PHAGOCYTOSIS OF YEAST CELLS *IN VITRO**

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Recently, during the examination of a "lupus cell" (LE) smear,† several intracellular yeast-like elements were observed within monocytic cells in a circumscribed portion of the preparation. At first glance, the intracellular yeast cells were mistaken for *Histoplasma capsulatum.* Thorough investigation of the two persons whose blood had been used in making the preparation yielded no evidence to indicate that either one had active histoplasmosis or logically could be considered a carrier of the organism in his peripheral blood. In view of this and the observations to be reported, it would appear that the intracellular yeast cells represented saprophytic organisms.

Histoplasma capsulatum is a yeast-like organism with slightly oval shape which measures 1 to 5 μ. It can be demonstrated occasionally in mononuclear cells and neutrophils in peripheral blood or bone marrow smears.[2] This is considered to be a highly suggestive observation and is accepted by many as a diagnostic feature of histoplasmosis. Most authorities agree, however, that successful cultural isolation of the Histoplasma is a preferable method of establishing the diagnosis.[2]

Since the chance occurrence cited above may mislead the casual observer, it is considered desirable to describe the general features of phagocytosis of yeast-like organisms *in vitro.*

MATERIAL AND METHODS

The organisms used were *Hansenula anomala, Saccharomyces carlsbergensis* (ATCC 9080), *Candida albicans* No. 1, *Candida albicans* No. 2, *Cryptococcus neoformans* (ATCC 10226), *Cryptococcus neoformans* (pigeon nest strain 48), *Cryptococcus neoformans,* variety innocuous, *Histoplasma capsulatum, Histoplasma duboisii Vanbreuseghem, Blastomyces dermatitidis,* and *Blastomyces brasiliensis. Histoplasma capsulatum, Blastomyces dermatitidis* and *Blastomyces brasiliensis* were grown in the yeast phase on Kurung's egg medium at 37° C.[3] All other organisms were cultured on Sabouraud's dextrose broth and kept at room temperature.

For direct observation of phagocytosis, oxalated blood was kept

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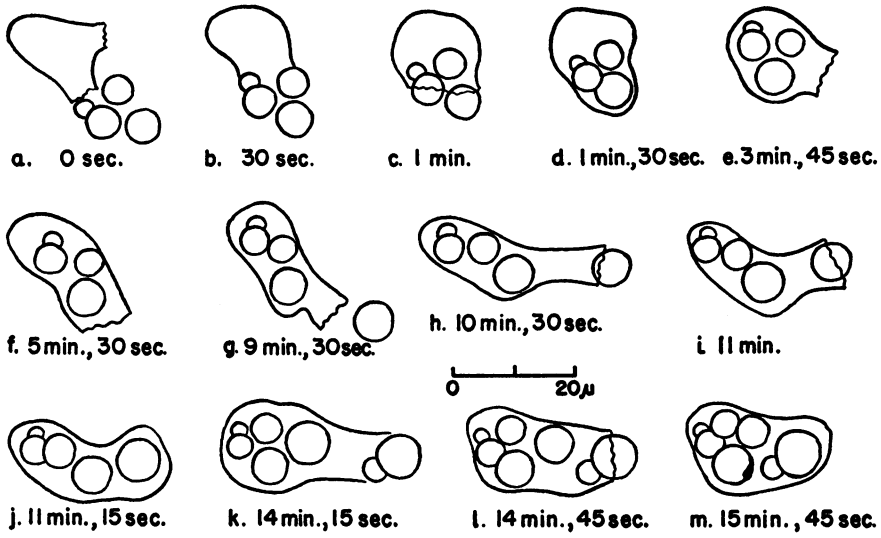
† The smear was prepared according to the "Snapper ring technique" for detecting the "LE factor" in patients with systemic lupus erythematosus.[1]

until the erythrocytes had settled, at which time a few loopfuls of the buffy coat were placed on a clean slide. A loopful of one of the cultures listed above was mixed with the suspension of leukocytes on the slide. A cover slip, elevated by applying Vaseline® around its edges, was then placed over each mixture, and the preparation was examined microscopically.

