Ultrastructural Comparison of Microbodies in Pathogenic and Saprophytic Hyphae of Fusarium oxysporum f. sp. lycopersici

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ABSTRACT

The fine structure of pathogenic fungal hyphae was examined in the vascular tissue of tomato plants inoculated with the Fusarium wilt organism, *Fusarium oxysporum* f. sp. *lycopersici.* Three to 5 days after inoculation, the hyphae were found localized in the metaxylem vessels of the tomato stems. In addition to the normal complement of fungal organelles, the cytoplasm of the fungus contained numerous, well-developed microbodies. A negative staining reaction with 3,3′-diaminobenzidine indicated that these microbodies did not have peroxidase activity. The fungus was also cultured on potato-dextrose agar and processed for electron microscopic observation. Examination of this material disclosed that saprophytic hyphae did not contain the large microbodies which were commonly found in the pathogenic hyphae.

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Additional key words: peroxisomes, glyoxysomes, Fusarium wilt of tomato.

Our knowledge of the fine structure of host-parasite interactions has increased considerably during the past few years; however, most ultrastructural investigations involving fungal pathogens have concentrated on the ultrastructure of haustoria (3). Recently, the cytoplasmic organelles found in the haustoria of the rust fungi were compared with those which occur in intercellular hyphae and axenic mycelium (4). Structural differences were observed for nuclei, mitochondria, endoplasmic reticulum, and microbodies. Since modifications in structure may be correlated with alterations in metabolism, comparative examinations of pathogenic hyphae and saprophytic hyphae could increase our knowledge about the structural and physiological changes associated with pathogenesis. Therefore, the purpose of the present study was to compare the fine structure of pathogenic and saprophytic hyphae of the Fusarium wilt organism (*Fusarium oxysporum* f. sp. *lycopersici* [Sacc.] Snyd. & Hans.) of tomato (*Lycopersicon esculentum* Mill.).

MATERIALS AND METHODS.–*Host inoculation.*–Stem cuttings of tomato (*Lycopersicon esculentum*, 'Improved Pearson') were inoculated with spores (bud cells) of *F. oxysporum* f. sp. *lycopersici*, race 1 (ATCC No. 16417). Culture of the host and pathogen and details of the inoculation procedure were according to a previous report (12). Stem cuttings ca. 2 cm below the cotyledons were taken from 4-week-old plants growing in a greenhouse at ca. 27 C. The severed ends of the cuttings were immersed in a suspension of spores (50,000 spores/ml) for 30 min. Following the inoculation, the cuttings were transferred to a one-tenth-strength Hoagland's solution for 3 days, then planted in sterilized soil and placed in a greenhouse for 2 additional days prior to sampling.

Artificial medium.–The isolate of *Fusarium* was grown in shake culture as previously described (12).

