

Proteinaceous Crystals in Cells of Virus Infected Plants

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Abstract. Crystal-containing organelles in cells of virus infected plants lying at chloroplasts and mitochondria are identical with single membrane-bound microbodies containing crystals of catalase described in healthy plants. Massive complex inclusions caused by turnip mosaic virus very frequently contain the same microbodies with crystal inclusions; that phenomenon may be related to some pathophysiological changes of virus infected plants.

Comparable proteinaceous crystals, but not lying within microbodies limited by a membrane, may also be found in cytoplasm of infected cells. These crystals are sometimes surrounded by a substance resembling the microbody matrix. Disintegrated cytoplasm of virus infected cells may also contain the same crystals lying free in "empty spaces". Cytopathological effects responsible for this phenomenon and possible artifacts as well are discussed.

Many papers describing the ultrastructure of virus infected plant cells recorded without any comment the fact of the occurrence of apparently proteinaceous crystals within special bodies in cytoplasm (MATSUI and YAMAGUCHI 1964, GEROLA and BASSI 1966, RUSSO *et al.* 1968, GEROLA *et al.* 1969, EDWARDSON 1974, ŠARIĆ and WRISCHER 1975, *etc.*). Comparable formations were observed in phloem cells of mycoplasma (MLO) infected plants (SHIKATA *et al.* 1969, ULRYCHOVÁ and JOKEŠ 1977). GOURRET (1970) described similar crystals in accompanying cells of periwinkle plants infected with MLOs; however, these crystals were not located in microbodies, but within the ergastoplasm cistern or within the vacuole of necrotizing cells.

Authors mostly reported the presence of crystal-containing bodies in uninfected control tissues as well (GEROLA *et al.* 1965, KOLEHMAINEN *et al.* 1965, CHAMBERS *et al.* 1965, VAN BAKEL and VAN OOSTEN 1972) and concluded that the crystal-containing bodies were not characteristic of virus infection. Nevertheless, many authors ascertained that crystal-containing organelles were considerably more abundant in the cytoplasm of diseased plant cells than in healthy plants (PETZOLD 1967, KISLEV *et al.* 1968, EDWARDSON 1974). ISRAEL and ROSS (1967) stated those bodies in mesophyll cells in the encircling zone around the tobacco mosaic virus (TMV) lesions; they found that these bodies in healthy tissues were always devoid of internal structure, and so suggested that the formation of microcrystals might reflect some aspect of the resistance

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mechanism. This idea appeared also in their following paper (ROSS and ISRAEL 1970): crystal-containing bodies were found in palisade cells, only in the areas shown to be immune by challenge inoculation. KANG-CHIEN LIU and BOYLE (1972) observed rhomboidal microcrystals contained in single membrane-bound organelles in the cytoplasm of cells infected with U-1 strain of TMV. No microcrystals were found by the authors in cells infected with the yellow TMV strain or in cells of uninfected potato plants.

The crystal-containing bodies were also observed more frequently in parenchyma cells of older leaves (PETZOLD 1967) and with a mosaic-like disorder not proved to be of virus origin (CHOD *et al.* 1973). However, the occurrence of needle-shaped cytoplasmic crystals in mesophyll was not confined to abnormal cells of white tissues in maize carrying the striate-2 gene (WILLIAMS and KERMICLE 1974). In tobacco cultured cells the crystal-containing bodies were abundantly seen in cultures in which shoots had been induced, *e.g.* in cells grown on a medium with IAA and kinetin (MATSUSHIMA *et al.* 1969).

The crystal-containing organelles in cells of leaf parenchyma have been called differently by different authors, *e.g.* spherosomes, lysosome-like bodies, dense bodies, cell components A, phragmosomes and cytosomes, PETZOLD (1967) used the term "Proteinvakuolen", CHOD *et al.* (1973) "intracytoplasmic inclusions". However, the most precise term for these organelles is microbodies and the authors who called them peroxisomes or glyoxysomes nearly reached the right name. TOLBERT (1971) characterizes the electron-microscopic face of plant microbodies (peroxisomes and glyoxysomes) as follows: Their profiles are nearly circular, they have 0.2 to 1.5 μm in diameter, a single limiting membrane (mitochondria have a double membrane), and a granular matrix (dense core) or a crystalline inclusion. Microbodies are in close association with chloroplasts and mitochondria.

