Histoplasma capsulatum in Tissue Culture.* (18708)

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In this laboratory certain tissues of the horse have been used in tissue culture to demonstrate infection with the virus of equine abortion(1). During the course of these studies, intracellular organisms identical in appearance with *Histoplasma capsulatum* were observed in certain horse tissues grown on film-coated cover-glass cultures. Details of the morphological study of this naturally infected horse tissue will be presented elsewhere(2).

Since no studies on growth of *H. capsulatum* (or other pathogenic fungi) in *in vitro* cultures of animal tissues have been reported, it seemed of interest to attempt the experimental cultivation of this fungus in tissue cultures of horse and chick embryo tissue. The results of this study are presented here.

Experimental Procedures. Horse tissues were aseptically obtained from a mare at 11 months gestation. Amnio-allantoic membrane and placenta were cultivated in flasks(3) and on cover-glasses placed in test tubes. Chick embryo tissue was cultivated by the latter method only. (The choice of the particular horse tissue used was determined by the findings in the naturally infected animal). Chick embryo extract (E.E.) and chicken plasma were prepared by conventional methods. The supernatant or liquid phase consisted of 50% human ascitic (cancer) fluid, 47% Hanks' balanced salt solution (B.S.S.), and 3% E.E., with the pH of the mixture adjusted to 7.6. Tissues from flask cultures were fixed in Zenker-acetic acid, and H and E stained paraffine sections prepared from them. Cover-glass cultures were fixed in Zenker-acetic acid, and stained by the hematoxylin-eosin-azure method.

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1. To be published.

2. To be published.

3. R. C. Parker, Methods of Tissue Culture, Paul B. Hoeber, Inc., 1950.

The following methods were used in preparing tissue cultures of horse amnio-allantoic membrane and placenta, and 12-day chick embryo muscle. The tissues were cut into 2 mm squares and immediately rinsed thoroughly with B.S.S. The rinsed fragments were then placed in 0.5 ml of B.S.S., to which was added 0.5 ml of a yeast cell suspension of *H. capsulatum* (Vanderbilt University strain No. 603) containing 30 million yeast cells per ml. The mixture of tissue fragments and organisms was then allowed to stand for two hours at 5°C. *Flask method.* Only horse tissues were used in this method. Two parallel sets of flasks were prepared, one series with amnio-allantoic membrane, and one with placental tissue. Three to four pieces of tissue, with a minimal amount of fluid, were transferred with a 5 ml pipette to a 50 ml Erlenmeyer flask containing 3 ml of supernatant, incubated at 37°C, and observed daily. Tissues were fixed for sectioning after 5 and 8 days of incubation. *Cover-glass method.* Three or 4 pieces of tissue were embedded in a plasma clot (equal parts of chicken plasma and E.E.) on a No. 1 12 x 50 mm cover-glass. This preparation was then introduced into a 16 x 150 mm pyrex test tube, and after standing for one hour, 2 ml of supernatant was added to each tube, and the cotton plug replaced with a rubber stopper. The cultures were slanted in a test tube basket at an angle which allowed the supernatant barely to cover the surface of the cover-glass, and incubated at 37°C. The following controls were prepared in the same manner: (1) tissue fragments without *H. capsulatum,* and (2) *H. capsulatum* without tissue fragments. All cultures were examined daily. The experiments dealing with placenta and amnio-allantoic membrane were terminated after five days of incubation; the chick embryo tissue experiments were terminated after 3, 4, 5 and 6 days of incubation.

Results. Flask method. Examination of H and E stained preparations of placenta and

