APPLIED MICROBIOLOGY, Feb. 1973, p. 309-312

Vol. 25, No. 2
Printed in U.S.A.

NOTES

Cryptococcus neoformans of Unusual Morphology

J. G. CRUICKSHANK, R. CAVILL, AND M. JELBERT

Department of Medical Microbiology, University of Rhodesia and Government Hospital, Umtali, Rhodesia

Received for publication 11 September 1972

A case of primary cryptococcosis of the lungs was caused by an isolate of *Cryptococcus neoformans* that assumes a giant form in tissue but which has a normal appearance on artificial culture. Electron microscopy revealed gross enlargement of the capsule and plasma membranes in the tissue form.

In infections caused by *Cryptococcus neoformans*, fungus cells are present in large numbers in the lesions. They are readily identified by their characteristic encapsulated, budding yeast form. Variations in morphology, which are normally confined to a range of sizes from 4 to 20 μm, also occur in the capsules which vary in size or may even be absent. Recently, a culture of *C. neoformans* (Coward isolate) was shown to develop branched septate hyphae (6) both in vitro and in infected mice. In another paper, the same authors state they have collected 11 isolates with this characteristic.

This note reports a rather unusual isolate which occurred in giant forms when in fresh pus from the lesions but which reverted to a normal appearance on artificial culture. The striking appearance of the pus caused considerable diagnostic difficulties. The two forms were examined by electron microscopy to determine the cause of the enlargement.

Case report. An African girl 8 months pregnant in her early twenties was admitted to Umtali hospital on 29 February 1972 with a history of haemoptysis for 1 month. Physical and X-ray examination revealed a massive right-sided pleural effusion. Thick brownish pus was aspirated, but no amoebae were found. The patient was placed provisionally on antibiotics with an underwater drain to relieve the pressure.

After diagnosis was made (see below), a course of amphotericin B was started. The patient failed to improve and was transferred to Harari Hospital in Salisbury where, in spite of continuous treatment, the course of the disease was progressive, and surgical intervention was considered.

Investigation. A wet preparation of the pus revealed large numbers of large yeastlike bodies, some of which were budding. Although the cells varied in size, the majority were 40 to 50 μm in diameter (Fig. 1). The large size of the organisms was suggestive of *Rhinosporidium seeberi*, but the budding characteristic suggested a cryptococcus species.

The pus was seeded onto Sabouraud dextrose agar and incubated at 38 C. Smears were prepared from typical looking colonies of Cryptococcus. The organisms now appeared normal and varied in size from 4 to 20 μm. Assimilation tests demonstrated the utilization of dextrose, sucrose, galactose, glucose, and maltose but not of nitrate or lactose. Starch was produced from glucose. The urease test was positive.

Some of this material was suspended in antibiotic-containing saline and was inoculated intracerebrally into mice. Within 7 days, the animals exhibited signs of meningoencephalitis and died. On autopsy, cryptococci in their original large form were found throughout the brain and meninges.

Pus obtained directly from the patient was differentially centrifuged until most of the residue consisted of yeast cells. The residue was fixed in glutaraldehyde and embedded in TAAB resin (TAAB Laboratories, Reading, England). Sections were cut on a Reichert ultratome, stained with lead citrate and uranyl acetate, and examined in a Hitachi H.U.11 electron microscope. Cryptococci from the cul-

