Recovery of *Cryptococcus neoformans* from Modified Dubos Liquid Medium Utilized for Isolation of Mycobacteria

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Received for publication 3 January 1975

*Cryptococcus neoformans* was isolated from nine pathological specimens cultured for mycobacteria. Five of these were recovered only in liquid TBC medium in the absence of growth on conventional substrates.

In patients with defenses compromised by underlying disease or by immunosuppressive drugs, there appears to be an increasing incidence of infection with *Cryptococcus neoformans* (11). This association has made the demonstration of this fungus in clinical specimens one of the most crucial tasks of the microbiology laboratory. Toward this endeavor, various bacteriologic techniques have been utilized, such as India-ink preparations, cultivation on a variety of bacteriological and mycological media, mouse pathogenicity studies, and assaying for cryptococcal antibody or polysaccharide (antigen), particularly in cerebrospinal fluid (2). Of the above named procedures, the serological approach is the most sensitive, especially in the absence of demonstrable cryptococci by India-ink or cultural methods (7). The value of this test, however, as a diagnostic tool for the detection of cryptococcal antibody is limited by negative results in the presence of culture-proven cases of cryptococcosis (1, 9, 16), and occasional nonspecific positive reactions due to rheumatoid factor. The latter, though, may be eliminated in most instances by pre-treating test sera with disulfide reducing agents (8).

In recent years, media have been developed for the selective isolation and differentiation of *C. neoformans* from various other fungi (3, 10, 13, 14, 15). As these media were tested predominantly with stock cultures or simulated clinical specimens prepared from a stock culture of *C. neoformans*, their value in the primary isolation of cryptococci from clinical specimens must await more extensive clinical trials. As the diagnosis of cryptococcosis is most reliably resolved by the demonstration of cryptococci by direct or cultural techniques, the availability of a medium that is capable of initiating the growth of cryptococci even under suboptimal circumstances is still desirable.

In our laboratory, during an approximate 2-year period, from 1972 to 1974, *C. neoformans* was isolated from nine pathological specimens (seven patients, two with duplicate specimens) submitted for possible cultivation of tubercle bacilli. These specimens, namely bone marrow, cerebrospinal fluid, pleural fluid, and lung biopsy, were not subjected to a decontamination procedure prior to direct inoculation into modified Dubos liquid TBC medium (5) (Pfizer), Lowenstein-Jensen and 7H11 agars (Pfizer), and onto routine conventional media such as 5% sheep blood and chocolate agars (BBL), Sabouraud and Mycophil agars (Difco), and into thioglycollate and glucose broths. Although yeast-like fungi were not observed in direct smears prepared in the TB laboratory, all nine isolates grew in the modified Dubos liquid medium, of which five, derived from four patients, were recovered only in this medium in the total absence of growth on conventional media even after incubation at 37 C for 2 weeks (Table 1). Three of the nine strains grew on 7H11 agar whereas none grew on Lowenstein-Jensen medium.

For reasons related to mycobacterial growth rate, examination of liquid TBC media was first performed on a weekly basis. Initially, growth observed with seven of the isolates occurred within 2 weeks in the form of a dense, smooth sediment which, upon shaking, could be uniformly dispersed. After these observations, two subsequently inoculated liquid media were examined daily, and the first signs of growth of *C. neoformans* were noted in 48 h. Direct microscopic examination of a sample from all flasks revealed the presence of budding, encapsulated yeast cells suggestive of *C. neoformans*. Subculture to sheep blood and Sabouraud agars produced characteristic buff-colored, smooth, mucoid colonies after 48 h of incubation at 37 C. Identification as *C. neoformans* was made ac-
TABLE 1. Comparison of recovery of C. neoformans from modified Dubos liquid and standard media

<table>
<thead>
<tr>
<th>Patient</th>
<th>Underlying disease</th>
<th>Diagnosis</th>
<th>Source</th>
<th>Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. C.</td>
<td>Renal transplant</td>
<td>Pulmonary infiltrate</td>
<td>Lung biopsy</td>
<td>+ +</td>
</tr>
<tr>
<td>E. G.</td>
<td>CLL*</td>
<td>Meningitis</td>
<td>Bone marrow</td>
<td>+ +</td>
</tr>
<tr>
<td>G. A.</td>
<td>Hodgkin’s Hodgkin’s</td>
<td>Meningitis</td>
<td>CSF*</td>
<td>+ +</td>
</tr>
<tr>
<td>S. F.</td>
<td>Reticulum cell sarcoma</td>
<td>Pleural effusion</td>
<td>Pleural fluid</td>
<td>+ 0</td>
</tr>
<tr>
<td>A. S.</td>
<td>CLL</td>
<td>Meningitis</td>
<td>CSF</td>
<td>+ 0</td>
</tr>
<tr>
<td>A. B.</td>
<td>CLL</td>
<td>Meningitis and pneumonia</td>
<td>Bone marrow</td>
<td>+ 0</td>
</tr>
<tr>
<td>E. L.</td>
<td>Chronic heart failure</td>
<td>Chronic pleuro-pulmonary infection</td>
<td>Pleural fluid</td>
<td>+ 0</td>
</tr>
<tr>
<td></td>
<td>Tuberculosis versus neoplasm</td>
<td>Chronic pleuro-pulmonary infection</td>
<td>Pleural fluid</td>
<td>+ 0</td>
</tr>
</tbody>
</table>

*CLL, Chronic lymphatic leukemia.
*CSF, Cerebrospinal fluid.

According to criteria outlined by Dolan and Woodward (4) and consisted of urease activity, lack of fermentative capability, and assimilation of dextrose, inositol, maltose, raffinose, sucrose, and trehalose, but not lactose or nitrate. Mouse pathogenicity was not assessed.

Culturally, cryptococci may be difficult to recover from clinical specimens particularly cerebrospinal fluid (6), and this may be attributed to the presence of a sparse number of organisms or to the difficulty cryptococci may encounter while making transition from in vivo to in vitro habitats. In this regard, liquid TBC medium may provide some essential growth-promoting factors capable of initiating and supporting the development of a small number of cells. In our laboratory, an attempt to establish the nature of particular growth-promoting factors was undertaken with three of the isolated C. neoformans strains. By sequentially depleting the complete medium of each enrichment component (albumin, oleic acid, dextrose, NaOH), no single factor was found to be stimulatory. However, these were not virg isolates but had been previously adapted to laboratory conditions by subculture.

Although cryptococcal infection was suspected in patients with meningitis, an unsuspected infection was disclosed through the use of liquid TBC medium by the recovery of C. neoformans from two consecutive pleural fluid specimens (patient E.L.) which had failed to yield this organism on any other medium.

In our experience as well as that of Neimeister and co-workers (12), C. neoformans has not been recovered from NaOH-treated specimens. However, with unmodified specimens inoculated directly into enriched media, laboratory workers should be aware of the potential recovery of unsuspected pathogenic yeasts or fungi.

We wish to express our gratitude to S. S. Schneierson for continued support and evaluation of the manuscript, as well as to Robert Swift of Pfizer Diagnostics for providing the various growth factor supplements.

LITERATURE CITED