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Title: ULTRASTRUCTURE OF DORMANT AND GERMINATING CONIDIA OF ASPERGILLUS NIDULANS

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Thomas C. Allen, Jr.

The cell wall of dry, dormant conidia of Aspergillus nidulans is three-layered. The wall of hydrated conidia is five-layered and the germ tube wall is continuous with the innermost layer. The outermost layer consists of rodlets measuring 10 nm in diameter. Mitochondria and endoplasmic reticulum increase in number and amount, respectively, during hydration and germination and vacuoles increase in size. The apex of the young germ tube is multivesicular.

Ultrastructure of Dormant and Germinating Conidia of *Aspergillus nidulans*

by

Edwin Ray Florance

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Associate Professor of Botany
in charge of major

Redacted for privacy

Head of Department of Botany and Plant Pathology

Redacted for privacy

Dean of Graduate School

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INTRODUCTION

Since Bracker's suggestion (3) that the ultrastructure of the fungus spore before and during germination had been studied in too few fungi to allow generalization, there has been a steady increase in the literature on this subject (2, 4, 5, 6, 16, 18). Border and Trinci (2) working with *Aspergillus nidulans* (Eidam) Wint., Tanaka (17) working with *A. oryzae* (Ahlb.) Cohn, Hawker (8) working with *A. niger*, V. Tiegh., and Remsen, Hess and Sassen (15) working with *Penicillium megasporum* Orpurt and Fennel have reached conflicting conclusions regarding the structure of the wall during dormancy and germination. This paper describes ultrastructural changes in the wall and protoplast of *A. nidulans* conidia from dormancy through hydration and hyphal tip extension.

MATERIALS AND METHODS

Aspergillus nidulans (Eidam) Wint. conidia were prepared for fixation in three ways:

1. Dry dormant conidia were harvested from seven-day-old cultures by suspending them directly in 20 ml of 4% glutaraldehyde buffered to pH 7.0 with 0.125 M sodium phosphate buffer, then centrifuged at 7500 rpm for 30 minutes.
2. Conidia were hydrated for 20 minutes in buffer, centrifuged ten minutes at 7500 rpm, and the resulting pellet placed in 4% glutaraldehyde. Therefore, total hydration time was 30 minutes prior to fixation.
3. Both germinating and hydrated conidia were removed from cultures grown on Czapek's Dox agar at 20C by cutting blocks approximately 0.5 cm square at hourly intervals for 11 hours starting at one hour post-inoculation.

Fixations were carried out by one of the two following methods:

1. Permanganate fixative was prepared after the method of Luft (13, p. 178). The final concentration of potassium in this fixative was 0.6% and it was adjusted with veronal acetate buffer to pH 7.5. Agar blocks with conidia were fixed for one hour at 4C, then given three 10-minute rinses in veronal acetate buffer, pH 7.0 and dehydrated.

2. Ten ml of 8% glutaraldehyde from a nitrogen sealed ampule were diluted to 4% with 0.125 M sodium phosphate buffer, pH 7.0. The conidial pellets or blocks were fixed for 24 hours in an icebath at 4C, rinsed in 0.125 M sodium phosphate buffer, pH 7.0, for at least 12 hours, and postfixed in 1% OsO_4 six hours at 4C. The samples were rinsed three times for ten minutes each in phosphate buffer and dehydrated.

Samples were dehydrated by three 10-minute (i.e., three washes of 10 minutes each) in 50% acetone, eight to ten hours in 70% acetone saturated with uranyl acetate, and three 10-minute washes in 100% acetone. After dehydration, the conidial samples were placed in a 2:1 epon-acetone solution and left until the odor of acetone could no longer be detected ($\simeq$ 12 hours at room temperature). A portion of each conidial sample was flat embedded in Araldite 6005-Epon 812 mixture using BDMA as a catalyst. Other portions were trimmed and placed directly in the pyramidal end of BEEM capsules. The samples were allowed to polymerize at 60C for 12 hours. They were sectioned with a Dupont Diamond Knife on a Sorvall "Porter-Blum" MT-2 Ultramicrotome. Sections in the 60-90 nm range were collected on Formvar coated grids; examined and photographed with a Philips EM-300 electron microscope operating at 60KV.

DORMANT CONIDIA

The cell wall of dormant (D) conidia is three-layered: D_1 = outed layer, D_2 = middle layer, and D_3 = inner layer (Figure 1). The D_1 layer is 7-14 nm thick, D_2 layer is 126-252 nm and D_3 layer is 140-224 nm thick. Total thickness of the cell wall ranges from 246 nm to 515 nm and averages 380 nm (averages are based on measurements of ten conidia). The conidial surface (D_1) layer is a network of rodlets (R). The rodlets, visible in sections of both dormant and hydrated conidia, are 10 nm diameter (Figure 2).

The protoplast of dormant conidia averages 2 μ diameter and contains a single nucleus bounded by a nuclear envelope. The vacuoles (V), measuring 245 ± 40 nm, are electron transparent and bounded by a unit membrane. Numerous vesicles (Ve), 70 nm or less in diameter, are bounded by a single limiting membrane and have a granular to fibrillar matrix of variable electron density. Endoplasmic reticulum is present in dormant conidia. Concentric membranous structures (CMS) are observed in the immature and mature dormant conidia fixed in glutaraldehyde (Figures 1, 6). Ribosomal material is abundant in all conidia fixed with glutaraldehyde. Mitochondria are either very elongate (Figure 7) or small and circular (Figure 1). This is presumably due to the plane of section.

HYDRATED CONIDIA

Dormant conidia averaged 3 μ diameter while conidia hydrated on agar for ten hours averaged 3.8 μ as a result of imbibition of water.

After 30 minutes hydration, the total thickness of the cell wall ranged from 414 nm to 498 nm and averages 456 nm. Two new layers or zones (N_1 and N_2) appeared in the cell walls of conidia fixed in glutaraldehyde (Figure 3). Each new layer measured 90-125 nm wide in conidia hydrated 30 minutes. More mitochondria were present in sections of hydrated conidia (Figure 10). Concentric membranous structures observed in immature and mature dormant conidia (Figures 1, 6) were not observed in hydrated conidia. Endoplasmic reticulum has been reported to be sparse or absent in dormant spores (8). It is more prominent in dormant A. nidulans conidia (Figure 1) and increases considerably in late stages of hydration just prior to germination (Figures 10 and 11). During hydration, electron transparent vacuoles (V) bounded by a unit membrane tend to increase in size and number (Figures 4, 10).

