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Electron Microscopy of Yeastlike Cell Development from the Microconidium of *Histoplasma capsulatum*

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Fine details of the sequential morphological events occurring during transition of microconidia (spores less than 5 μm in diameter) to the yeastlike phase of *Histoplasma capsulatum* as seen in ultrathin section are described and illustrated by electron micrographs. Masses of microconidia were obtained when the fungus was grown on a garden soil extract medium. Spores were incubated under in vitro environmental conditions conducive for phase transition (an enriched medium at 37°C). Within 48 h of incubation, the microconidia either germinated to give rise to a short mycelium or the germ tube process became a yeast mother cell without further extension. The wall of the yeast mother cell was thin and smooth, and its cytoplasmic content was ultrastructurally complex, consisting of numerous lipid bodies, vacuoles, glycogen-like deposits, and membrane systems. Within 96 h, the mother cell underwent multipolar budding to form simultaneously linear hyphal and/or ovate yeastlike daughter cells. During the transition, new cell wall materials of the germ tube, the mother cell, and yeastlike daughter cells arose by blastic action from the innermost layer(s) of the wall of the precursor form. Lomasome-like vesicles were often seen in association with areas of new cell wall formation. After organellar migration into and septation of the daughter cells, the yeast mother cell's cytoplasmic content underwent marked degenerative changes.

Certain morphological events associated with conversion of the mycelial to the yeastlike phase of *Histoplasma capsulatum* have been described by a number of investigators (8, 11, 12, 17, 19, 20). Each of these descriptions was based on interpretations made from either fixed or slide materials observed under the limitations of the light microscope. It is generally recognized that *H. capsulatum* may produce at least two types of conidia (1, 18). These conidia, termed micro- and macroconidia, are differentiated primarily on the basis of size. Whether or not these spore types possess different functions or physiological characteristics remains to be clearly demonstrated. Procknow et al. (20) described what was interpreted as the direct conversion in vivo of the macroconidium to a spherule-like structure containing numerous yeastlike cells. This observation remains to be confirmed. Dowding (8), Pine and Webster (19), and Goos (11) reported that the microconidia of *H. capsulatum* may give rise to yeastlike cells in vitro. Although sporulation of the fungus is dependent on a number of environmental and cultural factors (18), in general it is the microconidia (spores less than 5 μm in diameter) that predominate (1). Since it has been shown that particles greater than 5 μm in diameter rarely reach the deep alveoli (3), the ability of the microconidium to undergo phase conversion assumes epidemiological importance in the infectious process of pulmonary histoplasmosis.

In a recent study (10) using techniques of electron microscopy, we have described the morphological events occurring during germination of the microconidia of *H. capsulatum* when cultured at 25°C in a simple medium. In the present study, we describe a sequence of ultrastructural events occurring when microconidia were cultured under in vitro conditions conducive for mycelial-to-yeastlike phase conversion.

MATERIALS AND METHODS

Preparation of cultures. Two strains of *H. capsulatum* of unknown mating amd chemotypes, ATCC 24867 and Huff, were employed throughout. These strains were isolated from human infections and have been maintained in stock culture for a number of years; they are characterized by their ability to consistently undergo mycelial-to-yeastlike conversion on a variety of media.

Mycelial-phase cultures were grown for 21 days at 25°C on slants of the soil extract medium described

by Kwon-Chung (16). Under these cultural conditions, the fungi were seen to produce masses of microconidia as observed by light microscopy. The slants were flooded with sterile 0.9% saline, and the microconidia were dislodged by gentle scraping with a sterile wire loop. These suspensions consisted entirely of microconidia along with occasional hyphal fragments. The suspensions were harvested by centrifugation, washed once in sterile saline, and inoculated into 250-ml spinner flasks (Bellco Glass Inc., Vineland, N.J.) containing 100 ml of sterile brain heart infusion broth (BBL, Cockeysville, Md.) supplemented with 0.01% cysteine hydrochloride. Quantitative estimates of spores contained in the inoculum were not done. The flasks were incubated at 37°C. Specimens were taken at 24-h intervals and washed by centrifugation in 0.9% saline.

Preparation for electron microscopy. Cells were fixed at 4°C with 3% glutaraldehyde in 0.1 M s-collidine buffer (pH 6.8) for 24 h. After being washed in 0.9% saline, they were postfixed for 18 to 20 h at 4°C with 1% osmium tetroxide in the same buffer (pH 7.4), followed by prestaining with 3% aqueous uranyl magnesium acetate for 1 h at room temperature. Alternatively, portions of the cells were fixed in 0.6% aqueous potassium permanganate for 10 min at 4°C. After fixation, all specimens were embedded in 2% agar to facilitate handling. Small agar cubes containing the cells were dehydrated in a series of ethanol dilutions and embedded in Epon 812. Thin sections were cut with a diamond knife on an LKB Ultrotome III and picked up on 300-mesh copper grids. They were stained for 5 min with Reynold's lead citrate followed by 10 min with 3% uranyl acetate in absolute ethanol. Specimens were examined with a Hitachi electron microscope, model HU-11B-1, at 75 kV.

RESULTS

Microconidia of *H. capsulatum* are generally defined as smooth-walled spores which may be spherical, pyriform, or cigar shaped, and which range in size from 2 to 6 μm in diameter (18). Measurements of the microconidia of the strains employed in this study rarely exceeded a diameter of 3.0 μm.

