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Ultrastructure of Dimorphic Transformation in *Paracoccidioides brasiliensis*

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The fine structure of *Paracoccidioides brasiliensis* undergoing temperature-dependent transformation from mycelium to yeast and vice versa (M ⇌ Y) was studied. The transitional form to mycelium from the yeast appears as an elongated bud that extends from the yeast and which has a mixture of characteristics from both the yeast and the mycelium. The transitional form to yeast from the mycelium starts with enlargement of the interseptal spaces and cracking of the outer electron-dense layer of the cell wall of the hypha. Later the interseptal spaces tend to become round and separate. In M → Y only few interseptal spaces seem to transform. The yeast is produced by self-transformation of the hypha. In Y → M a new structure is formed and the yeast dies. Intrahyphal hyphae are observed during the transformation from M → Y, and intrayeast hyphae during the Y → M. Due to the high mortality and breakage observed in both types of transformations, we believe that wound of the yeast or the mycelium could elicit this phenomenon.

Paracoccidioides brasiliensis is a dimorphic fungus, which undergoes "thermal-dimorphism" (17), namely producing the mycelial (M) form at room temperature and a yeast (Y) form at 37 C. Drouhet and Zapater (7) studied the M form and the transformation from M to Y in cultures. Light microscope observations have been carried out on the transformation of M → Y and Y → M in cultures, and M → Y in experimental inoculations (5). The ultrastructure of the M and Y forms has been studied in cultures (4, 6) and the Y form in human tissue (9). Studies of the fine structure of this transformation could help not only in explaining the possible pathogenesis of the disease produced by this fungus, but also in explaining dimorphism in fungi.

MATERIALS AND METHODS

The particular strain of *P. brasiliensis* (IVIC-Pb9) was that used previously (5, 6). To obtain the M form, the Y form was inoculated into a GGY medium (glycine, 1%; glucose, 2%; yeast extract, 0.2% adjusted to *p*H 7.2 to 7.4 with KOH) and grown at room temperature (23 to 24 C) in 500-ml Erlenmeyer flasks which were placed in a reciprocal shaker at a speed of 120 oscillations per min with a stroke amplitude of about 5 cm. At least three successive passages of 10-day-old cultures were carried out to insure a pure filamentous culture.

The yeast was obtained from a mycelium grown in liquid Brain Heart Infusion Agar (Difco) at 37 C under the same conditions mentioned above. Different media were used because the forms at the end of the transformation grow better in them.

The transformed M → Y was harvested every 6 hr during the first day, and every 12 hr thereafter for a total of 5 days. Samples were fixed in 2% glutaraldehyde with 0.1 M phosphate buffer (*p*H 7.2) for 24 hr, postfixed in 1% osmium tetroxide in the same phosphate buffer for 3 hr, dehydrated in graded series of ethyl alcohol, and embedded in Maraglas (8). Ultrathin sections were cut with a MT-2 Porter Blum microtome with diamond knives and stained with uranyl (20) and lead (13). The electron microscope used was a Hitachi HU-IIB. The transformation was followed with a light microscope by using phase contrast.

RESULTS

The yeast or mycelium cells do not all transform at the same time. At a certain period, transitional forms to mycelium (TFM) or transitional forms to yeast (TFY) are intermixed with well-preserved mycelium or yeast, respectively.

Transformation from yeast to mycelium (Y → M). The TFM is characterized by a yeast with an elongated bud which has a larger diameter than the diameter of the normal hypha (0.8 to 1.7 μm; Fig. 1). The elongated bud is generally pear shaped and contains abundant glycogen, ribosomes, and multiple nuclei (Fig. 1 and 2). The neck between the elongated bud and the yeast is usually the same size as that of the normal bud (2), or broader (Fig. 2). Occasionally a complete septum with pore and Woronin bodies (19) can be seen separating the yeast from the elongation

(Fig. 3). It seems that the cell wall of the elongated bud is related in its growth to the inner zone of the cell wall of the yeast (Fig. 3). As the TFM grows, more interseptal spaces, in the zone of the hypha which is enclosed between two septa, are formed.

After 36 hr, TFM and abundant isolated elongated forms, bigger in diameter than the hypha and normal hyphae, are observed. Later there is an increase of normal hyphae and a decrease of the two other forms.

