Ultrastructure of Cell-Wall Thickenings and Paramural Bodies Induced by Barley Stripe Mosaic Virus

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ABSTRACT

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Examination of ultrathin sections of young barley leaf tissues systemically infected with the ND18 strain of barley stripe mosaic virus (BSMV) revealed many paramural bodies and associated cell-wall thickenings. Occurring along with paramural bodies and cell-wall thickenings were bulbous-shaped "extended plasmodesmata" and extraprotoplasmic sacs, which appeared to have a close association with, and in some cases terminations of, plasmodesmata. Nonmembrane bound, intra- and extraprotoplasmic osmiophilic bodies were observed in mesophyll cells from leaves infected with the virus.

During an ultrastructural study of the barley stripe mosaic virus infection process in three stages of symptom development in barley, we discovered unusual virus-induced modifications of the cell wall in the primary acute phase (17).

Several viruses have been associated with thickenings or protrusions of vascular plant cell walls. Among these were parsnip yellow fleck (19), oat necrotic mottle (6), potato virus X (1), tobacco ringspot (7, 26), carnation etched ring (15), bean pod mottle (10, 11, 12), cherry leaf roll (9), potato virus M (8), cauliflower mosaic (2, 3), tobacco mosaic (22), cowpea mosaic (12, 25), carrot mottle (18), and dahlia mosaic (13). In addition to changes in cell walls, several investigators reported modifications of plasmodesmata with or without the occurrence of vesicles between the cell wall and plasmalemma (1, 2, 3, 7, 11, 13, 15, 18, 22).

The purpose of this paper is to report the occurrence of modified plasmodesmata, accompanying cell wall outgrowths and paramural bodies in barley leaf tissue systemically infected with the ND18 strain of barley stripe mosaic virus (BSMV).

MATERIALS AND METHODS

The second emerging leaf of barley (*Hordeum vulgare* L.'Hudson Winter') seedlings in the three-leaf stage was inoculated with BSMV by the finger-wipe leaf rub method. All experimental plants were grown under greenhouse conditions. The virus isolate was originally supplied by R. G. Timian and has been designated as ND18 (20).

Barley leaf tissue exhibiting the primary acute symptoms described by McKinney and Greeley (17) was fixed in 5% glutaraldehyde for 10-16 hr at 4 C. Glutaraldehyde was buffered with 0.05 M potassium phosphate pH 7.1, with or without 2% sodium thioglycolate (14). Tissue was washed in cold buffer and postfixed in buffered 1% osmic acid at 4 C for 10-16 hr.

The embedding media consisted of a mixture of Spurr low-viscosity plastic (23) and modified Bo-Jax mixture (3:1, v/v) (5), with DMP-30 (tri-dimethylaminomethyl phenol) used as accelerator. Upon dehydration in an acetone series, tissue was soaked in acetone-plastic mixture (1:1, v/v) at room temperature for 3-4 hr, at 40 C for 1 hr, and transferred into fresh plastic and polymerized at 60 C for 26 hr. Noninoculated leaf tissue was treated in the same manner.

Thin sections were cut with glass knives on a Porter-Blum MT-2 ultramicrotome, placed on uncoated copper grids and stained with 2% uranyl acetate followed by lead citrate. Lead citrate stain was prepared by placing 0.5 g lead citrate (Polysciences, Inc., Warrington, PA 18976) along with 8 ml of carbonate-free, 2 N sodium hydroxide solution (Acculute, Anachemia Chemicals Limited, Champlain, NY 12919) in boiled, glass-distilled water. The final volume was brought up to 50 ml with water and then sealed with a layer of paraffin oil and stored at 4 C. Observations were made with a RCA EMU-3G electron microscope.

Thick sections (1.5-2.0 μm) for light microscope studies

were stained with an aqueous solution of toluidine blue. Embedding plastic, which had been stored at -10 C, was used as a medium to mount the thick sections on glass slides.

RESULTS

Symptoms characterized as the primary acute reaction appeared 5 to 7 days postinoculation. Symptoms began as chlorotic flecks in a green field which developed into uniform chlorosis across the leaf. The affected portion of the leaf blade eventually died.

