JOURNAL OF BACTERIOLOGY, Oct. 1968, p. 1349-1356
Vol. 96, No. 4

Ultrastructural Features of Host-Parasite Relationship in Oral Candidiasis

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Received for publication 15 July 1968

In oral candidiasis, many keratinized epithelial cells and cells of *Candida albicans* are shed. Scales from patients with oral candidiasis were used for electron microscopic study of the epithelial-fungal relationship. Scales, scraped from the tongue and oral mucosa, were fixed for fungi. Electron microscopic observations showed cells of *C. albicans* outside, penetrating, or within the epithelial cells. Extracellular fungi possessed a floccular material adherent to the outer surface of the cell wall. Intracellular fungi lacked the floccular material which appeared to detach as fungi invaded the epithelial cells. Large vacuoles, which sometimes contained myelin figures, occupied the cytoplasm of fungal cells. Epithelial cells frequently contained several fungi. Discontinuous plasma membranes marked sites of fungal entry. Cytoplasmic areas devoid of fungi showed many tonofibrils, but the cytoplasm adjacent to fungi often lacked tonofibrils. Micrographs suggested that fungal cells lysed the tonofibrils. Bacteria were abundant in the scrapings, but always occupied an extracellular position.

Although the fine structure of cultured cells of *Candida albicans* has been studied (13), the ultrastructure of this fungus growing in vivo has not been described. We recently undertook studies to observe, at the electron microscopic level, the host-fungus relationship in human candidiasis. As previously indicated (7), much additional basic knowledge of candidal infections is needed for therapeutic reasons. Oral candidiasis was studied because it is very common and large amounts of material can be easily obtained by innocuous scraping of the lesions. With this infection, large numbers of keratinized epithelial cells are shed along with numerous fungal cells (1, 2).

The present report describes ultrastructural features of the epithelial-fungal association in oral candidiasis. This is believed to be the first electron microscopic description of *C. albicans* in human tissues.

MATERIALS AND METHODS

Oral lesions were utilized from five patients with chronic mucocutaneous candidiasis of several years' duration. The patients were neither diabetic nor had history of antibiotic or corticosteroid therapy. They received no local or systemic anticandidal treatment for at least 6 months prior to this study. The degree of involvement ranged from discrete white patches to thick plaques of chronic hyperplastic candidiasis (1). With a scalpel, scales were scraped from the surface of infected areas on the tongue and buccal mucosa. Some scales were used for mycological cultures and the remainder for light and electron microscopy.

Cultures. Scales from each patient, when added to a test tube containing Mycobiotic (Difco), and Sabouraud's dextrose agar, gave positive cultures for *C. albicans*. Production of chlamydospores in corn meal-agar, germ tubes in human serum, systemic disease in mice, and sugar fermentation reactions confirmed the fungus to be *C. albicans*. *C. albicans* was the only fungus present.

Electron microscopy. Immediately after removal, the scales were fixed at room temperature for 2 hr in a mixture containing 3% glutaraldehyde and 3% acrolein buffered to *p*H 7.4 with 0.2 M sodium cacodylate (6). After initial fixation and washing in several changes of the buffer, scales were postfixed for 2 hr in ice-cold 1% osmium tetroxide, adjusted to *p*H 7.4 with 0.2 M sodium cacodylate buffer. They were then rinsed in the buffer and transferred to a 0.5% aqueous solution of uranyl acetate for 12 hr. Specimens were dehydrated in graded alcohols and embedded in Araldite (11). Thin sections (60 to 90 nm) were cut on a Porter Blum MT-2 ultramicrotome and mounted on naked copper grids for examination with a Philips EM 200 electron microscope operating at 60 kv. Some sections were stained with lead citrate (17) to enhance contrast and facilitate electron microscopic study.

Light microscopy. For light microscopy, 1-μm sections of the plastic-embedded material were cut

with an ultramicrotome and stained with Toluidine Blue or periodic acid-Schiff (15).

