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Rapid Conversion of *Histoplasma capsulatum*, *Blastomyces dermatitidis* and *Sporothrix schenckii* in Tissue Culture

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A simple method for positive identification of *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Sporothrix schenckii* is given. Primary tissue cultures of guinea pig peritoneal macrophage were inoculated with the mycelial phase of each organism and after 24 h the cells were stained and observed microscopically. The characteristic yeast phase could then be observed allowing for positive identification.

In the mycology laboratory it is often difficult and time consuming to identify the dimorphic pathogenic fungi. The process of isolation of *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Sporothrix schenckii* from clinical specimens requires at least 2 weeks, and in many cases up to a month of incubation at room temperature, 25 C. After isolation, definite identification cannot be made in the case of *B. dermatitidis* and *S. schenckii* until dimorphism is shown by conversion to the yeast phase. *H. capsulatum* also requires conversion if it does not develop characteristic tuberculation on the macroconidia, in which case its appearance, microscopically, is very similar to *B. dermatitidis*. In addition, growth of all the organisms on blood agar is usually atypical or nonsporulating and requires subculture to Sabouraud dextrose agar or conversion to the yeast phase. Therefore, the time required for identification is lengthened by the time required for conversion to the yeast phase.

The usual methods for conversion of *H. capsulatum*, *B. dermatitidis*, and *S. schenckii* to the yeast phase require inoculation of special media such as glucose-cysteine-blood agar and incubation at 37 C. (1) The process, however, involves repeated transfers to fresh media and in many cases weeks or months elapse before yeast can be demonstrated. An alternative method is inoculation of the mycelial phase into laboratory animals and recovery of the yeast on synthetic media. This process, however, requires a minimum of 2 to 3 weeks and is sometimes unsuccessful if the animal is able to overcome the infectious agent.

The purpose of this paper is to describe a method to considerably reduce the time required for conversion to the yeast phase, thereby allowing for a more rapid positive identification.

MATERIALS AND METHODS

Tissue culture growth medium. The medium used for tissue culture was Eagle 199 glutamine and HEPES buffer at pH 7.0. Fetal calf serum (15%) was incorporated into the medium along with 100 U of penicillin, 100 μg of streptomycin, and 40 μg of gentamicin per milliliter. This medium was dispensed in 100-ml serum bottles and kept refrigerated until needed.

Tissue cells. Peritoneal macrophages were collected from guinea pigs by the method of George and Vaughan (2) and washed twice in Eagle 199 medium at pH 7.0. Approximately 3×10^6 cells were collected from each guinea pig and stored at 4 C until needed (no longer than 1 week).

Monolayer formation. Lab-Tek tissue culture slides (4804, Lab-Tek Products, Div. of Miles Laboratories, Inc.) containing four chambers were inoculated with approximately 10^5 macrophages per chamber. The chambers were then filled with growth media (approximately 1 ml) and incubated at 37 C in a CO_2 incubator. After 24 h, the medium was removed aseptically by suction and fresh growth medium was added to the monolayer of cells. The tissue cultures were then ready for inoculation with the fungus.

Fungal inoculum. Hyphal elements from colonies of the mycelial phase of *H. capsulatum*, *B. dermatitidis*, and *S. schenckii* were added separately to a tube containing tissue culture growth medium and 3-mm glass beads. This mixture was then shaken 2 min with a vortex mixer. The concentration of mycelial particles was adjusted so that from 10^2 to 10^5 particles were added to the tissue monolayer in a small volume (ca. 0.05 ml). An exact concentration and size of the mycelial particles was found to be unimportant. A total of 10 isolates of *H. capsulatum*, 5 of *B. dermatitidis*, 2 of *S. schenckii*, and 1 of *H. capsulatum*

var. *duboisii* were used. Also, several saprophytes including *Scopulariopsis* sp., *Aspergillus* sp., *Chrysosporium* sp., *Monosporium* sp., and *Sepedonium* sp. were tested.