Microconidia of the two strains used here were observed to undergo similar changes in morphology in response to the cultural conditions employed for induction of mycelial-to-yeastlike conversion. After 48 h of incubation, numerous microconidia were observed to develop budlike structures resembling germ tubes. The wall of the budlike process appeared as an extension of the microconidial wall or one of its innermost layers (Fig. 1). In some instances, there was a partial disruption of the outermost layers of the

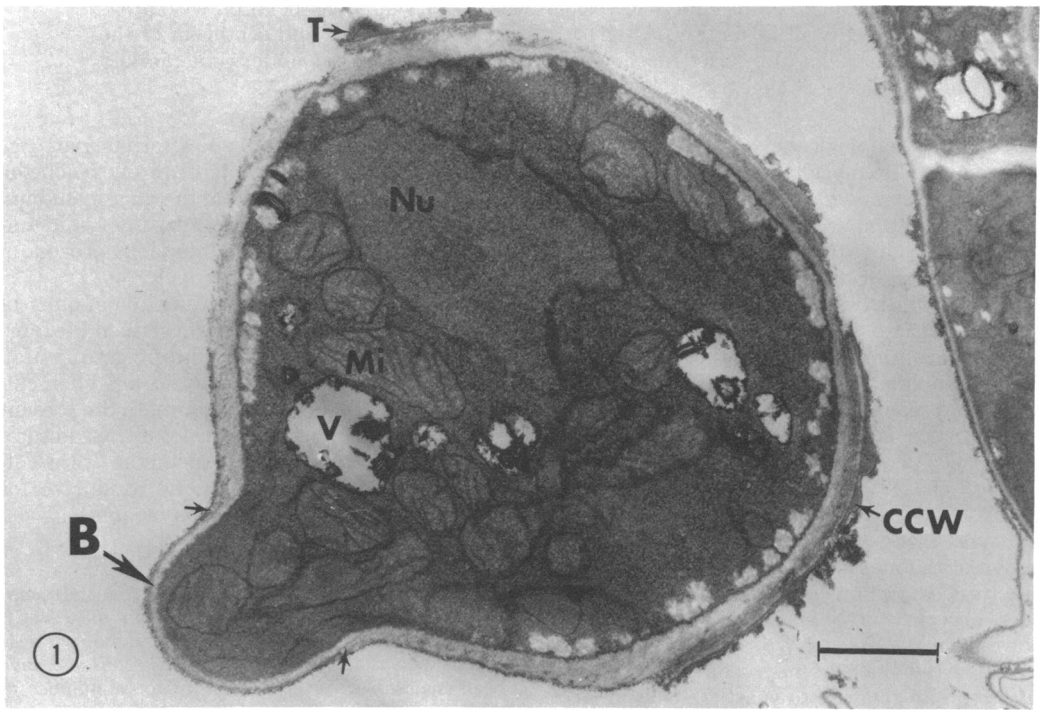

FIG. 1. *Germinating microconidium after 48 h of incubation at 37°C. Note the remnants of the conidial cell wall (CCW), the presence of tubercles (T), and the origin of the cell wall of the budlike germ tube (B) at the arrows. The cytoplasm contains a single nucleus (Nu), scattered mitochondria (Mi), and vacuoles (V). Fixation, Glutaraldehyde-osmium tetroxide. Bar indicates 0.5 μm.*

spore cell wall, perhaps as the result of swelling of the conidium. Such observations were similar to those reported for the hydrated germinating conidia of certain other fungi (9, 21). The outermost layer was somewhat more electron dense and possessed short tubercles that rarely exceeded 170 nm in length (see also Fig. 2, 3, and 8). Although these latter structures could not be seen on light microscopy of the spore suspensions used, they were readily demonstrated by electron microscopy to be extensions of the outermost layer of the spore cell wall.

Within 72 h under conversion conditions, one of at least two morphological events occurred. At times, the budlike structure functioned as a germ tube and elongated to form a short, septate mycelium a few cells in length (Fig. 2). In other germinants, the budlike structure was observed to undergo a marked enlargement without further extension. This enlarged, bulbous structure appeared analogous to the yeast mother cell (YMC) described by Pine and Webster (19).

A microconidial germinant and its yeast mother cell (conidial-YMC complex) are shown in Fig. 3. These cells measured about 1.8 and 3.6 μm in their respective diameters. In many cases, the YMC was seen to be approximately three times the size of the parent microconidium. In other cases, the YMC appeared much smaller (see Fig. 7, 9, and 10). Although perhaps the result of plane of section, this difference in size might suggest the direct conversion of microconidia to YMC. On observation by light microscopy, such small conidial-YMC complexes might represent what was described by Pine and Webster (19) as direct budding of yeastlike cells from the microconidial cell proper. Occasionally, septal formation occurred between the microconidium and the YMC. The YMC cytoplasm appeared highly organized and somewhat complex in its organellar content, with numerous lipid bodies, vacuoles, glycogen-like deposits, and occasional membrane systems scattered throughout. The thin YMC wall was smooth over its entirety and measured about 75 nm in total thickness. The YMC wall originated from an innermost layer(s) of the old spore wall. Lomasome-like vesicles were seen frequently in association with the plasma membrane. Although perhaps an artifact of orientation, a vesicle appeared in intimate contact with a lipid body (Fig. 4).

