of an artificial nature. Several recent reviews on the subject are available(12,13). Under conditions approximating as nearly as possible the *in vivo* situation, the spindle was photographed in an uninjured condition in living material, and the cells were seen to complete division. It is assumed that spindle formation is not an artifact produced by pressure, since colchicine-pretreated cells observed under similar conditions did not have spindles.

Rosza and Wyckoff(14) showed that in acid- or Flemming-fixed material the interzonal fibers were apparent in onion root tip, whereas they were absent in neutral formalin-fixed material. On the basis of our observations their findings on acid-fixed material may be considered "realfacts"(15) and the dissolution of the fibers by neutral formalin may be considered as an artifact. For the same reason the electron micrograph of the spindle prepared by Beams *et al.*(16) may be considered to be a "realfact" and not an artifact. Since we have observed and photographed the centriole, spindle fibers, and interzonal fibers, in living material, we conclude that a spindle in fixed tissue is a "realfact."

Summary. The observation that exposure to X irradiation causes partial dissolution of cell spindles and that chromosomes in the destroyed area become pycnotic led to this study of the living spindle. Photographs of grasshopper testis, living cells that have been immersed in Belar solution show spindle fibers and the attachment of some of these fibers from the centrosome to the chromosome. The reality of the spindle in fixed tissue, as revealed by phase contrast microscopy, is emphasized as a result of these studies. Prior to this experiment, Schmitt and Belar were also able to show the birefringence of spindle fibers.

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Detection by Tissue Culture of an Organism Resembling *Histoplasma capsulatum* in an Apparently Healthy Horse.* (19099)

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(Introduced by Roy C. Avery.)

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In this laboratory we have been concerned with the cultivation of the virus of equine abortion (E.V.A.) in certain tissues of the horse maintained *in vitro*(1). During the course of the investigation, intracellular organisms, resembling *Histoplasma capsulatum*, were observed in supposedly healthy horse tissue grown in tissue culture. As a result of these findings, Randall and McVickar(2) demonstrated the experimental development of *H. capsulatum* in tissue culture.

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1. To be published.

2. Randall, C. C., and McVickar, D. L., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 150.

The present paper deals with the detection by tissue culture methods of an intracellular parasite apparently indistinguishable from *H. capsulatum*. The observation is of interest, because the method is unique from the standpoint of diagnosis and histoplasmosis is a rarity in the horse, only one (unillustrated) case report(3) being found in the literature. It should be noted that the present paper is an outgrowth of the above problem(1), and only the pertinent details will be given here.

Experimental procedures. Horse tissues were obtained in the following manner: an apparently healthy 5-year-old mare of about 6 months gestation was instantaneously killed by a shot through the head; adult and fetal tissues were obtained with scrupulous attention to aseptic technic and sterile instruments were changed for each tissue. Portions of fetal lung, liver, spleen, heart; adult lung, liver, and spleen; that portion of the placenta with central mesoderm bordered by allantois on one side and chorion on the other and the amnio-allantoic membrane were taken for tissue culture. Representative portions were placed in Zenker-acetic acid for control studies. For reference, samples of each tissue were stored in a deep freeze at −40°C. All organs, fetal and adult, appeared normal grossly. All tissues were cultivated on cover-glasses placed in test tubes, and in addition, fetal spleen, lung, and liver were cultivated by the flask method(4). 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., containing 500 units of penicillin per ml. The pH of the mixture was adjusted to 7.6. Cover-glass cultures were fixed in Zenker-acetic acid and either stained *in situ* by the hematoxylin-eosin-azure method (H.E.A.), or were cut out of the clot and routine H. and E. stained paraffin sections were prepared. The ingredients of the clot and supernatant were cultured on blood agar and Brewer's thioglycollate medium for 10 days at 37°C without growth. As a number of the cultures were inoculated with E.V.A., the preparation of this material is mentioned, so as to eliminate a possible source of contamination. Virus containing material was centrifuged for 1 and ½ hours at 13,500 rpm at 4°C and 0.5 to 2 ml aliquots were cultured on the above media for 2 weeks. The technic was such as to preclude the likelihood of *H. capsulatum* being a contaminant.

