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Development of Woronin Bodies from Microbodies in *Fusarium oxysporum* f. sp. *lycopersici*

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With 11 Figures

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Summary

Woronin bodies are cytoplasmic organelles which commonly lie near the septa in ascomycetous fungi. Although these organelles were observed nearly 100 years ago, little is known about their origin and development. The present ultrastructural investigation describes the ontogeny of Woronin bodies in *Fusarium oxysporum* f. sp. *lycopersici* [Sacc.] Snyd. and Hans. In this fungus, Woronin bodies are produced by microbodies. Development of the Woronin body begins with the appearance of electron dense material within the microbody. This material aggregates adjacent to the membrane of the microbody and condenses into a single paracrystalline inclusion. Following its formation, the inclusion is gradually extruded and is eventually separated from the parent organelle by an exocytotic mechanism. After the separation, the paracrystalline inclusion is found at the septal pore. Although many recent electron microscopic studies have used various terms to designate these membrane bound organelles, in *Fusarium* these inclusions are believed to correspond to the Woronin bodies initially described by light microscopists.

1. Introduction

A class of round or oblong organelles has been frequently associated with the septal pores from ascomycetous fungi. These organelles, which were initially described in the nineteenth century by WORONIN, have been generally referred to as Woronin bodies by light microscopists (BULLER 1933). The application of the electron microscope to cytological examinations of fungi enabled investigators to characterize a type of organelle which was thought to correspond to the Woronin body (REICHLE and ALEXANDER 1965). However, other fine structural studies have designated the inclusions that lie near the septal pores as Woronin-like bodies (CAMP 1971, CUTLER and ERKE 1971), lipid (SACHS *et al.* 1970), lipid bodies (MOORE and MCALEAR 1962, MITCHELL and MCKEEN 1970), lipoid granules (ZACHARUK 1970), lipoidal inclusions (ZACHARUK 1971), lysosomes (WILSON, STIERS, and SMITH 1970), spherosomes (WILSON, STIERS, and SMITH 1970, MCCOY, GIRARD, and KORNFELD 1971),

crystal-containing microbodies (CAMP 1971, MAXWELL, WILLIAMS, and MAXWELL 1972), and granules (DICKSON 1963). Consequently, at the present time Woronin bodies may be erroneously designated by a host of ambiguous terms and/or could be closely accompanied by other organelles at the septal pore.

In addition to this apparent confusion that currently surrounds the Woronin bodies, very little is known about their origin and development. In *Ascodesmis sphaerospora*, Woronin bodies appeared to arise in membrane sacs possibly corresponding to dilated cisternae of the endoplasmic reticulum (BRENNER and CARROLL 1968). In *Polythrincium trifolii*, Woronin-like bodies were seen associated with crystal-containing microbodies (CAMP 1971). This observation led the author to speculate that either microbodies were the site of origin of the Woronin-like bodies or the two organelles occasionally fused with one another. However, a more recent study of two of the rust fungi, *Melampsora lini* and *Puccinia helianthi*, failed to demonstrate a direct developmental association between these two organelles (COFFEY, PALEVITZ, and ALLEN 1972).

