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Ultrastructural Changes During the Yeastlike to Mycelial-Phase Conversion of *Blastomyces dermatitidis* and *Histoplasma capsulatum*

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Fine details of the sequential anatomical events occurring during yeast to mold morphogenesis of the dimorphic fungal pathogens *Blastomyces dermatitidis* and *Histoplasma capsulatum* as seen in ultrathin sections are described and illustrated by electron micrographs. Discrete intracytoplasmic membrane systems intimately associated with the plasma membrane were observed to be formed within 6 to 8 hr after induction of the conversion process. Within 12 to 18 hr, an intermediate or transitional cell with Woronin bodies at the septum was formed from the converting yeastlike cell. Both cells were noted to contain increased numbers of mitochondria. At approximately 48 hr from the initial induction of the conversion stimuli, the newly forming hyphal cells were observed to produce postconversional intracytoplasmic membrane systems seen normally in the ultrastructural organization of the fully established mycelial-phase cell. These membrane systems appear to be associated with normal septal formation. Although minor variations of time were observed in the occurrence of the sequential events, it is suggested that yeastlike to mycelial-phase conversion of these two fungal pathogens proceeds via a similar mechanism of ultrastructural reorganization.

The phenomenon of dimorphism is remarkable in microbiological systems, and it has been speculated that this ability to undergo morphological alteration has enabled the dimorphic fungus to establish itself as an internal parasite of man and animals.

Although it is obvious that a profound alteration in morphological character occurs during the conversion process from one phase to the other, very little direct evidence at the subcellular level has been offered for the mechanisms involved in the biochemical or biophysical events associated with the conversion process characteristic of these pathogenic dimorphic fungi. The present study is concerned with the sequence of events occurring at the ultrastructural level during anatomical reorganization of the yeastlike to mycelial-phase transformation of the dimorphic fungal pathogens *Blastomyces dermatitidis* and *Histoplasma capsulatum*.

MATERIALS AND METHODS

One strain each of *H. capsulatum* and *B. dermatitidis* was employed in this study. *H. capsulatum* strain Huff was isolated locally from a patient with clinical histoplasmosis, and *B. dermatitidis* strain Sago was obtained from D. J. Guidry of the Louisiana State University School of Medicine. These strains were selected especially for the ability to yield excellent growth response of their respective yeastlike and mycelial phases when grown on solid or liquid Trypticase Soy (BBL) media at incubation temperatures of either 37 or 26 C. Yeastlike cells were derived from cultures incubated for 3 days at 37 C following rapid transfer. The use of high-viability log-phase inocula was necessary to obtain the greatest degree of synchrony during the conversion-induction period. Such cultures were found to have ca. 95% viable cells as determined by the method of Berliner and Reca (1). The yeastlike cells were harvested by washing the slants with a small amount of sterile liquid medium. Approximately 5.0 ml of a heavy cell suspension was transferred to 125-ml Erlenmeyer flasks containing 50 ml of sterile Trypticase Soy Broth. The flasks were placed on a reciprocal shaking apparatus maintained at 24 to 26 C. Beginning at 0 hr, specimens were taken at 6-hr intervals until cells competent of conversion had undergone transformation. The specimens were thoroughly washed and fixed in 3% glutaraldehyde with 0.2 M *S*-collidine buffer (*p*H 7.4) for 6 hr, postfixed in 1% osmium tetroxide in the same buffer for 6 hr at 2 to 4 C, dehydrated in a graded series of ethyl alcohol, and

embedded in Araldite. Ultrathin sections were cut on an LKB Ultrotome III with glass knives. The sections were picked up on 300 mesh copper grids, prestained for 5 min with Reynold's lead citrate, poststained with 3% uranyl acetate in methanol, and viewed in a Hitachi electron microscope (Type HU-11B-1).

