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Isolation of Highly Encapsulated *Cryptococcus neoformans* Serotype B from a Patient in New York City

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For the first time, *Cryptococcus neoformans* serotype B was isolated from a patient in New York City, not a region endemic for B/C serotypes. The isolate was morphologically unusual, with cells several times larger in infected tissue than those characteristic of the yeastlike pathogen. This anomaly may be a problem in the identification of similar isolates. Two serotype differentiation media and a slide agglutination test were used for definitive serotype identification.

Cryptococcus neoformans serotypes B and C have never been isolated from the environment and have rarely been associated with human infections, except in apparent endemic pockets, e.g., southern California (1, 5). We report here the first isolation, to our knowledge, of *C. neoformans* serotype B from a patient in New York City. This isolate was morphologically unusual: the cells and capsules were several times larger in infected tissue than those commonly described in the literature.

The patient, a 45-year-old woman, presented to Mount Sinai Hospital in April 1984 with shortness of breath, cough without hemoptysis, and fatigue. Results of an X-ray in 1981 had been negative, but a chest X-ray on admission showed an irregular density in the upper segment of the lingula, which was suspected to be a peripheral carcinoma. Because fiberoptic bronchoscopy, computed tomographic scans of the chest, brain, and bone, and other investigational procedures provided no definitive information, the patient underwent a thoracotomy. A 3-cm brownish green mass was found and removed from the lingula. The patient did well postoperatively and was discharged, without other treatment, 10 days later.

A portion of the excised mass submitted for frozen section was fixed in 10% Formalin, embedded in paraffin by standard techniques, sectioned, and stained with hematoxylin and eosin. Histologic examination revealed no evidence of malignancy, but extensive inflammatory changes, hyperplasia of the alveolar cells, lymphocytic infiltrates, and occasional multinucleated large cells were found. Numerous markedly encapsulated cells morphologically consistent with *C. neoformans* were observed in hematoxylin-and-eosin- and, subsequently, mucicarmine-stained sections (Fig. 1).

Concomitantly, microscopic examination of India ink preparations of exudate from the biopsy material confirmed the presence of *Cryptococcus*-like cells. Although the size of the capsule which surrounds the *C. neoformans* cell is highly variable, the cells themselves are generally subglobose to spherical, 2.5 to 8 μm in diameter (3). Cells in this specimen were 17.5 to 20 μm in diameter and surrounded by thick capsules, which increase the overall diameters of most of the cells to 40 to 55 μm (Fig. 2). These characteristics were generally observed even after mouse passage of the isolate.

Portions of the biopsy specimen were streaked onto each of three nutrient agars: 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.), chocolate, and Sabouraud glucose. All media were incubated at 37°C. Mucoid colonies, found on all media after 48 h of incubation, were further tested and identified as *C. neoformans* on the basis of API 20C assimilation patterns (Analytab Products, Plainview, N.Y.), positive urease activity on Christensen's urea agar, inability to assimilate KNO_3, and production of brown pigmentation on caffeic acid agar (6). No other fungal or bacterial growth was observed.

A subculture of the isolate was submitted for serotyping to the Wadsworth Center Mycology Laboratories. The serotype pair (A/D or B/C) of the isolate was initially determined by growth and color change on glycine-cycloheximide (GCA; 9) and glycine-canavanine-bromthymol blue (GCB; 7) agars. To inoculate these media, the yeast was first grown for 48 h at 37°C on Sabouraud glucose agar. A portion of growth was aseptically removed with a sterile transfer loop and streaked over the surface of GCA slants in screw-capped tubes (20 by 150 mm) and of GCB in 100-mm plastic petri plates. The change of GCA from yellow to red and of GCB from light green to blue after 3 days of incubation at 30°C indicated that the isolate was either a B or C serotype. Definitive identification of the isolate as a B serotype was accomplished by using specific capsular conjugates in a slide agglutination modification of the serotyping procedures of Evans (4).

Despite some variation in cell size, the vast majority of cells in the biopsy specimen were several times the characteristic size of *C. neoformans* cells. Even in Gram- and in methylene blue-stained smears, the cells appeared as large, poorly staining, subglobose structures. The unusual microscopic morphology and staining attributes could mislead laboratorians into dismissing the cells as artifacts or, at least, delay the presumptive identification of this yeastlike pathogen.