Proteinaceous crystals resembling crystals within microbodies in their shape, size, and inner structure, were also ascertained within mitochondria (LANCÉ-NOUGARÈDE 1966, PARTHASARATHY 1974a) and chloroplasts of diseased plants (FUJISAWA *et al.* 1967, KIM and FULTON 1969) or in sieve element plastids (BRAUN and SINCLAIR 1976). MILNE (1972) described abnormal pseudocrystalline structures in chloroplasts of isolated protoplasts and of incubated leaf discs obtained from tobacco leaves. In cultured spinach leaf discs CRAN and POSSINGHAM (1974) observed that chloroplast degeneration involved the loss of thylakoid membranes accompanied with a build up of osmiophilic droplets and membrane bound crystals. SCHÖRTZ *et al.* (1971) mentioned further references to crystals in the stroma region of plastids and described protuberances separated from chloroplasts and emitted into the cytoplasm; some of these bodies contained proteinaceous rhombic crystals. With *Oenotheras* about ten per cent of chloroplasts produced outgrowths, which might be formed by evagination — or they arose by segmentation of the bodies. Profiles of those bodies fully corresponded to microbodies identified as peroxisomes or glyoxysomes. However, microbodies may originate from the endoplasmic reticulum (TOLBERT 1971).

Crystal-containing bodies in leaf vascular tissues have already been described by BOUCK and CRONSHAW (1965). Investigating the ultrastructure of phloem in palms PARTHASARATHY (1974a, b) described proteinaceous crystalline inclusions in plastids and concluded that these crystals were released from plastids during injury. Thus the disassociated crystals are

displaced from plastids in disturbed sieve elements. Crystals within plastids were also found in maize *etc.*

Proteinaceous crystals like those mentioned were observed in ultrathin sections of some virus diseased plants, presented in our contribution.

Material and Methods

Specimens were taken (1) from *Nicotiana glutinosa* L. plants infected with turnip mosaic virus (TuMV) (syn. cabbage black ring virus); occurrence of microbodies in this material has been preliminarily mentioned by BRČÁK and KRÁLÍK (1977), (2) from plants experimentally infected with an incompletely identified virus isolated from *Anchusa officinalis* L., apparently a member of the Nepovirus group; two host plants of this virus were investigated, *Nicotiana megalosiphon* HEURCK et MUELL. (specimen abbreviation AoNm) and *Chenopodium murale* L. (specimen abbreviation AoCm), and (3) from naturally infected abutilon plants showing symptoms of abutilon mosaic (AbMV).

Specimens for ultramicrotomy were fixed with glutaraldehyde and osmium tetroxide, stained with uranylacetate and embedded in Maraglas. Ultrathin sections were contrasted with lead citrate. Methods by SPURLOCK *et al.* (1963) and RAYNOLDS (1963) were used for TuMV, AoNm and AbMV specimens (which were stained with uranylacetate in 70% ethanol and propylenoxide). The same procedure was employed with AoCm, but acetone was used for dehydration and solution of the embedding medium and contrasting was performed according to VENABLE and COGGESHALL (1965).

Results and Discussion

The great part of the crystal-containing organelles (Figs. 9 to 18, 20, and 22) are identical with microbodies (peroxisomes or glyoxysomes) which are known from healthy plants. They are limited by a single membrane, have a granular matrix and also the crystalline inclusions in them fully correspond to microcrystals within microbodies. Comparable structures were formerly described within plastids and proplastids and in lower plants; THORNTON and THIMANN (1964) found them in subepidermal cells of the *Avena* coleoptile. MARINOS (1965) believed that the presence of crystal-containing organelles in rapidly growing tissues could be due to conditions favoring rapid synthesis of proteins that are not immediately used in the growth of the cell but diverted into special storage sites. MATSUSHIMA *et al.* (1969) concluded that some new protein (enzyme) might be synthesized and stored in those bodies and in the later stage it might be released into the cytoplasm. Finally, FREDERICK and NEWCOMB (1969, 1971) proved catalase localization in microbodies which were identical to the catalase-rich peroxisomes. Their results suggested that the inclusions represented the progressive crystallization of catalase from the matrix. Thus, the equivalence of microbodies to peroxisomes as defined biochemically was shown cytochemically too. HILLIARD *et al.* (1971) concluded that the localization of catalase activity appeared to be preferential for the fibrous elements and the crystalline inclusions within the microbodies. They also found two tropical grasses *Saccharum officinarum* L. and *Digitaria decumbens* STENT. ("C₄" plants) containing fewer leaf micro-

bodies than temperate orchardgrass *Dactylis glomerata* L. (a "C₃" plant). FREDERICK and NEWCOMB (1971) stated that the average number of microbodies in the green parenchyma leaf cells was in general several times greater in grasses with high photorespiration than in those with low. Even formerly, the microbodies were tentatively suggested to be ancestral respiratory organelles. TOLBERT (1971) recorded eight enzymes found in isolated leaf peroxisomes and seventeen enzymes in glyoxysomes; nevertheless catalase is a major component of all microbodies. The above references may be important for elucidating the phenomenon observed by many authors who reported a higher occurrence of crystal-containing microbodies in virus diseased plants. Thus, we can presume that the greater number of microbodies in diseased plants may be related to their pathophysiological changes (*cf. e.g.* FARKAS and KIRÁLY 1958).