RESULTS AND DISCUSSION

Light microscopy. Sections (1 μm) examined with a light microscope showed many yeast cells and pseudohyphae, and to a limited extent their relation to the epithelial cells (Fig. 1). Fungal cells, characterized by periodic acid-Schiff-positive cell walls, were outside, penetrating, or within the epithelial cells. The cytoplasm, particularly that of some pseudohyphae, often stained intensely with Toluidine Blue, a basophilic dye with an affinity for ribonucleoprotein. Such areas of basophilia may contain ribonucleic acid which has been demonstrated by Gresham (5) to be present at the growing tip and constricted areas in pseudohyphae of *C. albicans*.

In addition to fungal and epithelial cells, numerous bacteria occupied the sections. Bacteria appeared to be extracellular, but their precise relation to the epithelial cells was difficult to ascertain by light microscopy. As previously reported for chronic hyperplastic candidiasis of the tongue and oral mucosa (2), leukocytes and parakeratotic cells were also present.

Electron microscopy. With the electron microscope, extracellular fungi were seen to possess a thick floccular material adherent to the external surface of the cell wall (Fig. 2 and 4). Intracellular fungi were devoid of the floccular layer. Those fungi in the act of penetrating epithelial cells contained floccular material only on their extracellular portion (Fig. 6). These findings indicated the material detached during invasion of the epithelial cells. It is not known whether this material is excreted by the organism or the epithelium.

Other than the external floccular coat, extracellular and intracellular fungi appeared fairly similar morphologically. The cell wall (Fig. 2, 6–8) ranged from electron-lucent to granular in appearance and consisted of at least two layers. The outer layer was more electron-dense than the inner layer, a feature more clearly shown in permanganate-fixed cells of *C. albicans* (13). In other yeast cells studied thus far, the outer layer contained mannan, and the inner layer glucan (14). The plasma membrane, situated immediately beneath the cell wall (Fig. 6 and 8), demonstrated the typical structure of a trilaminar unit membrane and sometimes invaginated to form mesosomes.

Large membrane-bound vacuoles in the cytoplasm constituted a conspicuous feature of cells of *C. albicans* (Fig. 2, 3, and 6). Their prominent size may indicate that cells of this fungus attain considerable longevity in chronic candidiasis, since vacuoles are known to increase in size with age (4). Vacuoles sometimes contained myelin figures (Fig. 8) similar to those in vacuoles of *T. utilis* (9) and in lysosomes of mammalian cells (3). Although the nature and significance of the vacuoles remain to be determined, they have been shown, in *S. cerevisiae* (12) and *C. albicans* grown in vitro, to contain hydrolytic enzymes corresponding to those in lysosomes. These enzymes, if present in the vacuoles seen in this study, may play an important role in the invasion of host cells by the fungus.

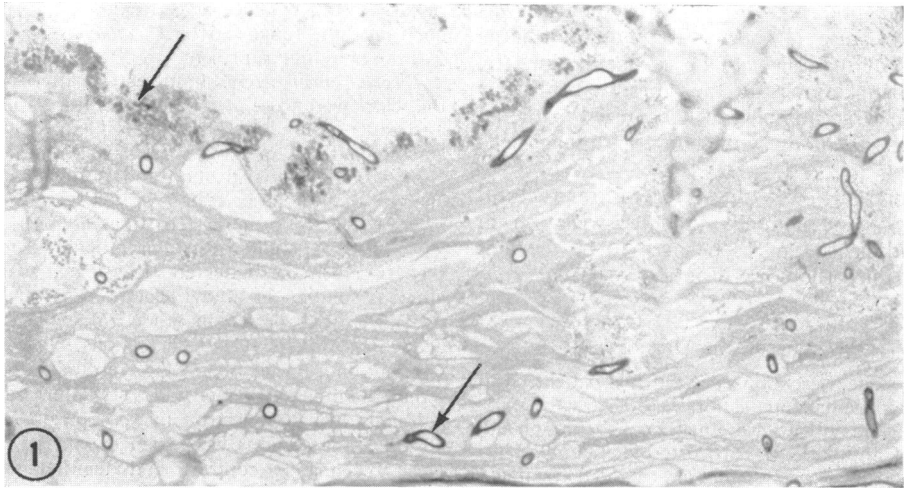

FIG. 1. *A 1-μm section of scraping from tongue of patient with oral candidiasis. Fungal cells have periodic acid-Schiff-positive cell walls (lower arrow). Many bacteria are present (upper arrow). Periodic acid-Schiff stain.* × *560.*