Cruickshank et al. (2) and Love et al. (8) reported similar-sized budding *C. neoformans* cells in aspirates from a pleural effusion and the brain, respectively. However, cells from the isolate of Cruickshank et al., like ours, reverted to the more common, smaller size when grown and maintained on artificial nutrient media. Apparently, the factors which promoted their unusual size in vivo were altered when the organism was grown in vitro.

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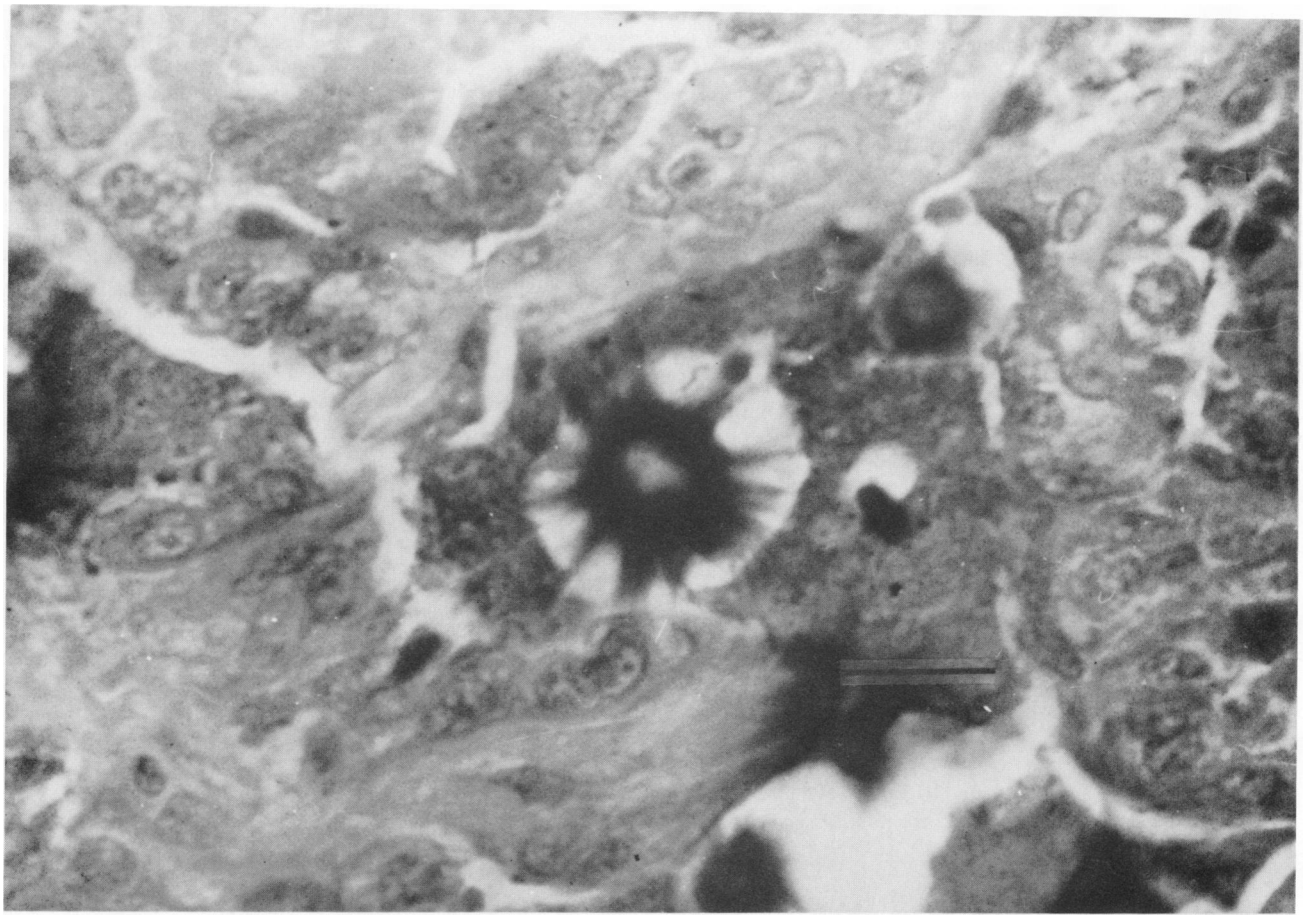

FIG. 1. Mucicarmine-stained section of excised pulmonary mass showing *C. neoformans* yeast cells encircled by capsular polysaccharide. Bar = 20 μm.