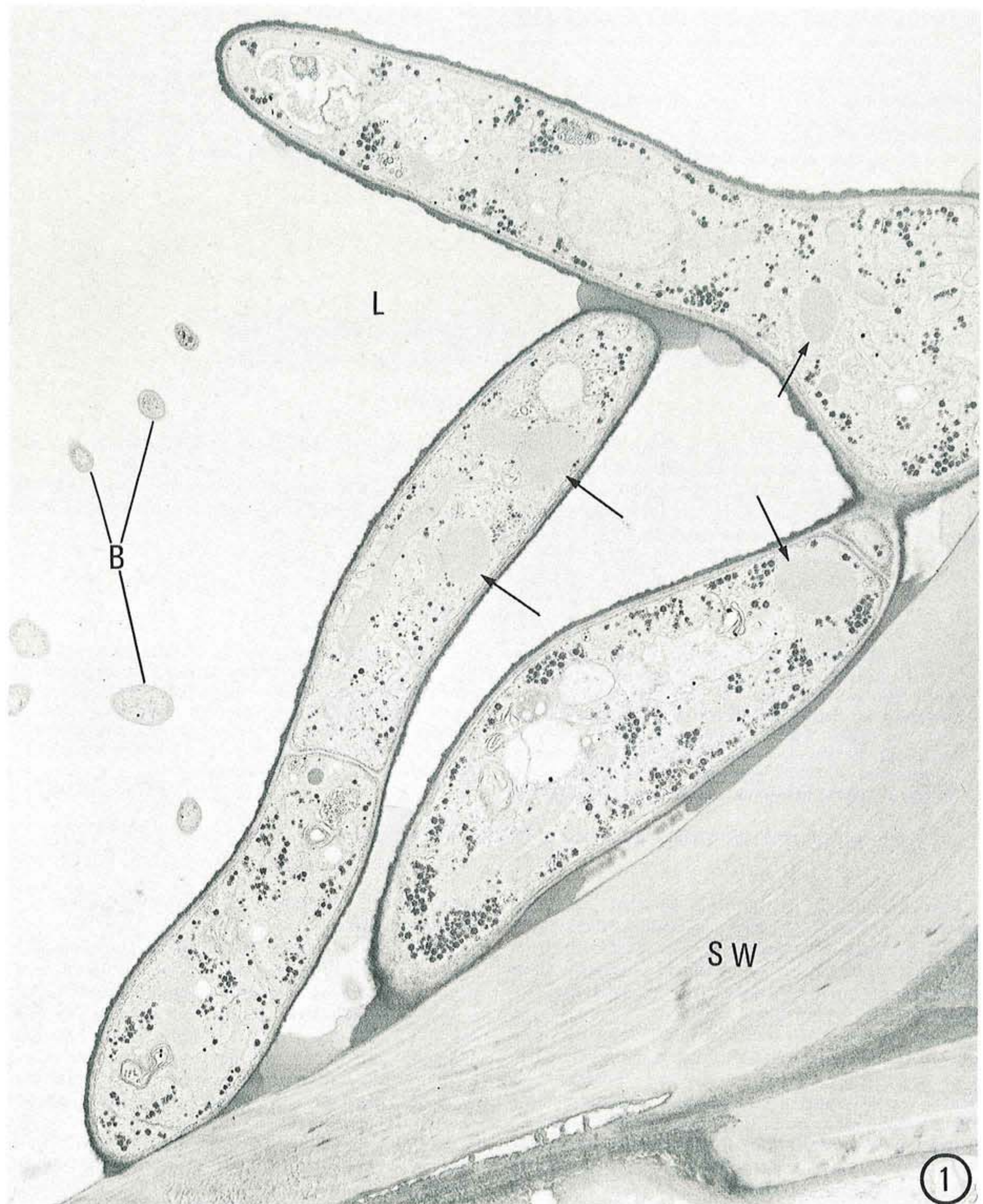


Fig. 1. Portion of a metaxylem vessel from tomato stem inoculated with *Fusarium oxysporum* f. sp. *lycopersici.* In addition to the organelles commonly encountered in fungal hyphae, several large microbodies (arrows) can be observed. SW = secondary wall of the vessel; L = lumen of the vessel; B = bacteria (× 12,000).

One ml of a spore suspension (50,000 spores) was added to the surface of potato-dextrose agar (PDA) in a petri dish. The plates were incubated for 3 to 5 days at 28 C; then the fungus was chemically fixed for electron-microscopic examination.

Preparation for electron-microscopic (EM) observation.—Cross sections of inoculated tomato stems, 2-3 mm thick and 1.5-0.5 cm below the cotyledonary node, were cut while immersed in 3% glutaraldehyde in a 0.05 M phosphate buffer and were transferred to a vial for further processing. Also, 2-3 ml of 3% glutaraldehyde were added to the surface of 3- to 5-day-old *Fusarium* cultures. Two- to 3-mm^3 sections of PDA bearing young fungal hyphae were removed from the plates and transferred to vials. Fixation, rinsing, and postfixation in osmium tetroxide were carried out in 0.05 M phosphate buffer (pH 6.8) contained in vials and at room temperature. Fixation for 1.5 hr was followed by washing in six changes of buffer over a period of 1 hr. The tissue then was postfixed in 2% osmium tetroxide for 2 hr, dehydrated in an acetone series, and embedded in Spurr's medium (16). Silver-gray sections were cut on a Sorvall MT-2 ultramicrotome with a diamond knife and mounted on uncoated 300 X 75 copper grids. The sections were stained with 2% aqueous uranyl acetate for 10 min, then with lead citrate for 5 min. Thin-sections were viewed in a Hitachi HU-11C electron microscope operating at 75 kv with a 30-μm objective aperture.