The following methods were used in preparing cultures of the various adult and fetal tissues. The tissues were cut in 2 mm squares and immediately rinsed thoroughly with B.S.S. (A) *Flask method.* Three parallel sets of flasks were prepared with fetal lung, spleen, and liver, respectively. Four to 8 pieces of tissue in a minimal amount of fluid, were transferred in a 5 ml pipette to a 50 ml Erlenmeyer flask containing 3 ml of supernatant, incubated at 37°C, and observed daily. A certain number of cultures were inoculated with 0.03 ml of the virus suspension within the first 24 hours of incubation. Tissues were fixed for sectioning after 5 and 8 days of incubation. (B) *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. one 12 x 50 mm cover-glass. The 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 was replaced by a sterile 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 cultures were observed daily for bacterial contamination and pH change. The clear supernatant was changed once weekly. At the end of the 14 days growth period, about ½ of each of the various sets of tissues was inoculated with the virus suspension, with the remainder considered as controls. Cultures were fixed and stained by H.E.A. method consecutively for 5 days, when the experiment was terminated. On the last day several controls and inoculated cultures of each tissue were also cut

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

out of the plasma clot, fixed in Zenker-acetic acid and H. and E. paraffin sections were prepared.

Results. (A) *Flask method.* Thorough examination of the numerous H. and E. stained sections failed to demonstrate any evidence of infection.

(B) *Cover-glass method.* The numerous explants of adult horse spleen stained by the H.E.A. method showed fair outgrowth of fibroblast-like cells. In 2 such 19-day-old spleen cultures, one inoculated with E.V.A., and one a control, a very rare cell contained intracellular organisms identical morphologically with the yeast cells of *Histoplasma capsulatum.* Numerous preparations of the other adult and fetal tissues appeared free of such forms. The results obtained from another group of cultures grown on film coated coverslips and subsequently cut from the clot, were most interesting. A study of two 19-day-old control cultures, one of adult spleen and one of amnio-allantoic membrane, revealed that the majority of cells contained many intracellular forms, identical with those described above (Fig. 1). Irrespective of the preparation, none of the infected cells showed any stigmata of degeneration.

When the intracellular forms were first noted in stained preparations the tissues that had been stored for 6 weeks in the deep freeze (with the exception of placental tissue) were ground with mortar and pestle and cultured on plasma agar at 37°C and room temperature. No growth occurred. Unfortunately all the tissue culture preparations had been fixed and stained. Attempts to demonstrate by histological study that the organism was present in the tissues removed at autopsy, also met with failure.

Discussion. A study of adult horse spleen and amnio-allantoic membrane cultivated by the cover-glass method, has revealed that many cells of the explant contained yeast cells in the cytoplasm apparently morphologically identical with *H. capsulatum.* Control H. and E. stained paraffin sections of autopsy material appeared free of infection. This fact, coupled with the demonstration that numerous cells of tissue culture material contained intracellular forms, seems to indicate that multiplication of such magnitude took place so as to make their detection possible by microscopic study. The organism was found only intracellularly, and the infected cells appeared healthy.