GERMINATING CONIDIA

Prior to the extension of the germ tube, the outer layers of the cell wall break down at the point of germ tube emergence (Figures 10 and 11, arrow). The new hyphal wall (NHW) is continuous with the innermost layer which was present in the conidia before germination (Figure 13).

As the germ tube emerges, the apex has a multivesicular appearance (Figure 12). The vesicles may be packets of wall material which migrate to the apical region and either become associated with or pass through the plasmalemma in regions where active extension and growth are occurring (6). This is indicated by the highly crenulate appearance of the edge of the cytoplasm at the apex of the germ tube and the vesicle (Ve) which appears to be in the process of uniting with the plasmalemma (Figure 12).

Thin sections of germinated conidia reveal an increase in the amount of ER and in the number of mitochondria (Figures 4, 8). Electron transparent vacuoles which measured 245 nm $\pm$ 40 nm in dormant conidia increase to 540 nm $\pm$ 100 nm during germination.

DISCUSSION

The cell wall of mature dormant conidia consists of three layers. The outer pigmented layer is composed of a network of rodlets (12). The protoplast of dormant conidia contains a single nucleus, mitochondria, abundant ribosomal material, small vacuoles, endoplasmic reticulum, and concentric membranous structures.

During hydration, two new layers appear in the conidial wall. Layering is probably due to changes within the existing wall brought about by imbibition of water and increased metabolic activity during development and not to de novo synthesis of new wall layers (1). The N_1 layer in the 30-minute-hydrated conidia (Figure 3), and the D_3 layer of the dormant conidia (Figure 1) have the same electron density. This suggests similar staining properties, thus similar chemical properties. The pattern in the 30-minute-hydrated conidia is what one would expect if swelling occurred on both sides of the D_3. Border and Trinci (2) reported that N_1 is about 10-15 nm wide and does not vary in thickness during subsequent development and germination. N_2 measured about 20 nm and increased in thickness during development and germination. Our micrographs show that each new layer measures 90-125 nm wide in conidia hydrated 30 minutes and both layers decrease during subsequent development (Figures 3, 13). The differences may be due to the fact that Border et al. did not show

stages less than two hours after initial hydration and that their material was fixed in Kellenberger's fixative.

Conidia undergoing germination and hyphal tip extension show the same general characteristics reported for other fungi (3). There is a localization of organelles on the side of the conidium from which the germ tube arises (Figure 11). The number of mitochondria and the amount of ER increase during germination (3, 5). Vacuoles increase in size and number in the area of the conidium away from the germ tube (4) (Figure 4). Prior to the extension of the germ tube, the outer layers of the conidial wall, around the area of the germ tube, break down (Figures 10, 11). Several workers report that wall breakdown is by mechanical rupture (7, 9, 10, 11), however, Marchant (13) believes enzymic degradation is involved.

Two origins have been proposed for the new hyphal wall. Tanaka (17), Remsen et al. (15), and Hawker (8) believe that the hyphal wall arises from a layer which was present in the dormant conidium. Border and Trinci (2) believe it arises from a layer that develops during hydration. The new hyphal wall is continuous with the innermost layer of the conidial wall (Figures 9, 13) and that layer was not present in the dormant conidium (Figure 1) but appears only after hydration (Figure 3). The discrepancy may be due to the fact that Tanaka (17) and Remsen et al. (15) either exposed the conidia to, or collected them in water, or water plus a wetting agent, prior to

fixation. Thirty minutes exposure to water causes layering in the conidial wall. However, these data still do not answer the question of whether the N_2 layer is synthesized de novo during hydration or whether it is derived from existing wall material.

As suggested by Border and Trinci, $KMnO_4$ fixation is inadequate for studying wall structure. However, glutaraldehyde fixed conidia had good cell wall detail (compare Figures 5 and 9).

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APPENDICES

Figure 1. Thin section of dormant Aspergillus nidulans conidium, showing ER, nucleus, concentric membranous structure (CMS), vacuoles, vesicles (Ve), mitochondria, ribosomes, three-layered cell wall (D_1, D_2, and D_3), and rodlets (R), glutaraldehyde, 70, 720X.

