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Scanning Electron Microscopy of Colonies of Six Species of *Candida*

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Thirty strains of six species of *Candida* isolated from patients were cultured for 60 h on Sabouraud agar, freeze-dried, and examined with a scanning electron microscope. The colonies were circular (*Candida albicans*, *C. guilliermondii*) or oval (*C. tropicalis*, *C. pseudotropicalis*, *C. krusei*, *C. parakrusei*) in outline, and those of *C. pseudotropicalis* and *C. krusei* had an irregular outline due to a peripheral pseudomycelium. The morphology of individual microorganisms was examined at the margins and apex of those species which lacked a surface coat (*C. pseudotropicalis*, *C. krusei*, *C. parakrusei*, *C. guilliermondii*), and through cracks in the surface coating of those which showed a surface coat (*C. albicans*, *C. tropicalis*). All species showed buds, bud scars, and interconnecting intercellular processes, but were generally spherical (*C. albicans*, *C. tropicalis*) or ovoid (*C. pseudotropicalis*, *C. krusei*, *C. parakrusei*, *C. guilliermondii*) in fixed preparations. In unfixed material, individual organisms were almost invariably indented. Fixation with 3% glutaraldehyde and washing before freeze-drying caused partial removal of the surface coating of colonies of *C. albicans* and *C. tropicalis*, which persisted only as irregular sheets or as a filamentous meshwork. This filamentous meshwork was also present among the organisms of colonies of *C. albicans*, *C. tropicalis*, and *C. pseudotropicalis*. It is concluded that these filaments represent the precipitation or unmasking of some component of the intercellular matrix of these organisms.

Scanning electron microscopy has been used to study the morphology of a variety of individual microorganisms, including *Actinomycetes* (9), various bacteria (2, 5, 7), dermatophytes (4), and *Candida albicans* (1). However, Whitaker and Drucker (8), who studied several species of *Streptococcus*, *Staphylococcus*, and *C. albicans*, appear to have been the only investigators to use this technique to examine the morphology of intact colonies of microorganisms.

In this paper, we describe and discuss the scanning electron microscopic appearances of both the colonies and the organisms within them of six species of *Candida* which may be pathogenic to man.

MATERIALS AND METHODS

Thirty strains of *Candida*, consisting of *C. albicans* (10), *C. parakrusei* (8), *C. krusei* (4), *C. tropicalis* (5), *C. pseudotropicalis* (2), and *C. guilliermondii* (1), were isolated from various clinical samples at the mycology laboratory of Auckland Hospital. These were cultured on Sabouraud agar at 37 C for 60 h. Occasional specimens were examined after 3 to 7 days of incubation. Two blocks of agar each containing four to ten discrete colonies of each strain were then cut from this agar and processed as follows. One block of each pair was quenched in liquid Freon cooled in liquid nitrogen to approximately −155 C and freeze-dried for 24 h at −60 C and at a reduced pressure of 10^{-2} Torr in an Edwards-Pearse freeze-drying apparatus (Edwards High Vacuum Ltd.). The other block was immersed in 3% phosphate-buffered glutaraldehyde (pH 7.0) for 24 h at 20 C. After fixation, they were washed quickly in four changes of deionized water, and then quenched and freeze-dried as described above.

All blocks were cemented onto aluminum stubs with a metal-containing glue (Solderene, Lambart and Smyth Ltd., London, England) and then coated first with carbon and then with gold to a thickness of approximately 30 nm while rotating in a vacuum of 10^{-5} Torr. The specimens were examined in a Cambridge 2A Stereoscan instrument (Cambridge Instrument Co.) with accelerating voltages of 5 to 20 kV.

RESULTS

When samples of *C. albicans* were immersed in glutaraldehyde, a milky material could be seen streaming from the colonies into the solu-