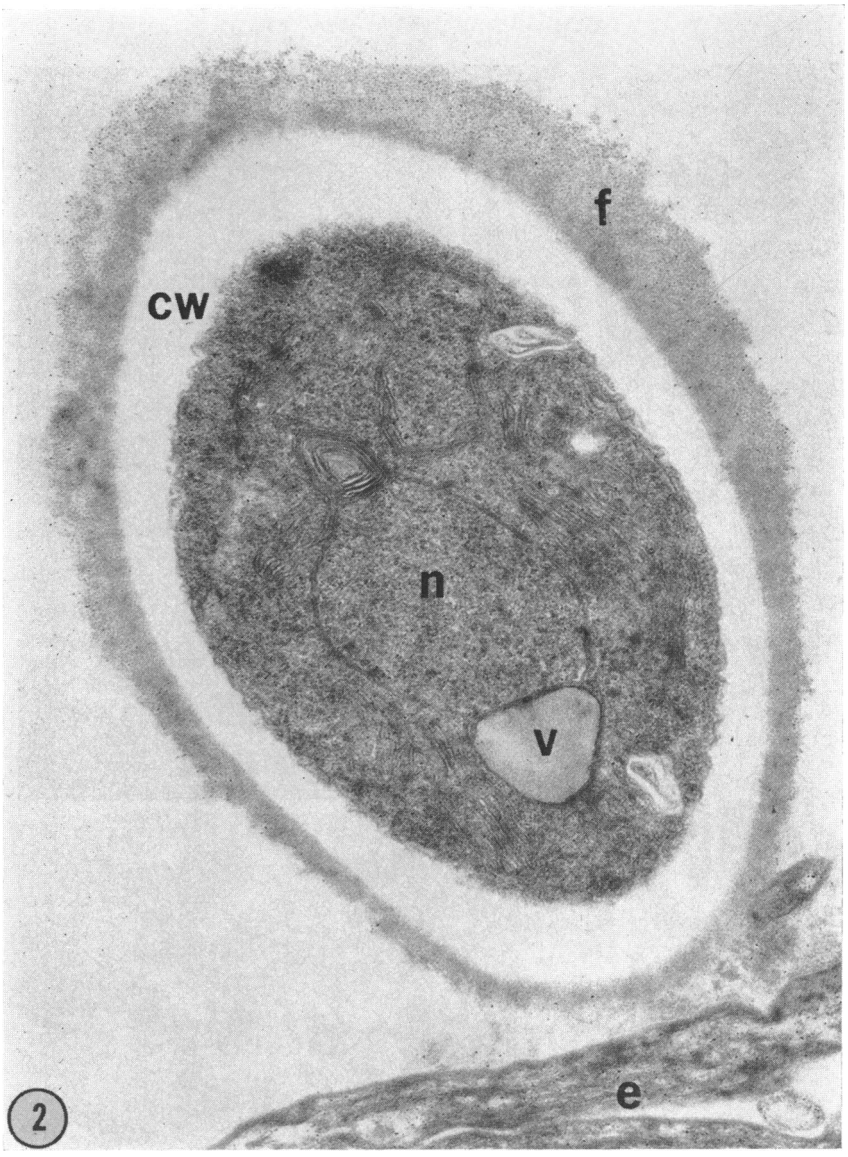

FIG. 2. *Cell of C. albicans on surface of epithelial cell (e). Floccular material (f), cell wall (cw), and a vacuole (v) adjacent to the nucleus (n) are shown.* × *23,000.*

The epithelial-host cells often contained several fungal cells (Fig. 9). Plasma membranes appeared discontinuous at sites of fungal entry (Fig. 6). Cytoplasmic areas devoid of fungi contained many tonofibrils sectioned at various angles and separated from each other by electron-lucent areas. In contrast, the cytoplasm adjacent to intracellular fungi often lacked tonofibrils (Fig. 7). It seems unlikely that absence of tonofibrils in the immediate vicinity of fungi results from techniques used to prepare the cells for electron microscopy, since the more peripherally located tonofibrils appear well preserved (Fig. 7). A possible explanation is that cells of *C. albicans* digest the keratin associated with the tonofibrils, producing homogenous areas at sites of their keratolytic action (Fig. 7). Studies by Kapica and Blank (8) show that *C. albicans* grown in vitro can digest keratin. They used human or animal keratin as the nitrogen source to grow