For permanent smears, the best preparations were obtained by mixing either the oxalated whole blood or the white cell layer with a loopful of one of the cultures, allowing 30 minutes for "incubation" at room temperature. A loopful of the leukocyte-yeast mixture was then spread on the slide and stained with Wright's stain.

Results

In wet preparations, phagocytosis was readily observed after the culture and white cells were mixed. Pseudopods projected from neutrophils, one or more at a time, and the cell moved in an ameboid fashion. As soon as the pseudopod came into contact with a yeast cell, the organism was engulfed by the flowing cytoplasm. Usually this process took 30 to 60 seconds. Examples of phagocytosis of yeast



Text-figure 1. *In vitro* phagocytosis of *C. albicans* No. 1. Drawn from wet preparation with the aid of the camera lucida.

forms are shown in Text-figure 1, in which various stages of ingestion of *Candida albicans* by a leukocyte are illustrated. Within one minute the leukocyte engulfed a cluster of 4 yeast cells simultaneously. (Text-figure 1, a to d.) Thereafter, pseudopods extended toward a nearby

yeast cell which was in turn engulfed a minute later (Text-figure 1, e to j). Figures a, k, l, m (Text-fig. 1) show the leukocyte ingesting a budding yeast form within a 90-second period. Thus, 7 yeast cells were completely engulfed in 15 minutes and 45 seconds.

A single leukocyte often ingested as many as 10 yeast cells in short periods of time if the concentration was a heavy one. This was the case with all the organisms tested. Occasionally the leukocyte came into contact with one portion of a pseudomycelium and moved along it in one direction.

Phagocytosis was observed in freshly drawn blood and in blood which had been kept for 3 to 5 hours at room temperature. Leukocytes in some samples appeared to be more active than in others. Neutrophils, monocytes, as well as eosinophils, demonstrated this property.

The yeast-like organisms found in the original LE preparation are shown in Figure 1. These cells were oval and measured from 1.5 to 2.8 by 2.8 to 5.5 μ. In some, darkly staining, crescent-shaped chromatin substance was encountered at one end; others were poorly stained and appeared devoid of chromatin. Many contained pink-staining cytoplasm with or without a dark-staining spot. Table I summarizes the histologic features of the organisms in their intracellular location.

TABLE I

Phagocytosis of Fungi: Intracellular Appearance

Organism	Appearance	Measurements (μ)
Hansenula anomala	Oval, oblong, cylindrical	3.6×2 (3 to 5.5) × (2 to 3.6)
Saccharomyces carlsbergensis	Oval, oblong, cylindrical	5.4×3.6 (3.6 to 7.2) × (2.7 to 4)
Candida albicans No. 1	Oval, spherical	4.5×3.6 (2.7 to 7) × (2 to 3.6)
Candida albicans No. 2	Oval, spherical	3.6×2.7 (2.7 to 4.5) × (1.8 to 3.6)
Cryptococcus neoformans No. 48	Spherical, oblong	5 (3.6 to 6) in diameter
Histoplasma capsulatum	Oval, oblong	3.5×2.8 (2.8 to 4.5) × (2 to 3)
Histoplasma duboisii	Oval, oblong, spherical	4×3.6 (3.4 to 6.4) × (2 to 3.6) 2 to 6.5 in diameter
Blastomyces dermatitidis	Spherical, oblong	6.4 to 9 in diameter
Blastomyces brasiliensis	Oblong, oval, spherical	(3 to 9) × (2 to 7)

Figures 2 to 12 illustrate some of the phenomena observed *in vitro*. The appearance of phagocytized *H. capsulatum* was similar to that observed in blood smears of patients with clinical histoplasmosis. The cells were oval in configuration and contained densely stained chromatin at one end. Most of the engulfed cells measured 2.8 by 3.5 μ (Table I).