RESULTS

Changes suggestive of reorganization of the normal ultrastructure of the yeastlike cells of both *B. dermatitidis* and *H. capsulatum* were observed to occur as early as 6 hr after initial induction of the conversion process, with the formation of an intracytoplasmic membrane system at the plasma membrane (Fig. 1). In some instances, a distinct layering of the plasma membrane adjacent to the invaginating system was observed as the system matured (Fig. 2). At this time, no other significant ultrastructural changes were noted in the organization of internal components of the converting yeastlike cell of either fungal organism. However, not all cells responded alike. A few cells appeared to initiate conversion only to abort and undergo degeneration, whereas others appeared to become nonviable.

Figure 3 illustrates a yeastlike cell of *B. dermatitidis* at 12 to 18 hr into conversion giving rise to an intermediate or transitional form. Separating the two cells is a septumlike structure, centrally located with Woronin bodies at either side. Randomly scattered throughout the primary yeastlike cell are numerous small intracytoplasmic membrane systems, mitochondria, and lipid bodies. Carbonell (Bacteriol. Proc., p. 34, 1969) reported the formation of a similar transitional cell with Woronin bodies at the septum during the yeast to mycelial transformation of *Paracoccidioides brasiliensis*. Adjacent to the septal-like structure in both yeastlike and intermediate cells are larger intracytoplasmic membrane systems, possibly associated with septal formation. In the intermediate cell, proliferation of other smaller membrane systems similar to those in the parent yeastlike cell can be seen. Numerous mitochondria are closely aggregated within the intermediate cell, possibly suggesting a high level of metabolic activity associated with this critical period of reorganization. The cell wall of the intermediate cell is thinner than that observed for the yeastlike cell.

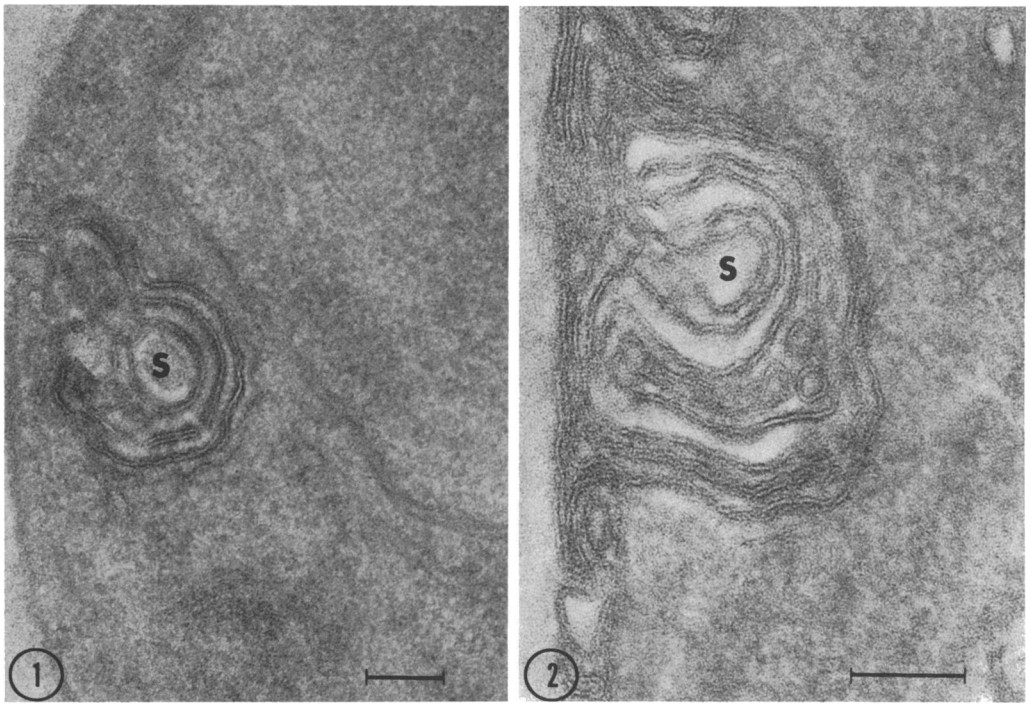


FIG. 1. *Yeastlike cell of Histoplasma capsulatum in early conversion, showing a small intracytoplasmic membrane system (S) developing from the plasma membrane. × 105,000. Marker represents 0.1 μm.*