Incubation with 3,3'-diaminobenzidine (DAB).—Stem segments from inoculated plants and 2- to 3-mm^2 sections of leaf from soybean (*Glycine max* L.) were incubated with DAB (8, 15, 21). Vials containing the tissue and a standard DAB medium (10 mg DAB; 5 ml 0.05 M propanediol buffer; and 0.1 ml 3% H_2O_2 adjusted to pH 9) were held 1 hr at 37 C. Then the tissue was rinsed 4 times in a 0.05-M phosphate buffer at pH 6.8, postfixed, and processed for EM observation as described above.

RESULTS.—*Observations of hyphae in host plants.*—Electron-microscopic examination of cuttings from tomato revealed that the fungus was localized in the primary xylem of the stem. Furthermore, hyphae are generally found only along the secondary walls and in the lumen of the large metaxylem vessels (Fig. 1).

Hyphae contain the normal complement of cytoplasmic organelles, which includes nuclei, nucleoli, ribosomes, mitochondria, smooth and rough endoplasmic reticulum (ER), vacuoles, and lipid droplets (Fig. 1). Glycogenlike aggregates occur as electron-dense particles in the peripheral region of the protoplast adjacent to the plasmalemma (Fig. 1, 2).

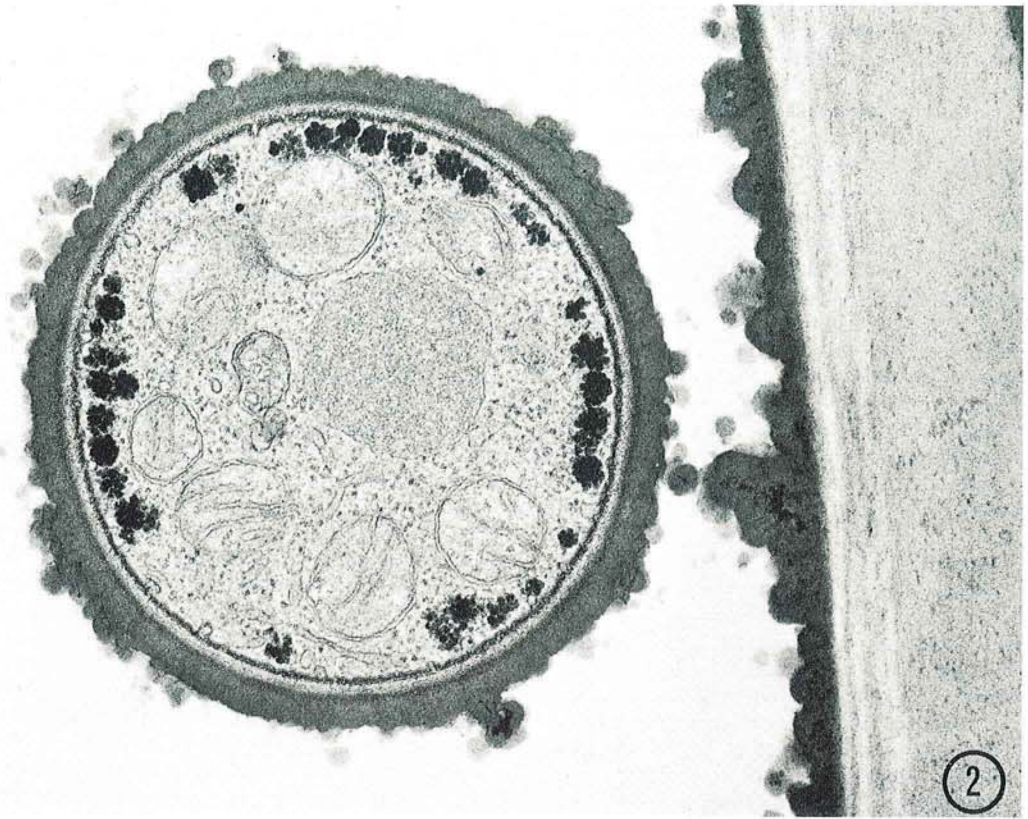
In addition to these normal cytoplasmic constituents, large microbodies also are frequently encountered (Fig. 1, 2). The microbodies, which appear nearly circular in cross sections, are bounded by a unit membrane (Fig. 2). The matrix of the organelle consists of homogeneous granular material. Discrete inclusions were not observed in the microbodies. No consistent associations between microbodies and other cytoplasmic structures were observed; however, the organelles occasionally are appressed to lipid droplets (Fig. 3). The most impressive feature of the fungal microbodies is their large size, which frequently approaches 1 μm. This measurement approaches the upper size limit commonly described for microbodies of higher plants (9, 14, 22).

