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Ultrastructure of Basidiospores and Mycelium of *Lenzites saepiaria*

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ABSTRACT

HYDE, JAMES M. (University of Mississippi Medical School, Jackson), AND CHARLES H. WALKINSHAW. Ultrastructure of basidiospores and mycelium of *Lenzites saepiaria*. J. Bacteriol. **92**:1218–1227. 1966.—Ungerminated and germinated basidiospores and 2-day-old mycelial cultures of *Lenzites saepiaria* were similar in their fine structure. Fixation with glutaraldehyde, followed by osmium tetroxide, was far superior to permanganate. Cell organelles were seen in cytoplasm of spores and hyphae, and clamp connections were abundant in hyphal elements. Numerous lomasomes, vesicular bodies, and complex concentric membranes occurred in the cytoplasm and were often associated with the cell membrane or the dolipore membrane (parenthesome) of the septum. Endoplasmic reticulum was not found, but numerous ribosomes were seen; polyribosome groupings were frequently noted. The nucleus was bounded by a double membrane which contained few pores. Germinating spores exhibited one or more large osmiophilic bodies in association with a vacuole and membranous elements. Other than possessing a thin wall, the emerging germ tube was similar in structure to the parent spore.

Lenzites saepiaria (Wulf.) Fr. is a brown-rotting basidiomycete which grows on, and decays, the cellulose portion of pine wood. Its requirements include a high average temperature, the presence of oxygen, and suitable moisture in the form of intermittent wetting. This species of *Lenzites* is one of the principal causes of decay of exposed pine lumber in warm climates (3, 20).

Only limited studies have appeared on this fungus. Physiological investigations conducted by Zeller (20) showed that mycelial cultures exhibited hydrolytic enzyme activity. Preliminary experiments in this laboratory have confirmed these observations and have further shown that the level of one acid hydrolase, acid phosphatase, is unusually high (C. H. Walkinshaw, H. W. Scheld, and J. M. Hyde, J. Cell Biol. **27**:110A, 1965).

This paper is a report of the fine structure of the fungal cell of *L. saepiaria*, both in the spore form and in the hyphal form. Special emphasis has been placed on a search for vesicular bodies and other cell organelles which might possibly serve as repositories of the acid hydrolases. The fine structure of germinated spores is compared with that of ungerminated ones, since other studies (18) showed a time-dependent response to succinic and other Krebs cycle acids during the process of spore germination. The fact that utilization of such acids was not demonstrated in the ungerminated spores implies a difference in metabolism that perhaps reflects a difference in the fine structure of mitochondria or other cell organelles.

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MATERIALS AND METHODS

Spore collection and germination. A dense layer of spores was collected from fresh sporocarps of *L. saepiaria* which were suspended over 100 mm petri dishes (100 by 15 mm) containing approximately 10 ml of 0.05 M maltose in distilled water. These dishes were incubated at 37 C and sampled at 1-hr intervals for electron microscopy. Initial studies showed that spore swelling and germ tube emergence occurred between 0 and 5 hr; thus, sampling was restricted to this time interval. Ungerminated spores were fixed immediately after their introduction into the plates.

Mycelial cultures. Mycelial cultures, 2 days old, were obtained from aeration cultures, originating from a polysporus culture of *L. saepiaria* collected and maintained on southern pine sapwood. The primary culture was made from fungus-infected wood on a malt-agar plate and was grown for 3 to 5 days. Then a small quantity of the fungus mat on the agar culture was transferred to a 16-oz (493.6-ml) prescrip-

tion bottle containing 250 ml of 1% malt extract (Difco) in distilled water. This liquid culture was harvested aseptically after 48 hr, was washed twice with sterile distilled water, and was blended as a 1:3 (v/v) suspension for about 45 sec at high speed in a Monel Waring Blendor according to the suggestions of Dorrell and Page (2). A 5-ml amount of this blended fungus suspension was then pipetted into a second prescription bottle containing malt extract. This second liquid culture grew as uniform pellets approximately 1 to 2 mm in diameter at 48 hr. These pellets were harvested and were fixed for electron microscopy.

Electron microscopy. In preparation for electron microscopy, materials were either treated directly with the fixative or were first embedded in 2% agar. Fixation for 5 min in 2% $KMnO_4$ was according to the procedure of Luft (9). A superior method of fixation had the following procedure. Materials were treated overnight (ca. 18 hr) at 4 C with 6.25% reagent-grade glutaraldehyde buffered with 0.1 M phosphate at *p*H 7.6 (14). After two rinses with cold buffer, samples were post-fixed with phosphate-buffered 1% osmium tetroxide for 1 hr at 4 C (10). Fixed materials were dehydrated in graded alcohols and were embedded in Dow 332-732 resin mixture (Dow Chemical Co., Midland, Mich.) as recommended by Lockwood (7). Sections were cut on a Porter-Blum model MT-2 ultramicrotome with glass or diamond knives and were stained with 2% uranyl acetate (16) for 45 min at room temperature. In addition, some sections were stained with lead citrate (13) for 1 to 3 min. All sections were examined in an RCA EMU-3G electron microscope.

