Cultivation of Yeast in Earle's "L" Strain Mouse Cells *in vitro*.* (20425)

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In this communication, we shall describe the use of Earle's strain "L" mouse cells(1) as an adjunct to the experimental study of histoplasmosis. This type of cell has been shown by Scherer(2,3) to support the growth of several viruses. Histoplasmosis is an important disease in some areas, particularly in the southern part of the United States, but studies of the experimental disease, in our experience, are handicapped for want of a uniformly susceptible experimental animal. Furthermore, the effect of fungicidal agents in infected animals is difficult to interpret. In an effort to find more suitable animal material in which to study the disease, cultures of Earle's strain "L" cells growing on glass were inoculated with the yeast cell phase of *Histoplasma capsulatum* (H.C.). We have shown previously that H.C. develops intracellularly in tissue cultures of horse and chick tissues(4) but the method is laborious and not as suitable as the one to be described.

Experimental procedure. The animal cells used in this study consisted of Earle's strain "L" cells derived from a single cell(1), grown on glass in Carrel flasks. The medium, changed 3 times weekly, consisted of 1 ml of the following composition: 40% horse serum, 20% chick embryo extract (E_{50}), and 40% Earle's balanced salt solution (BSS). Cultures 8 to 10 days old were scraped with curved-tip pipettes and thoroughly distributed in the liquid phase. Two drops of the resulting cell suspension were placed on a 6 x 22 mm coverglass, allowed to stand for ½ hour at room temperature, and the coverglass was inserted into a sterile rubber-stoppered 16 x 150 mm pyrex test tube containing 1 ml of nutrient fluid. The tube was slanted at an angle sufficient to bathe the surface of the culture and incubated at 37°C. After 2-5 days' incubation, the tissue cells were growing luxuriantly on glass and were judged satisfactory for inoculation of yeast cells of a laboratory strain of H.C. (strain 603 Vanderbilt). The inoculum was prepared by emulsifying two loopsful of a 7-day-old culture in 5 ml of normal saline. The animal cells were inoculated by two different methods. A) The nutrient fluid from one set of tubes was aspirated and 2 drops of the yeast cell preparation were pipetted on the coverglass. After standing for ½ hour at 4°C, the coverglass was rinsed thoroughly with BSS and reinserted into the standard test tube containing nutrient fluid. B) Another set of tubes was inoculated by adding 2 drops of yeast cell suspension directly to the nutrient fluid. All tubes, irrespective of the method of inoculation, were incubated at 37°C and were examined daily for pH and microscopic changes. Fluid change was made 3 times weekly. All preparations were fixed in Zenker-acetic acid at irregular intervals from 2 to 13 days after inoculation and stained by the hematoxylin-eosin-azure method (H.E.A.). Suitable yeast-free controls were stained at the same intervals. It was of interest to determine if infected cells could be subcultured. Accordingly, a group of 5-day-old "L" strain preparations were inoculated with yeast by the first method and the same routine of inspection and change of nutrient fluid was carried out as before, except that prior to each change of nutrient fluid the cultures were thoroughly washed with BSS. Four days after inoculation a number of slides were scraped and the cells pooled and placed on glass in the manner previously described. The subcultures were stained by the H.E.A. method 2-7 days after being initiated.

Results. Intracellular yeasts were observed in the cells growing on glass (Fig. 1), regardless of the method of inoculation. The preparations in direct contact with yeast cells were more heavily infected than those inoculated into the nutrient fluid. It is to be noted that "L" cells containing only a few yeasts did not

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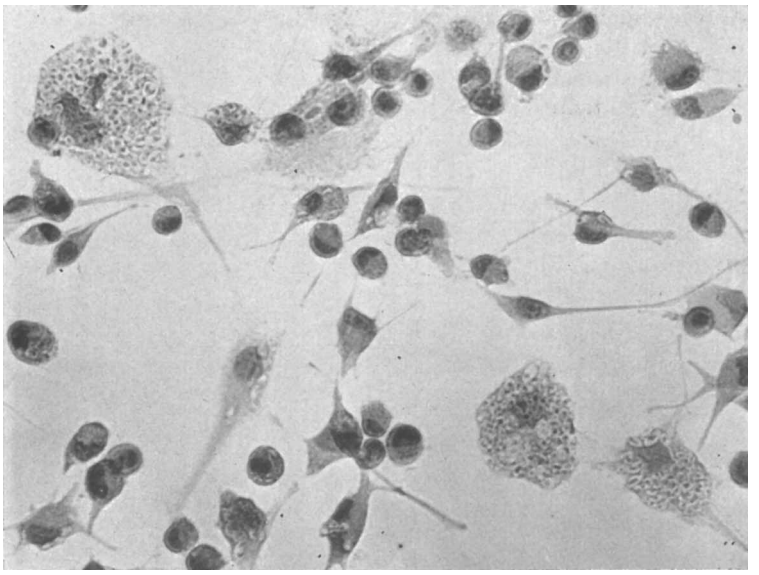

FIG. 1. Six-day-old culture of strain "L" mouse cells on glass inoculated with yeast cell phase of *Histoplasma capsulatum* on 2nd day. Stained with H.E.A. Note large cells filled with intracellular yeasts; other cells containing few yeasts are comparable in size to uninfected cells. × 300.

appear significantly larger than the cells of control cultures maintained under identical conditions. However, those containing large numbers of intracellular forms were much increased in size. A comparison of infected cultures of longer incubation periods with those maintained for only a few days showed that the older cultures were usually more heavily infected, though not invariably so. No attempt was made to estimate the relative number of infected cells. Infected cells generally showed little or no degeneration.

The cultures that were grown on glass, subsequently infected with H.C., and subcultured, became readily attached to the glass surfaces. The preparations stained at intervals of 2 to 7 days showed excellent growth of cells, many of which were parasitized.

Discussion. The mouse cells used in the investigation are extremely easy to cultivate and maintain and are easily infected with the yeast cell of *Histoplasma capsulatum*. This system may prove satisfactory in the cultivation of other pathogens, including fungi, and also provide a method of studying the effects of therapeutic agents both on the host cell and on intracellular pathogens.

Summary. It is shown that the yeast cell of *Histoplasma capsulatum* will develop intracellularly in Earle's strain "L" mouse cells cultivated on glass and that infected cells may be subcultured.

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