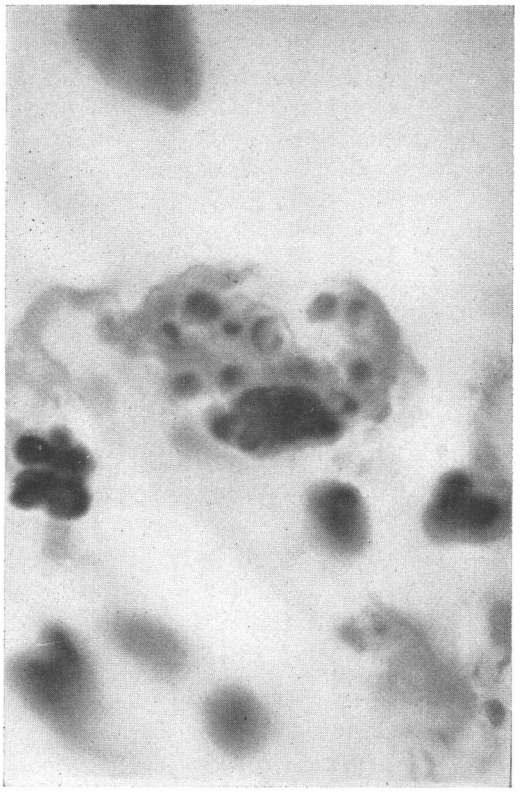

FIG. 1. Nineteen-day-old culture of horse amnio-allantoic membrane. Note the large cell filled with intracellular, apparently encapsulated forms, resembling intracellular yeast cells of *Histoplasma capsulatum.* Many of the cells of the explant are out of focus due to the thickness of the preparation. × 1425.

To our knowledge tissue culture methods have not, heretofore, been used as a method of detecting fungus infections. Histoplasmosis itself is apparently rare in the horse, and the demonstration of intracellular forms in the cells of the amnio-allantoic membrane suggests that placental transfer of *H. capsulatum* is possible.

Mention should be made of a disease of horses and mules known variously as farciminosus, epizootic lymphangitis or pseudofarcy, the causative agent of which is *Cryptococcus farciminosus.* A few doubtful cases have been reported in the United States, but

most of these, if not all, were cases of sporotrichosis(5). The clinical signs are characteristic and lack of them would tend to eliminate this possibility in the case in question. Da Rocha Lima(6) first made the interesting observation that the parasitic yeast cells of *Cryptococcus farciminosus* and *H. capsulatum* are very similar. Comparison of the two fungi in tissue culture might establish significant differences.

Summary. Intracellular organisms resembling *Histoplasma capsulatum* have been observed in apparently normal amnio-allantoic membrane and adult horse spleen maintained in tissue culture.

The significance of this finding and comparison with another fungus *Cryptococcus farciminosus* is discussed.

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Improvement of Protein Quality of Whole Wheat.* (19100)

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In the course of experiments designed to study the suitability of shredded wheat materials in low sodium diets some interesting observations on the improvement of the biological value of the protein of whole wheat have been made.

Experimental. The biological value of whole wheat products was assayed by the growth response of weanling albino rats fed a standard diet of the following composition: Wheat products 86%, corn oil 8%, cod liver oil 2%, salt mixture(1) 4%, choline 0.1% and contained per kilo of diet, thiamine 4 mg, riboflavin 8 mg, nicotinic acid 40 mg, calcium pantothenate 40 mg, pyridoxine 4 mg, biotin 1 mg, folic acid 0.5 mg, α-tocopherol 40 mg, and menadione (vit. K) 4 mg. On this diet the only variable element was the protein component (wheat product). Five groups of eight weanling albino rats, comparable as to weight, were put on the following diets: Control group: whole wheat; assay groups: whole wheat cooked; whole wheat cooked and fan dried; whole wheat cooked, fan dried, shredded, baked and oven-dried—commonly known as "shredded wheat"; comparison group: white flour. All of these wheat preparations were fed at a level of nitrogen such that they furnished 8.6% protein. Slight variations in the per cent of wheat product in the diet as a result of varying nitrogen content were equalized by the addition of the proper percentage of glucose. The rats were housed in individual cages and fed the above diets and water *ad libitum* for a period of five weeks. Records were kept of the daily food intake and the animals were weighed twice a week. The essential details of the heat treatment of the whole wheat are as follows: cooked, the whole wheat is cooked in boiling water for 38 minutes; fan dried, the cooked product is dried in a rapidly moving current of air at a tempera-

TABLE I. Average Gain of Wt of Weanling Rats Fed Wheat Products at Various Stages of Processing.

Basal diet with the following sources of protein, all fed at a level of nitrogen to give 8.6% protein	Avg gain in wt, g
Whole wheat	15.4
" " cooked	40.8
" " " and fan dried	24.8
" " " " " " shredded, baked and oven dried = shredded wheat	30.5
White flour	10.4

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