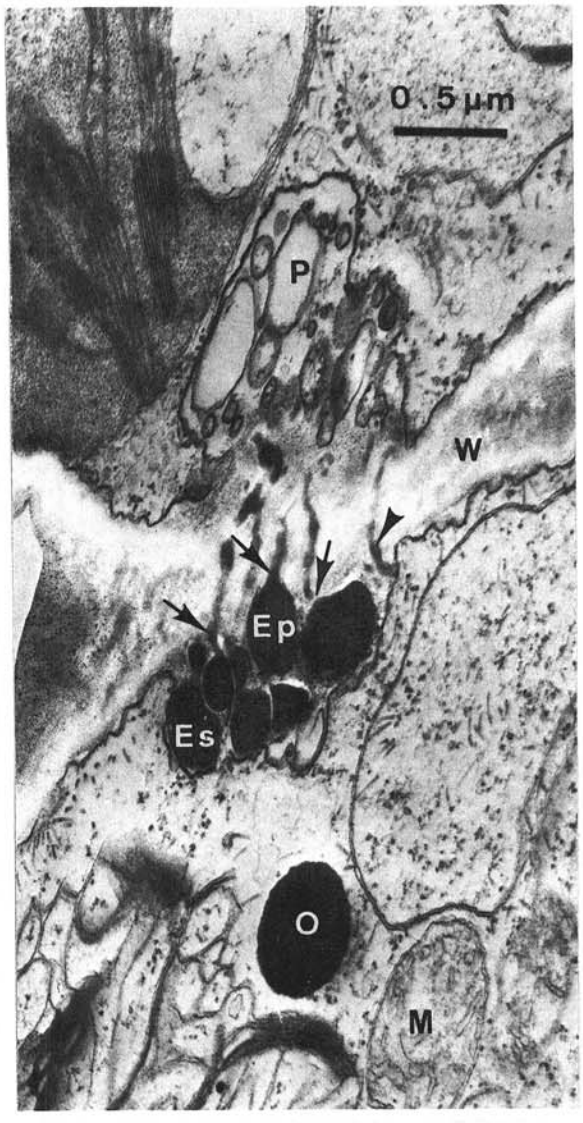

Fig. 1. Ultrastructural cytology of chlorenchyma tissue infected with barley stripe mosaic virus from uniformly chlorotic region in primary acute barley leaf. Note bulbous-shaped "extended plasmodesmata" and extraprotoplasmic sacs. Arrows indicate connection with plasmodesmata in wall. Arrowhead denotes 'normal' plasmodesma with possible plasmalemma connection. Legend: Ep = "extended plasmodesma"; Es = extraprotoplasmic sac; M = mitochondrion; O = osmiophilic body; P = paramural body; and W = cell wall.

The wall of infected mesophyll cells contained "extended plasmodesmata" with accompanying cell-wall outgrowths. These structures were observed in both chlorotic fleck and uniformly chlorotic portions of the leaf. Paramural bodies containing many vesicles or flattened tubules were located between the plasmalemma and the cell wall in this tissue (Fig. 1). Paramural bodies were not restricted to virus-infected tissue. They were occasionally observed in control tissue and noninfected experimental material.

"Extended plasmodesmata" were very electron-dense, membrane limited and terminated in bulbous protrusions (Fig. 1, 2-D, E). The protrusions were found between the plasmalemma and cell wall, causing invagination of the cytoplasm. Cell-wall outgrowths occurred around the plasmodesmata which contained less microfibrils than the primary cell wall (Fig. 2-E). Some cell-wall outgrowths extended toward the cytoplasm and contained plasmodesmata which did not terminate in bulbous protrusions. Wall outgrowths appeared as islands of cell-wall material in the cytoplasm surrounded by the plasmalemma in transverse or oblique sections.

Extraprotoplasmic sacs containing small spherical granules in close association with plasmodesmata were observed in cells from both chlorotic fleck and uniformly chlorotic tissue (Fig. 1, 2, 3). The sacs were always located between the plasmalemma and cell wall and were not observed in the absence of plasmodesmata. These sacs were membrane limited and appeared to be continuous with plasmodesmata (Fig. 3). Cell-wall material similar to that surrounding "extended plasmodesmata" occurred around the sacs (Fig. 2-C, 3-A, 3-B). "Extended plasmodesmata" and extraprotoplasmic sacs frequently appeared at the same location along the cell wall (Fig. 1, 2). Figure 2-A demonstrates that the modified plasmodesmata fields can be observed by light microscopy. Both types of abnormalities occurred only at cellular interfaces. These structures never were found in cell walls adjacent to intercellular spaces. Spherical or ovoid osmiophilic bodies frequently were found in chlorotic primary acute tissue either near cell-wall malformations (Fig. 1, 2-C) or scattered in cytoplasm. These structures were not limited by a membrane.

