FREE LIPIDS AND PHOSPHOLIPID PHOSPHORUS OF *HISTOPLASMA CAPSULATUM* AND OTHER PATHOGENIC FUNGI[1]

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Lipids represent a very important fraction of the cellular protoplasm of pathogenic fungi and are capable of inhibiting the proteolytic activity of the enzymes in the host (Peck, 1942; Jobling and Peterson, 1914). Baker (1942) found that the lesions in mice which were produced by the injection of *Blastomyces dermatitidis* phospholipid closely resemble those produced by the living organisms.

The quantitative composition of the lipids in the pathogenic fungi depends on the composition of culture media and on the methods of treatment of cells during harvesting and extraction (Peck, 1947). A literature search showed that a quantitative study of free lipids and phospholipid phosphorus has not been reported for *Histoplasma capsulatum*. It was believed that such an investigation with two other pathogens employing different media and methods of growth would contribute to an understanding of the biology and pathogenicity of these fungi.

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MATERIALS AND METHODS

Histoplasma capsulatum (Scritchfield), *Hormodendrum pedrosoi*, and *Microsporum canis* were used in this study. The yeast cells were grown for 4 days at 37 C, whereas the others were grown for 30 days at room temperature. Peck's medium, Sabouraud's agar medium, and asparagine liquid medium (Smith et al., 1948) were used with different conditions of growth. One batch of histoplasma yeast on Peck's medium was killed by

TABLE 1

Free lipids and phospholipid phosphorus of some pathogenic fungi

Organism	Free Lipids		Phospholipid Phosphorus	
	Weight, g	% by wt of dry organism	Weight, mg	% by wt of dry organism
Histoplasma capsulatum				
Yeast phase				
Peck's* medium	0.856	18.61	10.71	0.230
Peck's medium	0.458	18.23	4.85	0.190
Sabouraud's medium	0.196	19.54	2.14	0.210
Shake culture				
Asparagine liquid	0.178	2.64	2.35	0.035
Still culture				
Asparagine liquid	0.292	10.17	2.86	0.100
Hormodendrum pedrosoi				
Shake culture				
Asparagine liquid	0.027	1.98	0.11	0.008
Still culture				
Asparagine liquid	0.116	9.53	0.41	0.034
Microsporum canis				
Still culture				
Asparagine liquid	0.136	10.81	0.65	0.048

* Killed by heat (65 C, 3 hr).

heat (65 C for 3 hr), but all others were killed by 0.5 per cent formalin for 2 weeks (table 1). The cells were harvested, washed three times in distilled water by centrifugation and then harvested on a Büchner funnel. A random sample was taken for determination of dry weight prior to extraction. The remainder of the cells were used for the lipid extraction.

A known weight of wet cells was extracted in a Soxhlet apparatus for 36 hr with 200 ml of 2:1 chloroform-methanol at 65 C. The extraction was repeated for another 36 hr after grinding the residue with sterile white sand. The total of both extracts was considered as the total free lipids of the organism. The extract was evaporated to about 50 to 100 ml, the volume measured, and 8 ml were used for estimation of the amount of phosphorus, using amino-naphthol-sulfonic acid reagent as an indicator and the photometer as a detector (Hawk, Oser, and Summerson, 1947). The rest of the extract was evaporated completely and the amount of the free lipid was calculated from the residue.

RESULTS AND DISCUSSION

Data in table 1 show the weight and percentage of the free lipids and the phospholipid phosphorus of the fungi tested. The free lipids and the phospholipid phosphorus of the three batches of histoplasma were not significantly different, but they were higher than that of the mycelium of the same fungus and of the other two fungi tested. This could be due to the difference in the medium and the state of the fungus. Also it appears that the still cultures (which contain mycelium and spores) of two of the three fungi have higher lipid and phospholipid phosphorus than the shake cultures (which contain mycelium and almost no spores). This could be explained on the basis of the presence of the spores in the still culture.

The different fungi tested vary in the amount of phospholipid phosphorus. The percentage of the phosphorus in the shake culture of histoplasma is almost the same as that of the still culture of hormodendrum, but the percentage of their free lipid is almost in the ratio of 1:3.

Sufficient data are not available to permit comparison of these findings with other pathogenic fungi. There is a difference in the free lipid contents of organisms studied, however, and that described for *Blastomyces dermatitidis* (Peck and Hauser, 1938) and *Candida albicans* (Peck and Hauser, 1939). But the analyses of *Hormodendrum pedrosoi* and *Microsporum canis* were similar to those reported for *Trichophyton mentagrophytes* (Prince, 1960).

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SUMMARY

The yeast phase of *Histoplasma capsulatum* contains larger amounts of free lipids and phospholipid phosphorus than the mycelium of the same fungus and that of *Hormodendrum pedrosoi* and *Microsporum canis*. Also, the still cultures of *H. capsulatum* and *H. pedrosoi*, which contain mycelium and spores, have a greater amount of free lipids and phospholipid phosphorus than their shake cultures, which contain mostly mycelium.

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