FIG. 2. *Late stage development of a conversional intracytoplasmic membrane system (S) of yeastlike Histoplasma capsulatum. Note the multiple infolding and layering of and at the plasma membrane. × 145,000. Marker represents 0.1 μm.*

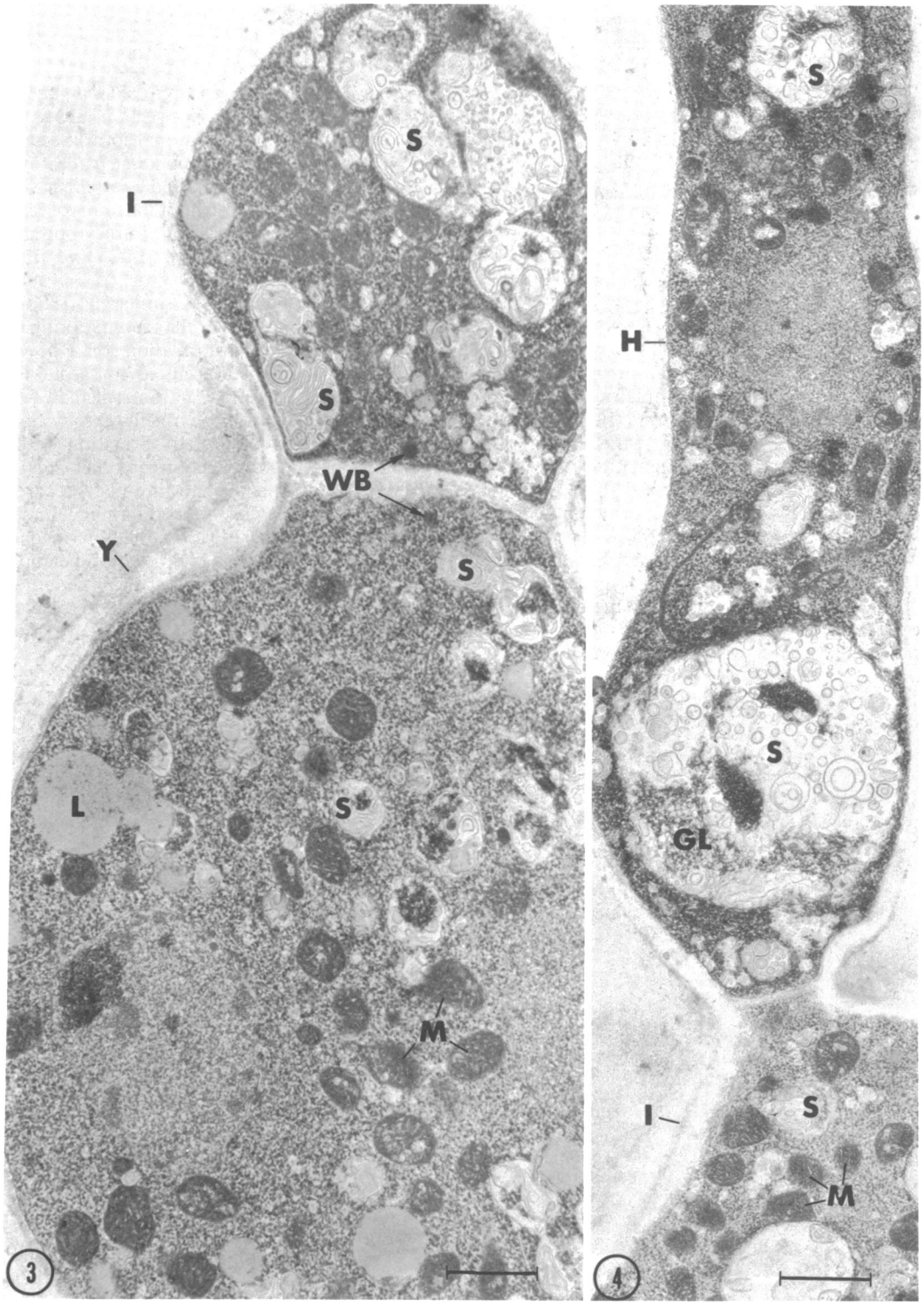


FIG. 3. *Intermediate conversional cell (I) arising from yeastlike cell (Y) of Blastomyces dermatitidis. Lipid bodies (L), mitochondria (M), and scattered intracytoplasmic membrane systems (S) can be seen in both cells. Woronin bodies (WB) appear in approximation with the septal-like structure separating the two cells. × 14,000. Marker represents 1.0 μm.*

FIG. 4. *Intermediate conversional cell (I) of Blastomyces dermatitidis giving rise to the primary hyphal cell (H). Note the large intracytoplasmic membrane systems (S) with early deposition of glycogenlike material (GL). × 13,500. Marker represents 1.0 μm.*