Transformation from mycelium to yeast (M → Y). After 18 hr almost all the hyphae are dead, displaying inside remnants of the intracytoplasmic membrane system (3, 6) and glycogen. Mitochondrial ghosts are frequent. The TFY begins with an increase in the diameter of the hypha at the interseptal spaces (Fig. 4). The outer electron-dense layer cracks at different places, that is, the outer layer disappears leaving the inner layer in direct contact with the surroundings. Inside the TFY, a prominent nucleus (Fig. 4) and abundant glycogen are observed. As the transformation to yeast continues, the thickness of the cell wall and the cracking of its outer layer increases (Fig. 5).

After the interseptal spaces increase in diameter, they separate and have a tendency to become round (Fig. 5). Most of the interseptal spaces do not transform. Usually hyphae with only one or two interseptal spaces transforming are observed; the others are empty or contain membrane remnants.

Prior to the formation of the typical yeast, the TFY resembles the former very much; however, the cytoplasmic membrane and the cell wall are undulated, and remnants of the outer electron-dense layer of the cell wall of the hypha are observed (Fig. 6).

At the beginning of the transformation from mycelium to yeast and coinciding with the high mortality of the mycelium, abundant intrahyphal hyphae are noted. A live hypha is seen entering a dead one (Fig. 8). Also various live hyphae can be seen inside a dead hypha. When the death rate of the yeast is at its peak, during transformation from yeast to mycelium, live hyphae were observed inside a dead yeast. This structure was called intrayeast hyphae (Fig. 7).

A constant relation between the so-called lomasomes (16) and the intracytoplasmic membrane system (6) with the newly formed cell wall of the TFY and TFM was not observed. However, this problem could only be elucidated by taking serial sections of this material (3).

DISCUSSION

Light microscope studies of dimorphic transformation of *P. brasiliensis* (5, 7) have not clarified

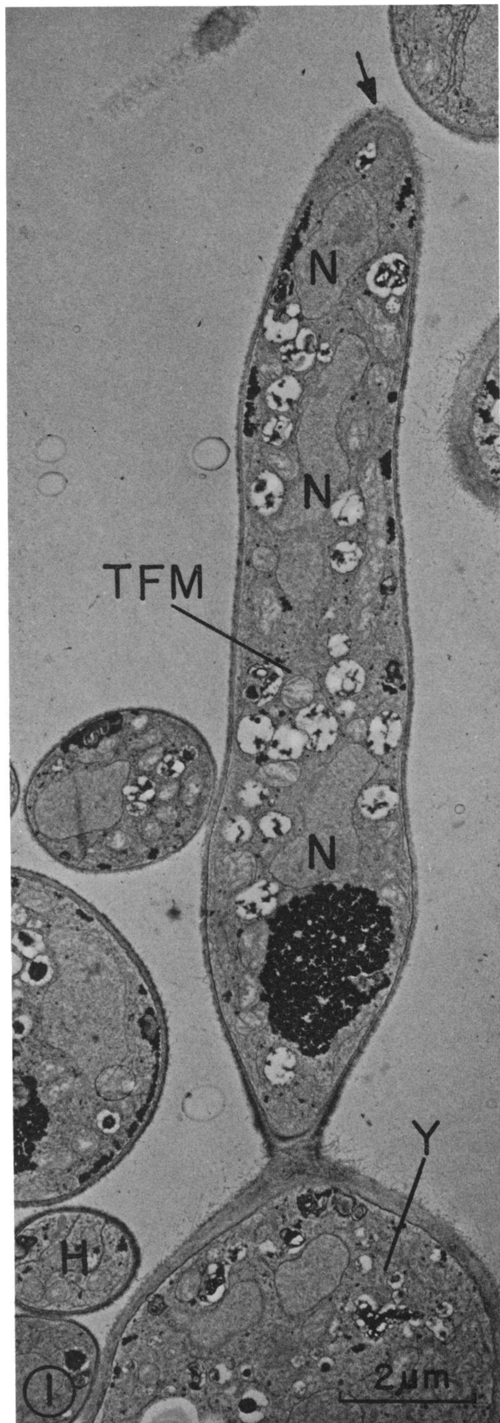
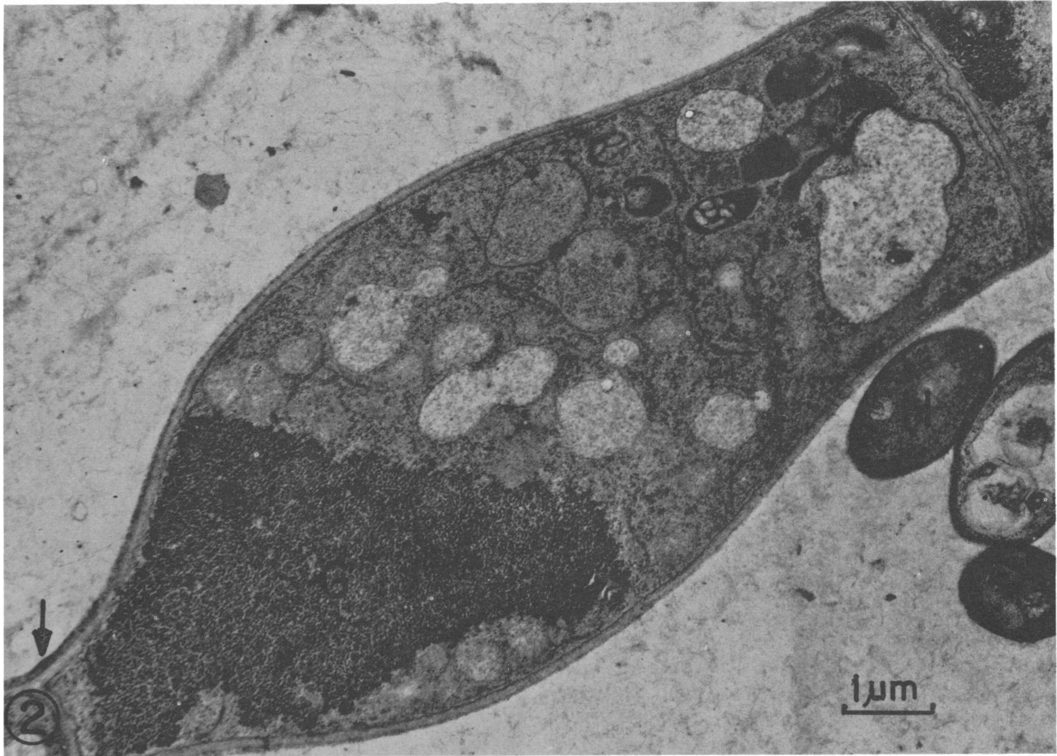