One chamber of each Lab-Tek tissue culture slide was left as an uninoculated control to determine if any artifact in the monolayer would resemble yeast. In addition, each organism used was inoculated into tissue culture growth medium alone to determine whether conversion would take place without the macrophage monolayer.

Incubation and staining. The inoculated tissue cultures and controls were incubated at 37 C in a CO_2 incubator for 24 h. The growth medium was then removed and the slides allowed to dry. After removing the chambers the slides were fixed in methanol and stained by periodic acid-Schiff using light green as a counterstain. Cover slips were then mounted permanently.

RESULTS

It was found that with each isolate of *H. capsulatum*, *B. dermatitidis*, and *S. schenckii*, conversion to the yeast phase occurred within 24 h. Although conversion was by no means complete in that amount of time, there were enough yeast present to determine that the organism was dimorphic (Fig. 1–4). Additional incubation time, from 3 to 5 days, will increase the number of yeast present (Fig. 5) and at least 3 days is necessary to establish a permanent yeast culture. This involves removing the growth

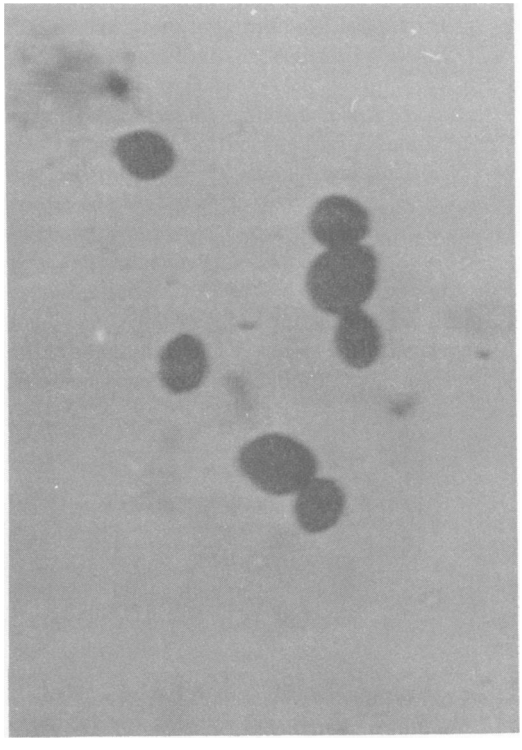

FIG. 2. *Budding yeast cells of H. capsulatum var. duboisii in tissue culture after 24 h at 37 C. ×340.*