RESULTS

Permanganate fixation. Longitudinal and cross sections of hyphae fixed with permanganate exhibited a coarse grain cytoplasm bounded by a complex, granular cell wall (Fig. 1). Nuclear and cytoplasmic membranes and particularly those of the mitochondria were distinct. Ribosomes and endoplasmic reticulum were not found, although vesicular bodies and small vacuoles containing electron-dense material were found in some sections. The term "vesicular body" is used in the operational sense. It describes those membrane bodies which are similar to lomasomes, complex concentric membrane elements, and invaginations of the cell membranes. All three of these entities may or may not be related. Invagination of the cell membrane was evident but often obscured by the coarse grain of the cytoplasm. The nucleoplasm appeared as an electron-dense material containing numerous irregular areas of low electron density.

Glutaraldehyde-osmium fixation. Spores and hyphae fixed in glutaraldehyde and postfixed in osmium tetroxide exhibited a well-preserved ultrastructure. Ungerminated spores possessed all the cytoplasmic elements found in hyphae (Fig. 2). The walls of the basidiospores were notably thin as compared with hypha walls and appeared uniformly thinner with this fixative than with $KMnO_4$. The cell membrane was sharply defined in all spore sections. Invaginations of the membrane occurred at irregular spacings along the spore's surface. Elaborate development of this process, in the form of complex elements, consisted of concentric rings or aggregated tubules, continuous with the cell membrane. One or two nuclei, bounded by double membranes containing occasional nuclear pores were present in the basidiospore cytoplasm. Cell organelles such as mitochondria, osmiophilic bodies believed to be lipid (hence referred to as lipid bodies), vesicular bodies, and ribosomes were more or less uniformly distributed throughout the cytoplasm. Endoplasmic reticulum was not found in any of the basidiospore sections, but polyribosome groupings were frequently observed.

Although the fine structure of the germinating spore (Fig. 3) was similar to that of the ungerminated one, certain changes were manifest after emergence of the germ tube. The most prominent feature of the germinating spore was one or, occasionally, as many as three large, osmiophilic bodies usually associated with a large vacuole possibly replacing lost cytoplasm in the parent spore. Another especially notable feature was the very thin wall of the germ tube. Spores incubated 4 hr or longer sometimes possessed two or more large vacuoles containing membranous elements and other materials of varying electron density. With up to 5 hr of incubation, there were no further significant differences in ultrastructure. Neither septum formation nor nuclear division was observed during this period.

Hyphal cells from 2-day mycelial cultures appeared similar to spores, except that the cell wall was notably thicker and the cell membrane, like that of the germinated spore, was less distinct (Fig. 4). As a result of the elongated nature of the hyphal cell, many sections lacked nuclei or mitochondria or both. Others showed only one large nucleus which occupied most of the cross section of the hypha. When longitudinal sections were obtained, cell organelles were found distributed throughout the cytoplasm. Most features were equally apparent in spores and hyphae; invaginations of the cell membrane were commonplace (Fig. 5), and structures containing vesicular material [lomasomes (11), Fig. 6] were numerous in all specimens. Mitochondria, ribosomes, and lipid bodies appeared equally distributed in both types of cells.

The septum observed in hyphal elements contained the complex pore type, referred to as the dolipore by Moore and McAlear (12) and treated