Cytochemical staining for peroxidase.—A possible functional relationship between fungal microbodies and biochemically defined microbodies in higher plants (i.e., peroxisomes and glyoxysomes) was investigated. Stem segments from infected tomato plants were stained for peroxidase activity with DAB. To serve as a control, leaf tissue containing peroxisomes was similarly treated with DAB. An intense electron-dense reaction product could be observed in the control tissue; i.e., the soybean peroxisomes (Fig. 4). However, no reaction product is present in the fungal microbodies (Fig. 3, 5). In the hyphae, only the mitochondrial cristae exhibited pronounced staining (Fig. 5). Similar mitochondrial staining has been reported in the cristae of yeast mitochondria where cytochrome c peroxidase was involved in the reaction (18).

Observations of hyphae in PDA culture.—The complement of organelles in saprophytic hyphae differs slightly from that in pathogenic hyphae (Fig. 1, 6). Greater numbers of ribosomes and rough ER are present in hyphae cultured on PDA. In addition, the mitochondria appear more ameboid in shape, and frequently contain paracrystalline inclusions. However, the most distinguishing feature of the saprophytic hyphae is the absence of well-developed microbodies. Large microbodies, comparable to those commonly present in the pathogenic hyphae from infected plants, were not found in saprophytic hyphae from PDA cultures. In contrast, the saprophytic hyphae occasionally contain small microbodies which have sparsely granular contents and are usually associated with the rough ER (Fig. 6).

DISCUSSION.—Microbodies are ubiquitous organelles in plant cells. Ultrastructurally, they consist