DISCUSSION

The term "paramural body" was proposed by Marchant and Robards (16) to refer to "all membranous or vesicular structures associated with the plasmalemma . . . regardless of their origin." Although paramural bodies occasionally were observed in healthy barley tissue, they were a common feature of diseased cells. Kim and Fulton (11) have discussed the possible functions of paramural bodies and their relationship to virus infections. Because of the close association between paramural bodies and cell-wall protrusions, it has been hypothesized that these vesicles and convoluted membranes are the result of increased plasmalemma activity in relation to extra cell wall synthesis (2, 11). This explanation of paramural body development seems well suited to ND18 BSMV-infected tissue since paramural bodies and cell-wall thickenings occur together.

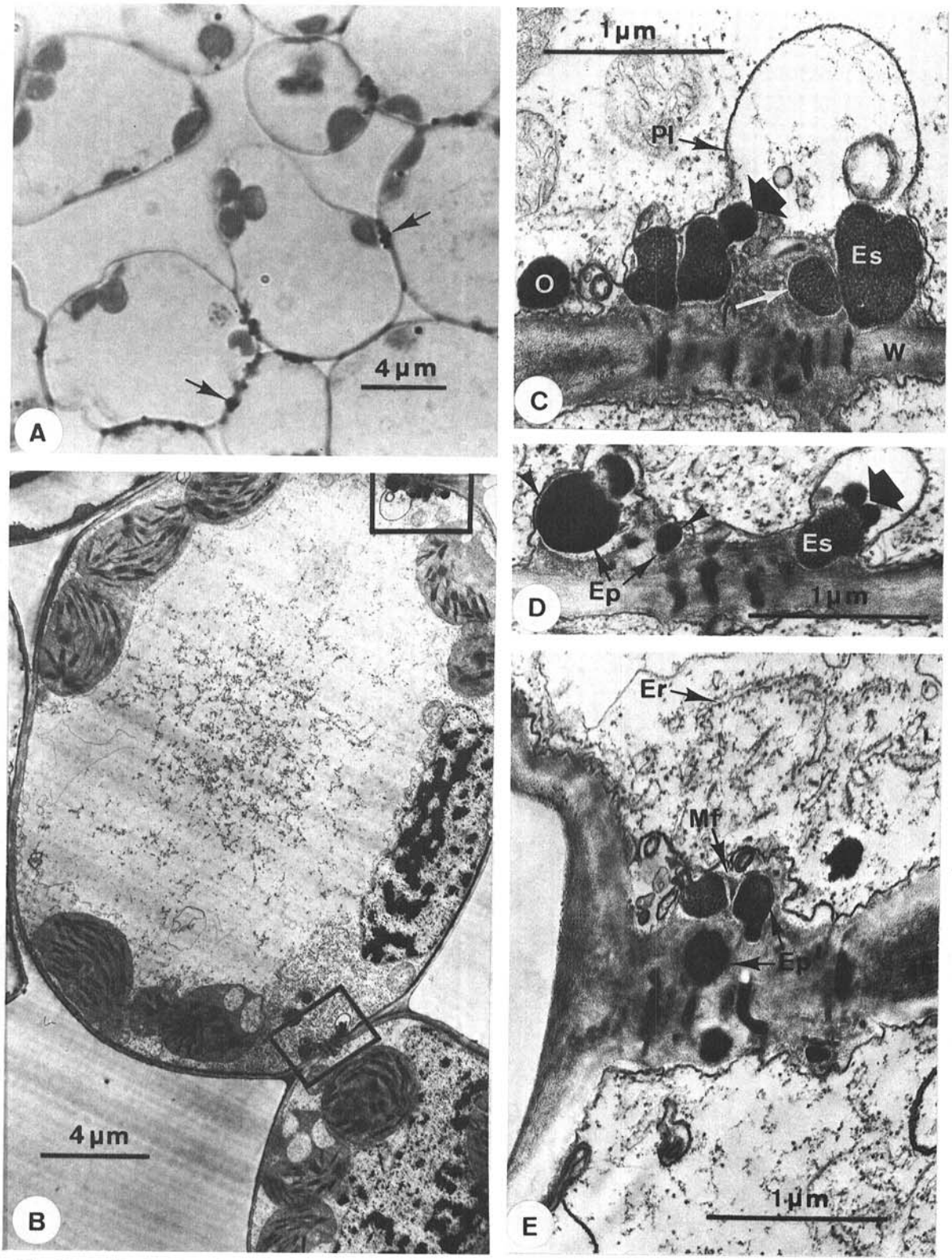

Fig. 2-(A to E). Cytology of cell-wall associated structures in primary acute barley leaf tissue infected with barley stripe mosaic virus. **A)** Light micrograph of structures in or near cell wall (arrows). **B)** Ultrastructural appearance of structures at cell junction (boxes). **C)** Higher magnification of upper boxed-in area of Fig. 2-B. Note occurrence of nonmembrane-bound osmiophilic bodies both between plasmalemma and cell wall (broad arrow) and in cytoplasm. White arrow indicates membrane-bound sac. **D)** Higher magnification of lower boxed-in area in Fig. 2-B. Note membrane-bound "extended plasmodesmata" (arrowheads). Broad arrow refers to osmiophilic bodies between the plasmalemma and the cell wall. **E)** Near median section of "extended plasmodesma" terminating in bulbous protrusion. Cell wall material surrounds protrusions, eventually isolating them. Legend: Ep = "extended plasmodesma"; Er = endosplasmic reticulum; Es = extraprotoplasmic sac; Mf = microfibrils; O = osmiophilic body; Pl = plasmalemma; and W = cell wall.

