THE UPTAKE OF LOW MOLECULAR WEIGHT SULFUR-CONTAINING COMPOUNDS BY HISTOPLASMA CAPSULATUM AND RELATED DIMORPHIC FUNGI*

by

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ABSTRACT

A number of low molecular weight organic sulfur-containing compounds were tested for their effect on the respiratory activity of yeastlike and mycelial `H. capsulatum. Of the compounds tested, L-cyst(e)ine was found to give maximum stimulatory effect on yeastlike phase respiration. The D- and meso isomers of cyst(e)ine as well as substituted derivatives were much less effective in the stimulation of respiratory activity of yeastlike `H. capsulatum. Respiration of homologous mycelial phase cell suspensions was depressed in the presence of L-cystine as substrate, while respiratory activity of yeastlike `B. dermatitidis and `S. schenckii was unaffected.

Whole cell suspensions of yeastlike `H. capsulatum actively transported $^{35}$-labeled L-cystine and methionine but apparently not $\beta$-mercaptoacetate-$^{35}$. Mycelial phase `H. capsulatum and the yeastlike and mycelial phases of `B. dermatitidis and `S. schenckii were observed to take up $^{35}$-labeled L-cystine to a much lesser degree than yeastlike `H. capsulatum as determined on a dry weight basis. These results suggest significant differences in the transport and subsequent intracellular mechanisms of metabolism of low molecular weight sulfur-containing $\alpha$-amino acids and related compounds by yeastlike `H. capsulatum and its corresponding mycelial phase as well as the dimorphic fungi `B. dermatitidis and `S. schenckii.

INTRODUCTION

There is a general agreement in the literature that an organic source of $-$SH or $-$S$-$ groups is essential for the in vitro growth of the yeastlike phase of Histoplasma capsulatum. The saprophytic or

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mycelial phase of this dimorphic fungus, as well as the yeastlike and mycelial phases of Blastomyces dermatitidis and probably Sporotrichum schenckii, do not appear to have this apparent requirement for organic sulfur (Gilardi, 1965). The amino acids cyst(e)ine are the most effective source of this apparent requirement for the in vitro growth of yeastlike H. capsulatum. Other sulfur-containing compounds vary considerably in their ability to support growth. Whether this requirement is due to the maintenance of an optimal O/R potential of the supporting medium or the satisfaction of a nutritional requirement as the result of a sulfur metabolism deficiency (or a combination of both) remains to be determined.

The present study concerns itself with certain comparisons of the uptake of sulfur $^{35}$-labeled compounds by the yeastlike and mycelial phases of H. capsulatum, B. dermatitidis, and S. schenckii as well as the subsequent effects of a number of structurally related low molecular weight sulfur-containing compounds on the respiratory activity of viable whole cell suspensions of these dimorphic fungi. The results of this study are reported herein.

**Materials and Methods**

Two of the strains of *Histoplasma capsulatum* (Huff and #105) were those employed in previous uptake studies (Garrison, 1961). *Histoplasma* strain no 12700 was obtained from the American Type Culture Collection, Rockville, Maryland. *Blastomyces dermatitidis* strains B-46 and Sago were obtained through the courtesy of Dr. D. J. Guidry of the Louisiana State University School of Medicine. *Sporotrichum schenckii* strain PH was from a recent clinical infection. The yeastlike phases of these dimorphic fungi were grown on trypticase soy agar (BBL) for 5 days at 37°C. Mycelial suspensions were obtained by seeding 1 liter flasks containing sterile trypticase soy broth with the appropriate yeastlike inocula, and incubating at room temperature for 7 days on a rotary shaking apparatus. This procedure permitted the conversion of the yeastlike inocula to finely divided hyphal suspensions of the homologous mycelial phase. Resting whole cell suspensions of each phase were prepared by washing the cells several times with cold sterile distilled water or appropriate buffer. Cell concentrations were determined on a dry weight basis by pipetting 1.0 ml aliquots of the cell suspensions in triplicate into tared stainless steel planchets. These containers were then dried to a constant weight at 100°C for 24 h.

**Uptake of sulfur-containing compounds as respiratory substrate**

Oxygen uptake of the resting whole cell suspensions was determined by the use of Warburg constant volume respirometers. All determinations were made in an atmosphere of air, and at tempera-