Fig. 2-3. 2) Transverse section through a hypha which lies near the secondary wall of a metaxylem vessel. A microbody, several mitochondria, ribosomes, and cisternae of the endoplasmic reticulum can be observed in the fungal cytoplasm. Electron-dense glycogenlike aggregates are also commonly found in the peripheral cytoplasm near the fungal wall (X 38,000). **3)** Section through a fungal hypha containing three microbodies (Mb) which exhibit a close physical association with lipid droplets (L). Although this tissue was treated with 3,3'-diaminobenzidine (DAB), the microbodies do not exhibit the intense electron-dense deposit which is characteristically observed in glyoxysomes and peroxisomes (Fig. 4). Membranes and ribosomes of the fungus are poorly defined after treatment with DAB (X 40,000).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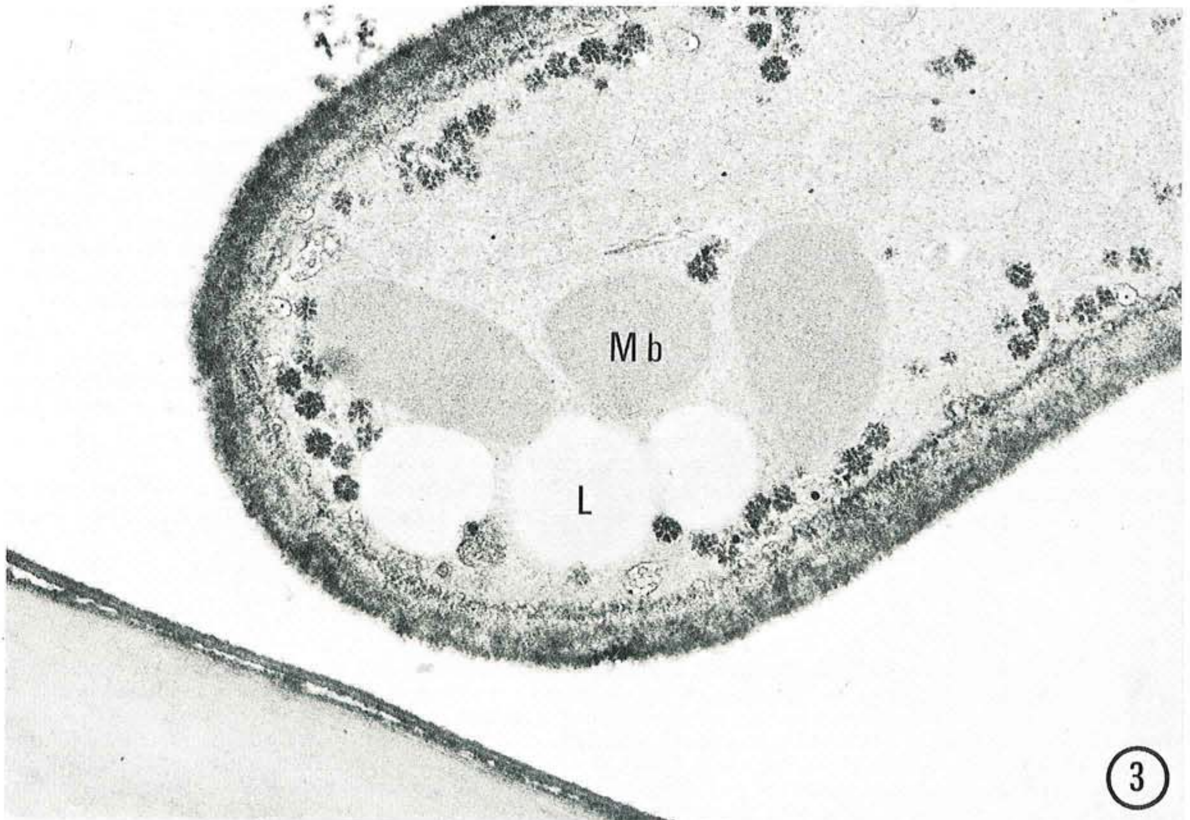
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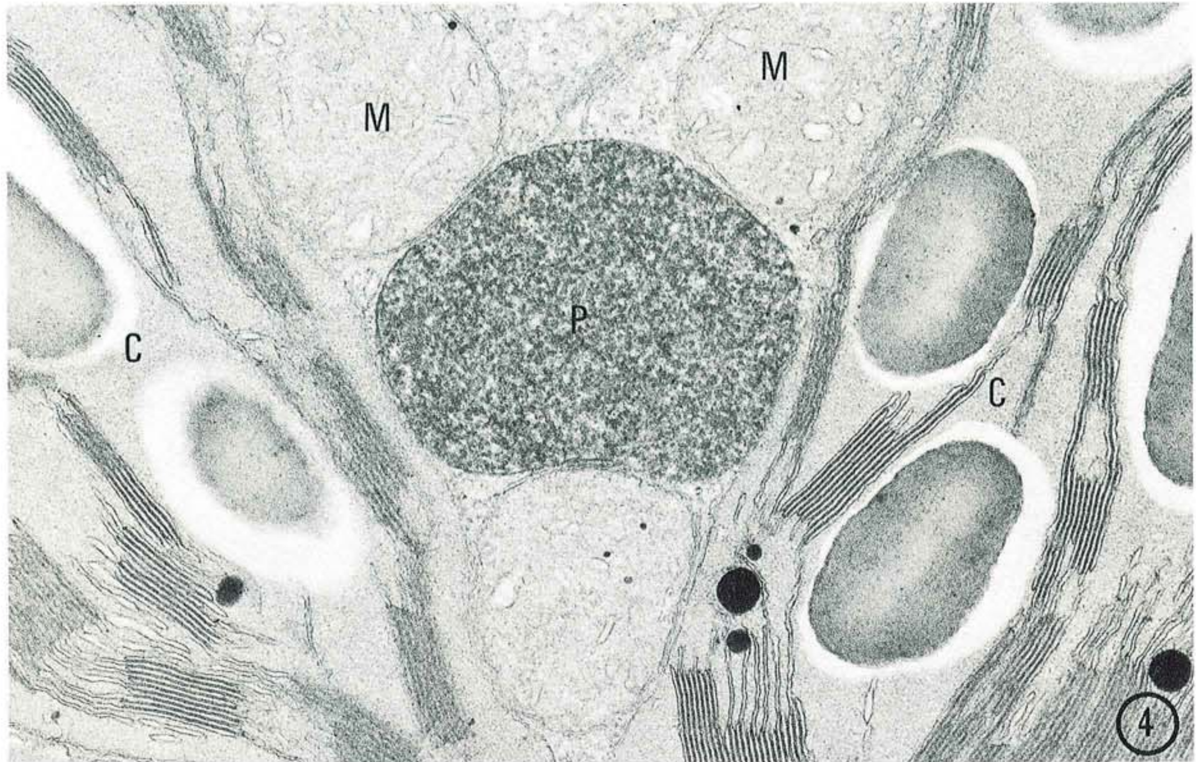

