial sections that were used to construct cylindrical inclusion in Fig. 2. These pinwheels correspond to the top planes of segments in Fig. 2. (C) Circular body; (PW) pinwheel; and (A) pinwheel arm.

in Maraglas plastic. Figure 1 was obtained from sections stained with 1 percent uranyl acetate for 1 hour followed by lead citrate for 5 minutes; Fig. 2 was drawn from serial sections stained with 1 percent uranyl acetate for 1 hour followed by lead citrate for 30 minutes. Connecting the ends of pinwheel "arms" in tracings from electron micrographs of nine serial sections (each about 60  $m\mu$  thick) produces a representation of a three-dimensional figure which roughly corresponds to the proposed cylindrical inclusion (Fig. 2). The connection of points was interrupted in two places in order to show more internal structure in the cylinders. Small solid circles are frequently

present in the centers of pinwheels (Fig. 3), and the solid central core in Fig. 2 results from connecting the tracings of these structures from serial sections. Because entire cylindrical inclusions have not been examined, it is not known whether central cores are continuous in some cylinders and discontinuous in others, or whether they are absent in some cylinders.

The length and width of plates in cylindrical inclusions are variable, and conformation of pinwheels in a series of serial sections also varies. This variation in conformation of pinwheels is a consequence of cross-sectioning plates that vary in length, which causes a change to appear in the num-

ber of pinwheel arms in serial sections. Changes in the length of pinwheel arms, as seen in serial sections, are a result of cross-sectioning tapering plates. When cylindrical inclusions (Fig. 2) are sectioned longitudinally, bands varying in thickness and length occur in groups that are characteristic of the bundle-type inclusions (Fig. 1).

Many pinwheels and bundles contain laminated aggregates (Fig. 1). Both these aggregates and the bundle-type inclusions contain electron-opaque linear components; however, the components of laminated aggregates are parallel and very closely aligned, while the linear components of bundles are neither parallel nor closely aligned. The laminated structure of the thick linear arms of the pinwheels in Fig. 3 is obscured by overstaining. Laminated aggregates contained in the pinwheels and bundles of Fig. 1 are the consequence of cross- and longitudinal-sectioning of closely aligned parallel flat plates. The blurred inclusions in this figure are assumed to result from tangential sections of laminated aggregates.

Many pinwheels and bundles that are clearly separated in an individual section appear to be interconnected in serial sections. The complexity of interconnections may make it difficult to determine accurately the lengths of entire cylindrical inclusions. The right half of Fig. 1 is interpreted as resulting from a longitudinal section through three or more interconnected cylindrical inclusions; the pinwheels in the left half of this figure are assumed to result from a cross section through at least three similarly interconnected cylindrical inclusions.

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#### References and Notes

1. A. Yamaguchi, T. Kikumoto, C. Matsui, *Virology* **20**, 143 (1963).
2. T. Hayashi, C. Matsui, A. Yamaguchi, *Phytopathology* **55**, 458 (1965).
3. C. Matsui and A. Yamaguchi, *Virology* **22**, 40 (1964).
4. M. Rubio-Huertos and F. G. Hidalgo, *ibid.* **24**, 84 (1964).
5. J. R. Edwardson, *Amer. J. Bot.*, in press.
6. P. E. Lee, *J. Ultrastruct. Res.* **13**, 359 (1965).
7. M. Weintraub and H. W. J. Ragetti, *Virology* **28**, 290 (1966).
8. M. C. Cremer, D. H. M. Van Slogteren, J. A. Van Der Veken, in *Proc. European Reg. Conf. Electron Microscopy, Delft, 1960* (1961), vol. 2, p. 947.
9. Florida Agricultural Experiment Station journal series No. 2409. Supported by AEC (contract AI 40-1-2583). I thank S. R. Christie for making the drawing and R. G. Christie for technical assistance.

19 May 1966