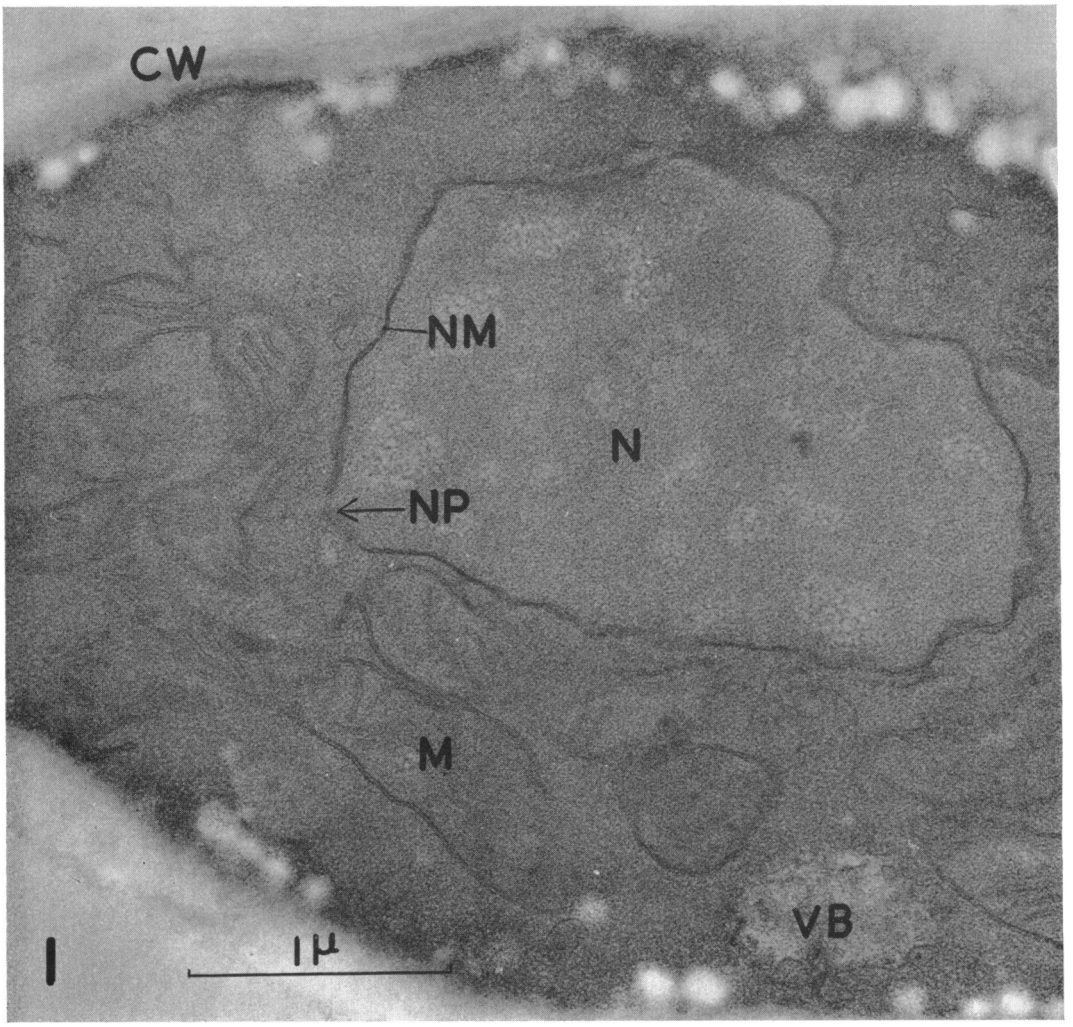

FIG. 1. *Permanganate-fixed hypha showing foamy cytoplasm adjacent to the cell wall (CW). Mitochondrion (M), nucleus (N), nuclear membrane (NM), nuclear pore (NP). A single large vesicular body (VB) is seen adjacent to the cell wall.* × *35,000.*

in detail by Giesy and Day (4). Two such dolipores are seen in Fig. 7, and part of another is shown at higher magnification in Fig. 8. The membrane surrounding the dolipore, the parenthesome, appears to be continuous with a network of membranes in the area of the septum (Fig. 8). Moreover, on close examination of the pore, a number of microtubules which appear to connect the cytoplasm on either side of the pore can be seen. The swollen area of the dolipore with its central plate or point of junction gives the impression that the pore may be formed by outgrowths from the ends of two adjacent cells. Degeneration of dolipores into simple pores was not observed.

Clamp connections (Fig. 9) were readily apparent in longitudinal sections of hyphae. The clamp cell contained numerous organelles and, in some cases, a nucleus in process of migration. A striking characteristic of the clamp cell was its highly vacuolated cytoplasm, particularly in the basal portion. This condition was evident in other cells as well, especially those believed to be in process of division (Fig. 10). Microtubules or parallel microfibrils were occasionally observed at the point of constriction in dividing cells, and