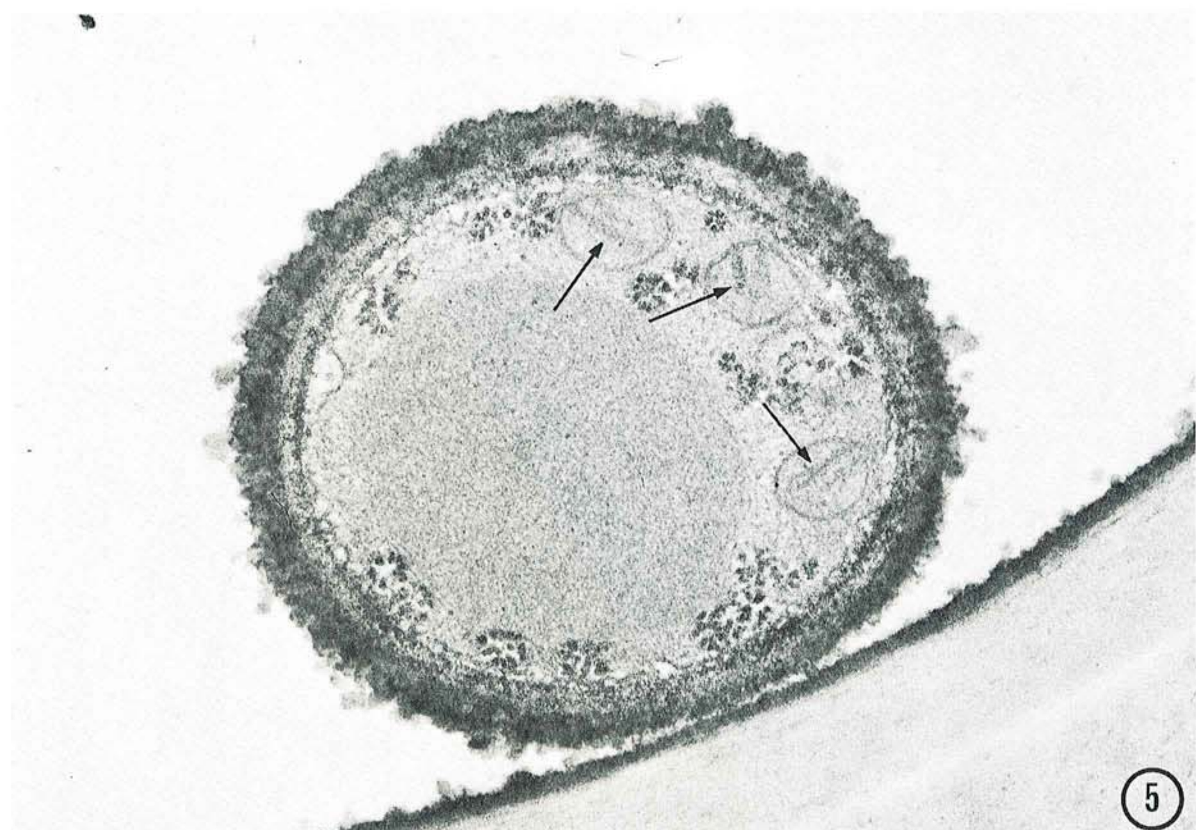

Fig. 4-5. 4) Portion of a mesophyll cell from soybean leaf. The intense 3,3′-diaminobenzidine reaction which occurs in microbodies (peroxisomes) from leaf tissue can be compared to the negative reaction observed in fungal microbodies (Fig. 3, 5). P = peroxisome; M = mitochondrion; C = chloroplast (× 38,000). **5)** Portion of a metaxylem cell that has been incubated in a medium containing 3,3′-diaminobenzidine. A fungal hypha, containing a microbody and several mitochondria, has been sectioned transversely. No reaction product is apparent in the microbody; however, some staining can be observed in the intracisternal space of the mitochondrial cristae (arrows) (× 40,000).