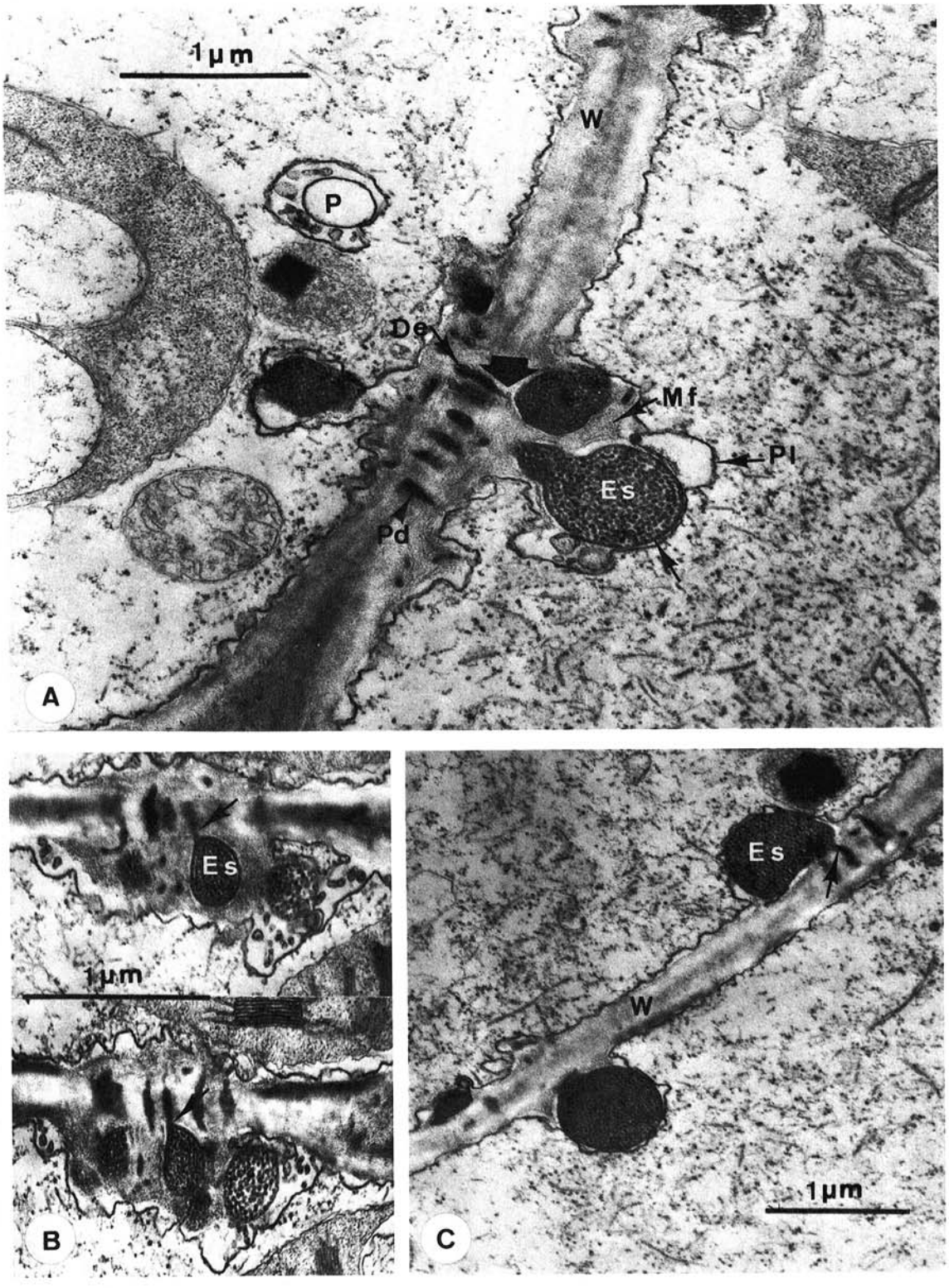

Fig. 3. Ultrastructure of extraprotoplasmic sacs in primary acute barley leaf tissue infected with barley stripe mosaic virus. **A)** Section of wall containing several sacs. Limiting membrane of the sac (unlabeled small arrow) is not continuous with plasmalemma. Note orderly rows of small spherical granules within the sac. **B)** Approximate serial sections illustrating connection of plasamodesma with sac (arrows). **C)** Section through sac protrusion and connected plasmodesma (arrow). Legend: De = desmotubule; Es = extraprotoplasmic sac; Mf = microfibrils; P = paramural body; Pd = plasmodesma; and W = cell wall.

Occurring with paramural bodies and cell-wall thickenings were membrane-bound structures termed "extended plasmodesmata" and extraprotoplasmic sacs. Since it was not demonstrated convincingly that the cavities of the "extended plasmodesmata" were lined by the plasmalemma (Fig. 1), the inclusion of the term plasmodesmata might be conjectural. However, structures resembling desmotublues were observed (Fig. 3-A), and the observance of few normal plasmodesmata in this tissue supported the hypothesis that these unusual structures were modified plasmodesmata. There is not agreement on the fine structure of normal plasmodesmata (21). As mentioned in the results, "extended plasmodesmata" were located between the plasmalemma and cell wall or embedded in thickened portions of the wall. Interestingly, this corresponds exactly with myelinic bodies reported recently by Kim and co-workers (12). The myelinic bodies were associated with tubules containing virus particles and "were composed of fine linear bands arranged concentrically." No ND18 BSMV particles or linear bands were found associated with "extended plasmodesmata."