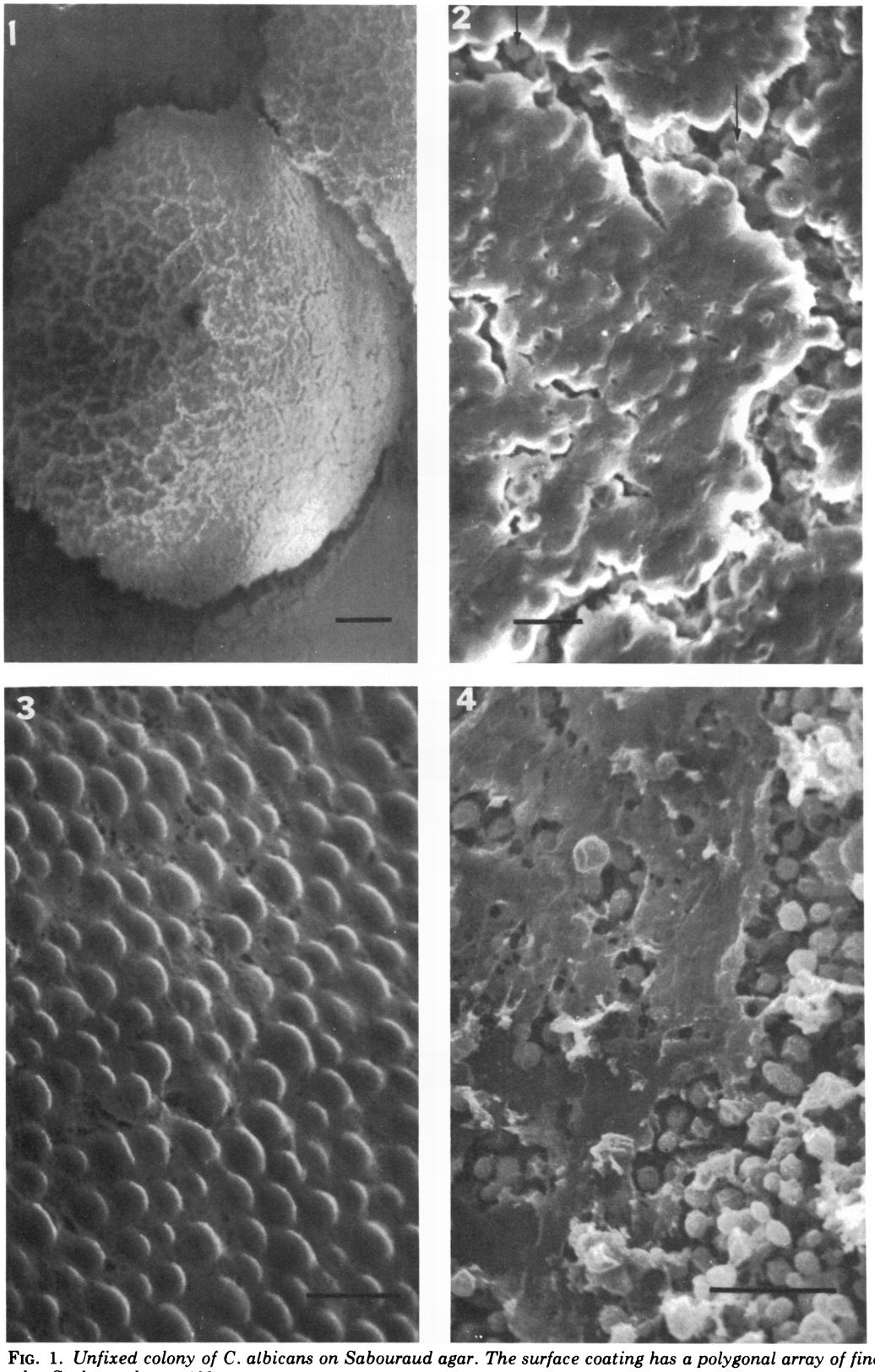


FIG. 1. *Unfixed colony of C. albicans on Sabouraud agar. The surface coating has a polygonal array of fine cracks. Scale marker = 100 μm.*

FIG. 2. *Unfixed colony of C. albicans. The smooth surface coating has cracked in several places to show individual microorganisms (arrows) beneath it. Scale marker = 10 μm.*

tion. This was not seen with other species. Colonies of *C. tropicalis* invariably fell off the underlying agar during the freeze-drying process, but they were carefully retrieved and mounted directly onto stubs for examination.

