

of Cartesian diver manostat (1,2), which maintains the system at any desired constant pressure. The extractor was found to work satisfactorily over extended periods when the pressure was adjusted to give an internal temperature of about 30° C.

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Microsporium gypseum and *Histoplasma capsulatum* Spores in Soil and Water¹

THE use of a membrane filter for recovery of bacteria from water has been described recently by Clark *et al.* (1). The method entails incubation of an exposed membrane on a sterile absorbent pad saturated with culture medium. We have found that, following exposure to liquids or gases, the membrane may be stained so as to render feasible direct microscopic examination for such relatively large structures as fungus spores and pollen. Such a procedure is, of course, effective only for those organisms producing distinctive and identifiable microscopic structures.

Following filtration of air, water, or other fluids, the membrane is removed to a Petri dish, and an adhesive, such as Haupt's gelatin, is added by dropper to the exposed surface area in order to prevent washing off the spores during the staining process. The membrane is then processed by a modification of the Hotchkiss-McManus (periodic acid-Schiff) technique (2), which stains hyaline fungus spores and mycelium a dark-red against a colorless background. The stained, moist membrane is mounted on a glass slide, under a cover glass, and examined under the microscope.

Application of this technique to aqueous soil extracts has resulted in the finding of numerous tuberculate spores typical of *Histoplasma capsulatum* (Fig. 1, A) in four of four soil samples from Williamson Co., Tenn., an area in which there is an extraordinarily high prevalence of histoplasmin sensitivity among the human population. All the samples tested had previously yielded *H. capsulatum* in culture by means of the mouse-inoculation test (3). That the tuberculate spores occur in soil had been demonstrated earlier by Emmons (4) in Virginia.

Also, by means of the filter method, a tuberculate spore morphologically identical with those obtained from soil and from artificial cultures of *H. capsulatum* has been recovered from a single 1-liter specimen of river water from Williamson Co. This is the first report of the natural occurrence of this organism in water.

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These results suggested the advisability of comparing the membrane filter method with the mouse-inoculation technique for effectiveness in the recovery of *H. capsulatum* from soil. Such a study is now under way.

From one of the above soils there were recovered, in addition, upon the membrane filter, several multi-septate spores typical in all respects of the macro-

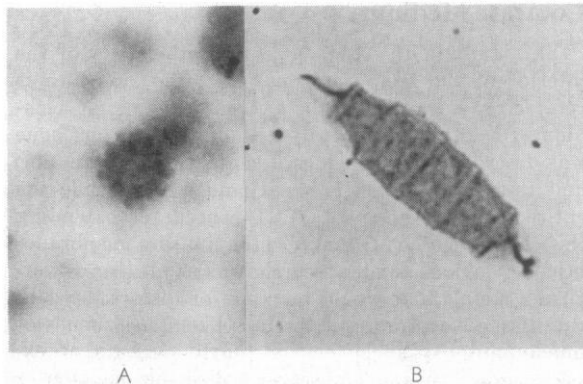


FIG. 1. A, tuberculate spore of *H. capsulatum* recovered from soil; stained on filter membrane. $\times 760$. B, macroconidium of *M. gypseum* recovered from water; stained on filter membrane. $\times 760$.

conidia of *Microsporium gypseum* (Fig. 1, B). Two strains of *M. gypseum* subsequently were isolated in pure culture from this soil specimen. This dermatophyte had been cultured previously by Mandels *et al.* (5) from wool cloth that had been buried in potted soil in an attempt to isolate keratinophilic fungi from the soil. Whether the isolate of *M. gypseum* had actually originated or was growing in the soil is not certain. According to information received from the laboratories in which the isolation was made,² the soil was composed of clay loam, sandy silt, humus, and manure, and had been used in indoor tests for several years. There is no record that the wool had been sterilized prior to burial.

We know of no report prior to the present one of the occurrence of macroconidia of *M. gypseum* in nature. Details of cultural procedures and of the use of filters in spore detection will be reported shortly.

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