FIG. 1. *Transitional form to mycelium. The diameter of the elongated form is bigger than that of the normal hypha (H). At the tip (arrow) the cell wall is thicker. Observe multiple nuclei (N). The cell wall of the yeast (Y) is thicker than that of the elongated form.*



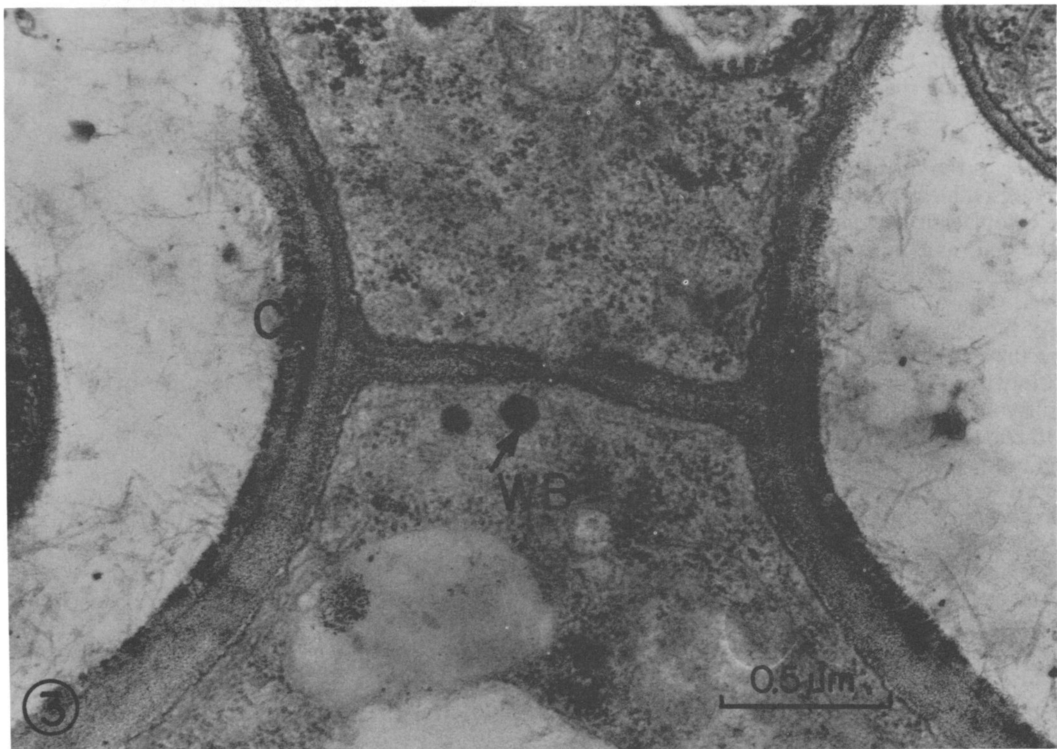
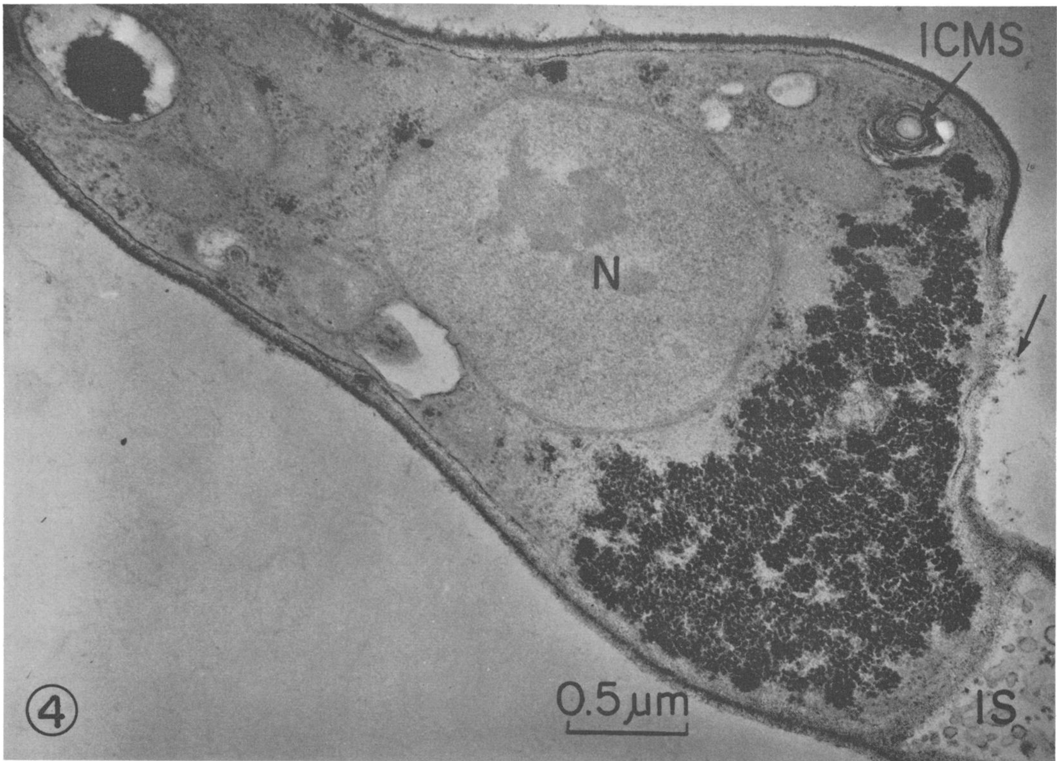


FIG. 2. *Transitional form to mycelium. Note (arrow) the narrow neck, the pear-shaped TFM, and the septum. Note the bigger diameter of the TFM and that of the normal hypha (H). G, glycogen.*

FIG. 3. *Transitional form to mycelium. Characteristics of the bud [lamination of the cell wall (CW) at the neck] and of the hypha [septum (S) and Woronin bodies (WB)] are mixed.*