Unfixed colonies of *C. albicans* were rounded, dome-shaped, and covered with a smooth layer of amorphous material of varying thickness which showed a polygonal pattern of fine cracks (Fig. 1, 2). In some cases this coating almost obscured the form of the organisms within the colonies (Fig. 2), but in other areas the rounded contours of underlying organisms could be clearly seen (Fig. 3). In fixed and washed colonies, this surface coat was much less uniform and appeared to have been removed in many places (Fig. 4). Where present, it consisted of irregular sheets (Fig. 4) or fine fibrillar networks (Fig. 5) superficial to the underlying organisms. A similar but less dense filamentous meshwork was also present within the colonies.

The individual organisms of *C. albicans*, which were observed through the cracks or deficiencies in the surface coating of colonies, were of various sizes (Fig. 6). Some organisms in each colony showed short interconnecting processes of varying thickness (Fig. 6), and organisms with buds or bud scars were relatively common (Fig. 6). Generally, the organisms were spherical or ovoid in form in fixed preparations (Fig. 6), but in unfixed material virtually all organisms had one or several concave depressions in their surface. Occasional organisms with an extensively convoluted surface were observed in fixed colonies (Fig. 7).

Colonies of *C. tropicalis* were oval in outline and larger (Fig. 8) than those of *C. albicans*, but like them they had a surface coat which was cracked in unfixed preparations (Fig. 9) but partly removed by glutaraldehyde fixation and washing. In *C. tropicalis* this coating was generally thinner than that observed with *C. albicans*, and it was thinnest near the apex of the colonies where the form of the underlying organisms was evident (Fig. 9). The organisms within the colonies were smooth and rounded, and many were interconnected by processes of different thickness. In fixed preparations these organisms lay in a fine filamentous meshwork (Fig. 10). In unfixed preparations many organisms showed one or more concave depressions.

The colonies of *C. pseudotropicalis* were also oval but had an irregularly fringed outline due to short filaments which emerged from their periphery (Fig. 11). Examination at higher magnification and after 3 to 7 days of incubation showed these structures to be composed of greatly elongated cylindrical organisms forming a pseudomycelium (Fig. 12). The colonies of *C. pseudotropicalis* appeared to lack any surface coat. They always appeared smooth-surfaced and undisrupted when examined at low magnification (Fig. 11), and at higher magnification (Fig. 13) the individual organisms at the surface of the colony were evident. These organisms were rounded or slightly elongated in form (Fig. 13). As with all species examined, interconnecting processes, buds, and bud scars were seen, but the filamentous meshwork seen in fixed colonies was not as dense as in *C. albicans* and *C. tropicalis* (Fig. 13). All unfixed organisms and occasional ones in fixed material showed one or more indentations.

The colonies of *C. krusei* were similarly oval, smooth-surfaced (Fig. 14), and lacking a surface coating (Fig. 15). However, after 60 h of incubation, the pseudomycelial filaments which emerged from the periphery of the colonies were generally shorter than those of *C. pseudotropicalis* and were composed of fusiform organisms of various diameters. Individual organisms elsewhere in the colonies were rounded or slightly elongated and had a smooth surface. Although buds, bud scars, and interconnecting processes were common, there was no filamentous meshwork within these colonies. The organisms in unfixed preparations showed one or more indentations (Fig. 15).

Colonies of *C. parakrusei* were also smooth-surfaced, ovoid in outline, and lacking a surface coat. However, even after 3 to 7 days of incubation, they did not form a typical pseudomycelium at their periphery. Whereas the majority of individual organisms were ovoid, many of those at the periphery of the colonies were greatly elongated (Fig. 17). Although interconnecting processes were present, a filamentous meshwork was not.

The colonies of *C. guilliermondii* were round and had a smooth, uncoated surface (Fig. 18). The organisms were round, oval, or elongated with interconnecting processes (Fig. 19). A filamentous meshwork was not present.

DISCUSSION

This paper describes the topography of colonies and individual organisms of six species of *Candida*, and it provides information about the

FIG. 3. *Unfixed colony of C. albicans. The surface coat is much thinner than that shown in Fig. 2, and the spherical form of individual microorganisms can be seen through it. Scale marker = 10 μm.*

FIG. 4. *Fixed and washed colony of C. albicans. The surface coat is irregular and discontinuous, exposing many of the underlying organisms. Scale marker = 10 μm.*