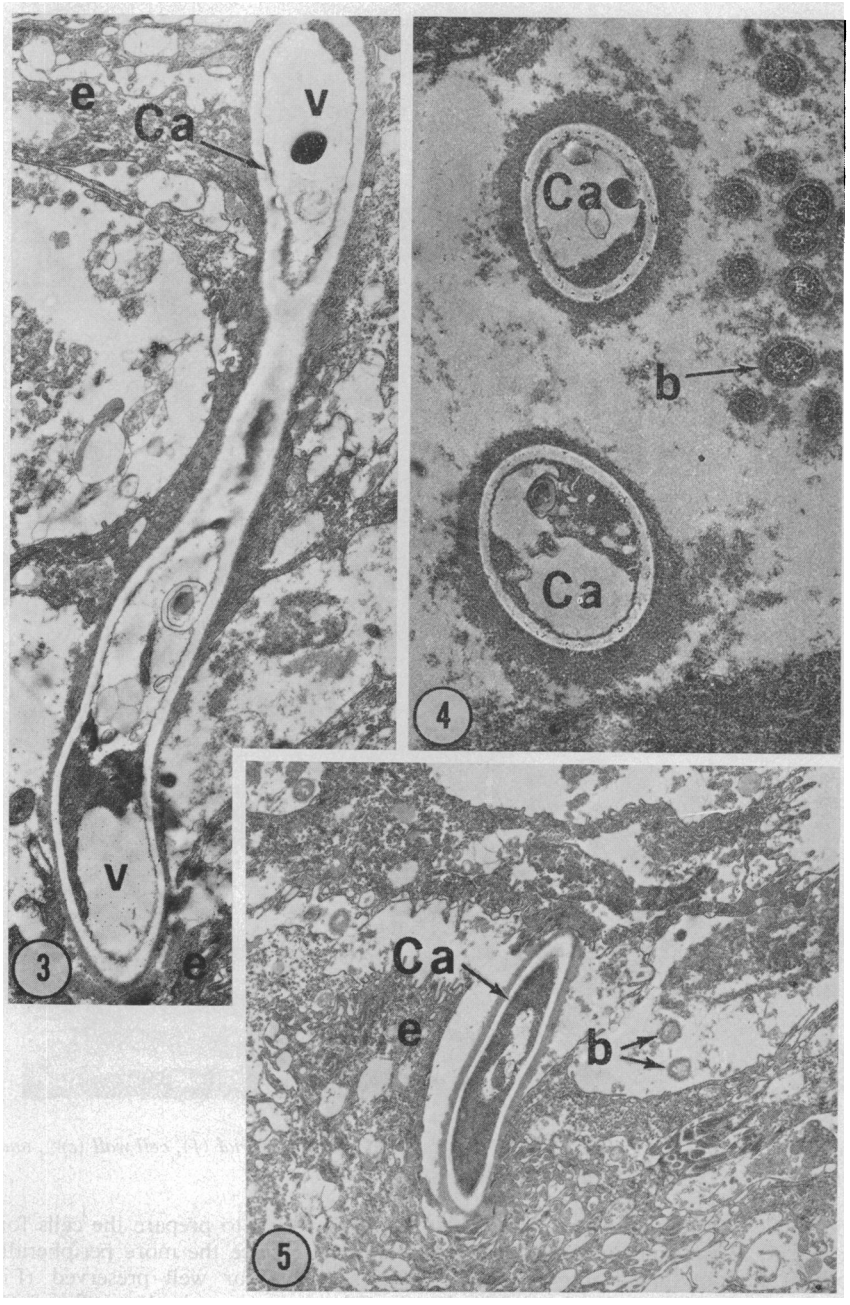

FIG. 3. *Pseudohypha of C. albicans. Note the large vacuoles (v) and portions of epithelial cells (e).* × *8,000.*