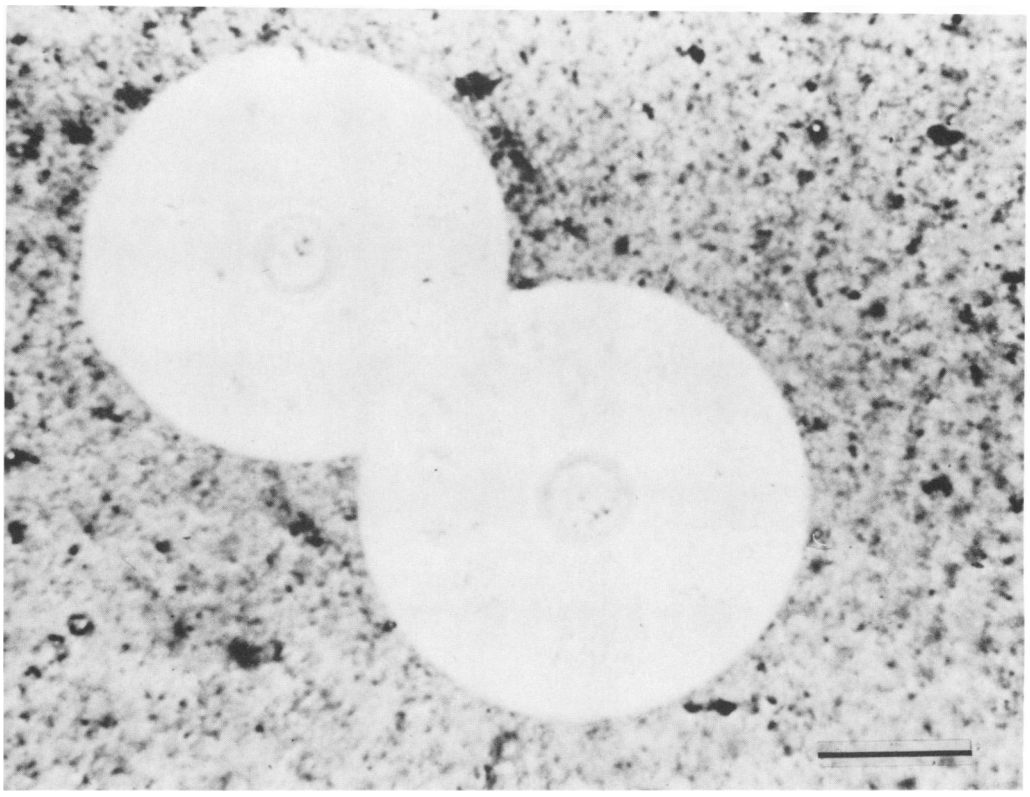

FIG. 2. Direct India ink preparation of fragment of excised pulmonary mass, showing markedly encapsulated cryptococcal cells. Bar = 20 μm.

Although the patient was a resident of New York City, she had visited southern California 13 years before admission to the hospital and Mexico in 1979 and 1980. In both areas, *C. neoformans* B and C serotypes have been reported in association with human infections (1). She recalled that, while in Mexico, she had a short-lived febrile illness without any respiratory symptoms. An X-ray performed in 1981 was negative. Nevertheless, although the exact origin of the present isolate is unknown, travel to these two known endemic areas does suggest acquisition from a reservoir(s) in these regions.

Although serotype differences have been related to the clinical response to therapy in five cases of cryptococcosis (D. K. Henderson, J.E. Edwards, Jr., W. E. Dismukes, and J. E. Bennett, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, F11, p. 315), there is still only limited information on the clinical and epidemiologic characteristics of *C. neoformans* serotypes. This lack of information is due, at least in part, to the lack of a simple, accurate serotype identification system. However, GCA or GCB medium provides a rapid, effective means for providing the physiologic reactions of *C. neoformans* isolates that correlate with A/D and B/C serotype pairs. We suggest that clinical laboratorians adopt the use of these two media as part of their standard identification procedures.

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