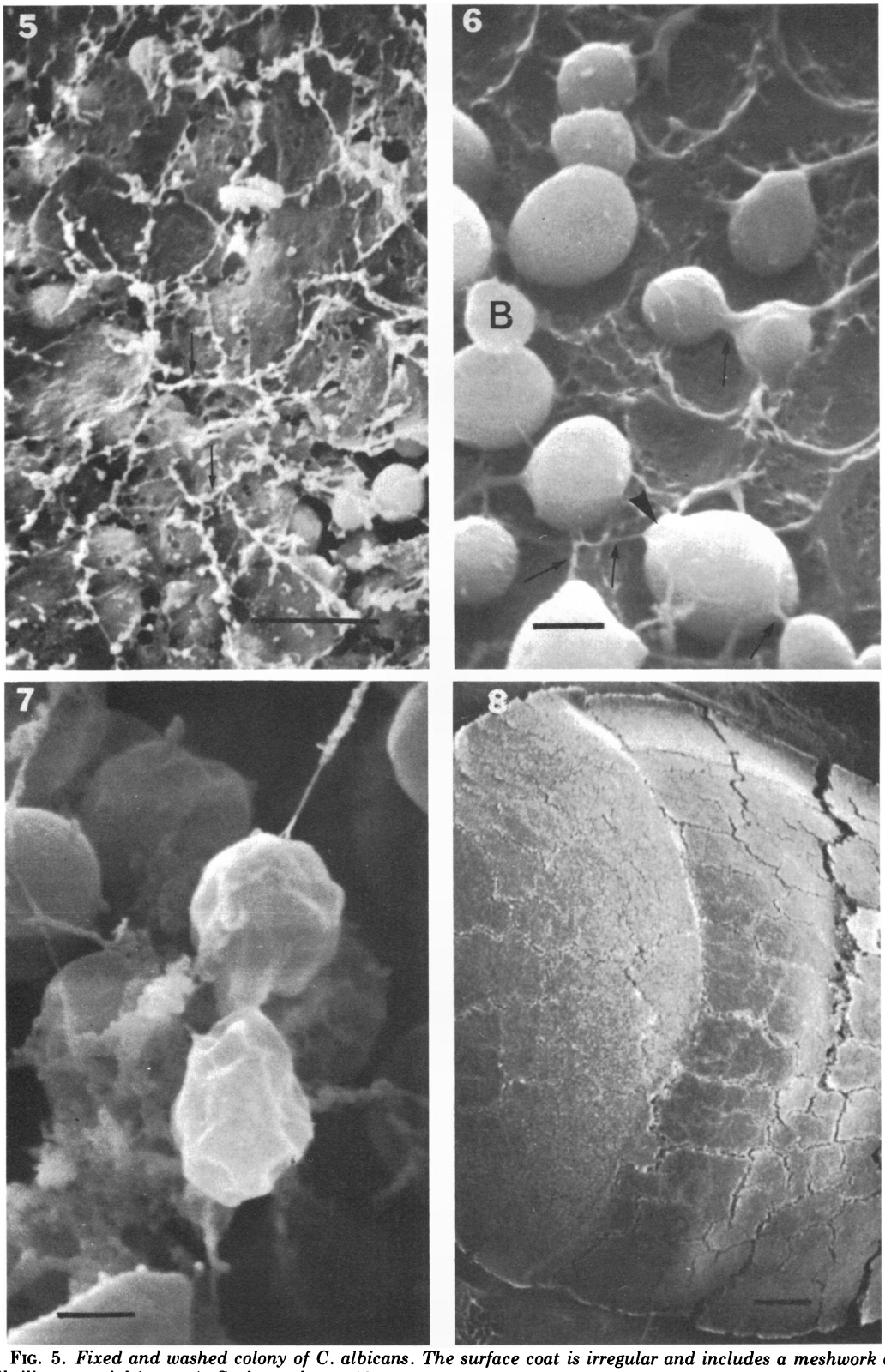
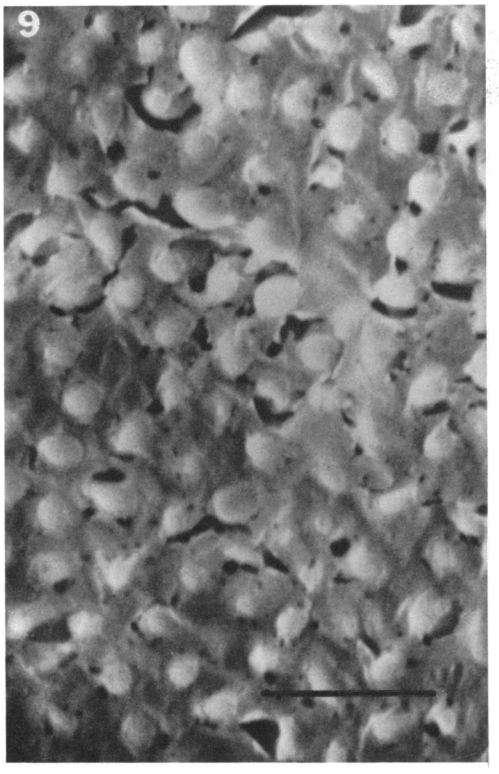