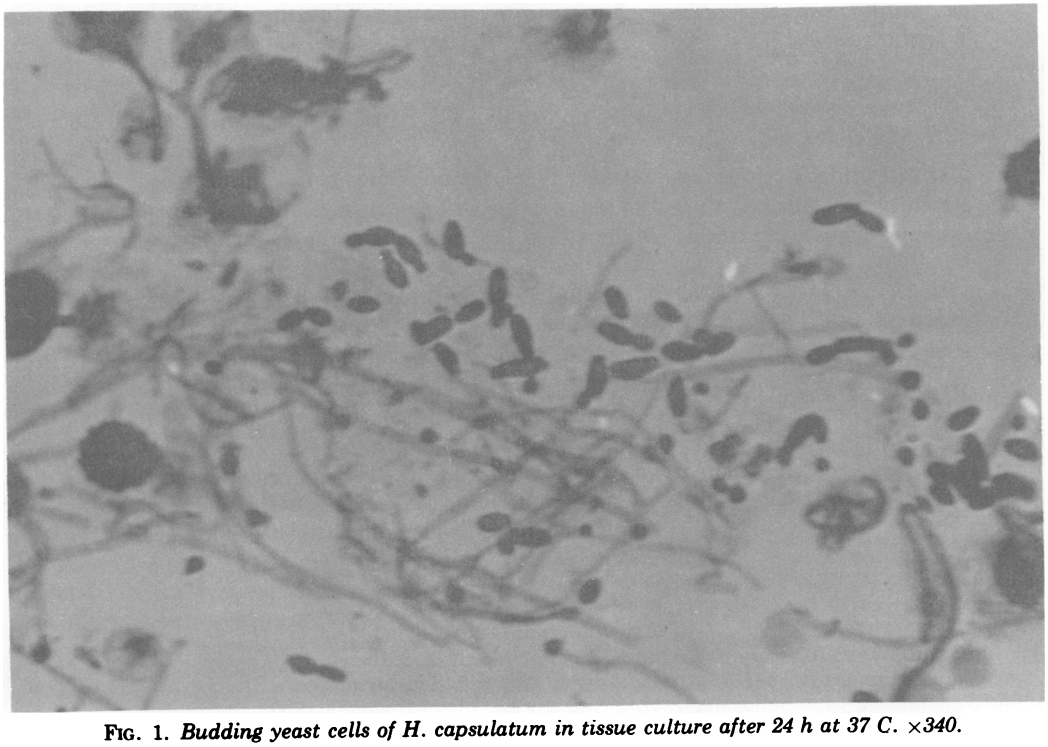

FIG. 1. *Budding yeast cells of H. capsulatum in tissue culture after 24 h at 37 C. ×340.*

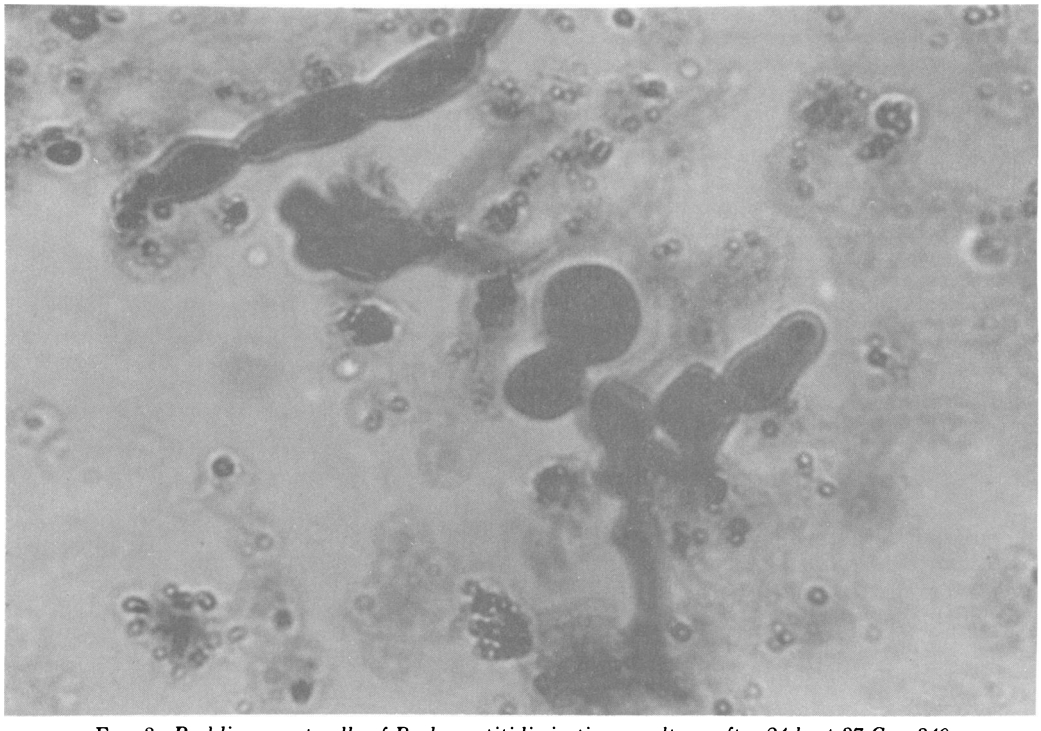

FIG. 3. *Budding yeast cells of B. dermatitidis in tissue culture after 24 h at 37 C. ×340.*