Depending upon the plane of section, the

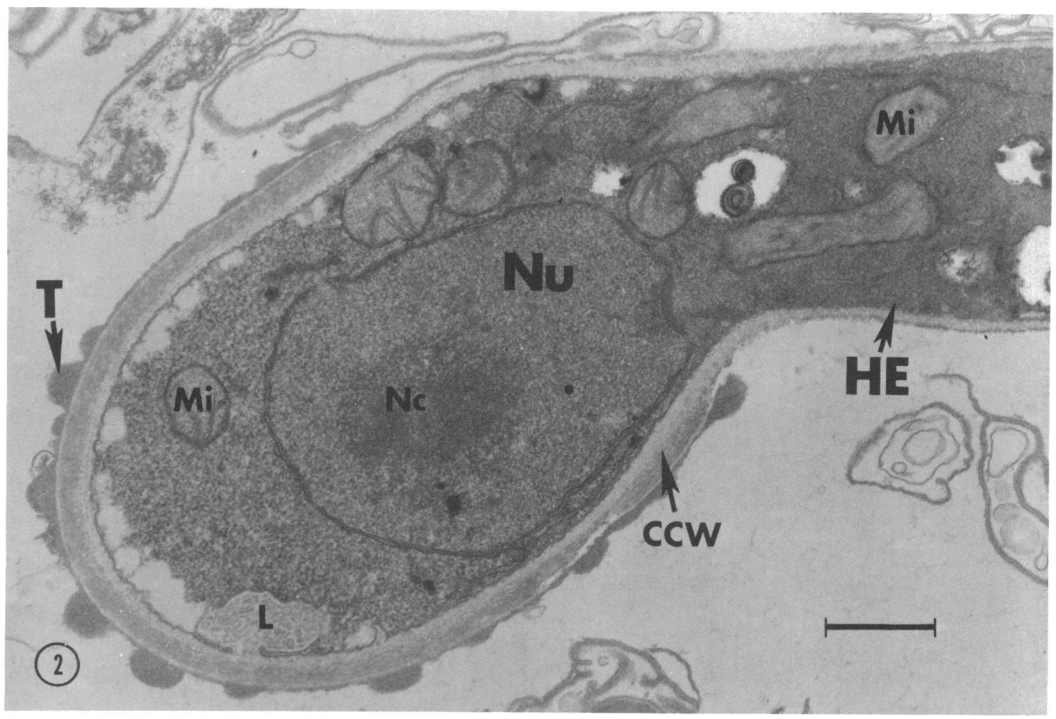

FIG. 2. *Portions of a germinating microconidium giving rise to a short hypha (HE). Note the presence of tubercles (T) at the outer limits of the conidial cell wall (CCW), a single nucleus (Nu) and nucleolus (Nc), scattered mitochondria (Mi), and a lomasome-like vesicle (L). Fixation, Glutaraldehyde-osmium tetroxide. Bar indicates 0.5 μm.*

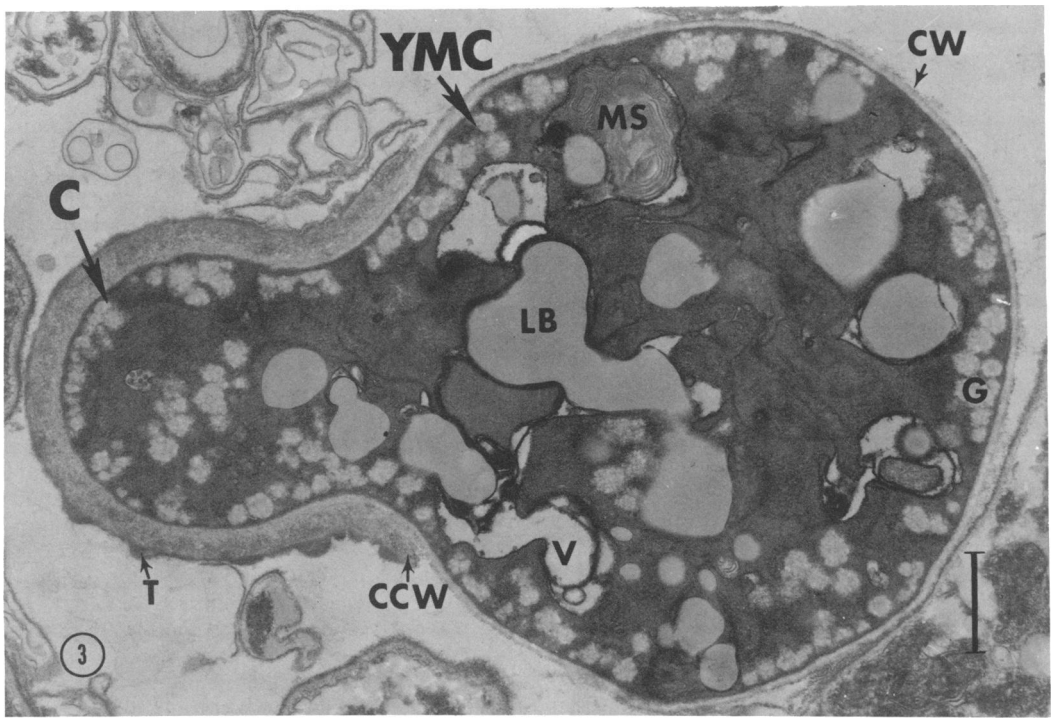

FIG. 3. *YMC development from the microconidium (C). Note the old conidial cell wall (CCW) with tubercles (T), the thin wall (CW) of the YMC, and the presence of lipid bodies (LB), vacuoles (V), glycogen-like material (G), and a membrane system (MS) in the cytoplasm. Fixation, Glutaraldehyde-osmium tetroxide. Bar indicates 0.5 μm.*