FIG. 4. *Two extracellular cells of C. albicans (Ca) also showing large vacuoles and thick floccular material surrounding their cell walls. Bacteria (b) are also present.* × *12,000.*

FIG. 5. *Pseudohypha of C. albicans (Ca); one end seems to be penetrating an epithelial cell (e).* × *6,000.*

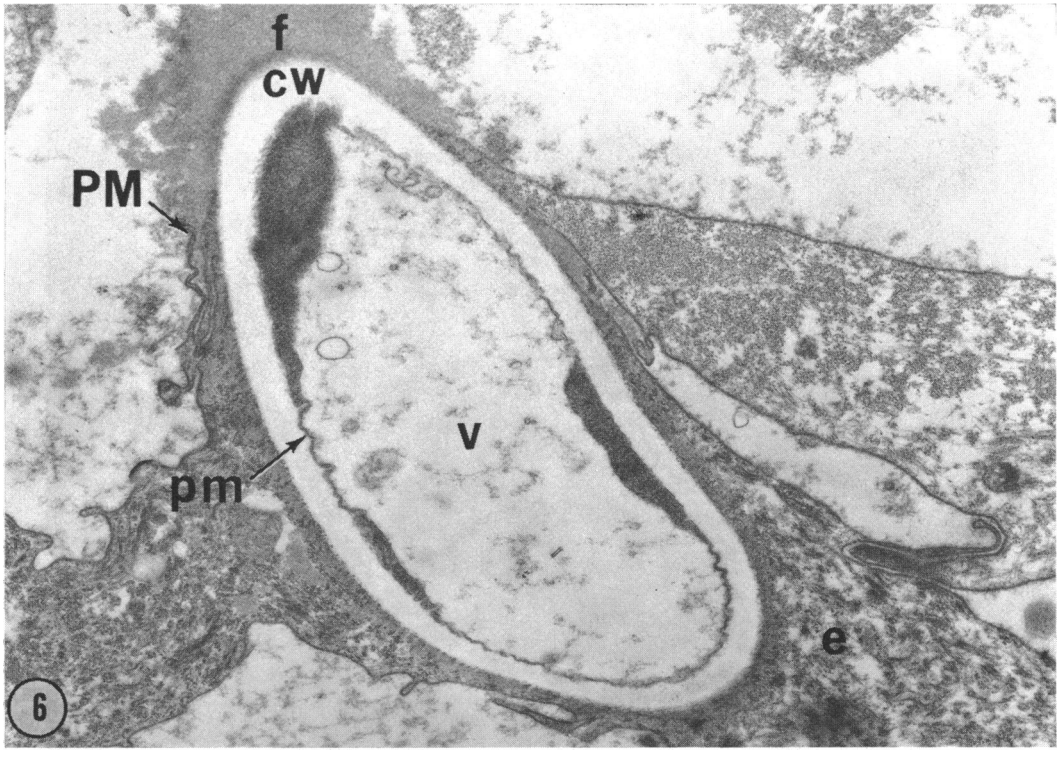

FIG. 6. *Cell of C. albicans invading an epithelial cell (e). A large vacuole (v) occupies most of the cytoplasm. Floccular material (f) is seen only on the extracellular portion of the fungus.* × *20,000.*

