FIG. 1. *Isolated plaques obtained by streaking bacteriophage-containing filtrates on host cultures of Pseudomonas aeruginosa. (left) Nonmetallic culture, (right) metallic culture.*

to isolate bacteriophages from large numbers of such preparations, this method is rather time-consuming and requires much glassware.

The following procedure for obtaining isolated plaques from such preparations is simpler than the above method. An agar plate is inoculated with 0.15 ml of a broth culture of the prospective host organism, and the broth is spread evenly over the agar with a sterile bent glass rod. A loop of the bacteriophage-containing liquid, prepared as above, is then streaked directly on the lawn of bacteria just as one would streak a bacterial culture on an agar plate to obtain isolated colonies. The plate is then incubated until bacterial growth and plaque formation occur. Further purification may be obtained by stabbing a plaque with a sterile needle and rinsing the needle in 1 ml of sterile broth. A loop of this broth is then streaked in the same manner. Figure 1 illustrates the effectiveness of this method for obtaining isolated plaques.

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ISOLATION OF A DRY VARIANT FROM A MUCOID STRAIN OF *CRYPTOCOCCUS NEOFORMANS* AND PRELIMINARY COMPARATIVE STUDIES

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The immune response of man and animals to *Cryptococcus neoformans* is extremely variable. Neill (J. Bacteriol. **59**:263, 1950) correlated the relationship between capsular size and antigenicity. This relationship was further demonstrated by Gadebusch (Naturwissenschaften **47**: 329, 1960), who noted that when the capsule was reduced in size by enzymatic action an increase in antigenicity could be detected.

In the present study, a small-capsule variant (ODH DV) and the large-capsule strain (ODH 127) from which it was derived were compared with respect to colonial morphology, capsule size, stability, virulence, antigenicity, and histopathological response. This variant was obtained after exposure of the parent strain (ODH 127) to Wescodyne G (Tamed iodine; West Chemical Products, Inc., Long Island City, N. Y.), 40 ppm,

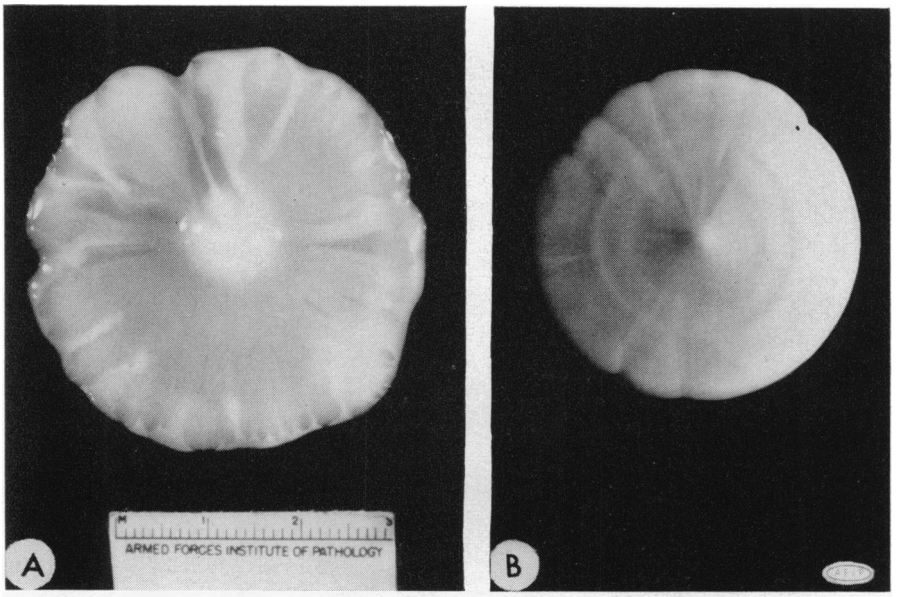

FIG. 1. (A) *Typical mucoid colony of parent strain (ODH 127).* (B) *Yellow, creamy colony of variant (ODH DV).*

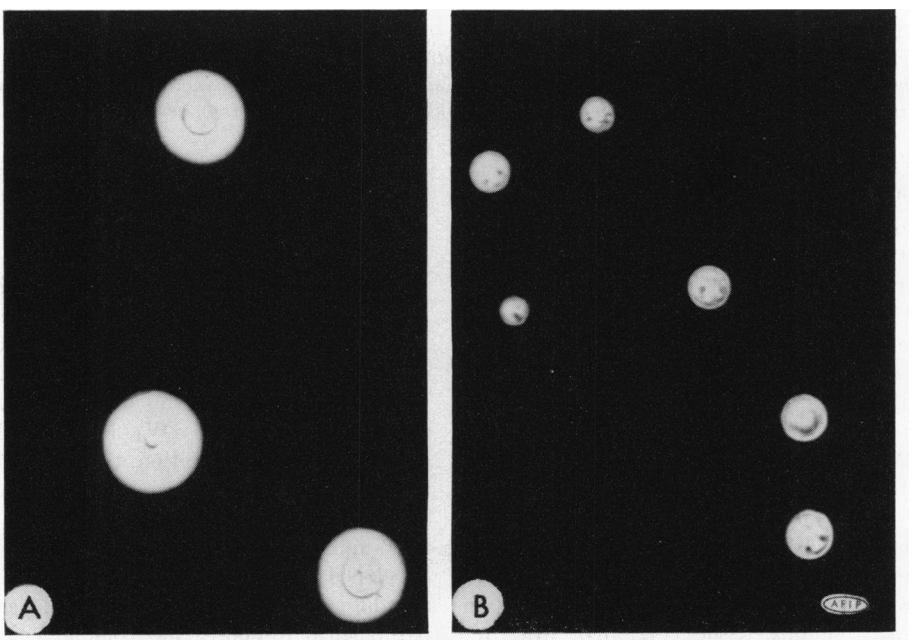

FIG. 2. (A) *India-ink preparation of parent strain with large capsule (1.14 to 17.11 μ).* (B) *India-ink preparation of variant showing small capsule (0.47 to 1.13 μ).*