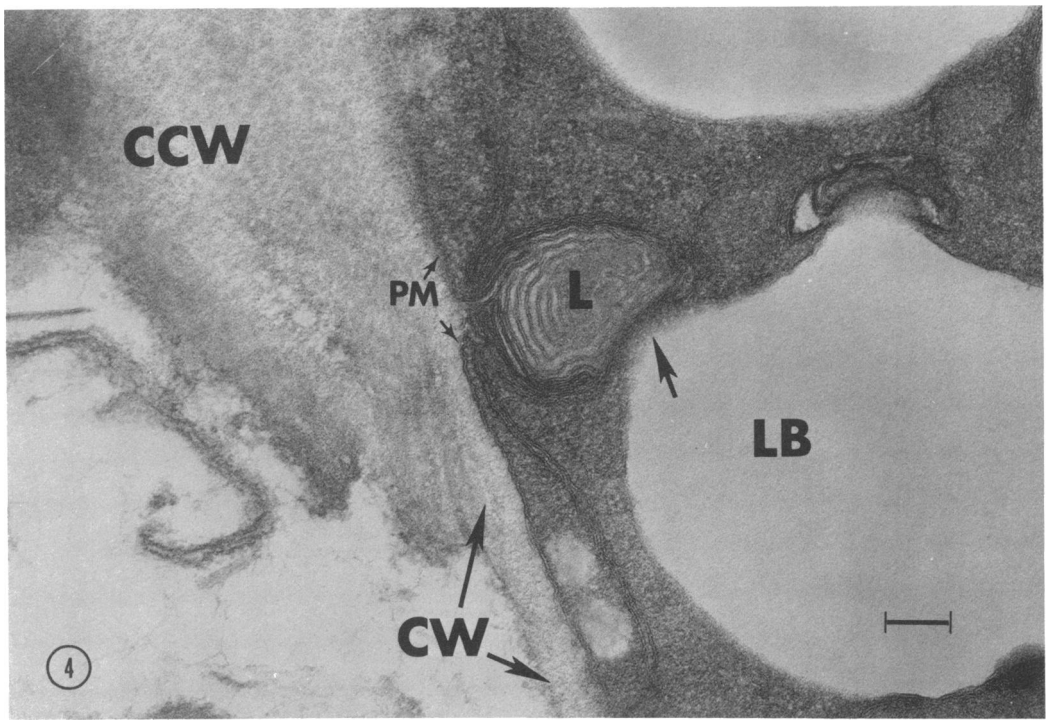

FIG. 4. *Portion of the conidial-YMC complex showing the relationship of the old conidial cell wall (CCW) to the wall (CW) of the YMC. Note the association of the lomasome-like vesicle (L) to the plasma membrane (PM) and the lipid body (LB) as marked by the arrow. Fixation, Glutaraldehyde-osmium tetroxide. Bar indicates 0.1 μm.*

YMC may give rise by blastic action to single or multiple buds located apparently at random sites along the periphery of the YMC wall (Fig. 5 and 6). The wall of the bud was observed to arise from an innermost layer of the YMC wall, while the outermost surface of the bud cell wall was somewhat thickened and of increased electron density. Certain of these buds appeared to enlarge to form short, ovate structures, which are interpreted as being newly forming yeastlike daughter cells. Figure 7 shows a thin section of a conidial-YMC complex after fixation in permanganate. Nuclear separation was clearly evident at the nearly completed septum. Remnants of the old microconidial cell wall remained adherent to the conidial portion of the complex. As is characteristic of cells fixed in permanganate, the cytomembranes were in sharp relief.

Elongate structures consistent with the fine-structural appearance of hyphal cells of *H. capsulatum* (10) were observed occasionally to arise simultaneously from the same YMC bearing the ovate yeastlike daughter cells (Fig. 8). The wall of the daughter hyphal cell appeared as an extension of the entire wall of the YMC, while that of the ovate yeastlike daughter cell appeared as an extension of only the innermost layer(s) of the parent YMC. After septation of the yeastlike and/or hyphal daughter cells, the cytoplasmic content of the conidial-YMC complex underwent marked degenerative changes (Fig. 9). After 108 h of incubation, numerous budding yeastlike cells characteristic of the parasitic form of *H. capsulatum* were found free in the cultures on examination by both light and electron microscopy. Rarely, budding yeastlike cells were seen still in intimate association with the now degenerate and probably nonviable conidial-YMC complex (Fig. 10).