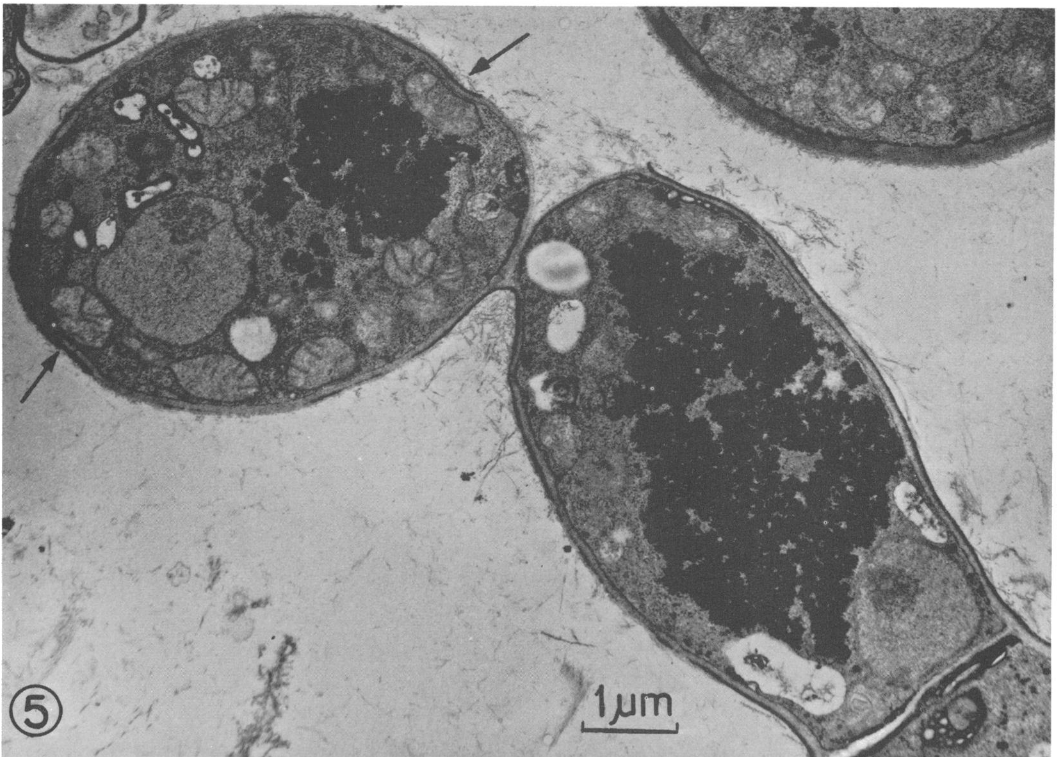
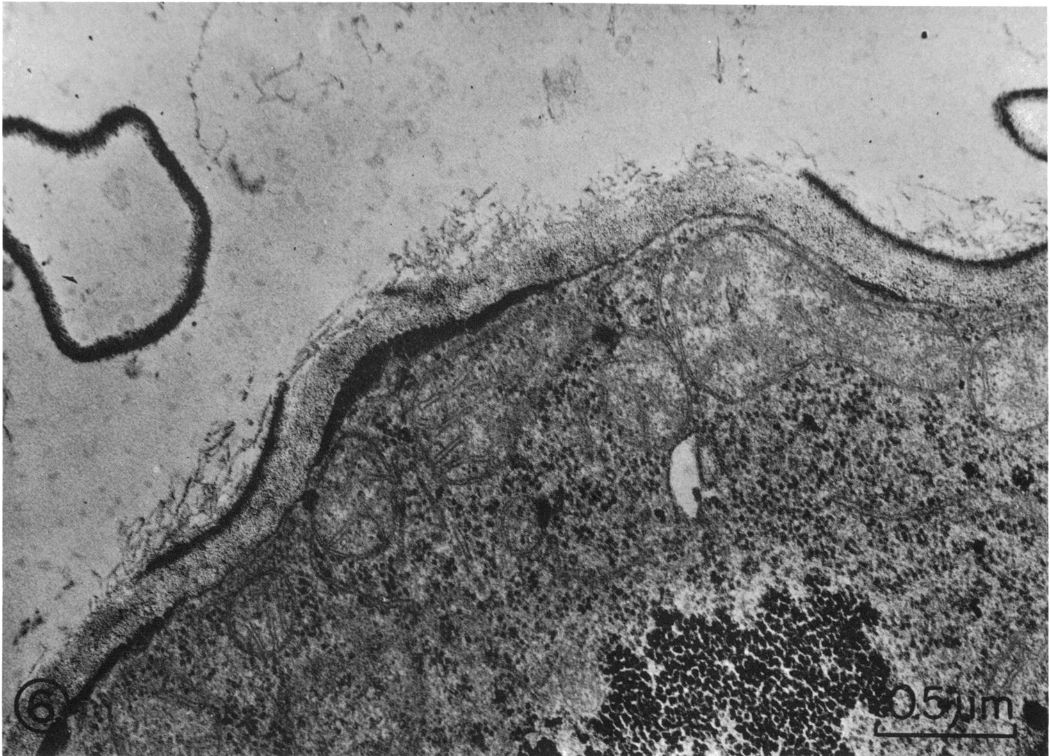