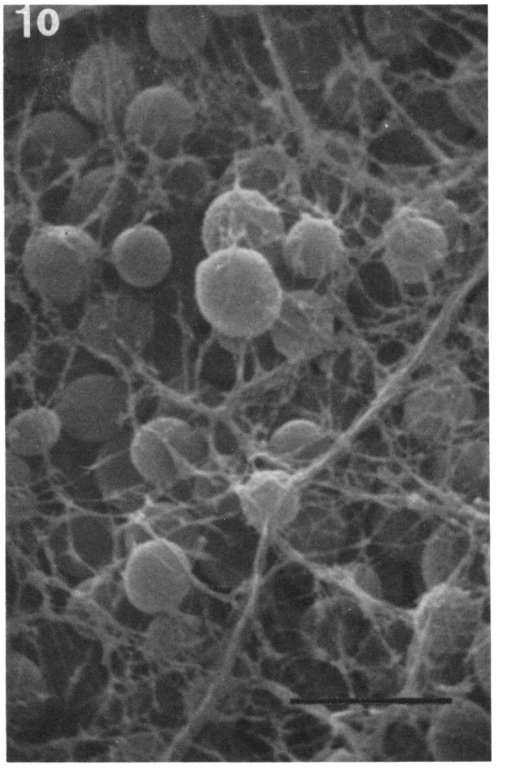
FIG. 5. *Fixed and washed colony of C. albicans. The surface coat is irregular and includes a meshwork of fibrillar material (arrows). Scale marker = 10 μm.*

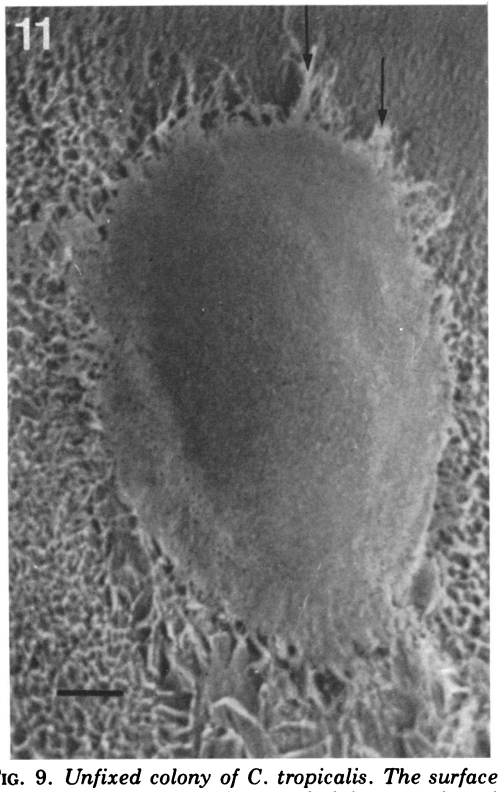
FIG. 6. *Variously sized spherical organisms within a fixed colony of C. albicans showing buds (B), bud scars (large arrow), and interconnecting processes of various sizes (small arrows). Scale marker = 2 μm.*

FIG. 7. *Organisms with an extensively convoluted surface within a fixed colony of C. albicans. Scale marker = 2 μm.*

FIG. 8. *Unfixed colony of C. tropicalis showing the oval colony form and extensive cracking of its surface coat. Scale marker = 100 μm.*