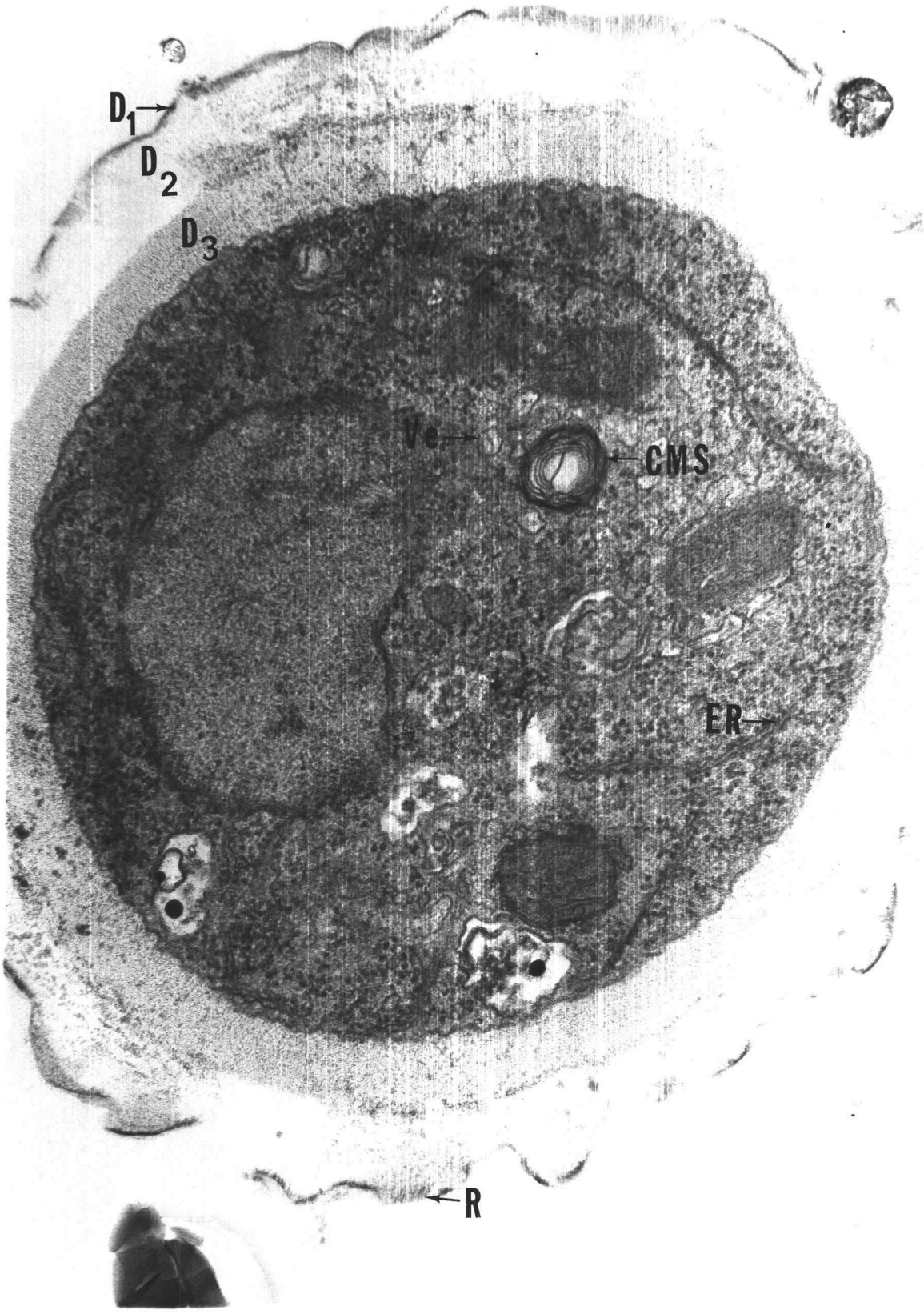

D1
D2
D3
Vc
CMS
ER
R

Figure 2. Portion of wall of conidium hydrated 10 hours. Note rodlets in outer layer, $KMnO_4$, 153, 920X.

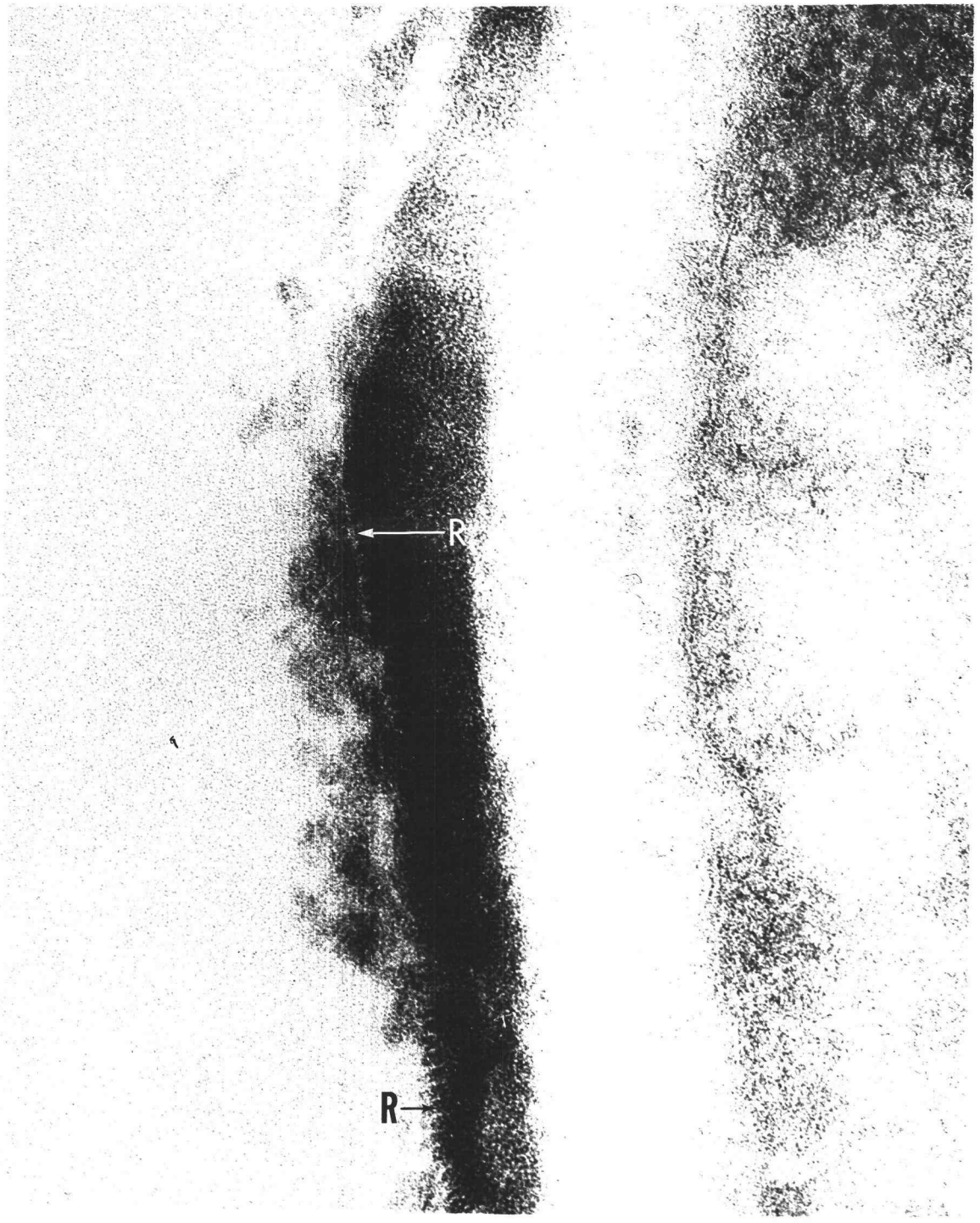

R
R

Figure 3. Portion of wall of conidium hydrated 30 minutes. Note new layers (N_1 and N_2), glutaraldehyde, 133, 200X.