FIG. 4. *Transitional forms to yeast. Observe the enlargement of the interseptal spaces of the hypha together with "cracking" (arrow) of the external layer of the cell wall. The other interseptal spaces (IS) had only cytoplasmic remnants. ICMS, intracytoplasmic membrane system; N, nucleus.*

FIG. 5. *Transitional forms to yeast. Observe the separation of the two TFY. Note remnants of the external layer of the cell wall (arrow). Observe the increased fibrillar peeling at the site of separation.*



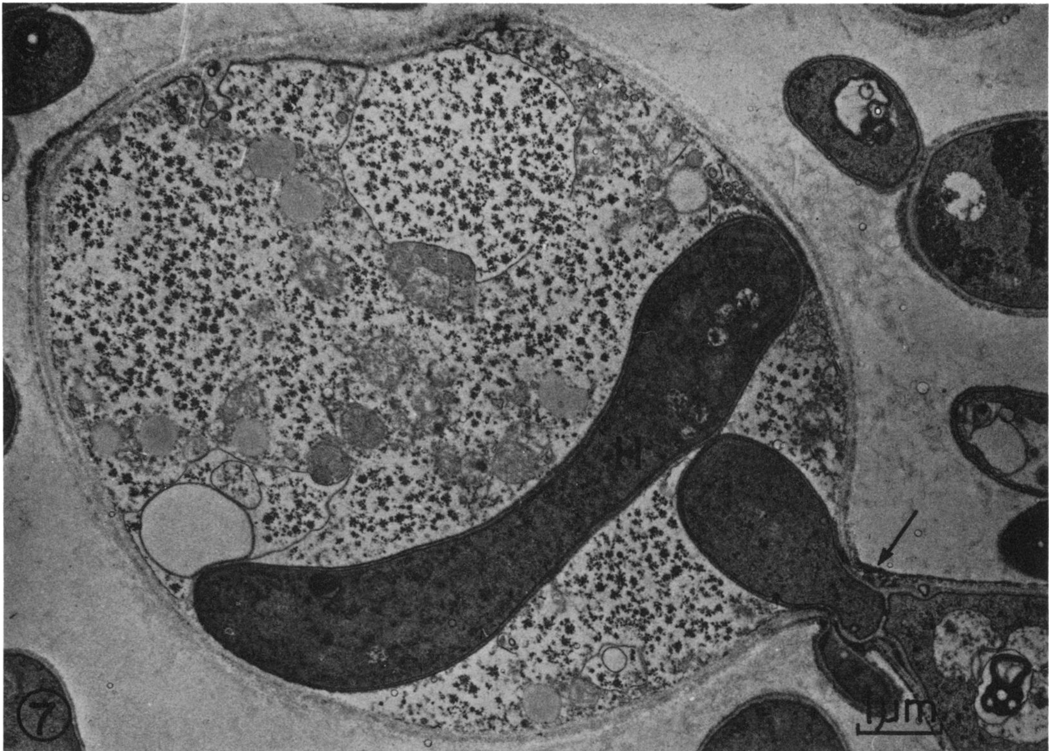


FIG. 6. *Transitional form to yeast. Observe the infoldings of the cytoplasmic membrane in relation to the newly formed cell wall. The fibrillar peeling is also related to the newly formed cell wall.*

FIG. 7. *Intrayeast hyphae. Observe the yeast identified by the neck of the bud (arrow), which has inside two hyphae (H). Cytoplasmic organelles are not easily identified in the yeast.*

whether the yeast will transform itself into mycelium. The data obtained here demonstrate that the yeast produces elongated structures that transform into mycelium, but the yeast itself dies and one frequently sees a completely developed hypha having at one end an empty yeast cell. In the transformation M → Y, it is the mycelium itself that transforms into yeast by enlargement of the interseptal spaces which later separate.