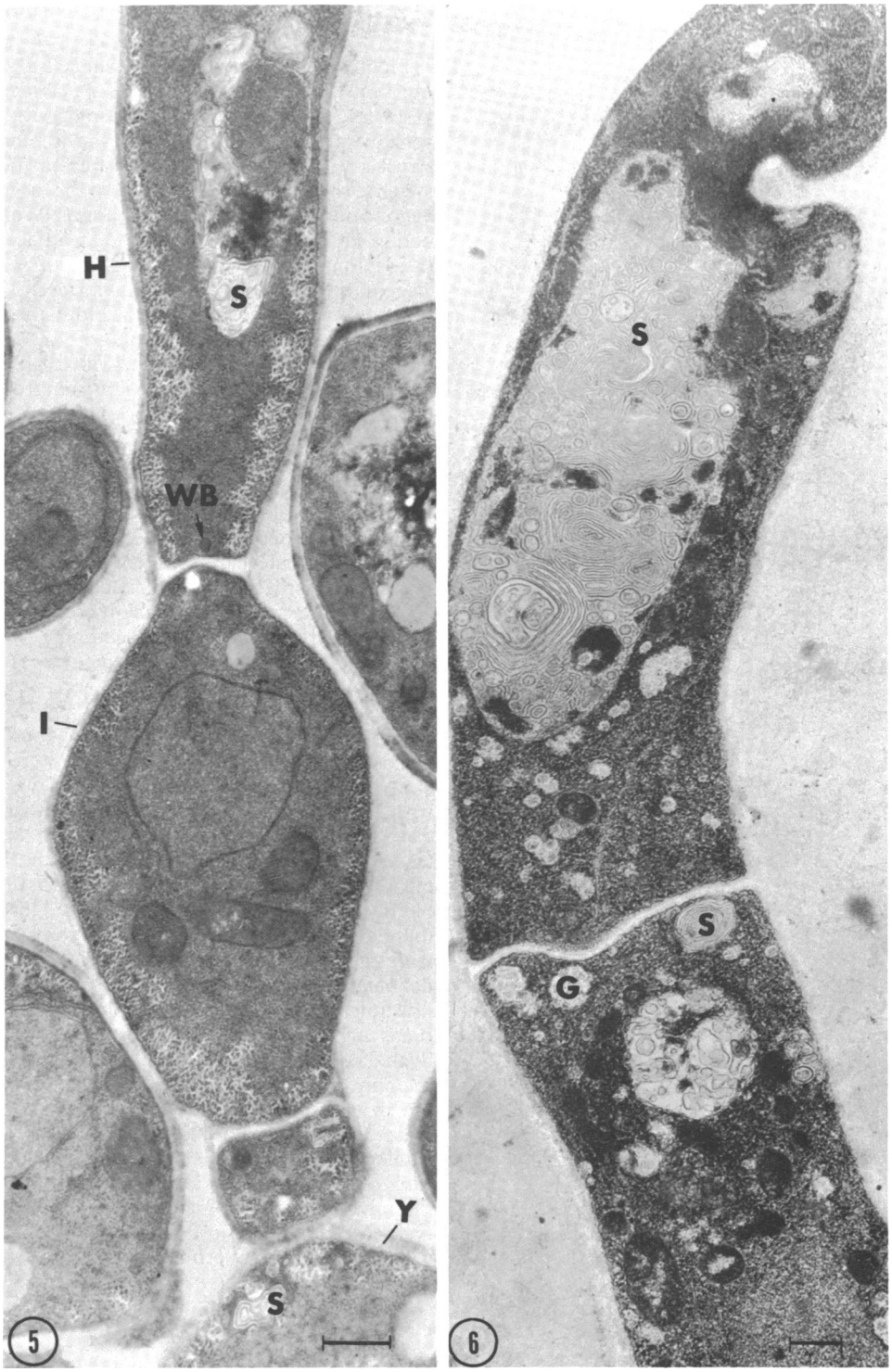


FIG. 5. *Composite views of the yeastlike (Y), intermediate (I), and primary hyphal (H) cell of Histoplasma capsulatum during the latter stages of conversion. Note the Woronin body (WB) and intracytoplasmic membrane systems (S).* × *19,500. Marker represents 0.5 μm.*

FIG. 6. *Longitudinal section of newly formed Blastomyces dermatitidis mycelial-phase cell with extensive tubular to laminated intracytoplasmic membrane systems (S). Glycogenlike granules (G) are present in approximation with the septum.* × *14,500. Marker represents 0.5 μm.*