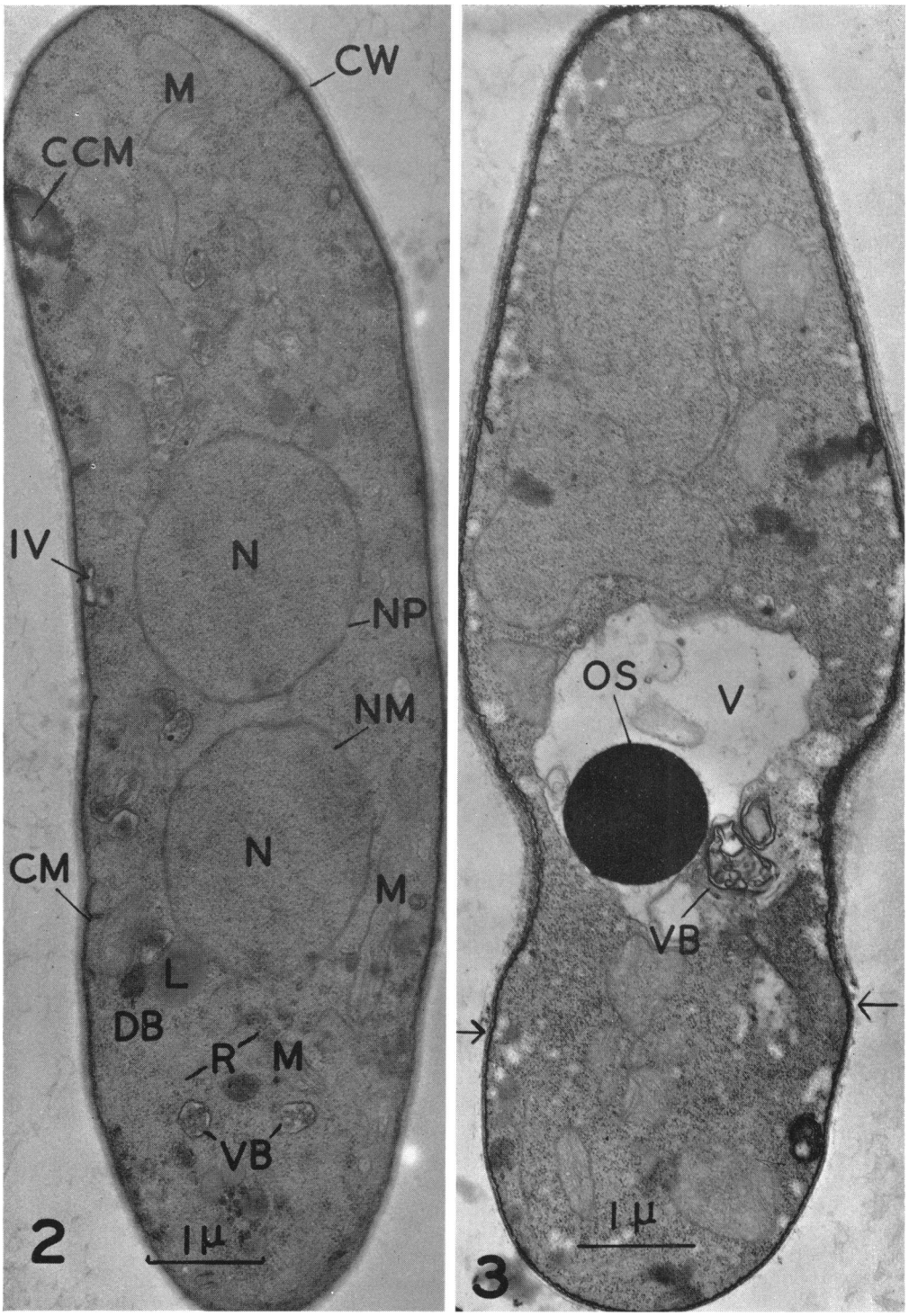

FIG. 2. *Longitudinal section of an ungerminated basidiospore illustrating cytoplasmic details. Cell wall (CW), cell membrane (CM) showing invaginations* (IV). *A complex concentric membrane (CCM) is evident. The two nuclei (N) are bounded by a double nuclear membrane (NM) containing nuclear pores (NP). Other cell organelles evident are vesicular bodies (VB), dense staining bodies (DB), mitochondria (M), osmiophilic bodies believed to be lipid (L) and other storage bodies. Ribosomes (R) are seen in groups throughout the cytoplasm.* × *18,000.*

FIG. 3. *Longitudinal section of a 5-hr germinating spore showing the germ tube (designated by arrows marking the cell wall boundary between spore and germ tube). Other notable features are the large osmiophilic body (OS), with its associated vacuole (V)* and *vesicular bodies (VB).* × *18,000.*