Crystal-containing microbodies in virus infected plants were usually localized normally as with healthy plants, *i.e.* at the cell wall, in the vicinity of chloroplasts and mitochondria (Figs. 17, 18 and 22). Then they occurred very frequently and were conspicuous especially within the cytoplasm of large complex (massive) inclusions originating due to TuMV infection (Fig. 20). TuMV belongs to the Potyvirus group representing lamellar inclusion forming (LIF) viruses; with these viruses several authors (*e.g.* MATSUI and YAMAGUCHI 1964, EDWARDSON 1974) ascertained a conspicuous occurrence of crystal-containing microbodies within massive inclusions. Presuming the functional identity of microbodies (within virus diseased plants and healthy plants as well), we could correlate their greater number in virus infected plants with the activation of some respiratory systems favouring the virus infection.

Infected cells frequently contain proteinaceous crystals which do not lie within microbodies. They were found in the cytoplasm and in the nucleus as well (*e.g.* MATSUI and YAMAGUCHI 1964). Such crystals are presented in Figs. 1 to 4, 8, 19 and 21. Figs. 5, 6 and 7 show crystals the surface of which is surrounded by a substance resembling the microbody matrix; however, no membrane limits these formations. Fig. 1 (= Fig. 21) and apparently also Fig. 19 demonstrate crystals lying in "empty" spaces. This (and the opaque crystal substance as well devoid of striation periodicity, as shown in Figs. 1 to 5, 8 and 21) may reflect either an artificial result (caused by unsuitable specimen preparation), or the destruction of protoplast due to the virus infection; *e.g.* Fig. 19 shows a very good striation of the crystal together with a strong degeneration of the cell content.

With similar crystals lying in massive inclusions WEINTRAUB and RAGETLI (1968) proved the protein nature of crystals by differential enzymatic digestion. Also RUSSO *et al.* (1968), KISLEV *et al.* (1968), HAMPTON *et al.* (1973), EDWARDSON (1974) and others mentioned crystalline bodies in the cytoplasm of virus infected plants not lying in microbodies and believed not to be of virus origin. CATHERALL and CHAMBERLAIN (1975) observed a greater frequency of crystalline inclusions in the tolerant genotype to agropyron mosaic virus than in the sensitive genotype. The above crystals were usually considered as virus-induced. HAMPTON *et al.* (1973) suggested that the crystals might represent deposits of crystalline protein accompanying terminal phases of virus-induced cytopathology.

The minimum estimated centre to centre distance of crystal lines was with AoNm of about 7.5 to 8 nm, with AbMV the striation periodicity was of about 26 nm. Various authors reported the mean lattice spacing with comparable crystals of 10 to 14 nm with maximum and minimum at 20 or 6 nm respectively; in one case a centre to centre distance of 3.5 nm. Of course, the direction of crystal sectioning is reflected in different striation periodicity.