Extraprotoplasmic sacs have been described previously (1). However, there are differences between the contents of sacs reported by Allison and Shalla (1) and those we observed in ND18 BSMV-infected cells in that the latter occurred only in early systemically invaded tissue and did not contain virus particles. Examination of many longitudinal sections indicated that the small spherical granules were not transversely sectioned filaments or rods. The origin and composition of the small spherical granules contained in the sacs of BSMV-infected cells were not determined. Extraprotoplasmic sacs appeared to have a close association with, and in some cases terminations of, plasmodesmata. Similar sacs also were detected in ND18 BSMV-infected corn cells (*C.* McMullen, W. Gardner, G. Myers, *unpublished*). It could be hypothesized that these structures are a protective response by the plant to restrict the translocation of virus. This possibility has been discussed for similar phenomena in local lesion hosts (1, 8, 22, 24) and in systemically infected tissue (11). As pointed out by Kim and Fulton (11), if this hypothesis is correct, these structures are a very inefficient constraint in systemically infected hosts, since virus particles were detected throughout the tissue. Previous investigators failed to find these wall abnormalities in BSMV-infected leaf tissue probably because the specific stage of virus infection was not examined.

Nonmembrane-bound, intro-, and extraprotoplasmic, osmiophilic bodies were located in ND18 BSMV-diseased tissue; they were also reported in tissue infected with either of two spherical viruses by Kim et al. (12). Esau (4) discussed the occurrence of similar structures in the cytoplasm of healthy cells. Kim et al. (12) hypothesized that osmiophilic bodies (globules) are transformed into myelinic bodies after they become extraprotoplasmic. We are not ready to suggest such a relationship exists between the osmiophilic bodies and extraprotoplasmic sacs or "extended plasmodesmata". It is conceivable that these structures are secondary effects of virus infection due to breakdown of cellular components or alteration of cellular metabolism.

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