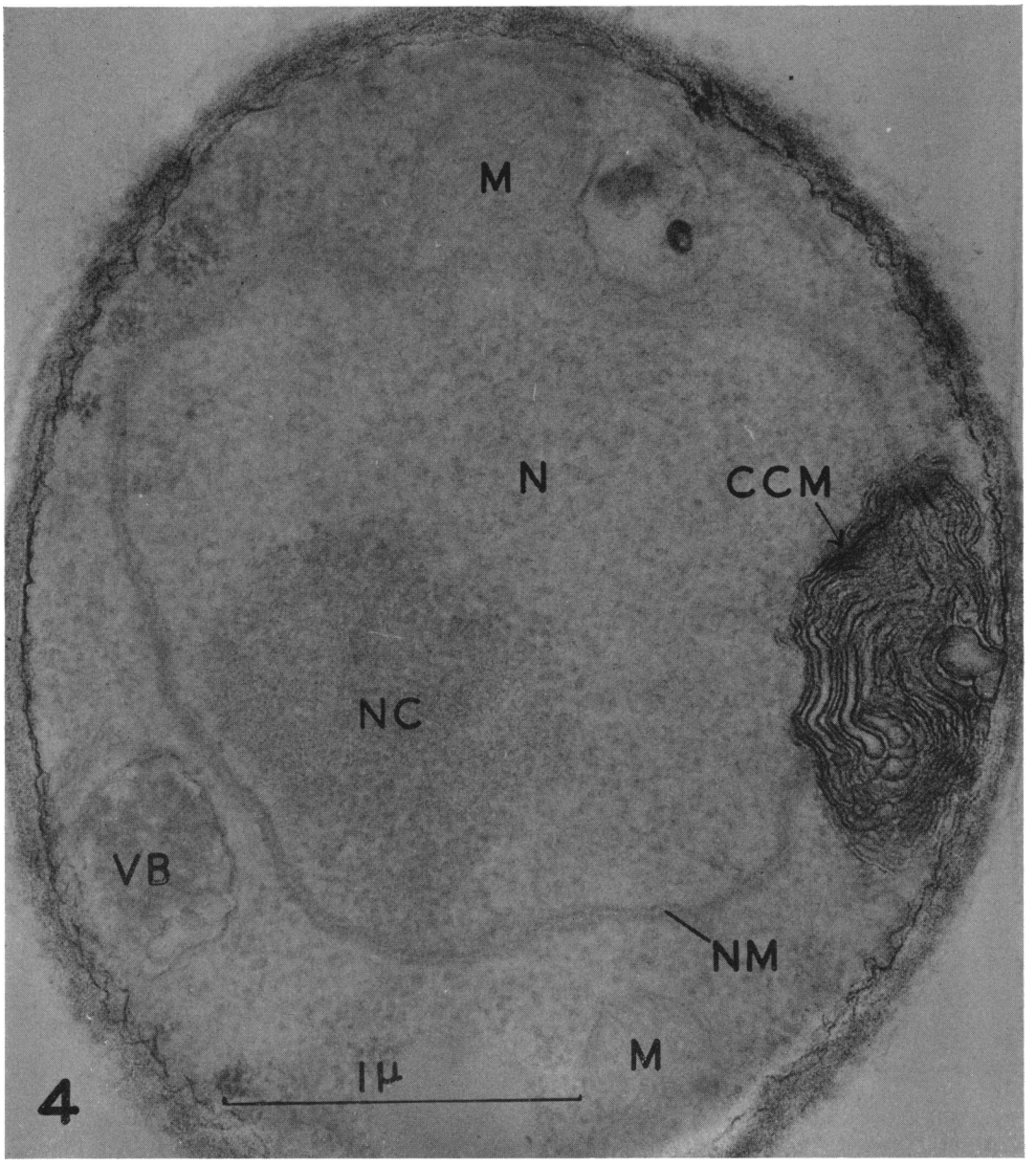

FIG. 4. *Cross section of a hypha of Lenzites saepiaria illustrating a nucleus (N), with a prominent nucleolus (NC), and a complex concentric membrane (CCM). Refer to Fig. 2 for abbreviations for other organelles.* × *50,000.*

were similar in appearance to those observed within the central region of the dolipore.

One feature seen in many preparations of hyphae, the foamy cytoplasm (Fig. 11), was much more evident in hyphal cells than in spores.

Hyphal degeneration, observed in many sections, was characterized by aggregation of ribosomelike particles into small clusters and by numerous vesicular bodies in a highly vacuolated cell. This eventually resulted in the final state of degradation, with convoluted cell wall and degenerate mitochondria (Fig. 12). The star-shaped clusters were noted only rarely in presumably healthy cells.

DISCUSSION

In this report we have demonstrated the advantages of glutaraldehyde-osmium fixation in elucidating the fine structure of fungi. Permanganate was found to be inferior, even though it