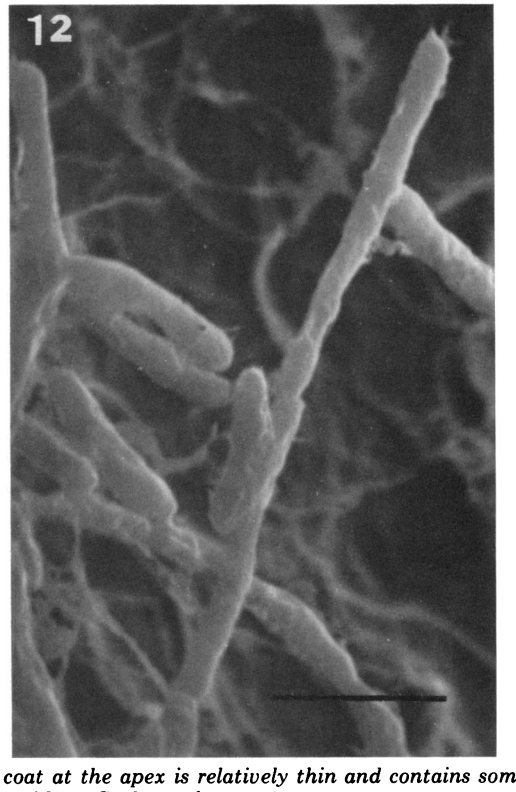


FIG. 9. *Unfixed colony of C. tropicalis. The surface coat at the apex is relatively thin and contains some discontinuities; the form of the underlying organisms is evident. Scale marker = 10 μm.*

FIG. 10. *Spherical organisms within a fixed colony of C. tropicalis lying within a filamentous meshwork. Scale marker = 10 μm.*

FIG. 11. *Unfixed colony of C. pseudotropicalis. They are smooth-surfaced and ovoid, with an irregular periphery due to a fringe (arrows) of pseudomycelium (Fig. 12). Scale marker = 100 μm.*

FIG. 12. *Pseudomycelium at the periphery of a fixed colony of C. pseudotropicalis. Scale marker = 10 μm.*