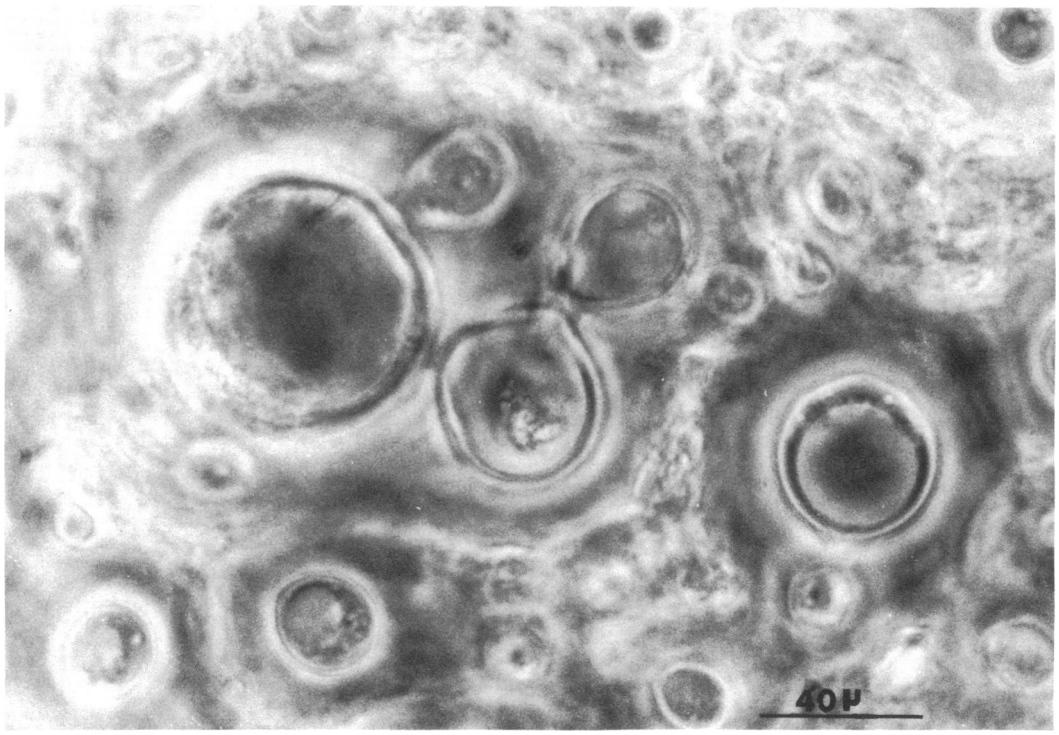

FIG. 1. *Phase-contrast photomicrograph of large cells of C. neoformans in original pus specimen.*

tured material were examined similarly.

Figure 2 is a budding form of the organism from the Sabouraud agar culture, and Fig. 3 is of the organism as it appeared in the aspirated pus. The first form (Fig. 2) conforms to the description to be found in the published work on ultrastructure of *C. neoformans* (1, 3, 4, 7–9, 11). The second form (Fig. 3), however, shows a number of unique features in its size, cell wall, and capsule. Most of the aforementioned authorities regard 4 to 10 μm as the usual diameter range with 2.5 to 20 μm as the extreme. The average size of our isolate in pus is over 40 μm and is associated with enlargement of the cytoplasm, cell wall, and capsule. Capsules are not infrequently as thick as the radius of the cell, but tend to be lost in preparation of specimens for electron microscopy (5). In this isolate, the capsule was as thick as the diameter of the cell and was stable throughout fairly rough handling (e.g. differential centrifugation) prior to embedding. However, upon culturing in artificial media, this capsule reverted to a normal appearance. The fine structure of the capsular material appeared to be the same in both preparations. The cell wall of the tissue form was up to 10 times thicker than that of the cultured strain

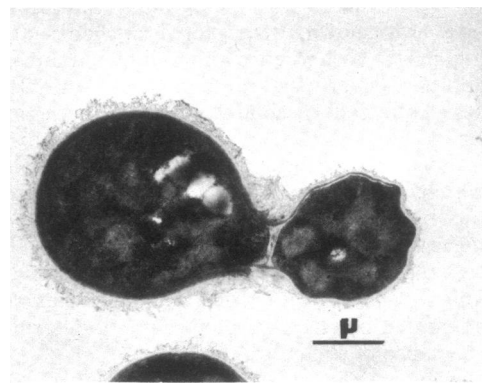

FIG. 2. *Electron photomicrograph of C. neoformans after culture on Sabouraud agar.*

and could be differentiated into a number of layers not hitherto described. At least five distinct bands could be seen and the central dark band seemed to be further subdivided. The intracellular organization of both forms was much the same and conformed to the classical descriptions.

Study of sections of affected lung removed at biopsy by light and electron microscopy revealed the characteristic gelatinous type of