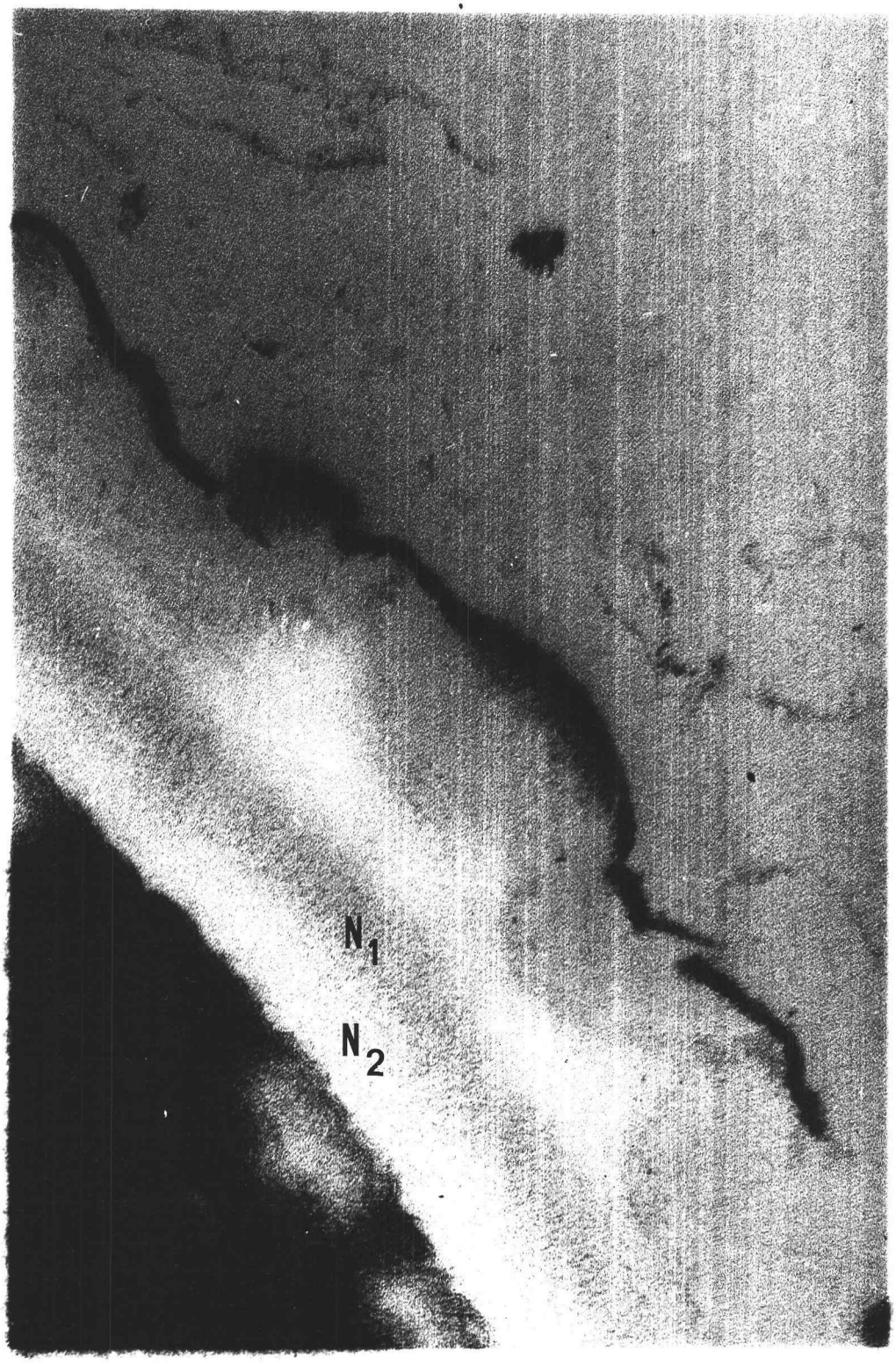

N_1
N_2

Figure 4. Germinating conidium. Compare wall structure detail to Figure 8. Note increased size of vacuoles (V) and localization of organelles, $KMnO_4$, 33,000X.