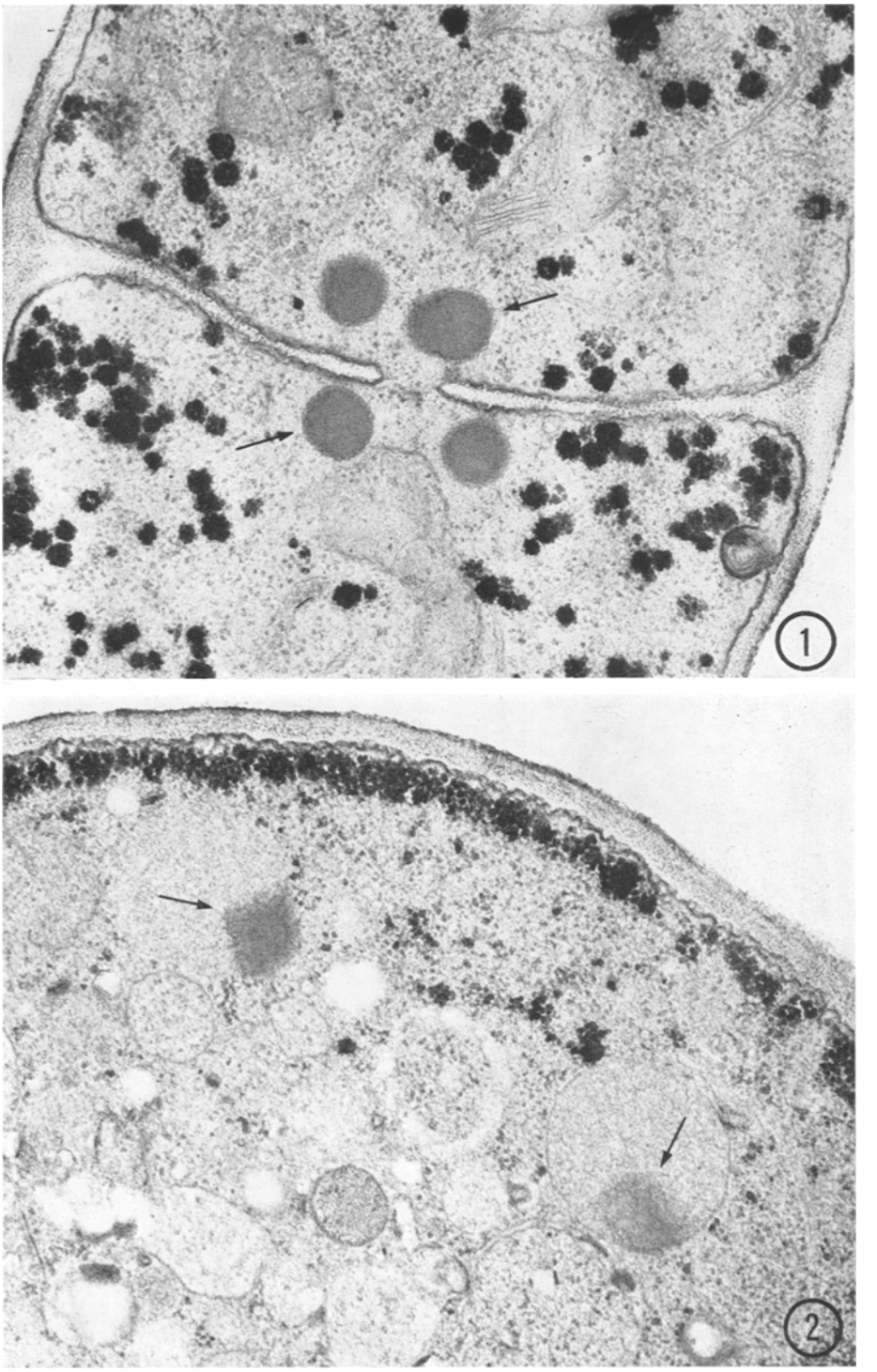
In an effort to clarify the origin, development and ambiguous terminology that presently surrounds Woronin bodies, an ontogenetic study of these organelles was undertaken in *Fusarium oxysporum* f. sp. *lycopersici* (WERGIN 1972 b).

2. Materials and Methods

An isolate of *F. oxysporum* f. sp. *lycopersici*, race 1 (ATCC No. 16417), was grown in shake culture as previously described (MACE, VEECH, and HAMMERSCHLAG 1971). After 3 days, spores (microconidia) were aseptically separated from the culture medium, washed several times in sterile distilled water and transferred to 4 ml test tubes containing medium B (ESPOSITO and FLETCHER 1961) amended with 0.1% yeast extract. The tubes containing spores and medium were incubated at 28 °C for 3 days and then centrifuged. The supernatant, consisting of the nutrient broth was removed and the pellets, containing hyphae from germinated spores, were embedded in molten water agar. Two-three mm^3 sections of agar bearing young fungal hyphae were removed from the tubes and transferred to vials. Fixation, rinsing, and postfixation in osmium tetroxide were carried out at room temperature in 0.05 M phosphate buffer at pH 6.8. Fixation for a period of 1.5 hours with glutaraldehyde was followed by washing in six changes of buffer over a period of 1 hour. The fungal tissue then was postfixed in 2% osmium tetroxide for 2 hours, dehydrated in an acetone series, and embedded in Spurr's medium (SPURR 1969). Silver-gray sections were cut on a Sorvall MT-2 ultramicrotome with a diamond knife and mounted on uncoated 300 × 75 copper grids. The sections were stained with 2% uranyl acetate for 10 minutes, then with lead citrate for 5 minutes. Thin sections were viewed in a Hitachi HU-11C electron microscope operating at 75 kv with a 30 μ objective aperture.