The structural characteristics of most of the intracellular forms of *Hansenula anomala* (Fig. 3), *Candida albicans* (Fig. 6), small *Saccharomyces carlsbergensis* (Fig. 4), *Histoplasma duboisii* (Fig. 7), and *Blastomyces brasiliensis* (Fig. 8) were very similar to those of *Histoplasma capsulatum* (Fig. 2). Large oblong and cylindrical forms of Hansenula and Saccharomyces (Fig. 5) were, however, readily distinguishable. *Histoplasma duboisii* was often characterized by a mixture of small and large spherical cells, a feature which served to differentiate this organism from *H. capsulatum*. Although the small forms of *B. brasiliensis* (Fig. 8) resembled Histoplasma, there were often larger forms (9 μ; Fig. 9).

Cryptococcus neoformans in the phagocytized state was usually spherical but occasionally assumed an oblong shape. Some of the organisms were darkly stained, or contained dense chromatin substance at one end (Fig. 10); most of them, however, were faintly stained and were characterized by broad capsules (Fig. 11). *Cryptococcus neoformans,* var. innocuous (Fig. 11) resembled *C. neoformans* in all respects. *Blastomyces dermatitidis* ordinarily exhibited characteristic features. The organism was large, measuring 6.4 to 9 μ in diameter, and was possessed of a darkly stained cell wall (Fig. 12). The cell membranes of the large forms of *B. brasiliensis* were thinner and occasionally exhibited multiple bud production (Fig. 9).

Discussion

Our observations indicate that the phagocytosis of yeast cells by leukocytes can be demonstrated readily *in vitro*. An outstanding feature, however, is the resemblance of many saprophytic yeast cells to *Histoplasma capsulatum*. This possible source of error certainly warrants consideration when blood smears or bone marrow or buffy coat preparations are used in efforts to establish the diagnosis of histoplasmosis. The requisites of sterile instruments, clean slides, and carefully washed skin are obligatory. In the instance provoking this investigation, in which yeast cells were observed within leukocytes in an "LE smear," it was apparent that the spores were contaminants on the slide at the time blood was deposited. In one preparation these organisms were indistinguishable from Hansenula, Saccharomyces or Candida. The size, contour, and staining properties of the yeast cells encountered under these circumstances should be carefully evaluated before a diagnosis of histoplasmosis is rendered on morphologic features alone. Of some interest is the narrow clear space or "halo" surrounding many of the organisms when in intracellular location. Milne[4] investigated the structure and cytochemistry of *H. capsulatum* and

concluded that this was not truly an indication of encapsulation. He observed that the "halo" appeared only in those instances in which alcohol was applied for purposes of staining or dehydration. In the present studies, Wright's stain was used; this, of course, is a methyl alcohol solution.

Conclusions

1. In the course of examining an "LE smear," yeast cells resembling *Histoplasma capsulatum* were encountered within leukocytes.

2. Phagocytosis of yeast cells by leukocytes was shown to occur in oxalated blood ***in vitro.***

3. The structural characteristics of phagocytized *H. anomala, C. albicans, S. carlsbergenis, H. duboisii,* and *B. brasiliensis* were similar to those of *H. capsulatum.*

4. Under the conditions utilized, *H. anomala, S. carlsbergensis, C. neoformans, C. neoformans,* var. innocuous, *B. dermatitidis, B. brasiliensis,* though readily engulfed by leukocytes, could be differentiated from *H. capsulatum* only if a large number of leukocytes containing engulfed organisms were examined. It was difficult to distinguish *H. anomala* from *S. carlsbergensis,* and *C. neoformans* from *C. neoformans,* var. innocuous.

5. Cleanliness and even sterility of instruments and glassware is an essential to the preparation of blood or marrow preparations to be used for the diagnosis of histoplasmosis.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

FIGS. 1 to 12. Various phagocytized organisms in leukocytes. All preparations are stained with Wright's stain. × 1,400.

FIG. 1. "LE smear," showing intracellular yeast-like organism.

FIG. 2. *Histoplasma capsulatum.*

FIG. 3. *Hansenula anomala.*

FIGS. 4 and 5. *Saccharomyces carlsbergensis.*

FIG. 6. *Candida albicans* No. 1.