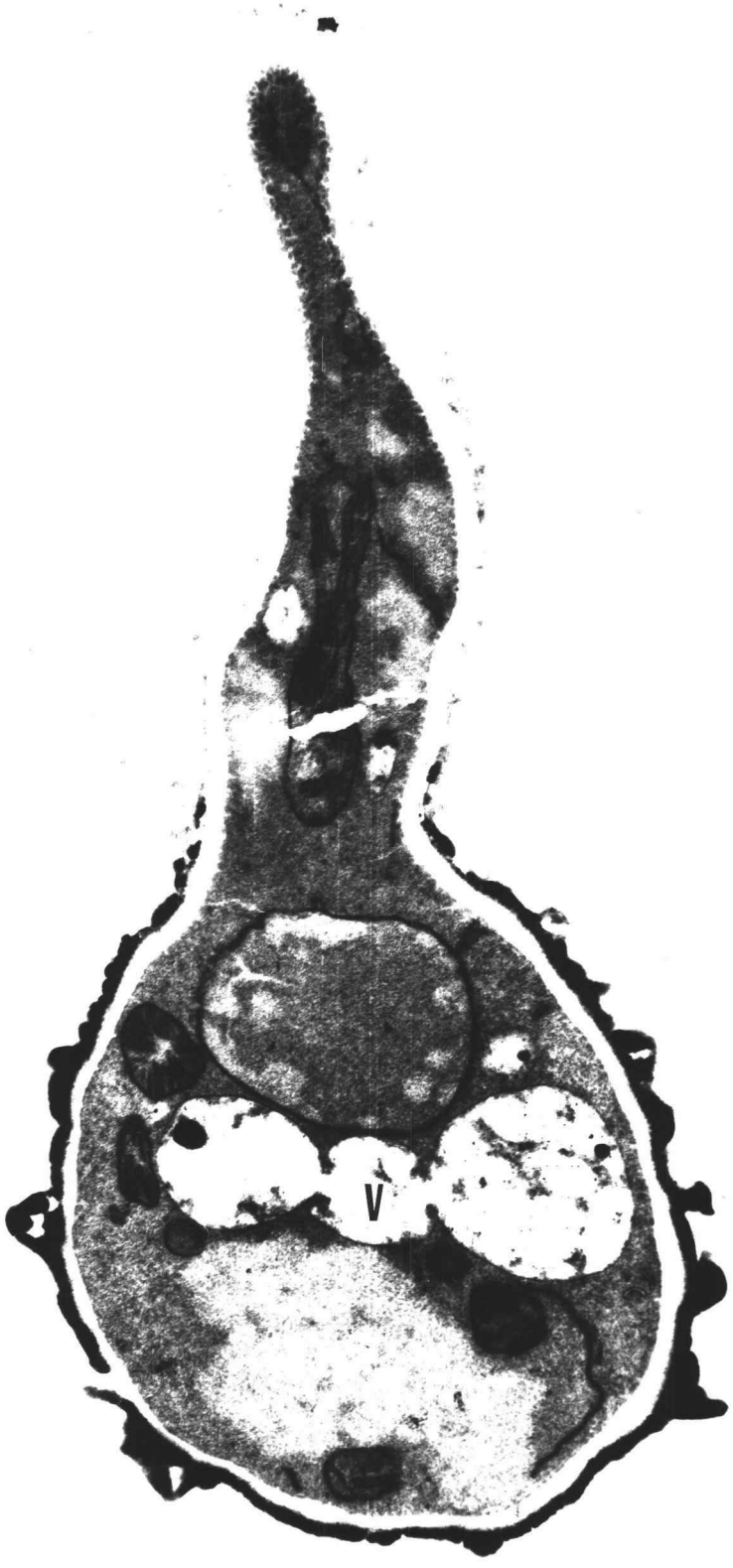

V

Figure 5. Same as Figure 4 except enlarged and printed darker to enhance wall detail, compare to Figure 9, $KMnO_4$, 39,300X.

Figure 6. Immature dormant conidium showing concentric membranous structures (CMS), glutaraldehyde, 108, 160X.

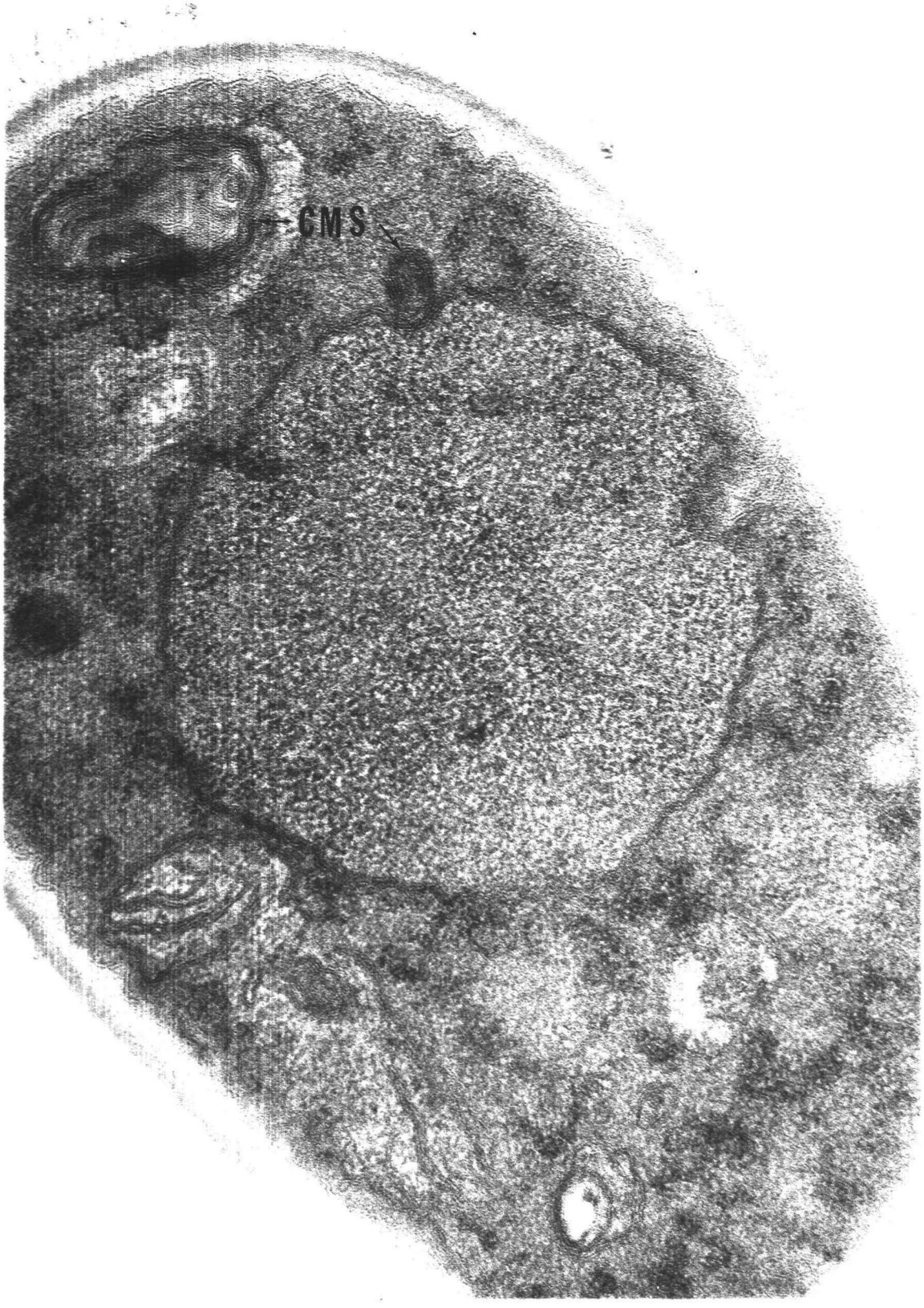

CMS

Figure 7. Dormant conidium showing elongated mitochondria (M), glutaraldehyde, 62, 100X.