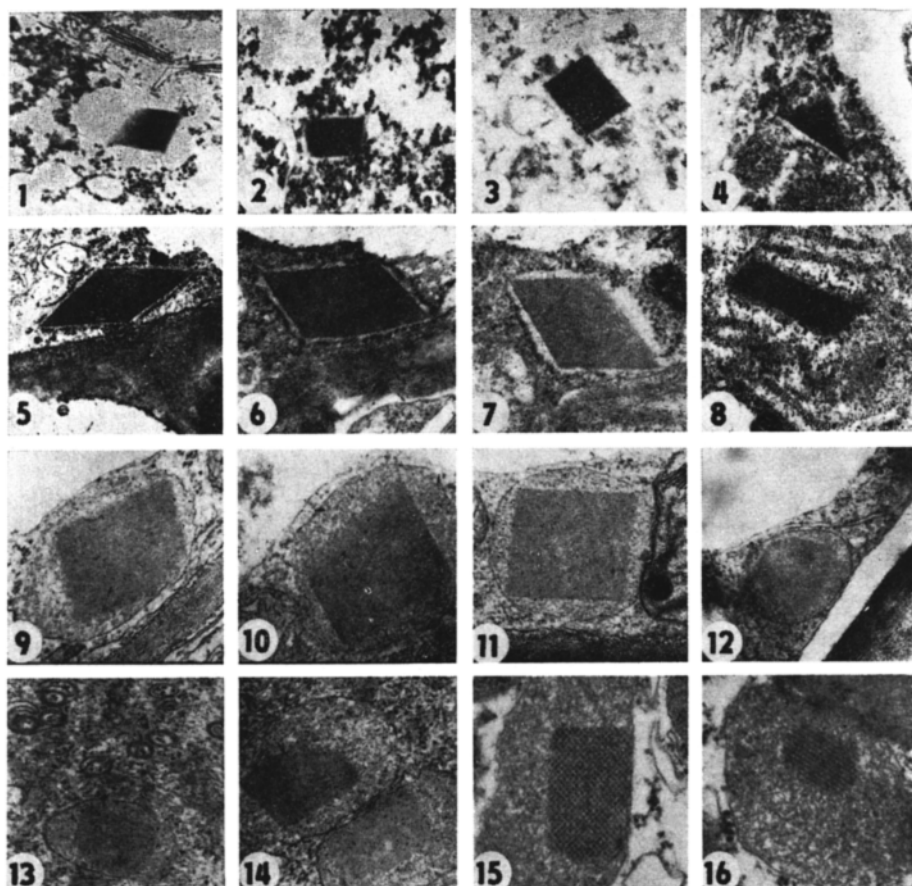
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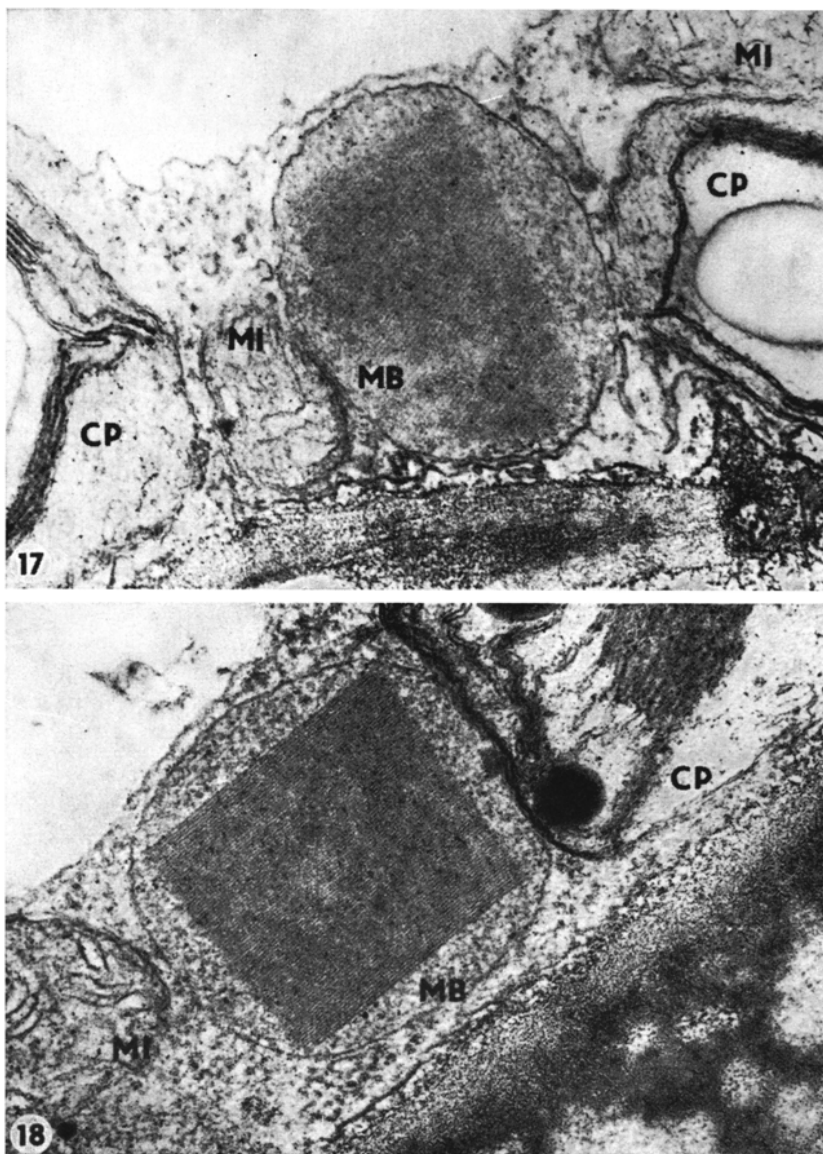
Figures at the end of the issue.