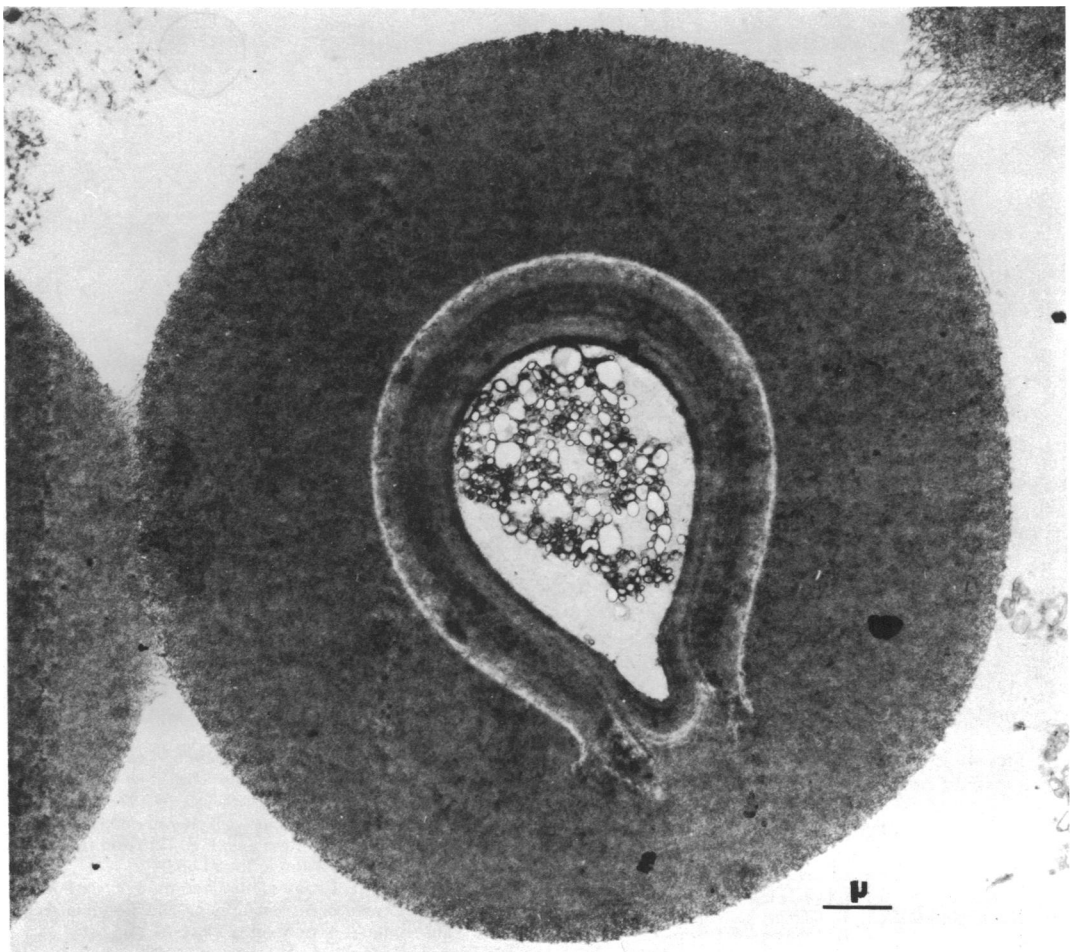

FIG. 3. *Electron photomicrograph of same C. neoformans as in Fig. 1 but separated without subculture from original pus specimen.*

reaction with large numbers of cryptococci, little cellular response, and progressive fibrosis. The banding of the cell wall was particularly well seen (Fig. 4).

Comment. Recognition of variants in morphology of organisms is important in laboratory diagnosis. The characteristics of *C. neoformans* are sufficiently clear for there to be little difficulty in recognizing the fungus which has hitherto been regarded as the only unicellular yeast causing systemic infection. Hyphal isolates have been reported. However, as they only produce this form in artificial culture, diagnosis from the tissue forms is unlikely to be compromised.

This isolate is of interest in that in clinical material it produced enormous forms which gave rise to diagnostic difficulties, but which resumed a typical appearance on artificial culture. The clinical picture did not indicate any particular degree of virulence, but localized cryptococcal disease is notoriously difficult to treat.

The infection was the rather unusual primary infection of the lung reported first by Sheppe in 1924 (12) and recently reviewed by Campbell (2). The lesions typically, as in this case, are subpleural, and the disease tends to become generally disseminated if the treatment fails to control it.

We thank the Secretary of Health for permission to publish this report. We are grateful to Dr. O'Connor of Umtali Hospital and C. Woodruff of the Public Health Laboratory, Umtali, for drawing our attention to the case.