Fig. 1. Longitudinal section through a portion of the septum. Woronin bodies (arrows) lie on either side of the septal pore. ×58,000

Fig. 2. Cross section through a young hyphal cell. Partially formed inclusions (arrows) can be observed in the microbodies. ×50,000



Figs. 1 and 2

3. Results

3.1. Appearance of Woronin Bodies

Woronin bodies are organelles that are generally found near the septal pores of vegetative hyphae (Fig. 1). In *Fusarium oxysporum* f. sp. *lycopersici*, the Woronin bodies are spherical, measuring approximately 0.2 μm in diameter, and are bounded by a single unit membrane. Because of their spherical shape and small size, the discrete unit structure of the membrane is observed only when the organelles are sectioned medially. In addition to these features, Woronin bodies characteristically contain a homogeneous, electron dense matrix. This distinctive trait enables one to distinguish Woronin bodies from other cellular structures and to trace their development as it occurs in the vegetative hyphae.

3.2. Formation of Inclusions in Microbodies

Microbodies which are ubiquitous organelles that are found in plant cells (MOLLENHAUER, MORRÉ, and KELLEY 1966, FREDERICK *et al.* 1968, VIGIL 1972) have been previously described in hyphae of *F. oxysporum* (WERGIN 1972a). These organelles, which measure approximately 0.5–1.0 μm in diameter, consist of a granular matrix that is surrounded by a single unit membrane. In young hyphal cells of *F. oxysporum*, small inclusions, having an electron density identical to that exhibited by the matrix of Woronin bodies, appear and develop in the microbodies (Fig. 2). A single inclusion appears to form in each microbody which lies along either the septal plate or the lateral walls near the plate. The inclusion initially forms at a localized site, tightly appressed to the inner surface of the membrane. At this site, the surface of the inclusion conforms to the rounded contour of the membrane of the parent microbody. However, the surface areas which do not oppose the membrane frequently appear angular (Fig. 3). At this early stage of formation, no distortion in the shape of the microbody is observed. Furthermore, no obvious cytoplasmic organelles or activities have been observed in association with the appearance of the inclusions in the microbodies.

3.3. Growth and Separation of the Inclusions

The initial appearance of the small inclusions is followed by their growth. The increase in size appears to result from the compaction or reorganization of the granular material, which comprises the matrix of the microbody, and