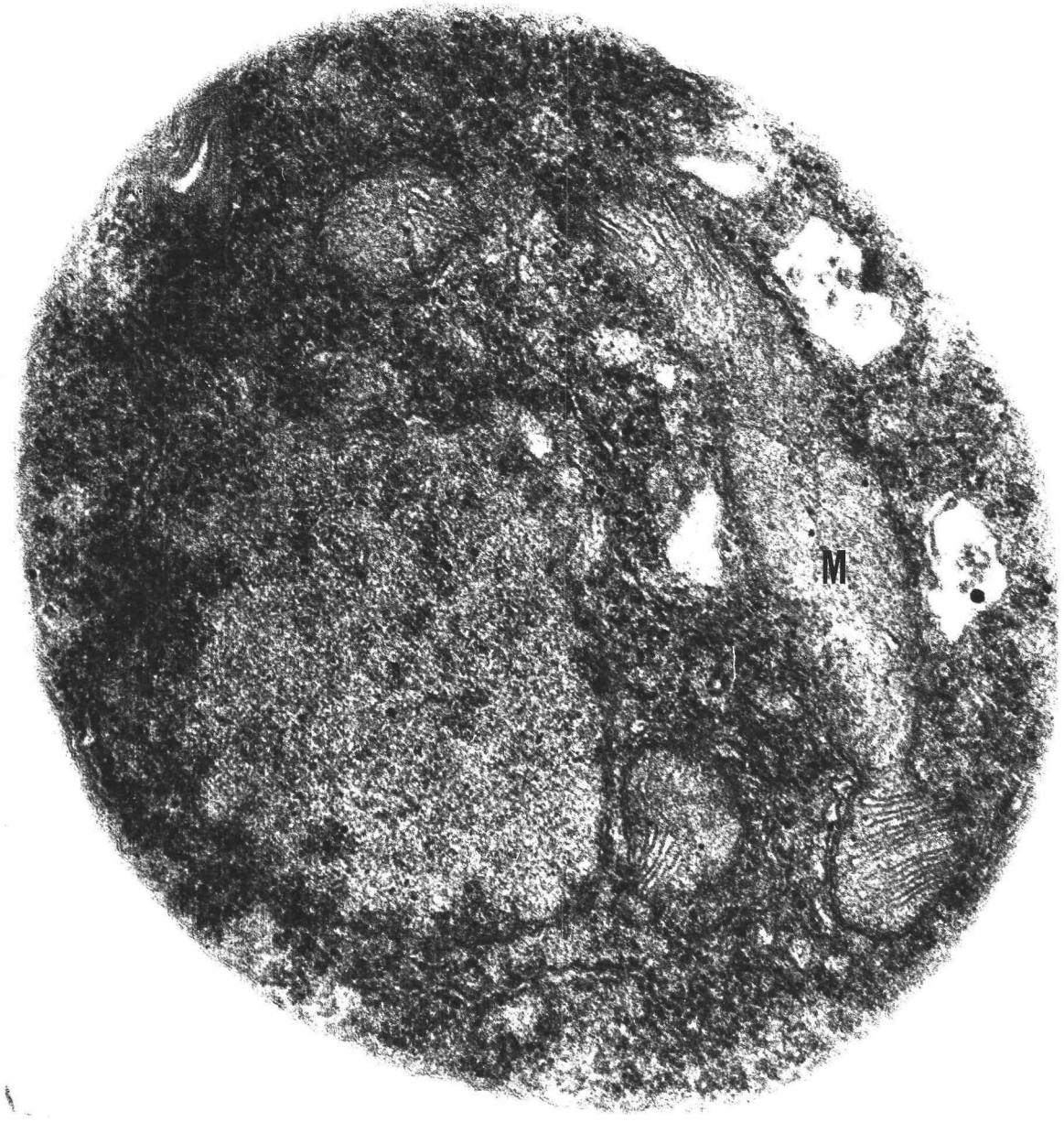

M

Figure 8. Germinating conidium showing localization of organelles toward germ tube tip and wall continuity between inner layer (IL) of conidium wall and germ tube wall, glutaraldehyde, 25,600X.

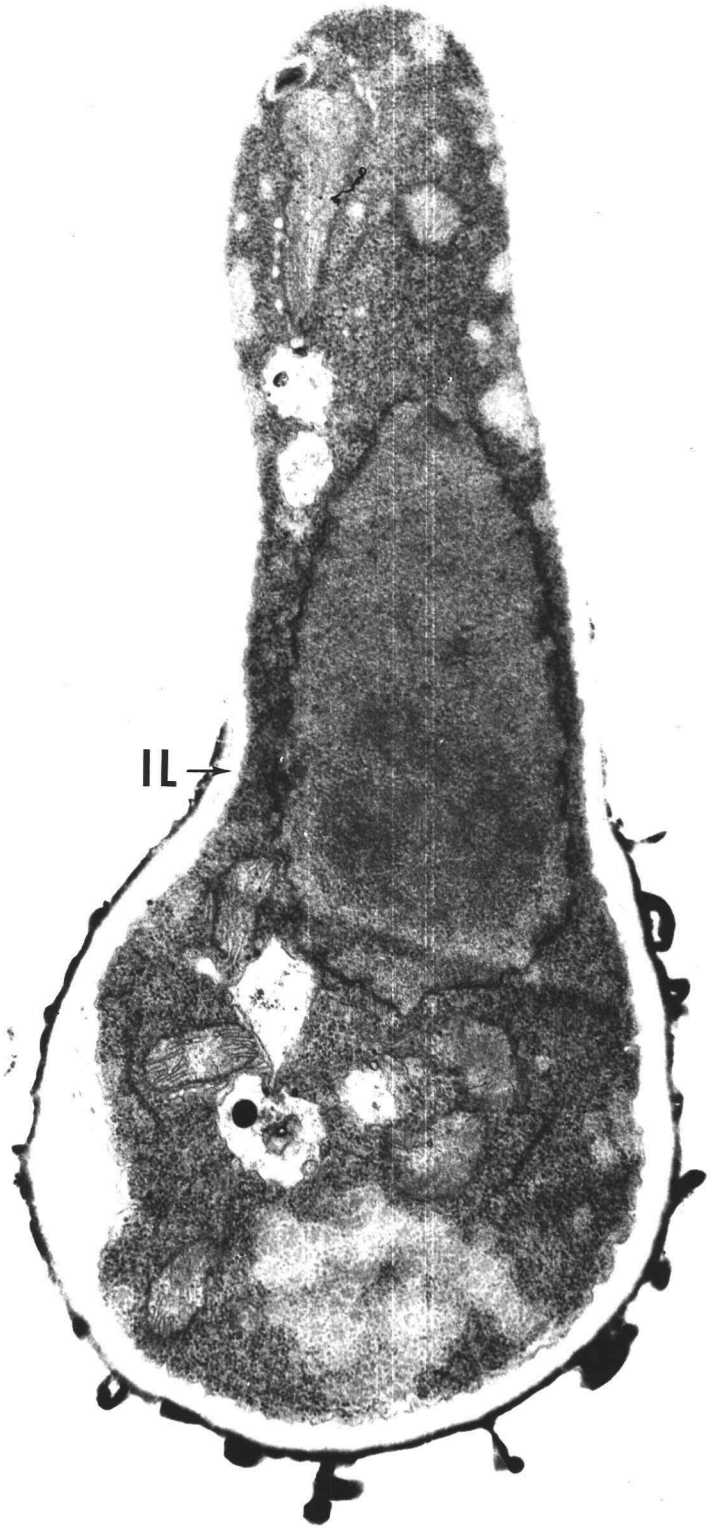

IL

Figure 9. Same as Figure 8 except enlarged and printed darker to enhance wall detail, compare to Figure 5, glutaraldehyde, 44,800X.