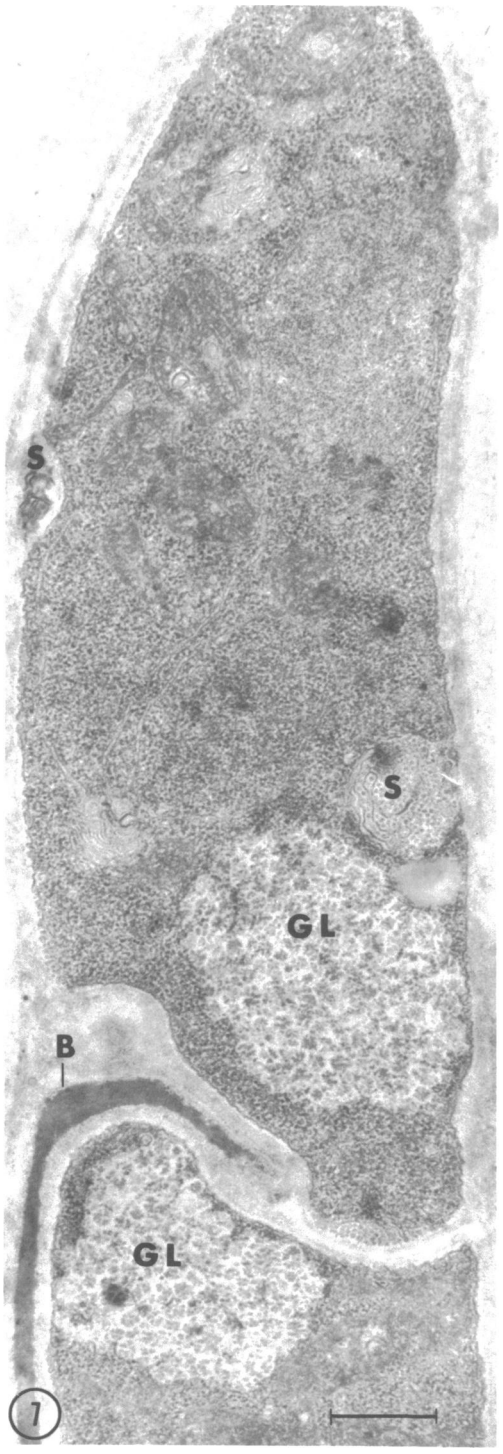


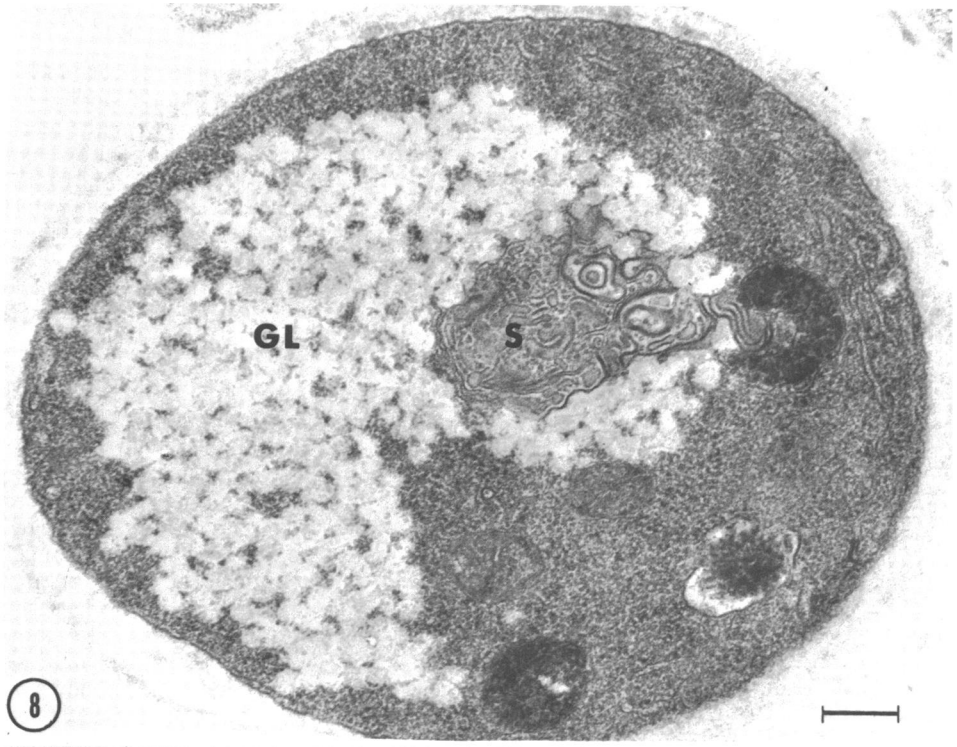
FIG. 7. *Longitudinal section of newly formed Blastomyces dermatitidis mycelial-phase cell, showing an aborted lateral branch (B) at the septal area, glycogenlike granules (GL), and developing intracytoplasmic membrane systems (S). × 28,000. Marker represents 0.5 μm.*

Figure 4 shows a primary hyphal cell developing from the intermediate cell of *B. dermatitidis* at 18 to 24 hr after initial induction of the conversion process. Adjacent to the septumlike structure is a large intracytoplasmic membrane system, showing what appears to be a sparse aggregation of glycogenlike bodies. Woronin bodies are not evident at the areas adjacent to the septum because of the low plane of section. The mitochondria of the newly formed hyphal cell are scattered diffusely throughout the cytoplasmic matrix. Figure 5 shows *H. capsulatum* at 22 to 30 hr into conversion, with both intermediate and primary hyphal cell arising from the converting yeastlike cell. In some instances, *H. capsulatum* was observed to produce multiple hyphal cells arising from either the yeastlike or intermediate cells.