DISCUSSION

The most comprehensive study available on the morphological events occurring during conversion of the mycelial to the yeastlike phase of *H. capsulatum* is that reported by Pine and Webster (19). Using techniques of static slide culture and fixed-slide preparation with subsequent light microscopy at various time intervals, these workers described four types of events involving morphological changes of mycelial-phase cells during the conversion process. These modes of ontogeny include: (i) the formation of yeastlike cells by budding of hyphal cells; (ii) the probable conversion of hyphal cells to the yeastlike phase by monilial chain formation; (iii) the formation of stalked yeastlike cells arising

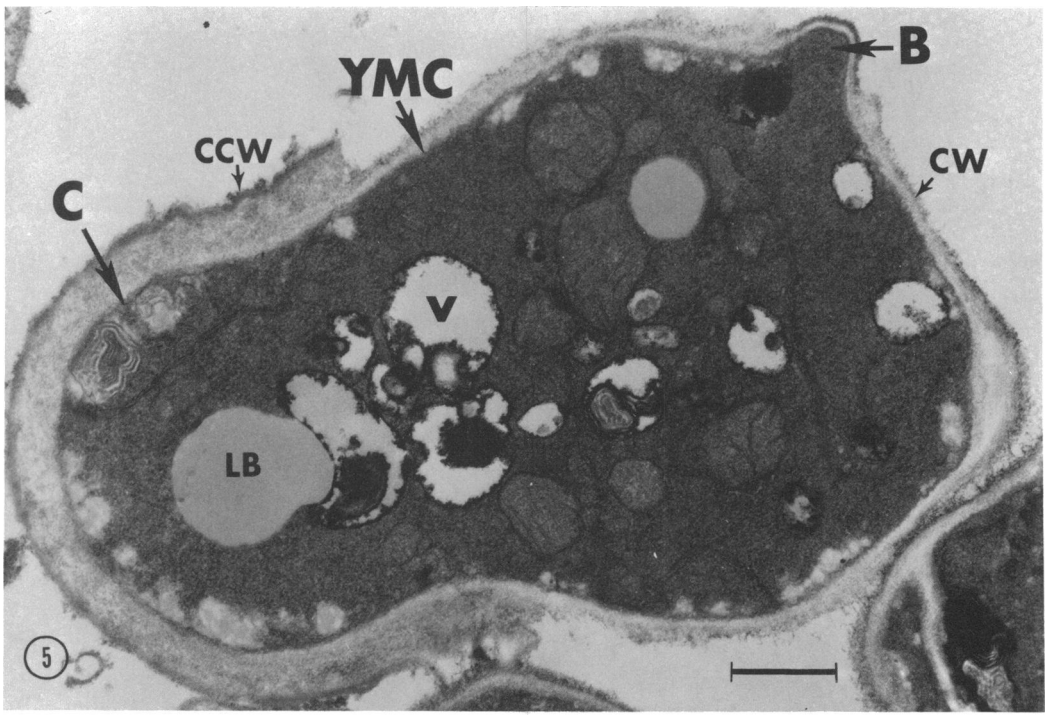

FIG. 5. *YMC and conidium (C) showing old conidial cell wall (CCW) and budlike structure (B) arising from the wall (CW) of the YMC. Fixation, Glutaraldehyde-osmium tetroxide. Bar indicates 0.5 μm.*