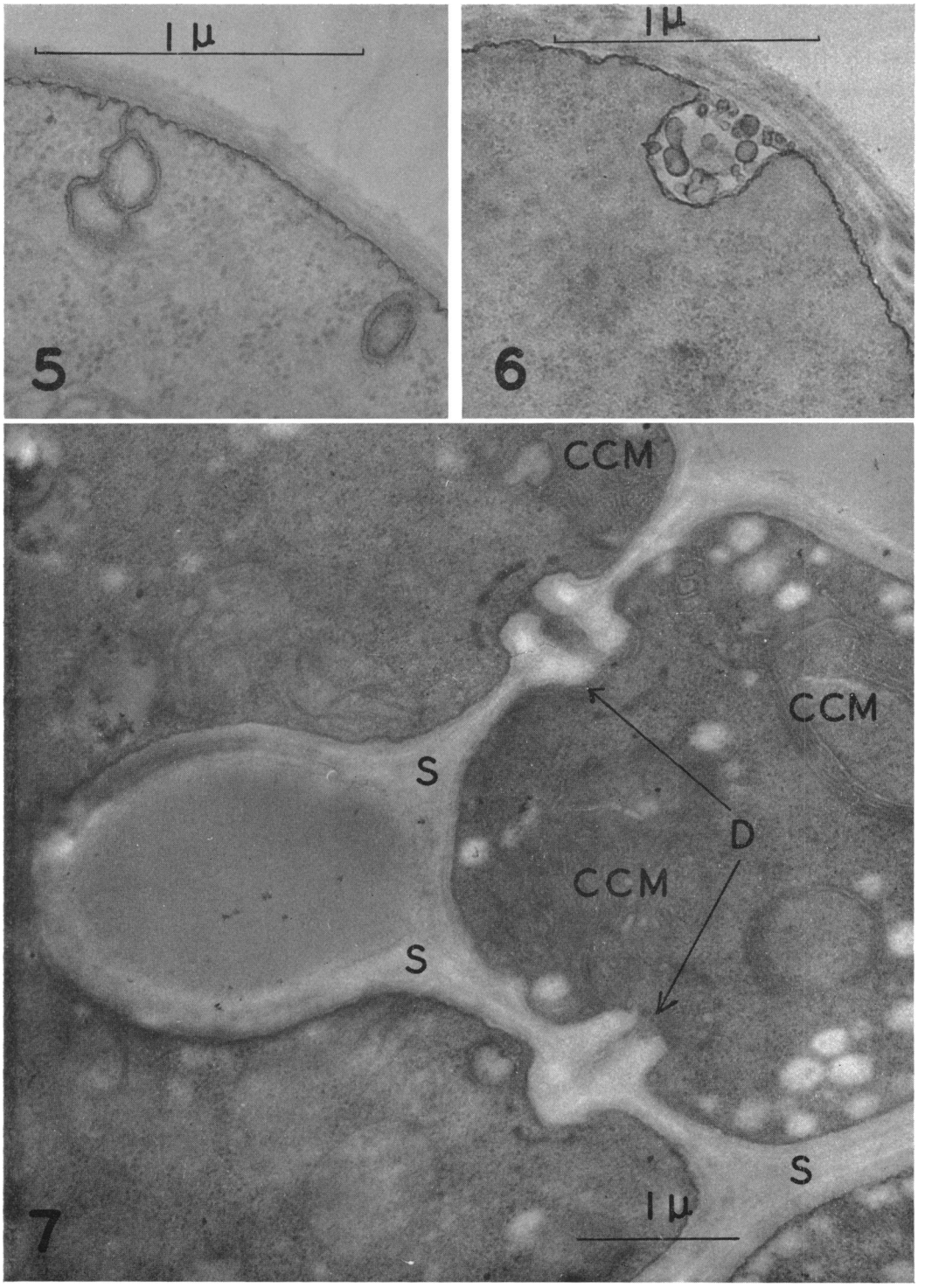

FIG. 5. *Vesicular body formation by invagination of the cell membrane.* × *50,000.*

FIG. 6 *Enlargement of typical lomasome showing the relation between this structure and the cell membrane and wall.* × *40,000.*

FIG. 7. *Section through a complex septum* (*S*) *in the region of a clamp connection showing two dolipores* (*D*) *and extensive complex concentric membrane systems* (*CCM*). × *25,000.*