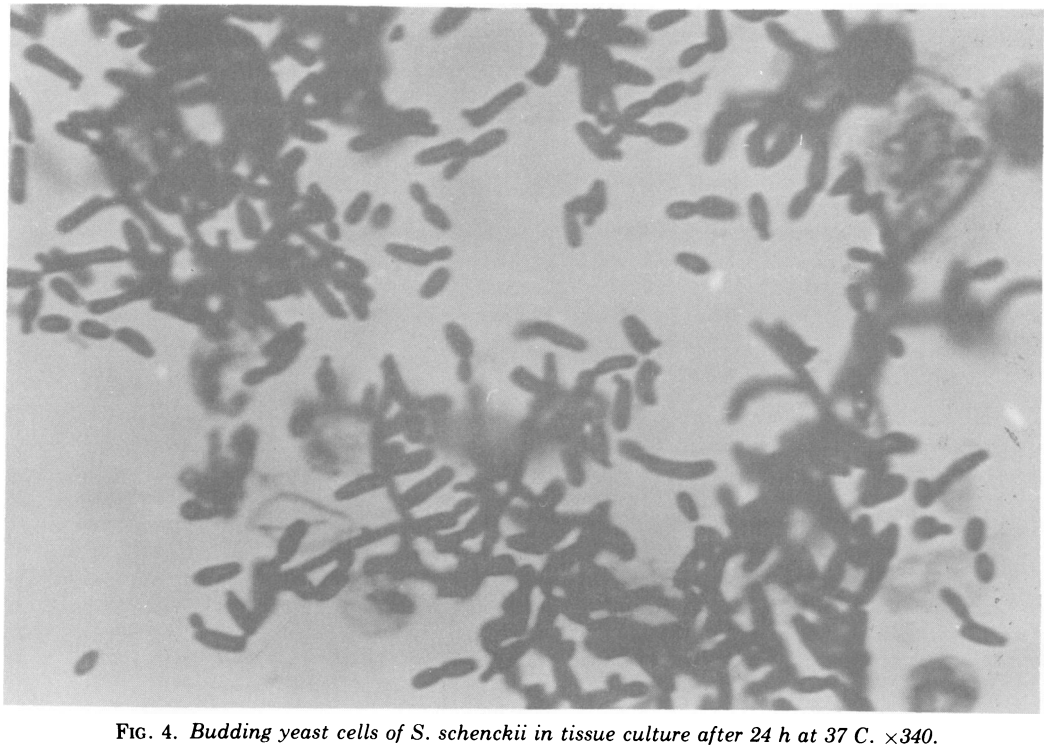

FIG. 4. *Budding yeast cells of S. schenckii in tissue culture after 24 h at 37 C. ×340.*