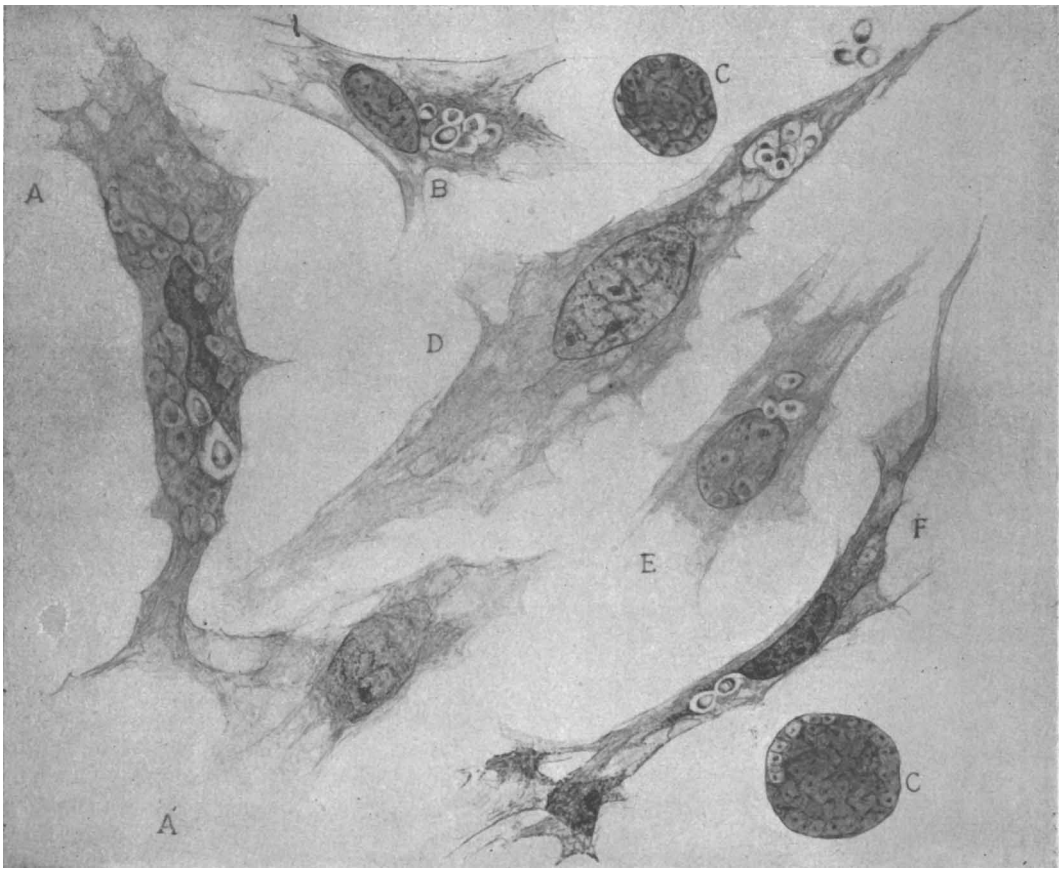

FIG. 1

Five-day-old cultures of 11-month-old horse placenta. A, B, E and F are fibroblast-like cells of the outgrowth containing yeast cells of *Histoplasma capsulatum*. A' is a normal cell included for comparison. C represents a highly diagrammatic sketch of the sac type of colony of *H. capsulatum*. (Photograph of camera lucida drawing; x1500.)

amnio-allantoic membrane revealed innumerable yeast cells of *H. capsulatum* multiplying within the interstices of the connective tissue. No intracellular yeast cells could be demonstrated unequivocally. The supernatant contained a heavy growth of yeast cells of the fungus; no mycelial development was noted.

Cover-glass method. In previous tissue culture experiments by the cover-glass method, with horse placenta and amnio-allantoic membrane of 6, 9 and 10 months gestation, it was found that the placental tissue produced only a scanty outgrowth of cells, whereas the amnio-allantois gave striking growth. In the present experiment with 11-month-old horse placenta, both in the presence and in the absence of *H. capsulatum,* the amount of outgrowth was commensurate with that obtained with younger placentas, and in this instance resembled fibroblasts. With 11-month-old amnio-allantoic membrane, however, no cells grew or migrated from any of the tissue fragments. Stained preparations of both chick embryo tissue and horse placenta, regardless of the age of the culture, revealed scattered fibroblasts or fibroblast-like cells with few to many intracellular yeasts (Fig. 1 and 2). The infected cells appeared without stigmata of degeneration.

Of particular interest in these cover-glass preparations was the striking form assumed by many of the colonies of *H. capsulatum* growing in the plasma clot. Scattered throughout the clot were numerous globose sacs con-

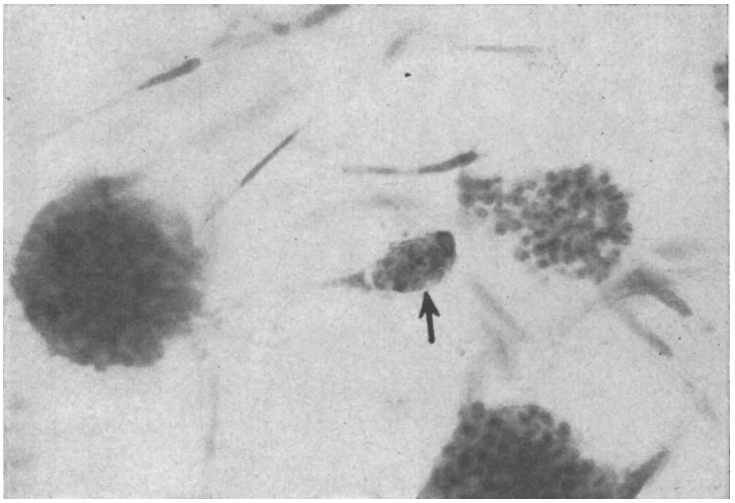

FIG. 2.
Five-day-old culture of 12-day-old chick embryo leg muscle. Intracellular yeast cell forms are indicated by an arrow. Note the compression of the cell nucleus. The large, dark-staining masses are the sac type of colony of *H. capsulatum* (see text and Fig. 3). Many cells of the explant are out of focus due to the thickness of the preparation. x800.