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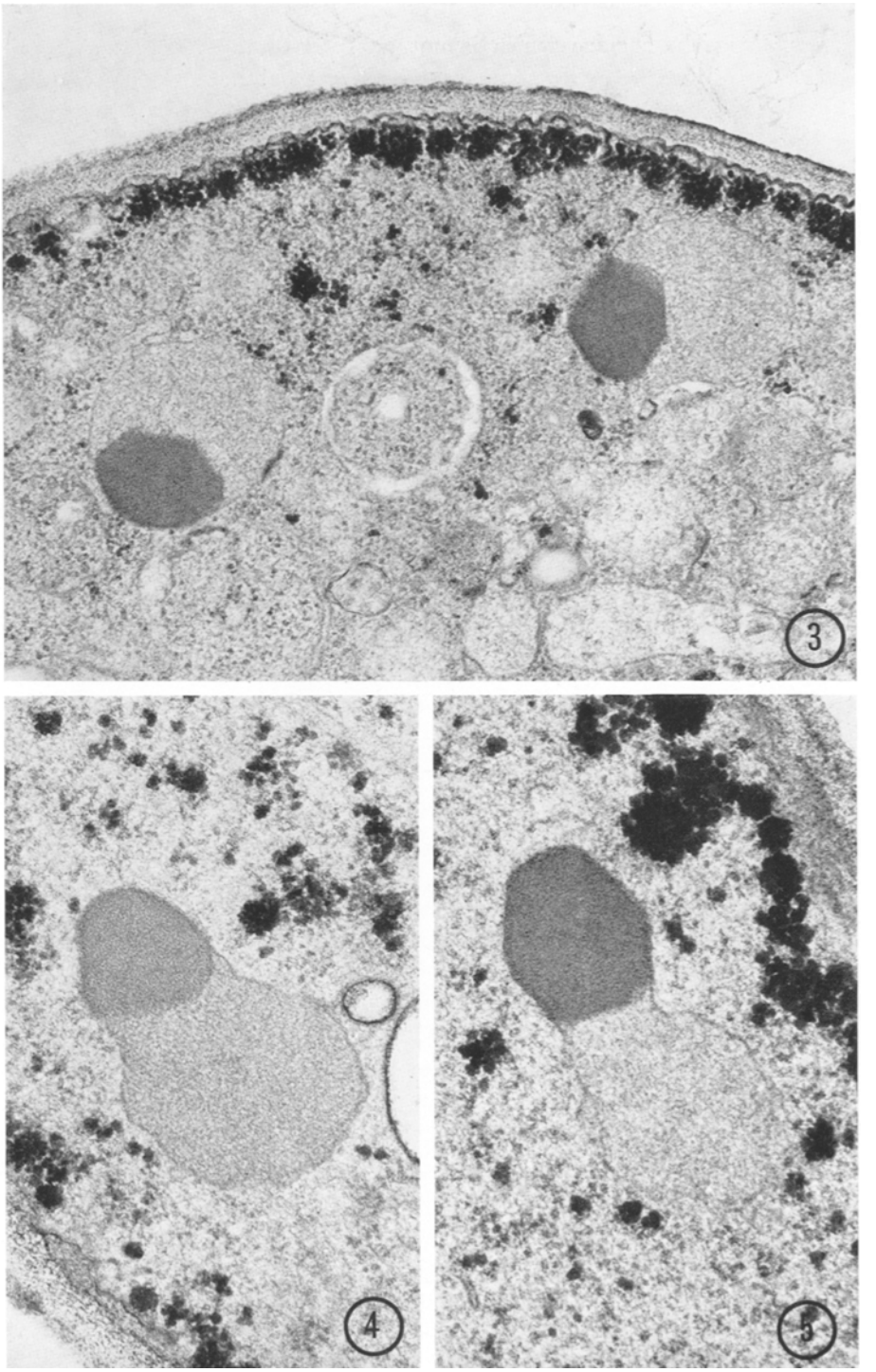
Fig. 3. Cross section through a young hyphal cell. In each microbody, a distinct inclusion lies tightly appressed to the inner surface of the membrane. ×50,000

Figs. 4 and 5. Sections through microbodies containing inclusions. The inclusions have begun to evaginate outward from the parent organelles. Although the inclusions tend to be round at this stage, occasionally six-sided structures (Fig. 5) are also encountered. Fig. 4, ×70,000; Fig. 5, ×70,000



Figs. 3–5

occurs along the surface which is not apposed to the membrane (Fig. 4). During this process, the dense inclusions tend to become spherically shaped; however, cross sections of six-sided forms have also been encountered (Fig. 5). In spite of this apparent compaction of material in the microbody, the resulting inclusions do not exhibit the distinctive subunits which are usually associated with a crystalline lattice.

Coincident with the growth of the inclusion is its separation from the microbody. At the localized site of its formation, along the membrane of the microbody, the inclusion begins to bud or evaginate outward from the parent organelle (Figs. 4–6). This process gradually continues until the membrane surrounding the inclusion eventually pinches off from that of the microbody (Figs. 7 and 8). Consequently, the two organelles, each with an intact limiting membrane, become physically separated from one another (Fig. 9).