Figure 10. Conidium just before germination. Note wall breakdown (arrow), increased amount of ER, and increased size of vacuoles (V), $KMnO_4$, 32,250X.

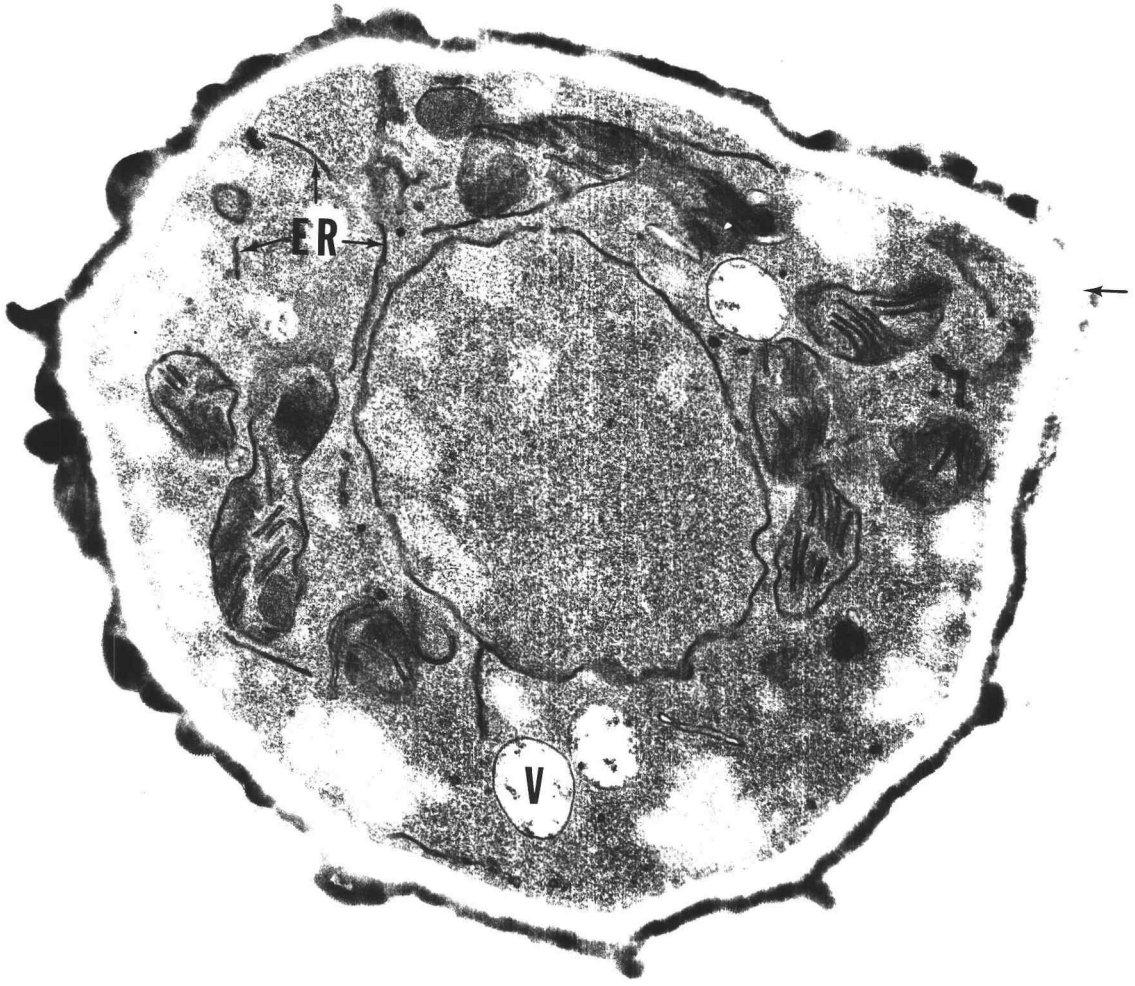

ER
V

Figure 11. Conidium just before germination. Note localization of organelles toward germ tube. $KMnO_4$, 32,250X.