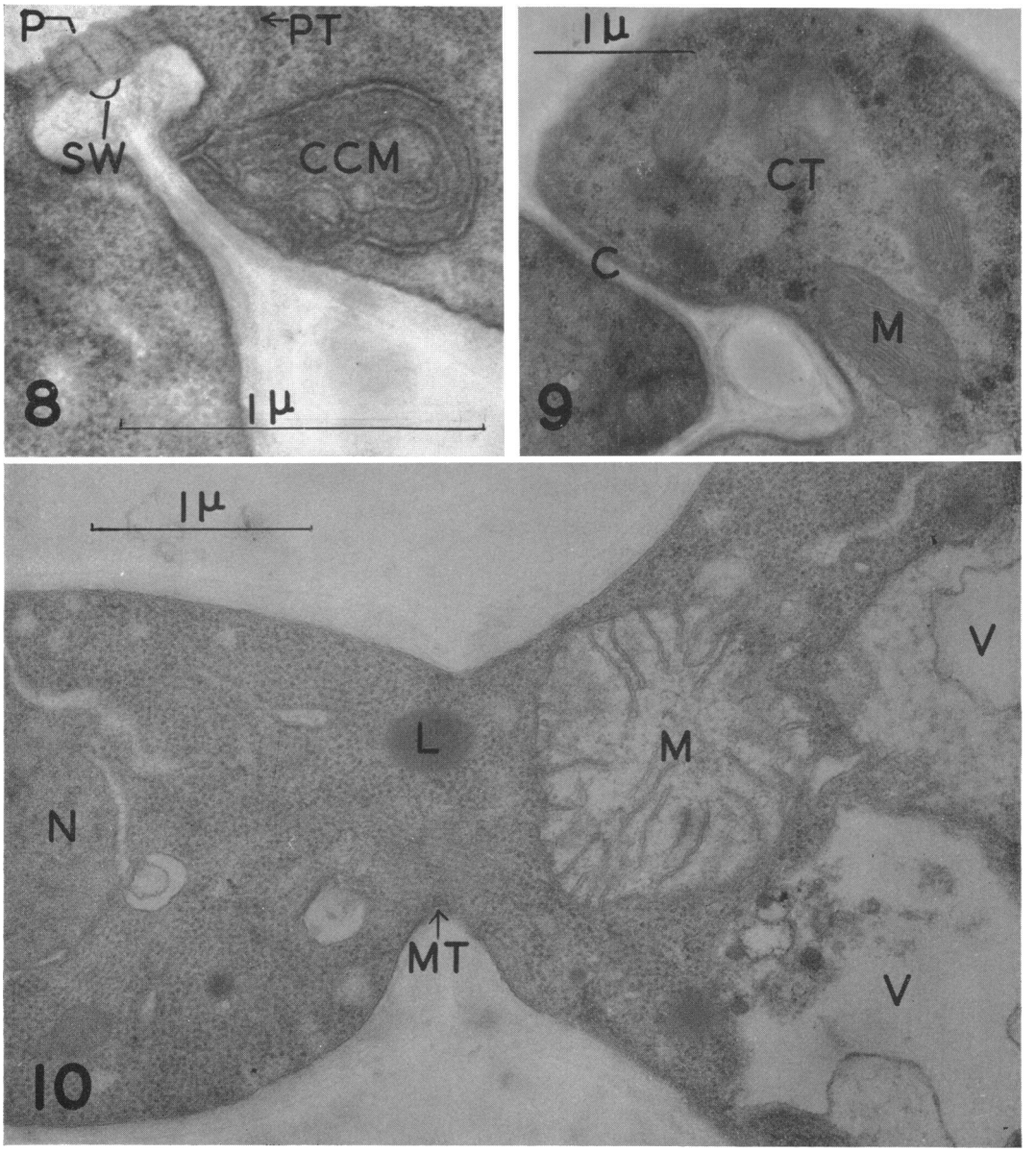

FIG. 8. *Detail of dolipore showing the parenthesome (PT) surrounding the dolipore and its connection to a membrane body (CCM). Note the microtubular appearance of the dolipore connection with swelling (SW) and central plate (P).* × *50,000.*

FIG. 9. *Section through a clamp connection (C) showing the clamp tube (CT) and mitochondria (M).* × *25,000.*

FIG. 10. *A cell, probably in the process of division, illustrating microtubules (MT) at the point of constriction. The nucleus (N) is visible on the left and a large mitochondrion (M) is evident in the vacuolated cell.* × *30,000.*

has been widely used as a fixative for fungal cells. Shatkin and Tatum (15), Blondel and Turian (1), and Luck (8) have previously used osmium tetroxide to advantage to fix fungus tissues. They reported that uranyl acetate was required either as a rinse after fixation or as an additive to the polymerization mixture. Therefore, it appears that certain existing fixation techniques are superior to permanganate, and that fungal cells do not present any unique problem in fixation. However, it was noted, during the course of our studies, that permanga-