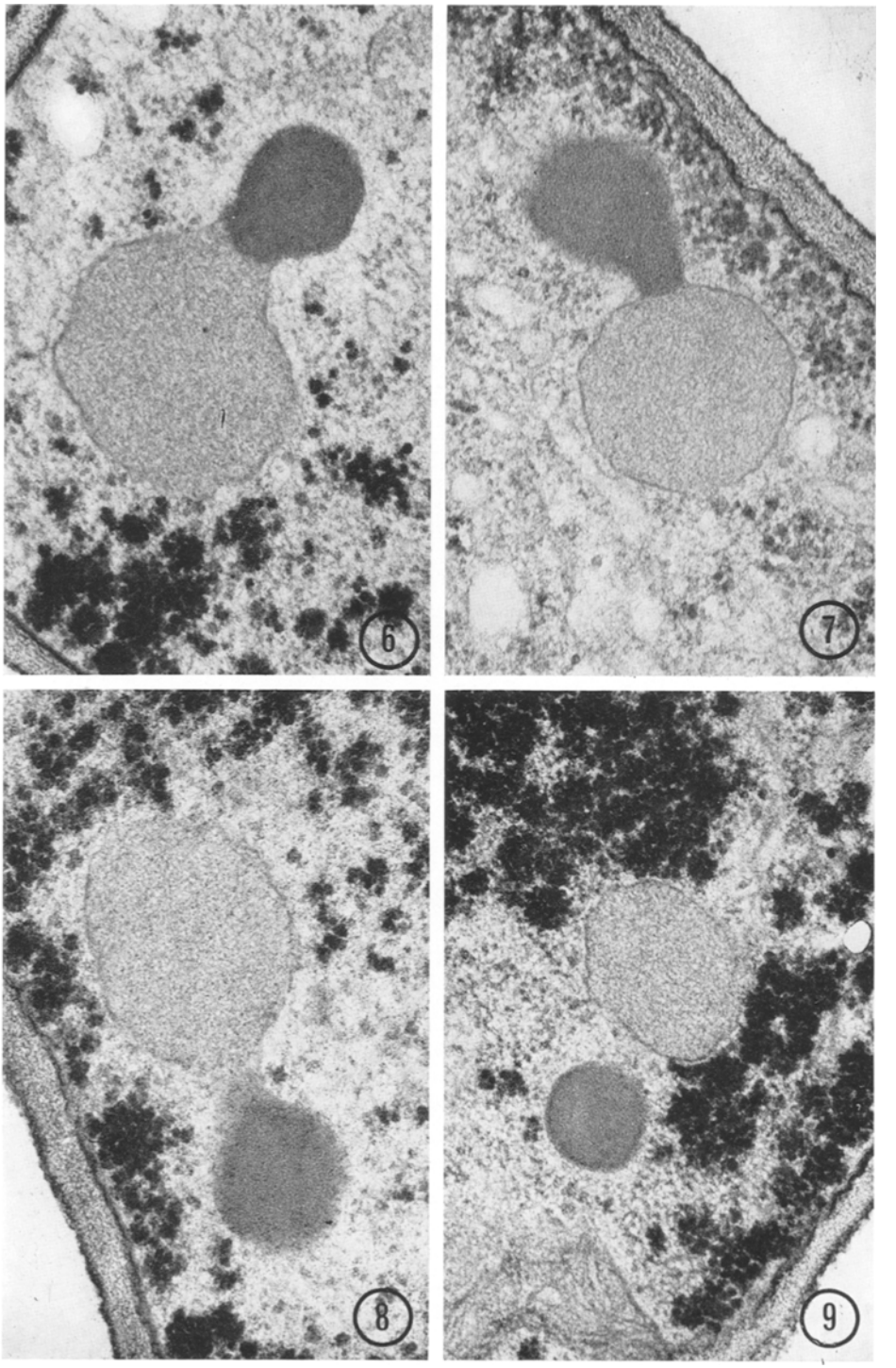
During the process of separation, the membranes of the departing spherical inclusions can be distinctly observed only when they are sectioned normally. Neither the membrane nor the discrete shape of the inclusion is apparent in oblique sections of the organelle.

3.4. Distribution of the Inclusions (Woronin Bodies) at the Septal Pore

After the inclusions have separated from the parent microbody, they become distributed near the septum where several of them commonly lie on either side of the septal pore (Fig. 10). These inclusions appear identical to the Woronin bodies previously described for *Fusarium* spp. (REICHLE and ALEXANDER 1965).

In *F. oxysporum*, the septal pores are occasionally plugged with a homogeneous electron opaque material (Fig. 11). This material, which has been previously described, is identical to the matrix of the Woronin bodies and is believed to be produced by them (REICHLE and ALEXANDER 1965, BRACKER 1967, BRENNER and CARROLL 1968, MCKEEN 1971). No spherosomes (lipid bodies) or unidentifiable granules were observed near the septal pores during the present study.