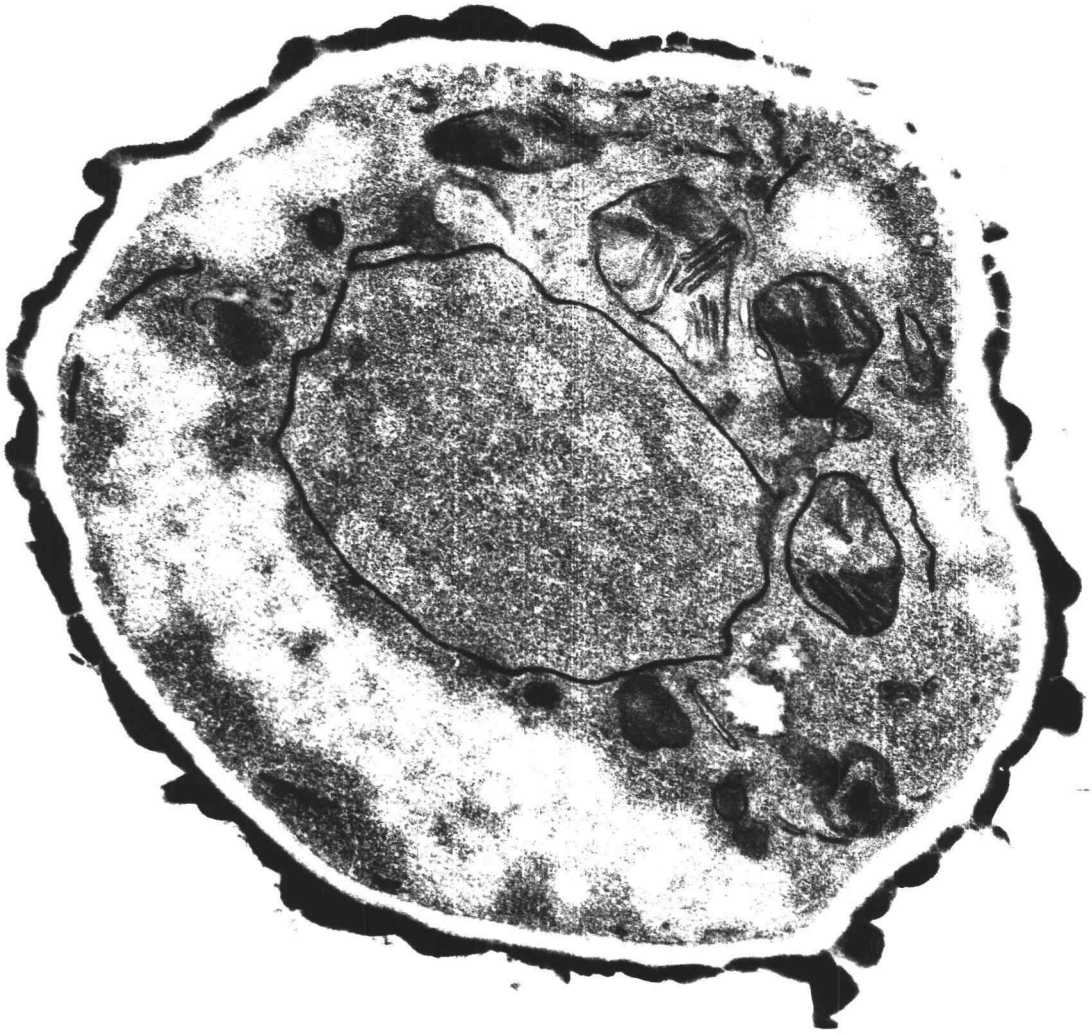
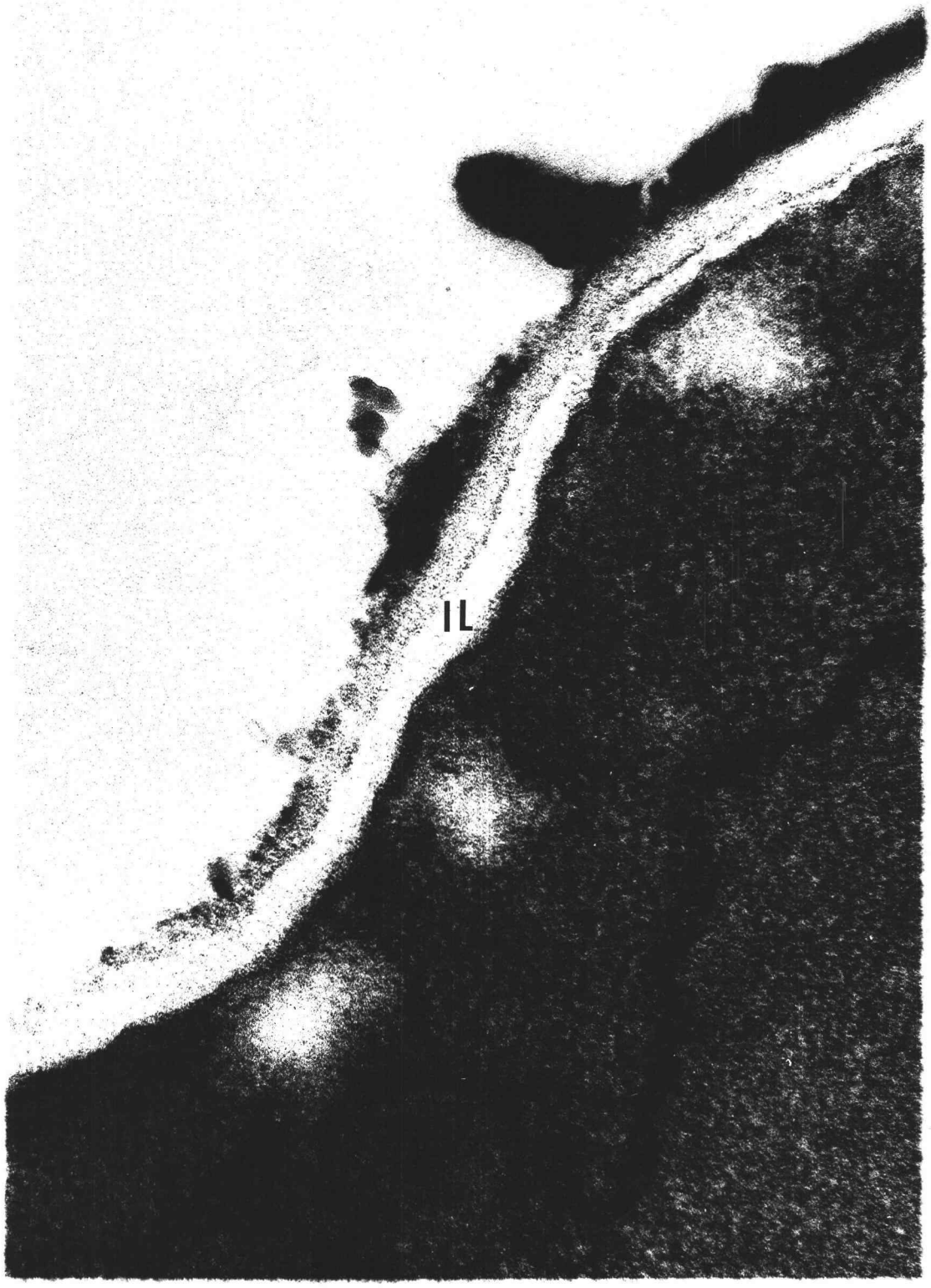


Figure 12. Hyphal tip showing numerous vesicles (Ve), one appearing to unite with the plasmalemma (arrow), $KMnO_4$, 83, 200X.


Ve
Ve

Figure 13. Portion of germinating conidium. Note continuity of germ tube wall and inner wall layer (IL) of conidium, glutaraldehyde, 146, 520X.


IL