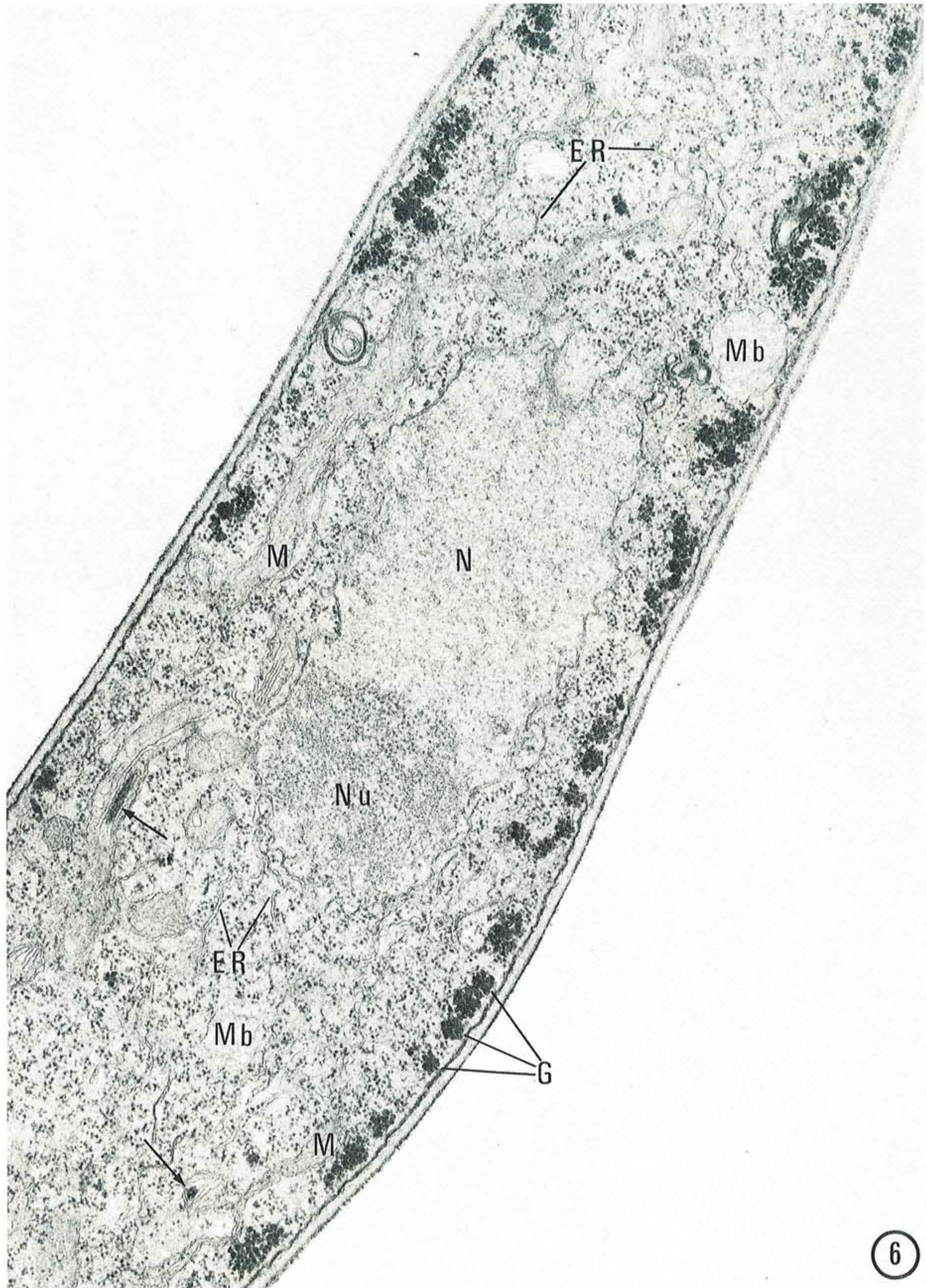

Fig. 6. Slightly oblique section through a fungal hypha cultured on potato-dextrose agar. As opposed to the observations of pathogenic hyphae, paracrystalline mitochondrial inclusions (arrows) and small, inconspicuous microbodies (Mb) were found in the saprophytic hyphae. N = nucleus; Nu = nucleolus; M = mitochondrion; ER = endoplasmic reticulum; G = glycogenlike aggregates (× 36,000).

of a homogeneous granular matrix which is bounded by a single unit membrane (9, 14, 22). However, crystalline, paracrystalline, globular, and fibrillar inclusions have been observed in these cytoplasmic structures (see 22). Our knowledge of the biochemistry and functional roles of microbodies has greatly expanded in recent years (see 1, 19, 22). As a result, biochemically characterized microbodies, designated as peroxisomes and glyoxysomes, have been described in plants. The mesophyll tissue from leaves contains peroxisomes which are associated with glycolate metabolism and photorespiration (20). In endosperm tissue, glyoxysomes contain enzymes of the glyoxylate cycle and are involved in gluconeogenesis (2). In yeast, peroxisomes contain several enzymes related to the glyoxylate cycle and peroxide metabolism (17). In all three cases, peroxidase activity, which can be demonstrated in these microbodies, is associated with the enzyme, catalase. The functional role of microbodies in *Fusarium* is unknown. Since microbodies in the pathogenic hyphae do not exhibit peroxidase activity, they apparently do not contain catalase. This lack of catalase in microbodies has also been reported for other fungi (4, 18) and for algae (10, 11).

Consideration of the postulated function of catalase and the environment of the pathogenic hyphae may provide a possible explanation for the absence of catalase in the microbodies from *Fusarium*. De Duve (5) has suggested that microbodies are primitive respiratory particles. In this capacity, they catalyze numerous oxidative steps in which oxygen serves as the primary electron acceptor, thereby giving rise to hydrogen peroxide. In the present study, growth of the fungus was restricted to the metaxylem vessels. These cells have a very low oxygen tension (6). Under this condition, perhaps oxygen does not serve as the primary electron acceptor; hence, hydrogen peroxide would not be produced and the induction of catalase synthesis would not occur.

Other environmental factors can affect the enzyme complement of microbodies and the frequency and distribution of these organelles in plant tissues. Several enzymes of the glycolate and tricarboxylic acid cycle can be either repressed or induced by growing cultures of yeast on different carbon sources (see 1). Light intensity induces enzyme synthesis of several microbody enzymes in leaf tissues from wheat (7). In *Sclerotinia*, a threefold difference in the frequency of microbodies can be observed by alteration of the growth media (13). In the present study, differences in the structure and frequency of microbodies have been correlated with the pathogenic and saprophytic states of fungal growth. However, the in vivo and in vitro factors responsible for producing these differences have not been identified.

The differences in morphology obtained by comparing the nature and occurrence of microbodies in pathogenic and saprophytic hyphae have been presented. Since these morphological differences may be associated with alterations in metabolism, this aspect should be considered when the fungus is studied in the absence of its host.

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