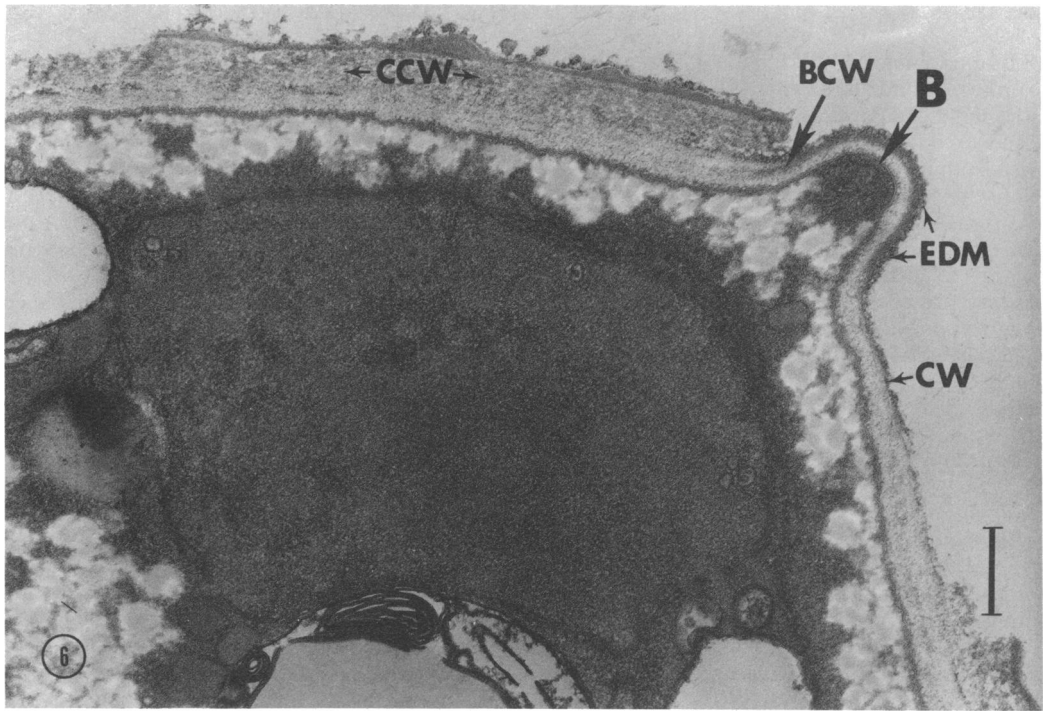

FIG. 6. *Portion of a conidial-YMC complex showing the origin of wall (BCW) of the bud (B) from an inner layer of the wall (CW) of the YMC. Note the remnants of the old conidial cell wall (CCW) and the electron-dense material (EDM) at the outer surface of the bud. Fixation, Glutaraldehyde-osmium tetroxide. Bar indicates 0.25 μm.*

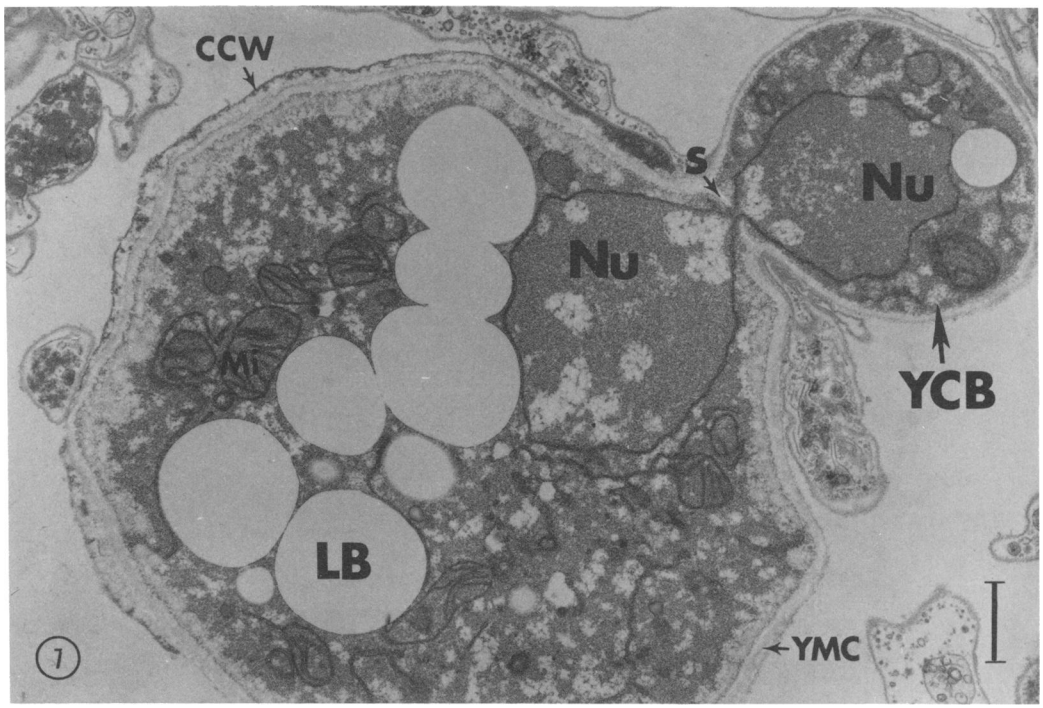

FIG. 7. *Yeastlike daughter cell bud (YCB) and YMC showing remnants of the old conidial cell wall (CCW), multiple lipid bodies (LB), scattered mitochondria (Mi), and separation of the nuclei (Nu) at the developing septum (S). Fixation, Potassium permanganate. Bar indicates 0.5 μm.*