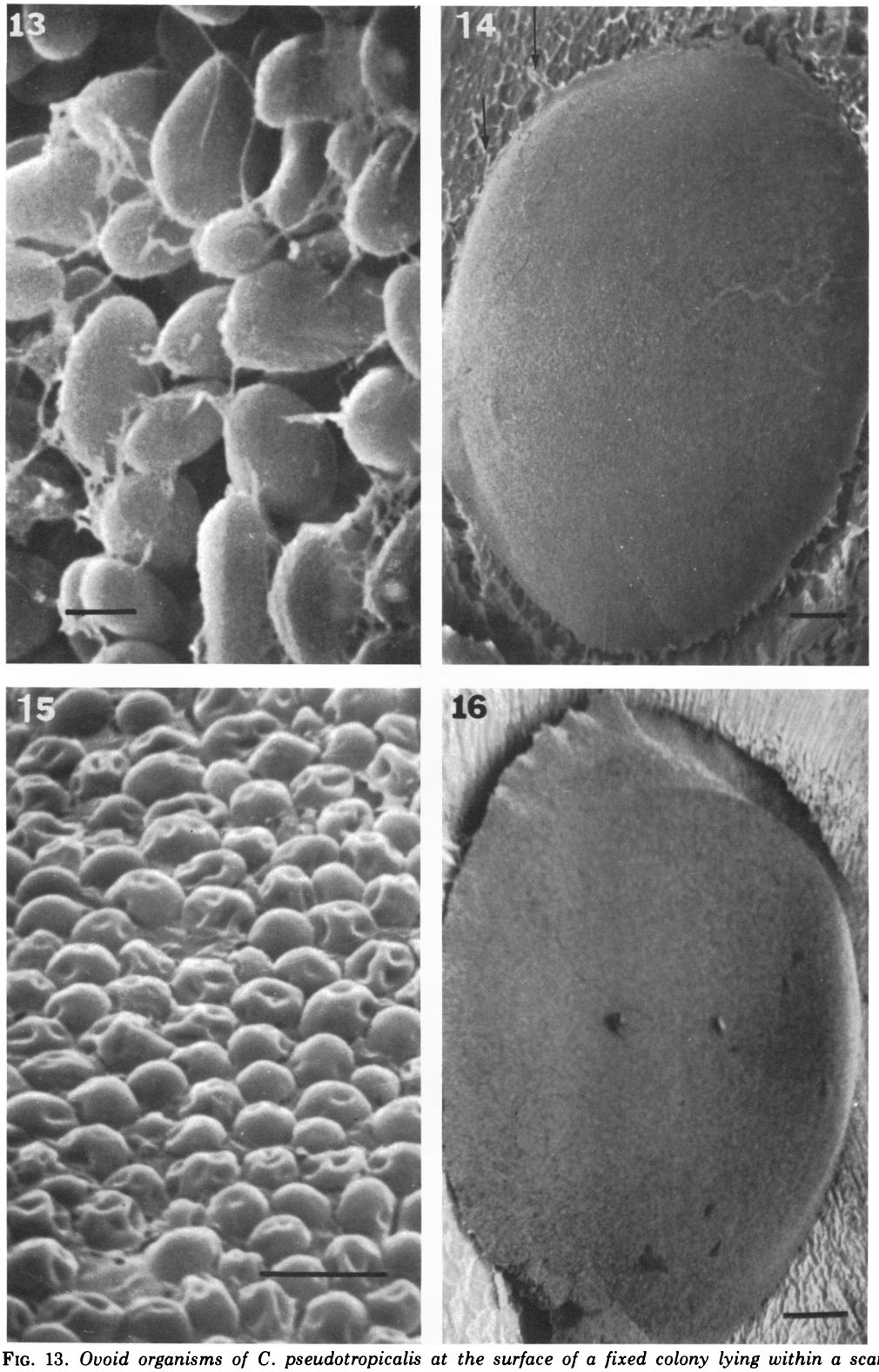
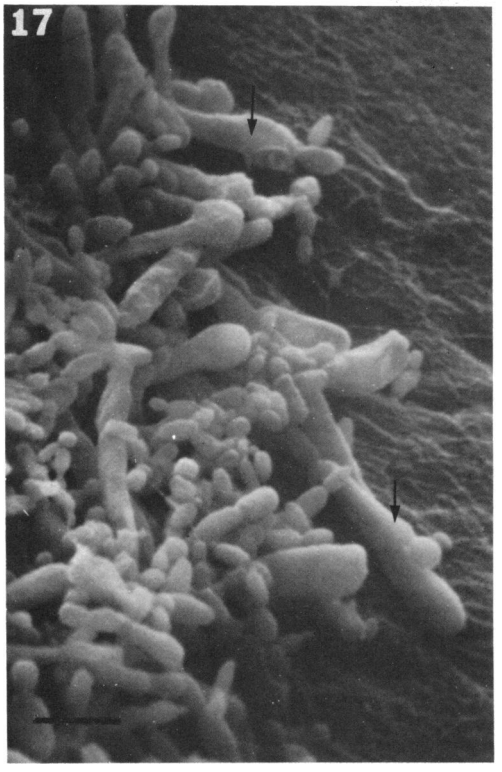


FIG. 13. *Ovoid organisms of C. pseudotropicalis at the surface of a fixed colony lying within a scanty filamentous meshwork. Scale marker = 2 μm.*

FIG. 14. *Oval unfixed colony of C. krusei appearing smooth surfaced with delicate pseudomycelium (arrows) extending from its periphery. Scale marker = 100 μm.*

FIG. 15. *Organisms at the surface of an unfixed colony of C. krusei showing a spherical indented form. Scale marker = 100 μm.*

FIG. 16. *Unfixed oval colony of C. parakrusei showing a smooth surface. Scale marker = 100 μm.*



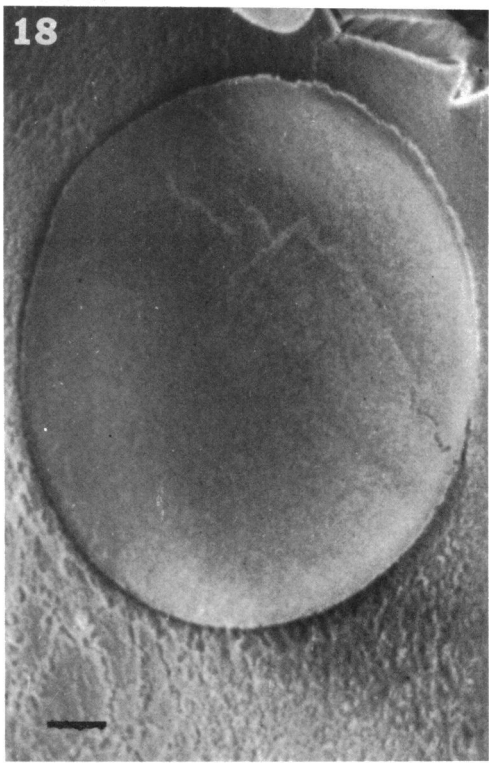


FIG. 17. *Periphery of a fixed colony of C. parakrusei showing elongated organisms (arrows). Scale marker = 10 μm.*

