

NOTES

Cryptococcus neoformans: Pitfalls in Diagnosis Through Evaluation of Gram-Stained Smears of Purulent Exudates

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The recognition of *Cryptococcus neoformans* in Gram-stained smears of purulent exudates may be hampered by the presence of the large gelatinous capsule which apparently prevents definitive staining of the yeast-like cells. In such stained preparations, *C. neoformans* may appear either as round cells with gram-positive granular inclusions impressed upon a pale lavender cytoplasmic background or as gram-negative lipid bodies.

The diagnosis of cryptococcosis may be achieved culturally, serologically, or by visualizing the fungus directly in infected tissues or body fluids. Presumptive identification in the latter instances is enhanced by demonstrating encapsulated yeast cells in India ink preparations.

Although negative staining of the capsule is the most widely used method for detecting *Cryptococcus neoformans* in various body fluids and exudates (1, 10) little data exist regarding the utility of the Gram stain as a diagnostic adjunct. In this regard, because of two experiences relative to the appearance of cryptococci in Gram-stained preparations of exudates, potential pitfalls in diagnosis were revealed. In both instances, specimens had been submitted for bacteriological analyses and were not directed by the clinician for mycological studies.

In the first instance, *C. neoformans* was initially overlooked in Gram-stained smears of bronchoscopic specimens from a renal transplant patient. In these preparations, the organism appeared as pale lavender to red, round globules resembling fat droplets. Because the constancy in size and sharpness of the contours suggested a more definitive structure, wet and India ink preparations of the specimen were examined, and encapsulated yeast-like microorganisms were seen, later identified as *C. neoformans*.

In the second encounter, surgically obtained pus and biopsy material from a lytic lesion of the right eighth rib of a patient was submitted for microbiological analysis. In the first microscopic field of a Gram-stained smear, gram-negative, round, budding yeast cells were seen. In other fields, single round cells measuring 4 to 6.5

μm were observed which showed a symmetrical arrangement of gram-positive granular inclusions; the remainder of the cell had a lavender hue. Occasionally, two such stippled cells were seen that were adjacent to each other (Fig. 1). These speckled cells were enveloped by a faint

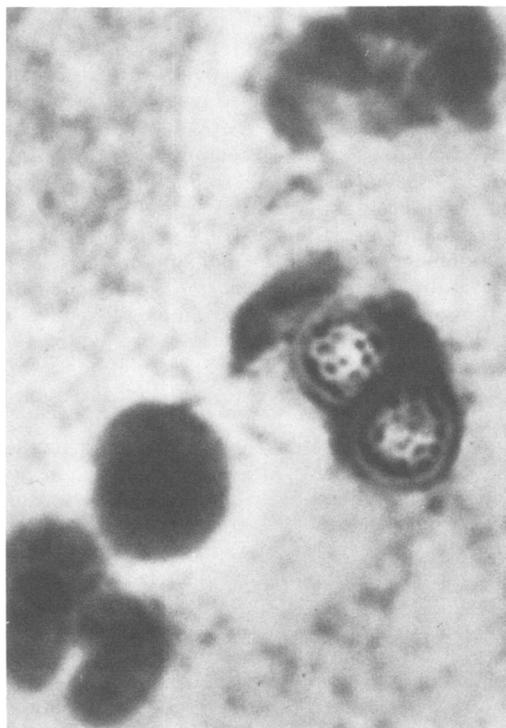


FIG. 1. Gram-stained smear of rib exudate showing a pair of stippled cells of *C. neoformans* (original magnification, $\times 1,000$).

pink halo. Hematoxylin- and eosin-stained frozen sections of the bone biopsy, prepared at the time of surgery, failed to reveal the presence of fungi.

The pus and biopsy specimens from the second patient were plated on 5% sheep blood agar (BBL Microbiology Systems), MacConkey agar, and Mycophil agar (BBL Microbiology Systems). After 48 h of incubation at 37°C, numerous smooth glistening colonies developed on the blood and Mycophil agar which were shown to be composed of encapsulated yeasts, later identified as *C. neoformans* through standard methods (10). After this disclosure, the Gram-stained smears were reexamined, and after an extensive search, several more gram-negative budding yeast cells were observed, some within mononuclear cells. Histological confirmation of cryptococcosis was subsequently made by observing encapsulated mucicarmine-stained budding yeast cells in histological sections. Budding yeast cells were seen also by the Gomori methenamine silver stain.

A search of several texts (1, 2, 4, 7-10) has disclosed only two references to the Gram staining characteristics of *C. neoformans*: In one, a textbook of medical microbiology, *C. neoformans* is described as a gram-positive yeast-like microorganism (2). In the other, a standard text of mycology, it is stated that "staining of [histological] sections by Gram's method shows that the central portion of the organism inside the mucinous capsule stains intensely blue" (1). In the former instance, information is lacking as to the age and source of the organism, state of capsulation, or cultivation conditions, if applicable, before staining. In the latter, the Gram stain was modified specifically for staining of histological sections and applied for a much longer period of time. Additionally, it would seem that preparations of biopsy or clinical materials for histological examination might alter the permeability properties of the organism, perhaps relative to the degree of capsulation and, hence, the intensity of staining.

As Gram-stained smears of purulent exudates are frequently evaluated in clinical microbiology laboratories, the recognition of this fungal pathogen in such preparations is critical to diagnosis and patient management. It is especially important to emphasize that cryptococcal meningitis may render a purulent exudate (6). As bacterial agents are readily sought on Gram stains of such

material, it is conceivable that cryptococci, when present, can easily be overlooked if the microscopist is unaware of the staining subtleties of cryptococci in such preparations. Definitive diagnosis may thus be delayed or even missed as cryptococci may not grow in culture (3, 5).

It is therefore suggested that when round lipid bodies or stippled unicellular structures are observed in Gram-stained smears of body exudates and fluids, a search for cryptococci should be undertaken through other standard techniques, e.g., mucicarmine stain. Although India ink preparations may be adequate for demonstrating cryptococci in cerebrospinal fluid, this technique is not useful for specimens such as sputum, tissue homogenates, or any viscous specimen containing large amounts of tissue materials. In such specimens, capsules can be readily detected microscopically in a wet preparation without ink or stain, inasmuch as small tissue particulates tend to adhere to the capsule surface, outlining the capsule in much the same way as do particles of India ink.

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