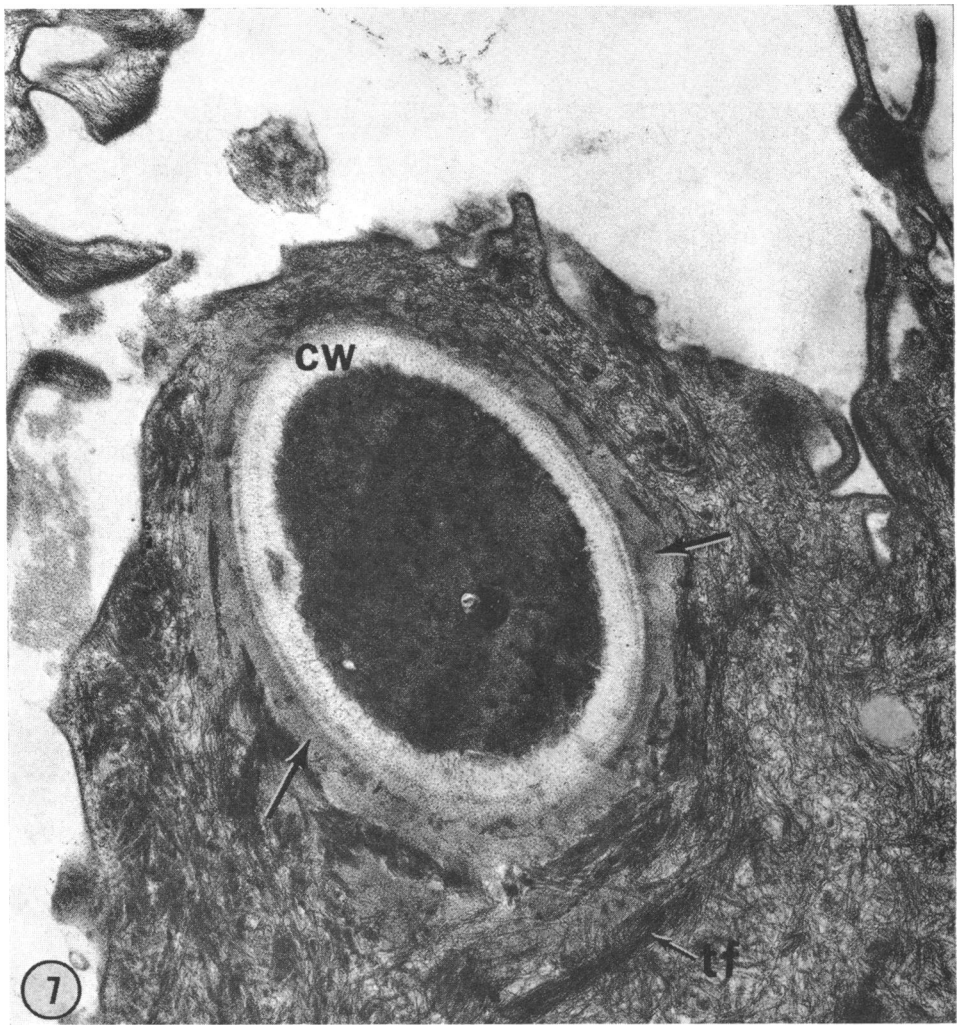

FIG. 7. *Cell of C. albicans within an epithelial cell. Outer part of the cell wall (cw) is more electron-dense than the inner part. Tonofibrils (tf) are absent in the immediate vicinity of the fungus (arrows).* × *23,000.*