for 10 min. The parent strain had previously failed to yield a small-capsule variant, despite repeated subcultures on artificial media and animal inoculation over a period of 4½ years.

Organisms surviving the exposure to Wescodyne were of two colonial types: the predominating typical glistening mucoid colony of the parent strain (Fig. 1A) and an occasional yellow, creamy colony of the variant (Fig. 1B).

The radius of the capsules in the variant ranged

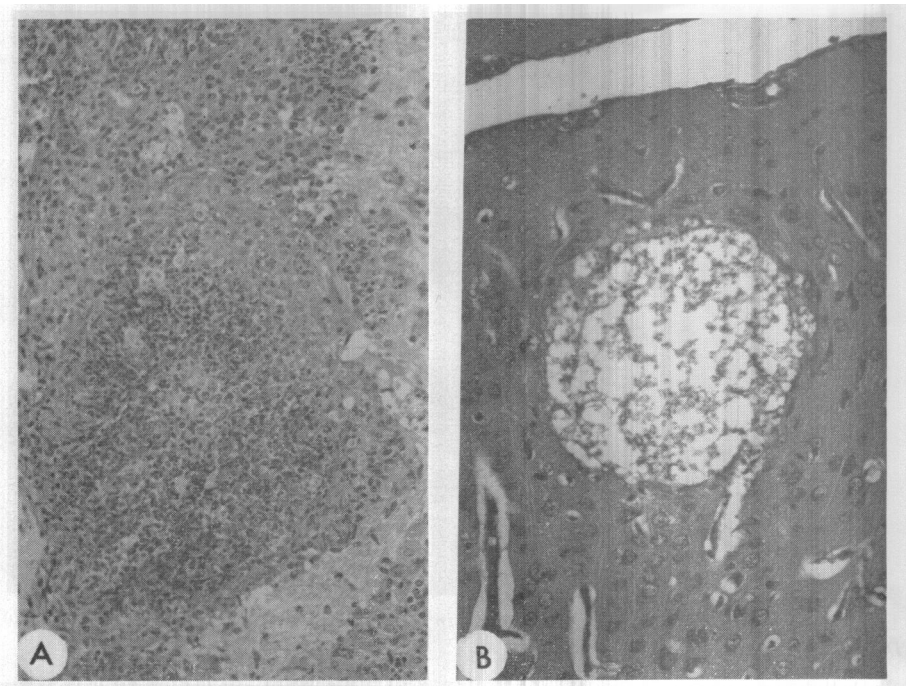

FIG. 3. (A) *H & E-stained section of mouse brain inoculated with parent strain; mice killed at onset of CNS symptoms. A characteristic field of multiple necrotic areas with dense inflammatory infiltrate containing many mononuclear cells.* × *180.* (B) *H & E-stained section of mouse brain inoculated with variant; mice killed at onset of CNS symptoms. A small cystic area filled with organisms is in evidence.* × *180.*

from 0.47 to 1.13 μ in India-ink preparations (Fig. 2B), while that of the parent strain varied from 1.14 to 17.11 μ (Fig. 2A). The small-capsule variant has remained stable through repeated subcultures on artificial media and numerous animal passages.

Differences in the virulence and histopathological responses of the variant and parent strains were also observed. When Swiss mice, in two groups of 15 each, were inoculated intravenously with 200,000 organisms, those receiving the variant strains developed central nervous system (CNS) symptoms in 7 to 12 days. Histological sections of the brains in these animals with CNS symptoms revealed multiple small cystic areas filled with organisms. There was little reaction in the surrounding tissue (Fig. 3B). Mice receiving the parent strain, in contrast, did not develop CNS symptoms until the 14th to 120th day. Histological sections of these brains were characterized by multiple necrotic areas with a dense inflammatory infiltrate containing many mononuclear cells but few organisms of *C. neoformans* (Fig. 3A).

Finally, the parent and variant strains differed widely in their capacity to stimulate antibody in rabbits. Two groups of eight mature albino rabbits were immunized over a 2-week period with ten intravenous injections, containing a total of 9×10^9 organisms of the parent (ODH 127) or variant (ODH DV). In sera collected 7 days after the last injection, the tube agglutination titers in animals receiving the variant were high, and varied from 1:640 to 1:2560; none of the animals immunized with the parent strain developed a titer exceeding 1:2. In addition to the differences in capsule size, virulence, histopathological response, and colonial morphology noted, it thus also appears that the dry variant provides a highly antigenic strain for the production of immune sera against *C. neoformans* in rabbits.