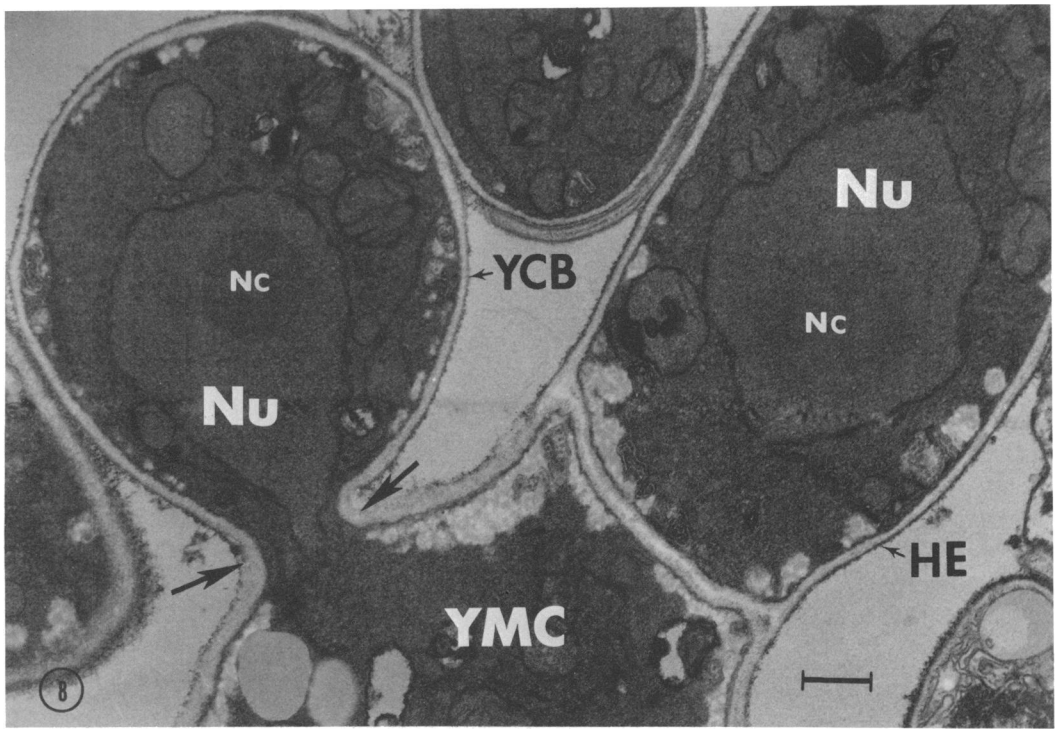

FIG. 8. *Linear hyphal element (HE) and ovate yeastlike cell bud (YCB) arising from the YMC. Note the nuclei (Nu), the nucleoli (Nc), and the origin of the wall of the YCM from an inner layer of the wall of the YMC as indicated by the arrows. Fixation, Glutaraldehyde-osmium tetroxide. Bar indicates 0.25 μm.*

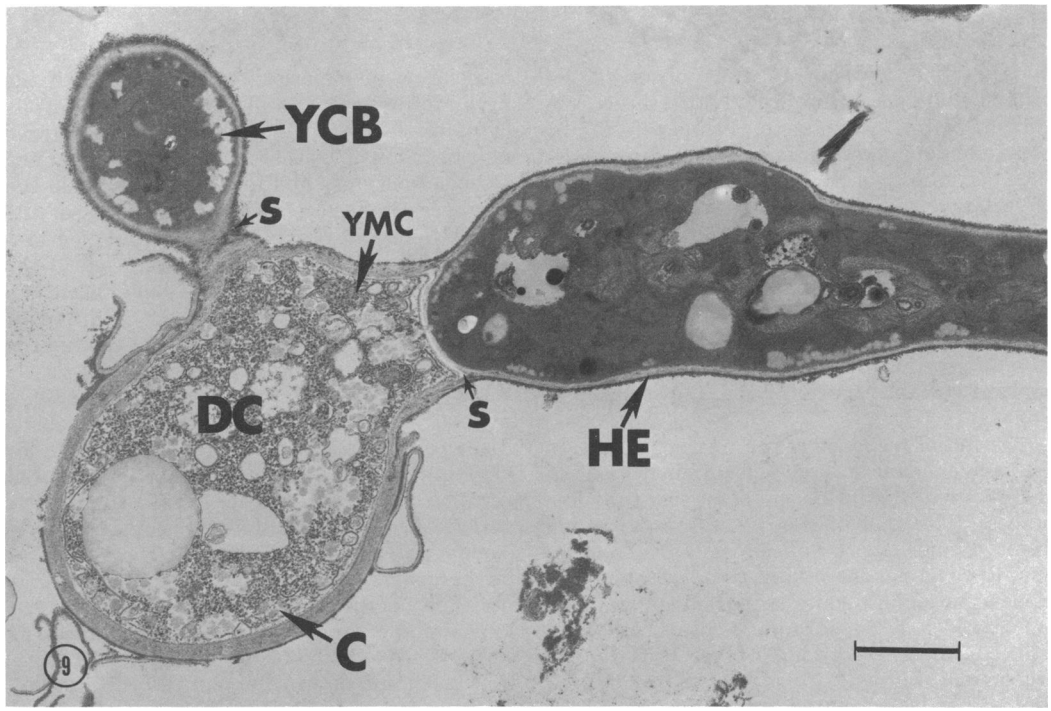

FIG. 9. *Conidial-YMC complex showing both hyphal element (HE) and yeastlike cell (YCB) daughter cells. Note the septal areas (S) and the degenerate cytoplasm (DC) of the complex. Fixation, Glutaraldehyde-osmium tetroxide. Bar indicates 1.0 μm.*