Large, complex intracytoplasmic membrane systems were observed frequently in the newly forming mycelial phase cells as hyphal extension proceeded from the intermediate cell (Fig. 6). At 36 to 48 hr into the conversion process, newly developing intracytoplasmic membrane systems suggestive of lomasome-like structures as seen in other fungal organisms (9, 11) were observed arising from the plasma membrane (Fig. 7). The large membrane systems seen previously in association with septal development were generally observed to be almost totally obscured with aggregations of glycogenlike material. Carbonell and Rodriguez (4) noted a similar association of glycogen granules with the intracytoplasmic membrane systems of 5- to 18-day-old mycelial-phase *B. dermatitidis* cells. However, these granules were seldom seen in direct contact with the membrane systems; instead, a clearly delimited space was observed. Figures 8 and 9 illustrate similar transverse sections of *B. dermatitidis* and *H. capsulatum*, respectively, through hyphal cells approximately 6 to 10 cell-units from the converted yeastlike-phase cell. Both sections are in close approximation to developed septal areas, and show extensive aggregations of glycogenlike bodies, possibly representing the structures seen previously in Fig. 7. At 48 to 72 hr past the initial induction of conversion, the mycelial-phase cell showed a more typical septal formation with well-defined Woronin bodies and septal-associated membrane systems (Fig. 10).