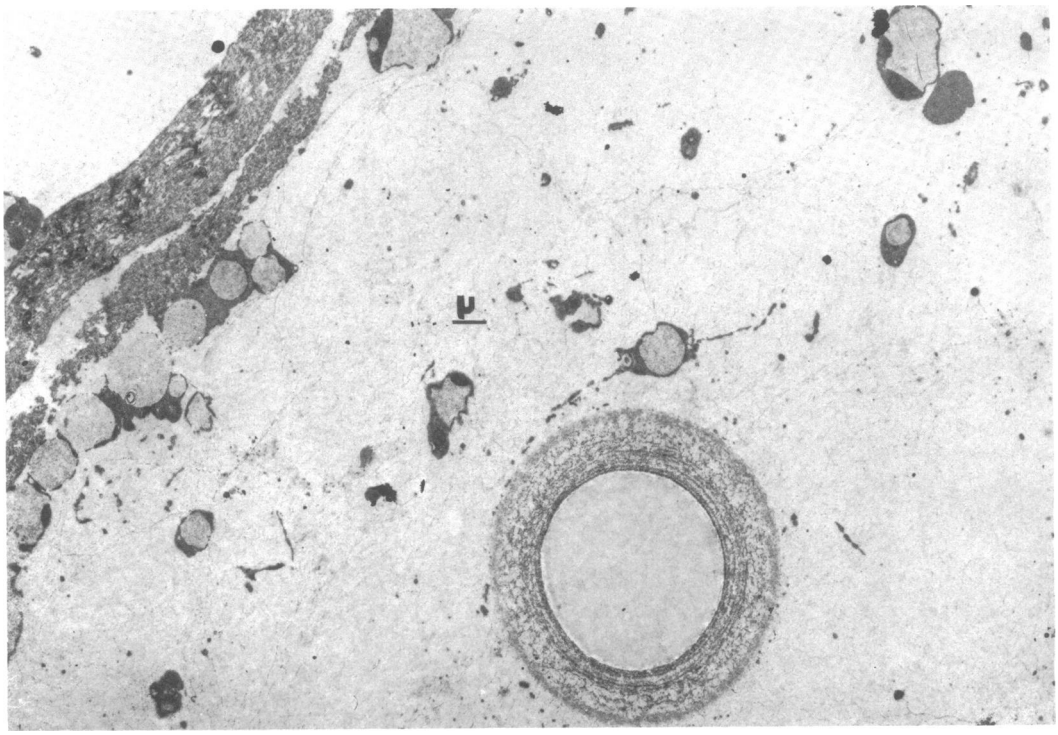

FIG. 4. *Electron photomicrograph of a section of the lung biopsy showing little reaction in the tissue and the layered cell wall of a C. neoformans cell.*

LITERATURE CITED

1. Al-Doory, Y. 1971. Ultrastructure of Cryptococcus neoformans. Sabouraudia **9**:114–118.
2. Campbell, C. D. 1966. Primary pulmonary Cryptococcosis. Amer. Rev. Resp. Dis. **94**:236–243.
3. Carbonnel, L. M. 1971. Ultrastructure of human pathogenic fungi and their mycoses, p. 38–66. *In* R. D. Baker (ed.), Human infection with fungi, actinomycetes and algae. Springer-Verlag, New York.
4. Edwards, M. R. 1966. The internal and external fine structure of the yeast Cryptococcus neoformans. Sixth International Congress for Electron Microscopy, Kyoto, p. 783.
5. Edwards, M. R., M. A. Gordon, E. W. Lapa, and W. C. Ghiorse. 1967. Micromorphology of *Cryptococcus neoformans*. J. Bacteriol **94**:766–777.
6. Lurie, H. I., H. J. Shadomy, and W. J. S. Still. 1971. An electron microscopic study of Cryptococcus neoformans (Coward strain). Sabouraudia **9**:15–16.
7. Matele, P., H. Moor, and C. F. Rebinard. 1969. Yeast cytology. *In* A. H. Rose and J. S. Harrison (ed.), The yeasts. Academic Press, Inc., London.
8. Niki, T. 1967. Ultrastructural studies of Pathogenic fungi, Cryptococcus neoformans and Candida tropicalis. Shikulu Acta Medica **23**:42–54.
9. Ruinen, J., and M. H. Deinoma, 1968. Cellular and extracellular structures of Cryptococcosis laurentis and Rhodotorula. Can. J. Microbiol. **14**:1133–1137.
10. Salfelder, K. 1971. Cryptococcosis in human infection with fungi, actinomycetes, and algae, p. 383–464. Springer-Verlag, New York.
11. Shadomy, H. J., and H. I. Lurie. 1971. Histopathological observations in experimental cryptococcosis caused by a hyphae producing strain of Cryptococcus neoformans (Coward strain) in mice. Sabouraudia **9**:6–9.
12. Sheppe, W. M. 1924. Torula infection in man. Amer. J. Med. Sci. **167**:91–108.
13. Tsukahara, T. 1963. Cytological structure of Cryptococcus neoformans. Jap. J. Microbiol. **7**:53–67.