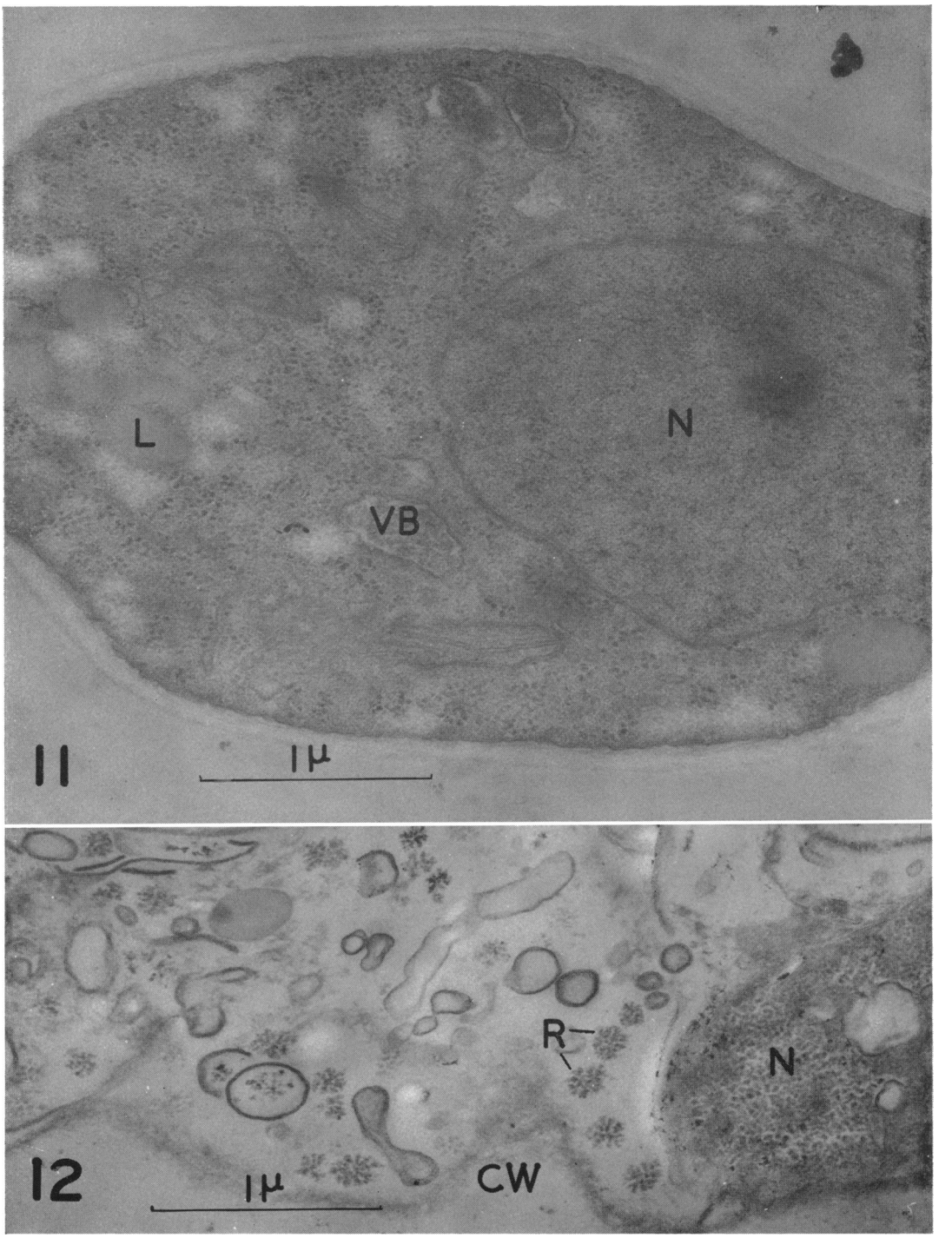

FIG. 11. *Cross section of a hypha of Lenzites saepiaria exhibiting foamy cytoplasm. Note polyribosome grouping.* × *35,000.*

FIG. 12. *Longitudinal section of a degenerating hypha. Granular material, possibly ribosomal, is aggregated into starshaped clusters. The cell membrane has disappeared, and the cell wall has been crenulated.* × *35,000.*

nate-fixed cells were more easily infiltrated by the epoxy embedding medium.

The foamy cytoplasm was seen in many preparations. This appearance was largely limited to hyphal elements and was seldom conspicuous in spore preparations. In addition, the spongy appearance was noted in material fixed in either glutaraldehyde-osmium or permanganate. It is probable that these areas represent glycogen or other storage material leached out during specimen preparation. Further study is indicated by this observation.

The fine structure of ungerminated and germinating basidiospores of *L. saepiaria* was very similar to that of hyphal cells. The large osmiophilic bodies seen in germinating spores possibly were formed by coalescence of the smaller lipid bodies, which appear to be fewer in number in germinating spores. The fact that the osmiophilic bodies were always associated with a large vacuole and vesicular membrane system suggests that this entire complex may represent the mobilization of nutrients for the emerging germ tube. A second possibility is that the vacuole in the spore may be formed by loss of cytoplasmic constituents into the germ tube. Voelz and Niederpruem (17) compared the ultrastructure of late stages in the germination of another wood-rotting fungus, *Schizophyllum commune*, to ungerminated spores. Basidiospores in the ungerminated state had poorly defined mitochondria compared with germlings. At 18 hr of incubation, numerous vacuoles were apparent. Weiss (19), on the other hand, reported on studies with *Neurospora crassa* in which no significant difference in the ultrastructure of germlings and ungerminated conidia was found. She noted the occurrence of vacuoles in both conidia and germlings. Mitochondria did not differ in appearance in germinated spores compared with resting conidia.

The presence of elaborate membrane figures in association with the cell membrane and in the cytoplasm of fungus cells has now been established for a number of species of fungi (1, 5, 6, 15). Shatkin and Tatum (15) have proposed that the invagination of the cell membrane is associated with cellular activity, and Hohl (6) has clearly shown this to be the case for the slime molds. That such membrane systems are functional is further evident from the current study which established the intimate relationship between the dolipore membrane and the concentric membrane structures. Since fungi, such as *L. saepiaria*, probably depend upon the action of hydrolytic enzymes for their utilization of wood, it then is reasonable to postulate that such fungi possess an efficient means of moving substrates or enzymes, or both, through the cytoplasm, possibly involving the dolipores and concentric membranes. That the membranous structures are high in lipid is indicated by their pronounced uptake of osmium during fixation. The membranes appeared in several different forms, and it seems probable that lomasomes, vesicular bodies, and concentric complex membranes are different manifestations of a single type of entity.

The degree of vacuolization noted in hyphae and old clamp connections suggests that certain portions of the hyphal elements may exist in a highly degenerate form compared with the advancing hyphal tip. Extensive degeneration of hyphal elements might result in their functioning mainly as conducting tubes and not areas of active metabolism. Some evidence for aging of mycelial cultures was reported in a preliminary communication (C. H. Walkinshaw, H. W., Scheld, and J. M. Hyde, J. Cell Biol. **27**:110A 1965) in which it was shown that the level of hydrolytic enzymes increased over a 12-day culture period, whereas other enzymes, such as catalase and cytochrome oxidase, decreased significantly in cultures older than 3 days. Thus, it would appear that measurement of hydrolytic enzyme activity in mycelial cultures may be an expression of degradation of the fungus cells as well as that of the host substrate.

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