FIG. 18. *Unfixed round, smooth-surfaced colony of C. guilliermondii. Scale marker = 100 μm.*

intercellular matrix of these organisms when growing in colonies. In the only comparable study, Whitaker and Drucker (8) described the colony form of *C. albicans*. These investigators reported difficulties in maintaining the integrity of colonies of *C. albicans* during glutaraldehyde fixation, and they concluded that quenching and freeze-drying unfixed material was a satisfactory method of preparation of colonies for examination by scanning electron microscopy. Our observations indicate that careful chemical fixation should also be included in investigations of this type. Although this may cause some overall reduction in the size of individual organisms, it greatly reduces the indentation and distortion seen in the unfixed organisms and, by the partial removal of extracellular material, it also demonstrates more clearly the morphology of the organisms within the colonies. Although the shrinkage and distortion of organisms were clearly related to preparative procedures, no differences were recognized which could be attributed to strain differences within any of the species examined.

Colonies of *C. albicans*, as previously shown (8), and also those of *C. tropicalis* have a well-developed surface film or coating which was not evident in the other four species examined. It is interesting that these two species are the most pathogenic of those studied. These two species also showed the richest filamentous meshwork. The presence of this meshwork both within the remnants of the surface coat and also between the underlying organisms in fixed material indicates a similarity between the surface coat and the intercellular matrix. Of the other species studied, only *C. pseudotropicalis* showed a filamentous meshwork, but this was less well-developed and a clearly defined surface coat was not apparent.

Although the origin of these filaments is not known, their observation only in fixed preparations suggests that they may represent either a precipitation of extracellular material by the fixative, or the unmasking of a fibrillar component by the dissolution of amorphous material. This filamentous meshwork was clearly distinguishable from the interconnecting processes between cells which were present in all species examined and which had a variable diameter

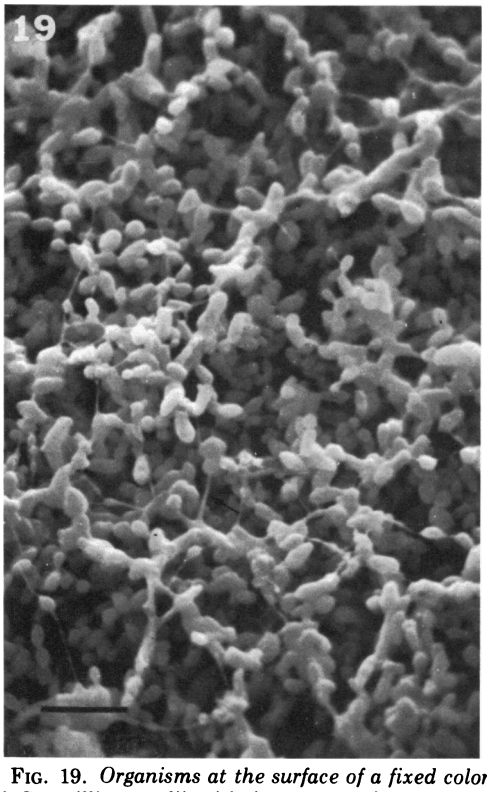


FIG. 19. *Organisms at the surface of a fixed colony of C. guilliermondii with interconnecting processes (arrow). Scale marker = 10 μm.*

corresponding to the stage of cell division and separation. These latter connecting processes probably correspond with the intercellular bridges discussed by Whitaker and Drucker (8) and illustrated by Montes et al. (6) in *C. albicans*.

Peripheral pseudomycelia were observed in all of the relatively small number of strains of *C. pseudotropicalis* and *C. krusei* examined. Hazen et al. (3) have described similar pseudomycelia in colonies of *C. tropicalis*, but this was not the case in the present study.

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