DISCUSSION

Our results suggest that ultrastructural reorganization during the yeastlike to mycelial-



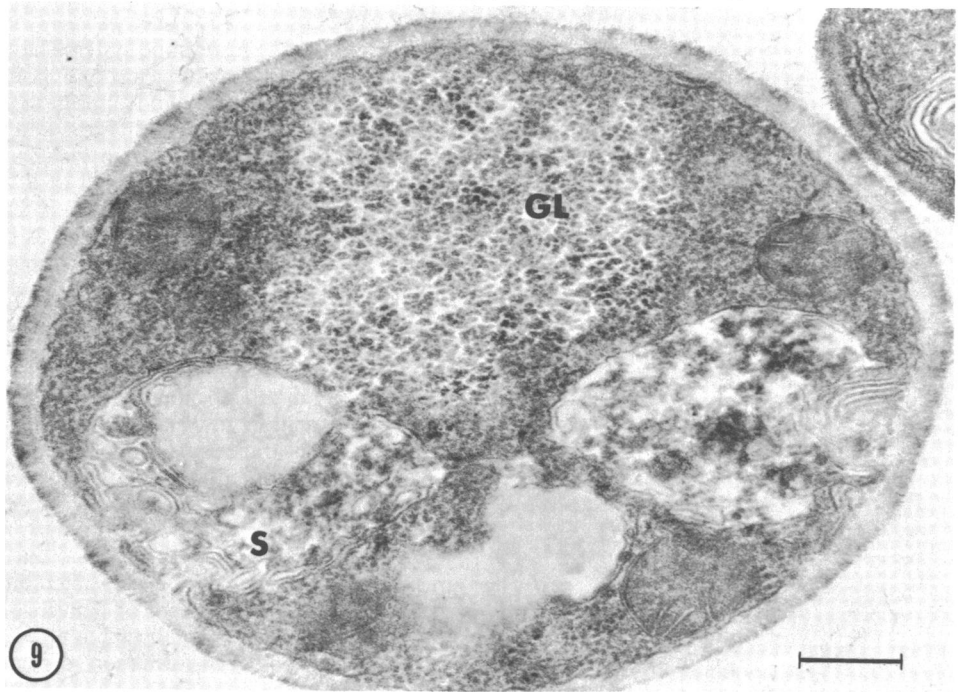


FIG. 8. *Transverse section of hyphal cell of Blastomyces dermatitidis through an area close to a developed septum, showing a postconversional intracytoplasmic membrane system (S) in association with glycogenlike bodies (GL). × 48,000. Marker represents 0.2 μm.*

FIG. 9. *Transverse section through a hyphal cell of Histoplasma capsulatum with a postconversional intracytoplasmic membrane system (S) in association with glycogenlike bodies (GL). × 64,000. Marker represents 0.2 μm.*