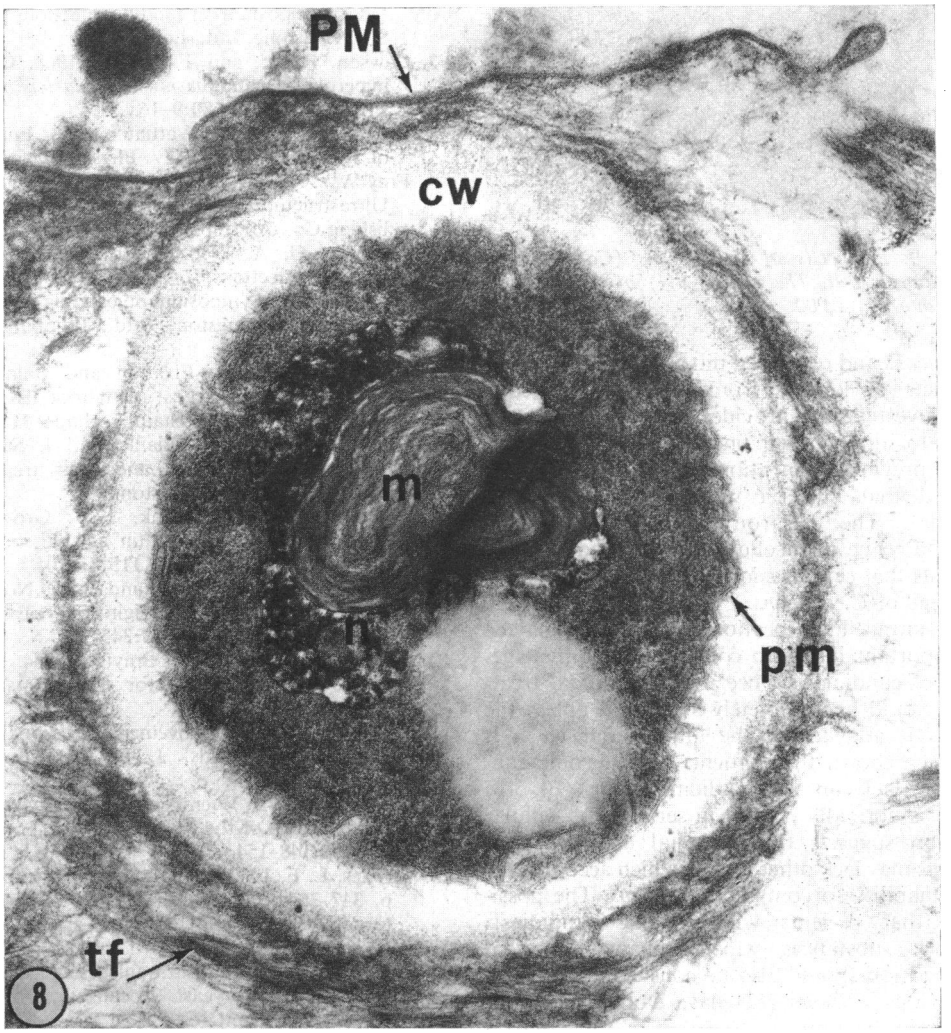

FIG. 8. *Cell of C. albicans located within an epithelial cell. Note large myelin figure (m) lying next to the nucleus (n) and filling most of a vacuole. Tonofibrils (tf) adhere to the external surface of the cell wall (cw).* × *44,000.*

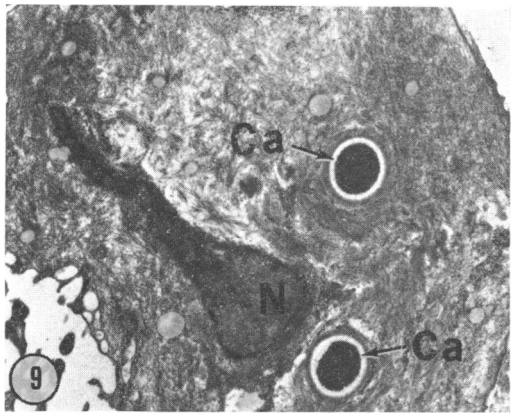

FIG. 9. *Two cells of C. albicans (Ca) within a parakeratotic cell. The nucleus (N) of the epithelial cell is shown.* ×*7,000.*

C. albicans and recovered nitrogenous breakdown products of keratin from the culture media. Our investigation provides ultrastructural evidence to support their findings.

As previously mentioned, light microscopy showed that bacteria were abundant in this material. The electron microscope revealed bacteria to be extracellular (Fig. 4 and 5). This suggests that cell invasion in oral candidiasis is a privilege of *C. albicans*.

The intracellular location of *C. albicans* may be an important factor to consider in the management of candidiasis since no anticandidal treatment would be completely successful unless the fungus is attacked at the intracellular level. It has been shown that patients with chronic candidiasis lack an anticandidal factor (10, 16) which is normally present in serum. The cellular invasion suggests the epithelial cells of these patients may lack other factors which accounts for their inability to combat *C. albicans*. The possibility that patients with chronic candidiasis produce substances which enhance candidal proliferation should also be considered.

Previous workers (13) have emphasized difficulties in fixation of *C. albicans* for electron microscopy. The results shown here suggest that the fixation procedure introduced by Hess (6) for ultrastructural study of host and pathogen in fungal infections of plants can be applied satisfactorily to the study of candidal infections.

ACKNOWLEDGMENT

This study was supported by Public Health Service Research Career Development Award 5-K3-AI-31, 210-03 (L.F.M.) and research grants AI 07635-02, DE 02110, and 2M01 FR-32-08.

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