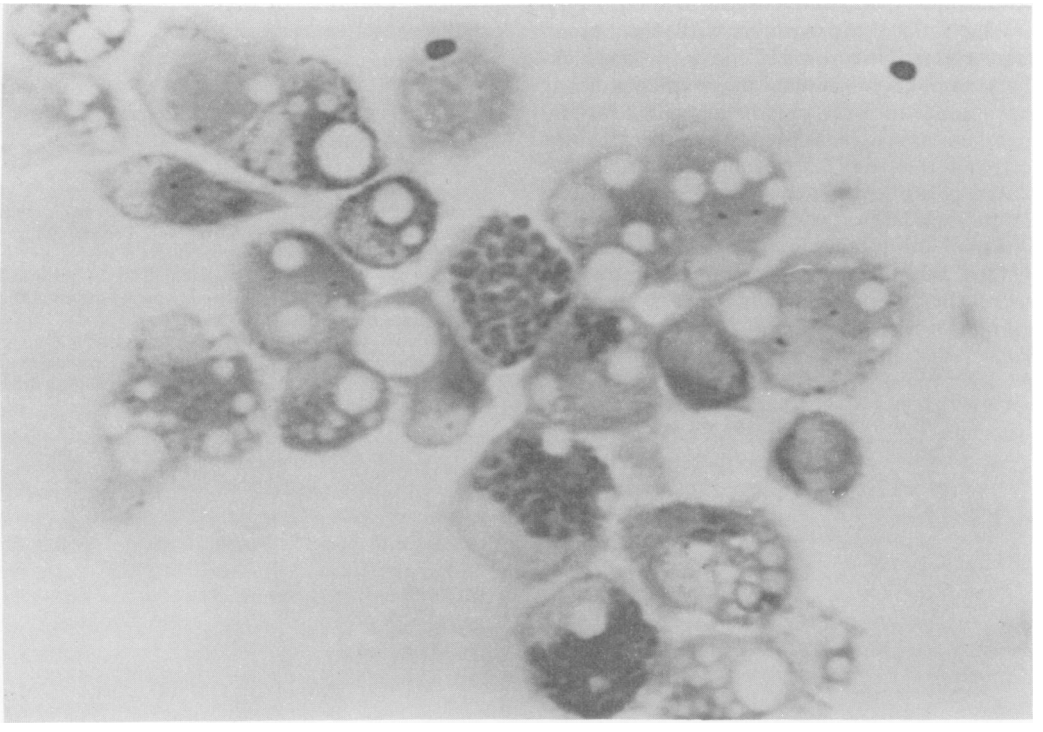

FIG. 5. *Budding yeast cells of H. capsulatum in tissue culture after 72 h at 37 C. ×340.*

media and monolayer and inoculating a slant of brain heart infusion agar with blood at 37 C. The yeast observed were budding and characteristic in size and shape. Mycelial fragments or spores would not be confused with yeast, since only budding cells were considered as yeast. In controls, where the tissue cultures were left uninoculated, there was nothing observed that resembled yeast. Also, tissue culture growth medium alone gave no conversion with any organism after 7 days incubation.

Other fungi including *Scopulariopsis* sp., *Aspergillus* sp., *Chrysosporium* sp., and *Monosporium* sp. were tested with this tissue culture method to determine if they might be confused with the yeast phase of the dimorphic fungi. In each case there was nothing present after 24 h incubation that resembled yeast. In fact, permanent slides were difficult to make, due to the large amount of growth consisting of sterile mycelium that would not adhere to the slide. Also, the macrophage monolayer was completely disrupted and most of the growth medium was lost.

Sepedonium sp. was also tested with this tissue culture method. In this case there was not a large amount of growth present and the monolayer was not completely disrupted after 24 h incubation. Germ tubes were formed from the large verrucose aleuriospores and there was a slight hyphal growth but there was nothing present resembling budding yeast after 24 h incubation at 37 C.

DISCUSSION

Conversion of the dimorphic pathogenic fungi from the mycelial to the yeast phase in tissue culture is not a new procedure, since Larsh et al. (3) had previously reported doing so with HeLa cells. However, their procedure was designed to give complete conversion and maintenance of the organism and hence considerably more time was required in tissue culture. Also, the cost of maintaining a continuous line of HeLa cells is impractical for most clinical laboratories. The method presented here is not expensive and provides a permanent slide of the yeast phase of the isolate for future reference.

Conversion is required with *B. dermatitidis*, and *S. schenckii* and often with *H. capsulatum* when growth is atypical or consists of sterile mycelium. Conversion is necessary to differentiate *H. capsulatum* from *H. capsulatum var. duboisii* since the difference in size of the yeast is the distinguishing characteristic. This tissue culture method can save much time in establishing a definite laboratory diagnosis over conventional methods of conversion in cases of

histoplasmosis, blastomycosis, and sporotrichosis. The total time required with the macrophage tissue culture is 7 days; 5 days for collection of the peritoneal macrophages, 1 day for preparation of the monolayer, and 1 day for conversion of the yeast phase. This time can be shortened to 2 days if a source of previously isolated peritoneal macrophage is available. An immunology laboratory that routinely tests for migration inhibitory factor would provide a constant supply of peritoneal macrophages. Hence, this method, which reduces the time required for identification of *H. capsulatum*, *B. dermatitidis*, and *S. schenckii* by conversion to the yeast phase from a minimum of 2 weeks (possibly several months) to 7 days, is inexpensive and practical.

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