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Size of the Spores of *Histoplasma capsulatum*. (19937)

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Cozad and Furcolow(1) recently reported the results of their measurements of a large number of spores from several strains of *Histoplasma capsulatum*. These authors reported that most of the spores were small and nontuberculated. Since these findings were contrary to the usual concepts, it appeared worthwhile to make an independent check of their observations.

Materials and methods. Cultures of 5 of the isolates of *Histoplasma* (Traub, Spitzli, Guest, Perdue, and White) studied by Cozad and Furcolow were obtained, and spore counts of each strain were made. The isolates were grown in screw-top test tubes on potato dextrose agar slants. They were incubated at room temperature for approximately 6 months. In each instance the entire culture was scraped from the slant and was macerated by grinding in a sterile tissue grinder. Microslide preparations were made from each of the suspensions using lactophenol without the dye. Semi-permanent mounts were made by ringing the coverslips with a preparation of paraffin and vaseline. Microscopical observations were made with the high power oil immersion lens. Three traverse sweeps across each slide preparation were followed with no attempt to select areas, but all of the spores were counted as they appeared in the fields of observation. Therefore, differences in technics of Cozad and Furcolow's studies as compared to ours were as follows: 1) The cultures were approximately 6 months old when studied in our laboratory; their measurements were made on 1 and 5 months old cultures. 2) Our measurements were made with a 95x high power oil immersion lens (approximate magnification 1200 diameters); theirs were made with a 44x high dry lens (approximate magnification 500 diameters). 3) In our studies the entire culture was macerated by grinding in a sterile glass tissue grinder and the mounts then prepared from the suspension; theirs were made from a selected area of the culture. We hoped to secure, in this manner, a more representative sampling of the entire colony than could be obtained by the method of Cozad and Furcolow. We measured 1000 spores from each strain; they measured 250. Aside from the specific differences numerated above, technics and methods of observations were the same.

Results and interpretations. Fig. 1 presents a comparison of the results obtained in our laboratory to those obtained for the same strains of *Histoplasma* by Cozad and Furcolow(1). Although the curves shown in Fig. 1 are similar, our measurements show a greater frequency of small spores and fewer numbers of tuberculated spores. Only 6.8% of the 5000 spores measured by us exceeded 4.8 μ in diameter, and only 3.4% were tuberculated. This compares with 31.1% larger than 4.8 μ by their measurement and 11.2% tuberculated. It is also interesting to note that the largest percentage of tuberculated spores observed by us was 8.7% in strain 5, whereas strains 14 and 1 showed more than 20% tuberculated spores according to their measurements.

The greatest difference in measurements of the same strain occurred with strain 14 where

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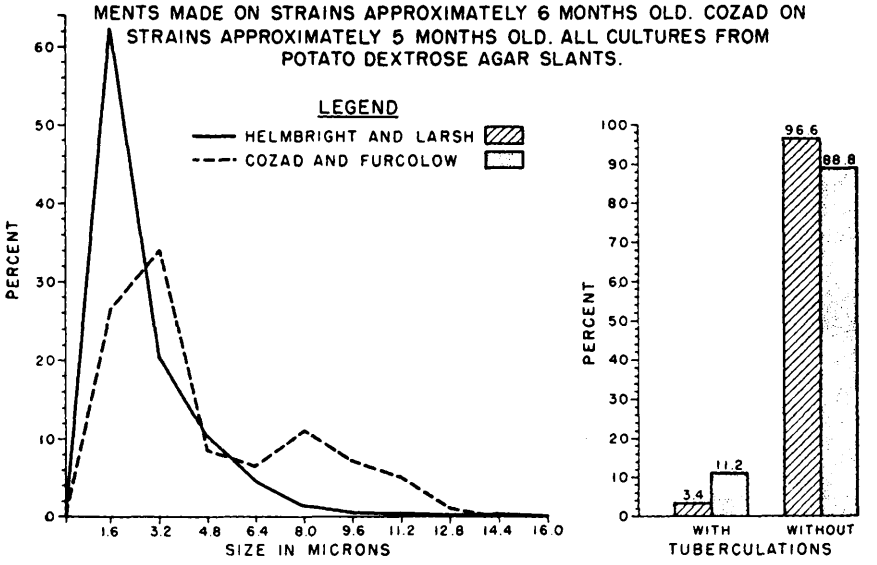
FIGURE 1

COMPARISON OF THE SIZE AND PRESENCE OF TUBERCULATIONS OF THE SPORES OF THE SAME 5 STRAINS OF *H. capsulatum* BY TWO OBSERVERS. HELMBRIGHT MEASUREMENTS MADE ON STRAINS APPROXIMATELY 6 MONTHS OLD. COZAD ON STRAINS APPROXIMATELY 5 MONTHS OLD. ALL CULTURES FROM POTATO DEXTROSE AGAR SLANTS.



we found 96.4% of the spores 4.8 μ or less in diameter, whereas the other workers found only 37.2% in this size range.

The nearest agreement of our measurements with theirs was noted in strain 2 where we found 95.2% of the spores to measure 4.8 μ or less, and they found 93.6% in this size range.

It appears that most of the discrepancies noted are due to the increased frequency of small spores recorded in our measurements. This we interpret to be due largely to the employment of the oil immersion lens in our measurements. In employing the oil immersion lens it was feasible to get greater accuracy in measurements, particularly of the small spores, which may account for the reported difference between our results and those of the other investigators. It is also interesting to note that we found no secondary rise in frequency of the spores about the 8 μ point compared to that reported by Cozad and Furcolow. Actually only 110 of 5000 spores measured (2.2%) were 8 μ or larger in size.

Summary. 1. In an effort to determine the size of the spores in *Histoplasma capsulatum,* 1000 spores from each of 5 strains were measured. To obtain a homogeneous suspension the entire culture was scraped from the potato dextrose agar slant and macerated in a sterile glass tissue grinder. The measurements were made using a high power oil of immersion lens. 2. On the whole, our results confirm those reported by Cozad and Furcolow and substantiate the high frequency of small spores in the strains of *Histoplasma capsulatum* observed.

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