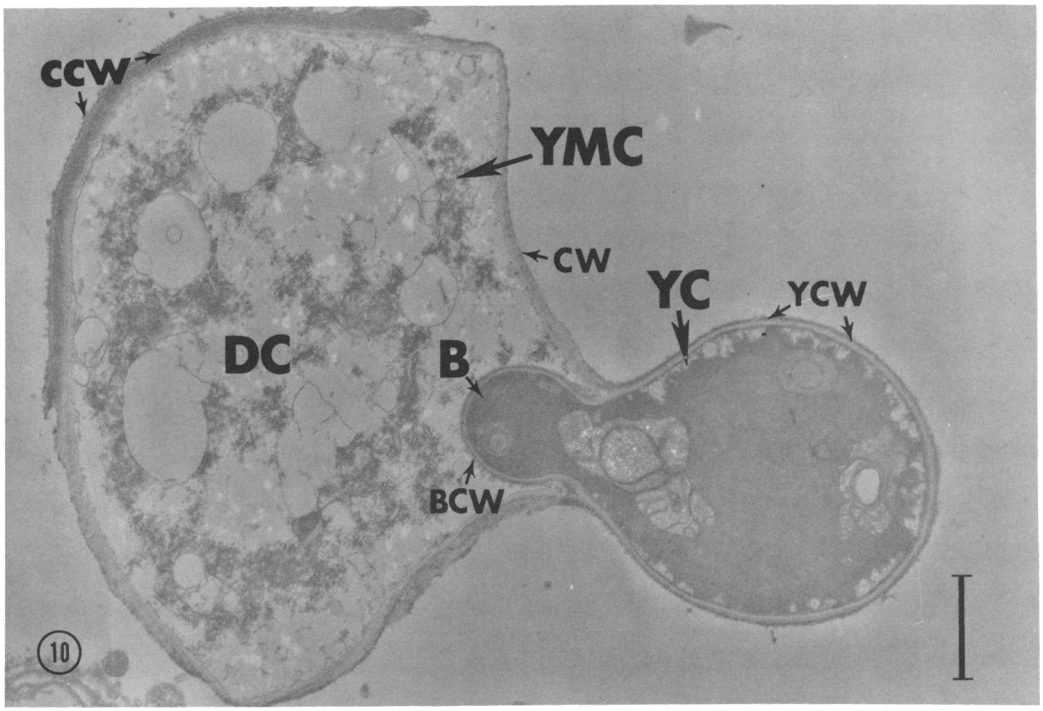

FIG. 10. *YMC showing degenerate cytoplasm (DC) and remnants of old conidial cell wall (CCW). Note the yeastlike cell (YC) and its bud (B) and the relation of the yeastlike cell wall (YCW) to the bud cell wall (BCW) and the cell wall (CW) of the YMC. Fixation, Glutaraldehyde-osmium tetroxide. Bar indicates 1.0 μm.*

in a manner similar to that observed for microconidial formation; and (iv) the direct conversion of microconidia to yeastlike forms by polar and nonpolar budding. The microconidium then becomes a yeast-phase mother cell directly, or forms yeastlike-phase cells by budding and gradually loses its identity as a microconidium. This latter division does not take into account the obvious alternative, in which spores germinate to form hyphae which then may form yeasts by method i, ii, or iii. Using techniques of electron microscopy, the results of the present study confirm certain aspects of the above observations.

The initial response of the microconidium to the conversional stimuli (an enriched medium at 37°C) was the formation of a germ tube-like process. The wall of this process was derived from an inner layer(s) of the wall of the germinant in a manner resembling that described previously for similar spore suspensions germinating at 25°C in Sabouraud-glucose broth (10). This continuity of the inner layer(s) of the microconidial wall with the wall of the germ tube process has been pointed out by Bartnicki-Garcia (2) as being the most common mode of genesis, and appears throughout the fungi. The association of lomasome-like vesicles with the newly formed YMC wall and lipid bodies of its cytoplasm suggests that lipid storage material may be taken up by these membranes containing attached enzymes and used as substrate in the synthesis of new cell wall components of the YMC. Such utilization of stored lipid could result in the extensive vacuolization seen in the YMC cytoplasm. Lomasomes have been described in a number of fungi, and it is thought that they perform secretory functions in the formation of new cell wall material (15, 22).

No microconidium was observed to undergo hyphal-cell and YMC formation simultaneously. Although the regulatory factors responsible for directing the mode of morphogenesis after initial germination at 37°C are not known, such observations suggest basic differences between microconidia in their initial physiological responses to the conversional stimuli. At the same time, the ability of the YMC to give rise to both linear hyphal and ovate yeastlike cells simultaneously suggests the possibility of two different regulatory mechanisms directing pathways of synthesis associated with phase transition of the intermediate conidial-YMC complex.

Preliminary studies with macroconidia de-

rived from our respective strains indicate that only occasional macroconidia germinated to form a short hypha, but YMC formation from the macroconidium was never observed under the in vitro cultural conditions employed for microconidial germination. These results are similar to those reported by Pine and Webster (19). They did not observe yeastlike development from macroconidia, although they suggest that yeast formation may result from conversion of a hypha formed by the germinating macroconidial cell. In this regard, we have observed that hyphal cells arising directly from the microconidium or the intermediate YMC or contained in the original inoculum appear to undergo ultrastructural changes suggestive of yeastlike transition consistent with methods i and ii as described by Pine and Webster. However, further study is necessary before these observations can be described in detail.

On the basis of ultrastructural evidence alone, it is difficult to speculate on the nature of the regulatory mechanisms involved or the biochemical events that occur during phase transition of the *H. capsulatum* microconidium. The sequential ultrastructural changes associated with morphological alterations occurring during conversion of the microconidial cell might suggest possible changes in the biochemical nature of the cell wall during the various stages of morphogenesis. It is known that the cell wall of *H. capsulatum* consists predominantly of chitin and glucan (5, 6, 7, 12, 14). The ratio of chitin to glucan (7), the configuration of the glucan as α- or β-linked polymers (4, 7, 13), and the presence of autolytic extracellular or wall-associated glucanases (4) may be important factors in determining whether the cells grow as a yeast or a mycelium. The development of techniques employing electron cytochemical methods might be useful in localization studies associated with cell wall synthesis or degradation that might occur during the conversion process of cell systems such as those described here. Such studies are in progress.

ACKNOWLEDGMENTS

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