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PROTEINACEOUS CRYSTALS IN VIRUS INFECTED PLANTS



Figs. 1 to 16. Proteinaceous crystals *in situ* ($\times 23\ 500$): 1 to 4 — AoCm, 5 to 12 — AoNm, 13 and 14 — TuMV, 15 and 16 — AbMV. Only Figs. 9 to 16 show crystals within single membrane-bound microbodies. Note the differences between crystal sizes, densities and lattice spacings.

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Figs. 17 and 18. AoNm. Crystal-containing microbodies (MB) lying at chloroplasts (CP) and mitochondria (MI). Fig. 17 — $\times 44\,300$, Fig. 18 — $\times 56\,500$.

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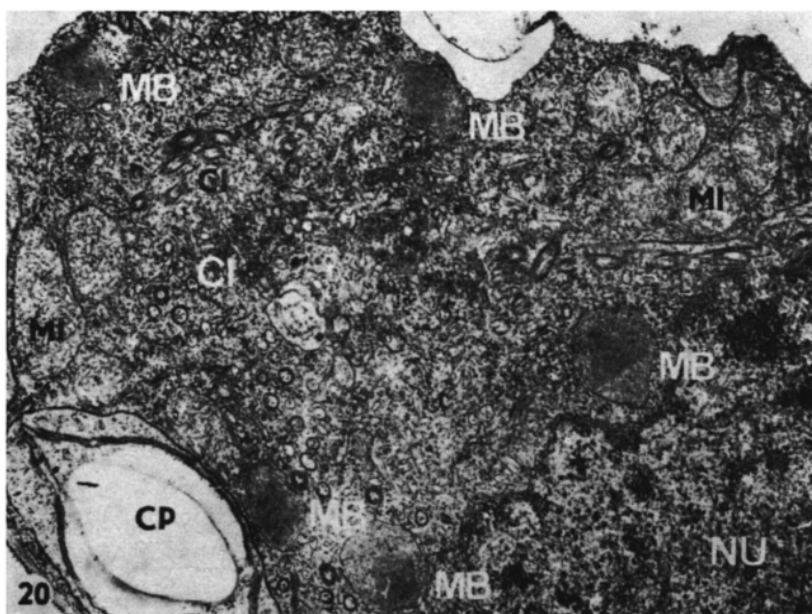
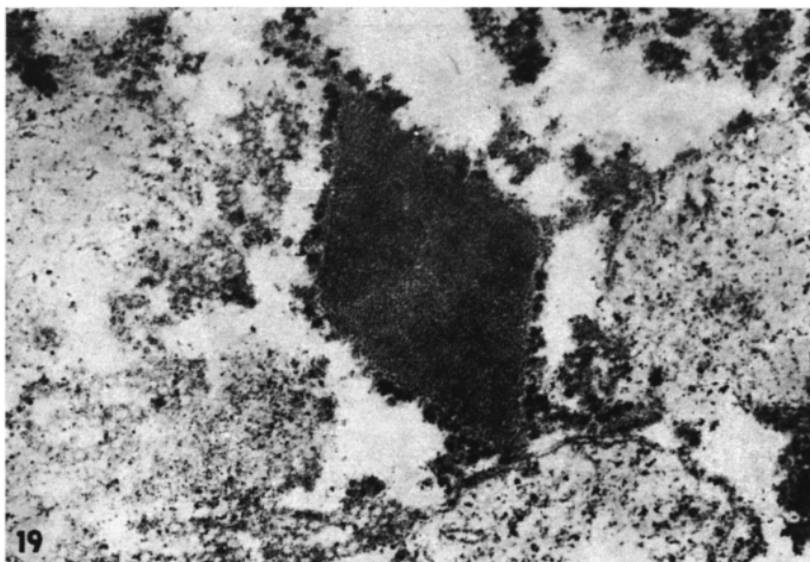


Fig. 19. AoNm. A proteinaceous crystal within a portion of the disintegrated cytoplasm ($\times 68\,500$).

Fig. 20. TuMV. Part of a massive inclusion at the nucleus (NU) caused by TuMV infection in a *Nicotiana glutinosa* cell. All microbodies (MB) contain single crystals. The massive inclusion also contains mitochondria (MI), a chloroplast (CP) and a large amount of cylindrical proteinaceous inclusions (CI). (Approxim. $\times 15\,000$).