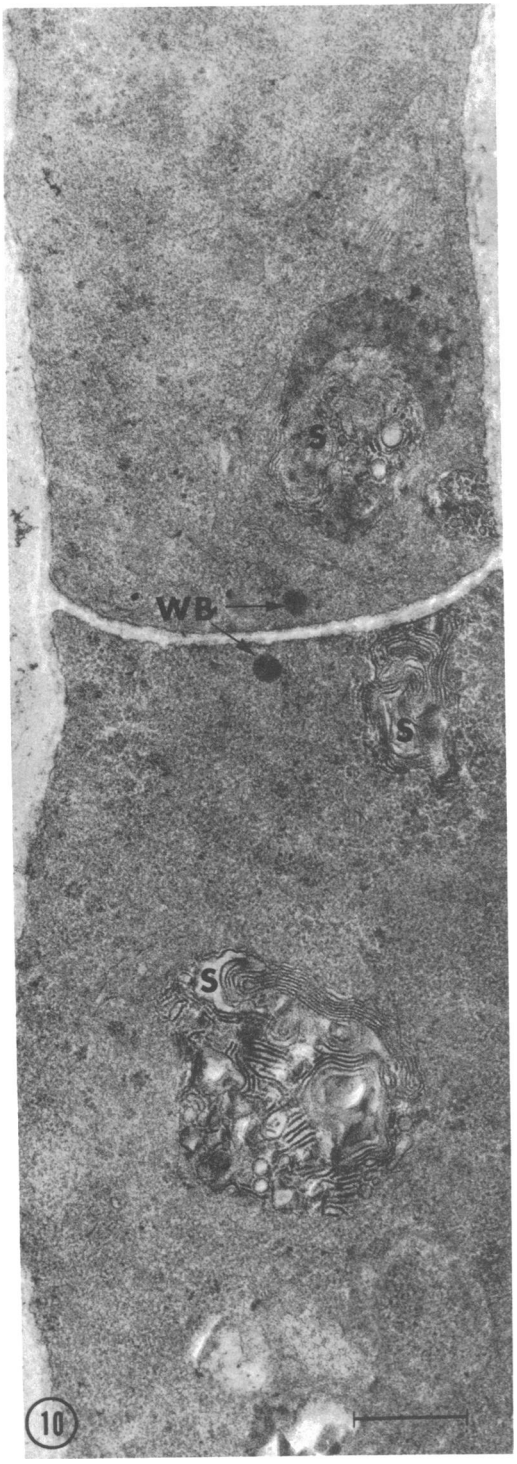


FIG. 10. *Segment of maturing hyphal cell of Blastomyces dermatitidis, showing intracytoplasmic membrane systems (S) along with a formed septum with Woronin bodies (WB). × 31,000. Marker represents 0.5 μm.*

phase transformation of *B. dermatitidis* and *H. capsulatum* proceeds via a similar sequence of events, and that these events may be reflective of a common conversional mechanism. Significant changes at the ultrastructural level in both fungal organisms occurred rapidly after the induction of conversion, with the appearance of numerous small intracytoplasmic membrane systems and increased numbers of mitochondria. The early appearance of these membrane systems following the change in thermal environment is suggestive of a response to physiological stimuli occurring during this period of transformation. These membrane systems do not arise from pre-existing organelles of the yeastlike phase, but develop de novo from the plasma membrane and proceed rapidly to maturity during the active period of yeast- to mycelial-phase conversion. Both fungal organisms were observed to form a small intermediate or transitional cell which appeared to be premycelial in character, as evidenced by the presence of Woronin bodies adjacent to the septal-like structure. As in the parent yeastlike cell, numerous small intracytoplasmic membrane systems, as well as increased numbers of mitochondria, were present typically in the transitional cell.

Intracytoplasmic membrane systems have been described in several pathogenic and nonpathogenic fungi (2–5, 7, 10), and appear to be associated generally with cell division and septal formation (6, 8). The membrane systems in the fully established mycelial-phase cells of *B. dermatitidis* as well as *P. brasiliensis* have been described previously as resembling the mesosomes of bacteria in that they originate from the plasma membrane, contain cell wall-like material, and form vesicles or tubules. The lomasomes described by Moore and McAlear (9) may be part of the intracytoplasmic membrane system. Such structures appear to be associated with the ascospore cell wall formation in *Penicillium vermiculatum* (11). The appearance of the numerous small intracytoplasmic membrane systems during the early stages of the induction period of yeastlike to mycelial-phase conversion in both *B. dermatitidis* and *H. capsulatum* suggests that these membrane systems may be associated in some manner with the mechanism of conversion, and that they may be functionally unique during the critical period of transformation of these dimorphic fungi.

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