The transitional forms to mycelium or to yeast have characteristics of both forms. There is, for instance, enlargement of the interseptal spaces in which portions of the cell wall have characteristics of the cell wall of the yeast as well as a tip growth typical of the hypha. Most interesting is to find a yeast with an elongated bud in which the separation is a septum with pore and interseptal bodies (Woronin bodies; 19). The fact that these bodies, which are characteristic of the mycelium, are found in the cytoplasm of the yeast suggests that important alterations occur in the metabolic process of these transitional forms.

The morphological changes of the cell wall during the transformation and the biochemical differences between the cell wall of the mycelium and that of the yeast (12) point out important metabolic changes related to the synthesis of the components of the cell wall.

Diverse opinions regarding the layers of the cell wall of fungi have been postulated (1). The fact that the outer electron-dense layer of the hypha cracks and disappears reinforces the theory that the cell wall of the mycelium has at least two layers.

There are some similarities between the formation of microconidium in *Neurospora crassa* (10, 14, 18), the germination of conidia in *Botrytis cinerea* (11), and the transformation Y → M in *P. brasiliensis*. In all three it seems that the inner portion of the cell wall of the yeast, microconidiophore, or conidia contributes to the origin of the cell wall of the transitional hypha, microconidium, or germ tube, respectively.

Very few fungi have been studied, and, consequently, it is difficult to generalize about the ultrastructure of these alterations or to assess the relation of the specific changes to their corresponding metabolic changes.

The appearance of intrahyphal hyphae (15) and intrayeast hyphae together with the high mortality of the mycelium and the yeast, due to the adverse conditions in which they are cultured, suggest that wound or intoxication of the myeclium or yeast is the main factor that elicits this phenomenon.

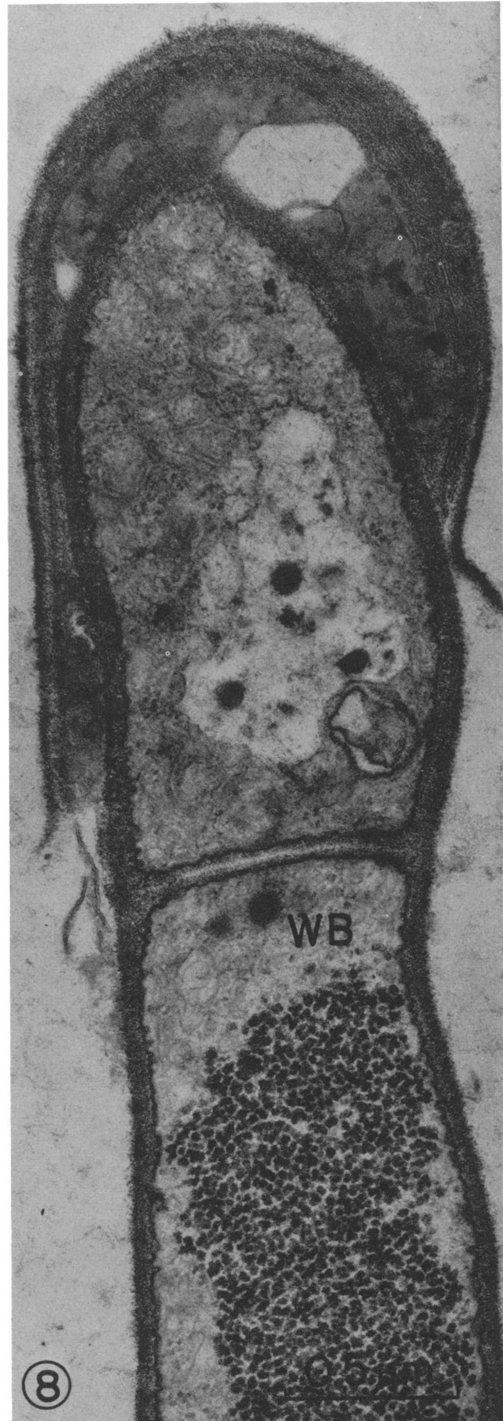


FIG. 8. *Intrahyphal hyphae. Observe how the tip of the hypha penetrates inside a dead hypha in which it is difficult to identify the cytoplasmic organelles. WB, Woronin bodies.*

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