Figs. 6–9. Sections through microbodies and their respective inclusions. These figures are believed to represent the successive stages which occur during the separation of the inclusion from the parent organelle. During this process, the inclusion evaginates (Fig. 6), the membrane between the two bodies constricts (Fig. 7), and finally, the inclusion is "pinched off" (Fig. 8). Two distinctly separate organelles, the microbody and the Woronin body, result from this process (Fig. 9). Fig. 6, ×72,000; Fig. 7, ×66,000; Fig. 8, ×70,000; Fig 9, ×65,000



Figs. 6–9

4. Discussion

4.1. Origin of Woronin Bodies

In *Ascodesmis sphaerospora,* Woronin bodies were shown to originate in a pouch-like membrane system, which was thought possibly to represent dilated cisternae of the endoplasmic reticulum (RRENNER and CARROLL 1968). However, close examination of the micrographs from this earlier investigation, and the observations made in the present study, suggest that the pouch-like membranes described in *A. sphaerospora* probably correspond to the microbodies observed in *F. oxysporum.*

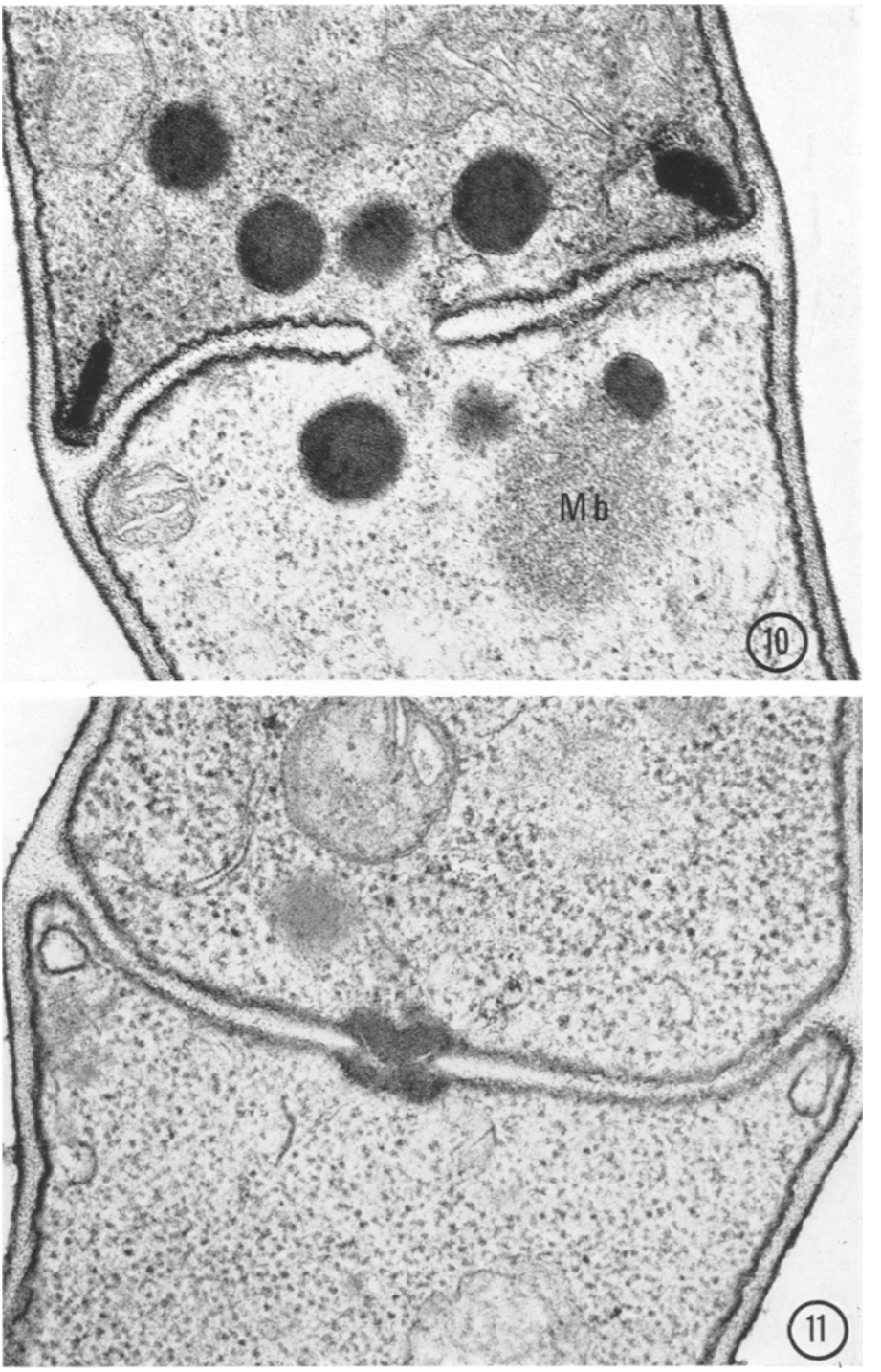
Microbodies have been previously observed near the pore in septate fungal hyphae (CAMP 1971, COFFEY, PALEVITZ, and ALLEN 1972, MAXWELL, WILLIAMS, and MAXWELL 1972). In two of the rust fungi, *Puccinia helianthi* and *Melampsora lini,* microbodies surround a dense cytoplasmic matrix in the pore region (COFFEY, PALEVITZ, and ALLEN 1972). Although dark-staining bodies are sometimes observed in this matrix, the authors obtained no evidence that these arise from the microbodies. In *Polythrincium trifolii,* a physical association between microbodies and Woronin bodies influenced the author to speculate that either the former gave rise to the latter or the two organelles were fusing with one another (CAMP 1971). In the present study of *Fusarium oxysporum,* the distribution of the two organelles and the configurations between them suggest that the Woronin bodies originate as inclusions within the microbodies and later separate from the parent organelle by an exocytotic mechanism. Although inclusions are commonly observed in microbodies from higher plants and animals, apparently no other examples of extrusion and separation of the inclusions from the parent organelle have been reported.