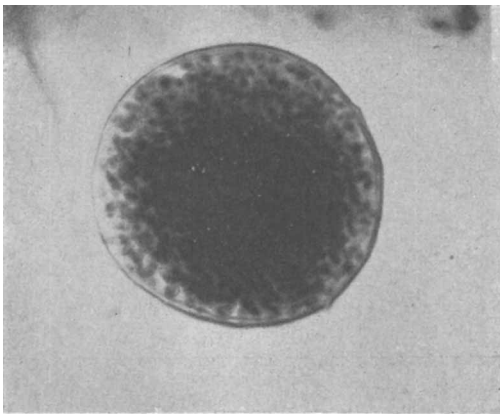

FIG. 3.
Sac type of colony of *H. capsulatum*, with yeast cells enclosed within a well-marked membrane. From a 5-day-old culture of 11-month-old horse placenta. x 400.

taining myriads of encapsulated yeast cells surrounded by a well-defined, sharply-delimiting membrane (Fig. 3). These sacs, which bore a striking resemblance to the *in vivo* form of *Coccidioides immitis*, varied in size from 20 to 300 μ in diameter. Further simulation of the sporangia of *C. immitis* was seen in sacs which had burst, allowing their contents to scatter into the surrounding plasma (Fig. 2). Under these circumstances, the individual, characteristic, budding yeasts of *H. capsulatum* could be readily observed. Not all of the colonies of the fungus assumed this bizarre form; many were the usual flatly-convex colony, with no delimiting membrane. The sac type of colony was far more abundant in the cover-glass cultures containing tissue fragments than in the cultures lacking tissues. Growth of *H. capsulatum* in the mycelial form was negligible in the former type of preparation, marked in the latter. In neither type of preparation, however, was there any growth of organisms in the supernatant.

Discussion and conclusions. A study of chick embryo and horse placental tissue, when cultivated in tissue culture with *H. capsulatum* by the cover-glass method, has revealed that fibroblasts or fibroblast-like cells of the outgrowth contain yeast cells of this organism in the cytoplasm. It is of interest to note that sections of infected tissues from systemic histoplasmosis rarely, if ever, show yeast cells in fibroblasts. Whether or not these intracellular yeasts can continue to multiply within the fibroblast, or are ultimately destroyed, remains to be determined. Similarly, whether or not the fibroblast which contains yeast cells can divide further, or undergoes degeneration, is not yet known. An explanation for the unique type of colonial morphology of

H. capsulatum seen in the cover-glass cultures, cannot be given at this time. This phenomenon is not dependent upon the presence of living tissues, since the sac-like type of colonies were observed in the controls which lacked tissue fragments. Of possible significance is the fact that this type of colony occurred only when the inoculated yeast cells were fixed *in situ* and surrounded by clotted plasma; in the supernatant of the flask cultures, only free, individual yeast cells were seen. Further work is in progress in an attempt to clarify these problems.

ADDENDUM. Since submitting this paper for publication an article by O. H. Duque has come to our attention. Writing in *Bol. Clin., Univ. Antioquis,* 1937, v9, 312, he reports the results of the cultivation of several pathogenic fungi with fibroblasts grown *in vitro*. The only positive findings concerned a culture inoculated with the mycelial phase of *H. capsulatum* which developed intracellular bodies interpreted as spores.

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Effects of Cortisone and Adrenocorticotrophic Hormone on Wound Healing in Normal and Scorbutic Guinea Pigs. (18709)

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Evidence suggests that ascorbic acid may influence the secretory activity of the adrenal cortex(1,2). However, the rôle of this vitamin in adrenal metabolism is still obscure. A possible mode of action was indicated by Lowenstein and Zwemer(3), who isolated an ascorbic acid-adrenal corticosteroid conjugate. Their work is yet to be confirmed. Symptoms of adrenocortical insufficiency resemble those of scurvy(4). Both conditions are characterized by hypersensitivity to stress(5) and may be favorably modified by adrenocortical hormone or by ACTH(4,6-9). Furthermore, increased utilization or destruction of ascorbic acid has been observed in stress(10). Recent investigations have demonstrated that adrenal cortical hormone may inhibit the growth of connective tissue, thus simulating, in some respects, the pathologic changes seen in scurvy(11-13). Moreover, like ACTH and Cortisone, vit. C in massive doses(14) or in conjunction with desoxycorticosterone(15,16)

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