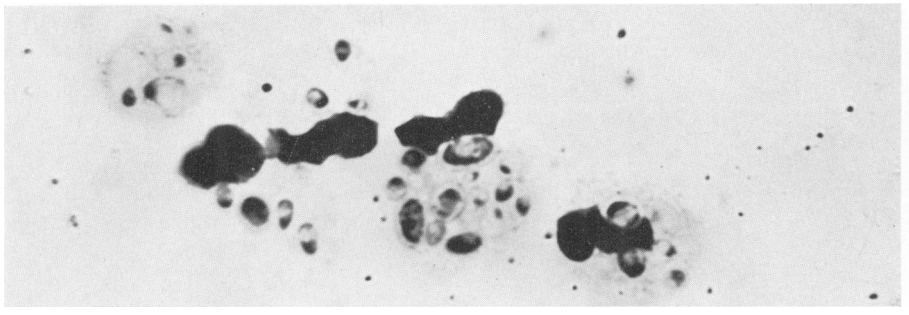
FIG. 7. *Histoplasma duboisii.*

FIGS. 8 and 9. *Blastomyces brasiliensis.*

FIG. 10. *Cryptococcus neoformans.*

FIG. 11. *Cryptococcus neoformans,* var. innocuous.

FIG. 12. *Blastomyces dermatitidis.*



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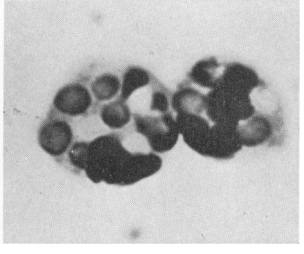

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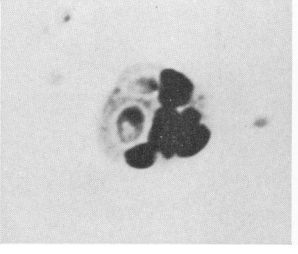

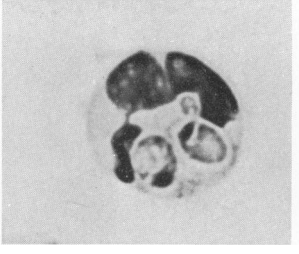

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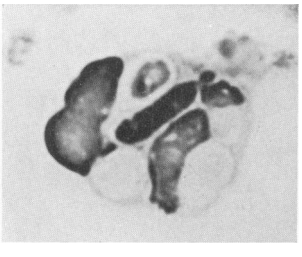

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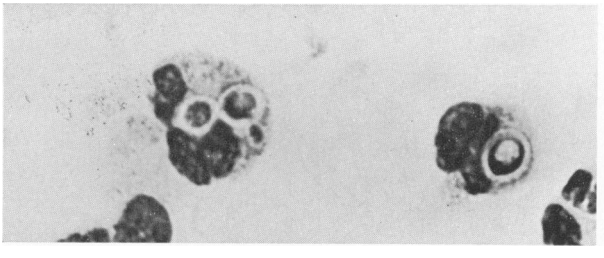

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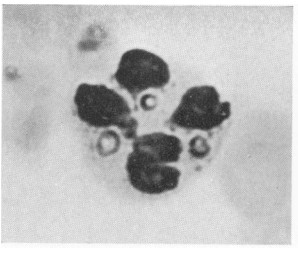

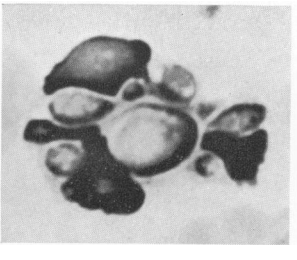

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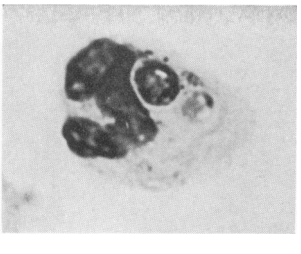

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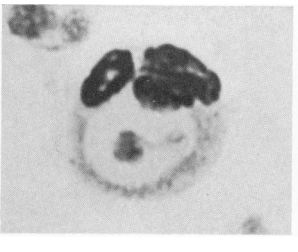

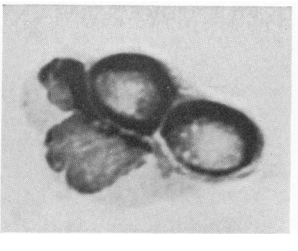

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