4.2. Function of Woronin Bodies

The proximity of Woronin bodies around the pore of septate hyphae has been previously documented (REICHLE and ALEXANDER 1965, BRACKER 1967, BRENNER and CARROLL 1968, SCANNERINI 1968, MCKEEN 1971) and was also observed in the present study of *F. oxysporum.* Because of their occurrence at this site, Woronin bodies are believed to act as plugs, whose presence or absence in the pore regulates cytoplasmic flow between adjacent cells. However, their ontogenetic association with microbodies may indicate a physiological function as well.

Fig. 10. Longitudinal section through a portion of the septum. Several Woronin bodies are apparent on either side of the septal pore. A microbody (*Mb*) containing an evaginating inclusion lies to the lower right of the pore. ×62,000

Fig. 11. Portion of the septum which has been sectioned longitudinally. The septal pore is occluded with an electron dense structure believed to be derived from the Woronin bodies. ×67,000



Figs. 10 and 11

Enzymatic activity has been demonstrated for inclusions which occur as amorphous, paracrystalline and crystalline structures in microbodies from plants and animals (see review, VIGIL 1972). Since the Woronin bodies that occur in *F. oxysporum* are extruded inclusions, perhaps they also contain enzymatic activity. Recently, a biochemically defined organelle has been described near the septum in *Ceratocystis fimbriata* (WILSON *et al.* 1970). This organelle is bounded by a unit membrane and contains hydrolytic enzymes. To conform with the accepted terminology, this biochemically defined organelle was referred to as a lysosome. However, the distribution and fine structure of the lysosomes in *C. fimbriata* (see Fig. 3 in WILSON *et al.* 1970) are consistent with those described for the Woronin bodies in *F. oxysporum*. Consequently, the morphologically defined Woronin bodies in *F. oxysporum* may be identical to the biochemically defined lysosomes in *C. fimbriata*.

4.3. Terminology for Woronin Bodies

Three different types of organelles, which are similar in size and shape, have been described in ultrastructural investigations of *F. oxysporum*. In the present study, an organelle consisting of a granular matrix which is bounded by a unit membrane was observed in young hyphal cells. The morphology of this structure corresponds to that of the microbody which is commonly found in plant cells (MOLLENHAUER, MORRÉ, and KELLEY 1966, FREDERICK *et al.* 1968, VIGIL 1972). A second type of organelle has a homogeneous electron dense matrix that is surrounded by a unit membrane. This body is similar to the Woronin body described in a previous fine structural investigation (REICHLE and ALEXANDER 1965) and is believed to correspond to the structures initially described by WORONIN. Finally, a third type of structure, previously described in *Fusarium* spp. (REICHLE and ALEXANDER 1965, WERGIN 1972 a), consists of a homogeneous electron translucent matrix which is not bounded by a unit membrane. This organelle corresponds to the spherosome (lipid body) which is commonly found in higher plants and has been recently morphologically and biochemically characterized (YATSU, JACKS, and HENSARLING 1971). These three terms, microbody, Woronin body, and spherosome have historical precedence, are widely accepted and have been morphologically characterized. If they were used to designate the comparable structures which appear in fungi, the confusion resulting from the use of numerous ambiguous terms, such as Woronin-like body, lipid, lipid body, lipoid granule, lipoidal inclusion, granule, and crystal-containing microbody could be avoided.

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