A RAPID, SIMPLIFIED MEDIUM FOR CONVERTING THE MYCELIAL PHASE OF BLASTOMYCES DERMATITIDIS TO THE YEAST PHASE 1)

by

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Blastomyces dermatitidis, a diphasic, pathogenic fungus, resembles many saprophytic fungi when grown in the mycelial phase at room temperature (25°C). In clinical work it is often difficult to distinguish it from Histoplasma capsulatum, the causative agent of histoplasmosis. Therefore, to obtain a positive identification of B. dermatitidis, it is necessary to convert it to its tissue phase which is a thick-walled, budding yeast-like organism 8—15 microns in diameter and in appearance is identical to fungus cells found in tissues infected by B. dermatitidis. The large budding yeast-like cells bear little resemblance to the oval 1.5 × 3.5 micron yeast cells of H. capsulatum.

There are several different artificial culture media which have been used by many investigators to obtain the "in vitro" conversion of B. dermatitidis. Among these are brain heart infusion agar (1), blood agar (2), starch agar enriched with 5—10 per cent serum (3), beef extract agar (4) and Sabouraud’s dextrose agar (5). When mycelial elements are transferred to one of these media and incubated at 37°C, conversion usually occurs but often the time interval varies with each different media and in some instances with the same media. The use of laboratory animals to establish an identification is a common procedure, but animal inoculation usually requires several weeks for positive identification.

The purpose of this investigation was to determine:

1) The most efficient medium for the conversion of B. dermatitidis by comparing cottonseed agar 3), brain heart infusion agar, brain

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3) Pharmamedia produced by the Traders Protein Division of Traders Oil Mill Company, Fort Worth, Texas. Names of commercial manufacturers and trade names are provided for identification purposes only, and their inclusion does not imply endorsement by the Public Health Service; nor does exclusion of commercial manufacturers’ names imply nonendorsement by the Service.
heart infusion agar with 5 per cent human blood, and a conversion medium proposed by KELLEY (3).
2) The effect of variation in the pH level on the conversion process of the cottonseed agar.
3) The effect of variation in the dextrose concentration on the conversion process of the cottonseed agar.

**MATERIALS AND METHODS**

**Inoculum:** The inoculum used in this investigation consisted of 26 isolates of *B. dermatitidis* from both human and animal specimens. Isolations were accomplished at the Kansas City Field Station. These isolates were grown at 25°C on Sabouraud’s dextrose agar, slanted in 2 ounce perfume bottles for a period of two weeks before use. Blocks of agar, 1 mm square, containing a mycelial mat were taken from the edge of the colony and used to inoculate slants of the comparison media to be incubated at 37°C and to inoculate a slanted perfume bottle of Sabouraud’s agar for determining the viability of the colony.

**Media:** The four media used in this investigation were prepared as follows:
1) Cottonseed agar: 2 per cent pharmamedia; 2 per cent dextrose and 2 per cent agar, final pH 6.0.
2) Brain heart infusion agar (Difco) prepared according to instructions for rehydration, final pH 7.4.
3) Brain heart infusion agar (Difco) with 5 per cent outdated human blood, final pH 7.4.
4) KELLEY’s medium (3) consisting of .03 per cent beef extract, 1.0 per cent peptone, .05 per cent sodium chloride, 1.0 per cent starch, 2.0 per cent agar and 5.0 per cent calf serum, final pH 7.0.

The cottonseed agar and brain heart infusion agar were dispensed into 20 × 150 mm culture tubes with cotton stoppers, autoclaved for 15 minutes at 15 psi, and slanted.

KELLEY’s medium and brain heart infusion agar were prepared and autoclaved for 15 minutes at 15 psi, then cooled to 47°C using a water bath; the calf serum or blood was added aseptically. The media were then dispensed into 20 × 150 mm tubes and slanted.

**pH Variation:** The final pH of the cottonseed agar was varied, using Na HCO₃ to 6.0, 6.5, 7.0 and 7.5 to study the effect of pH on the rapid conversion of *B. dermatitidis.*

**Dextrose Variation:** The concentration of dextrose was varied in cottonseed agar from 0 per cent, .05 per cent, 1 per cent, 1.5 per cent and 2 per cent to study the effect of dextrose on the rapid conversion process.

All tubes were inoculated, as stated above, sealed with a Parafilm cap and incubated in a 37°C incubator for a period of from 4 to 7 days with daily observations.