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Virulence Differences in Mice of Type A and B *Histoplasma capsulatum* Yeasts Grown in Continuous Light and Total Darkness

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Type B yeasts were more virulent for mice than type A under most experimental conditions. Mice infected with type B yeasts grown in the light lived significantly longer than those with type B yeasts grown in the dark. Virulence differences of type A yeasts grown in continuous fluorescent light versus total darkness were not statistically significant.

Possible virulence differences between the yeast phases of the A and B mycelial phenotypes of *Histoplasma capsulatum* have been looked for in rabbits (2) and in mice (3) with equivocal results. In an attempt to further clarify this point as well as to better standardize yeast inocula used for various types of experiments, we investigated the effects which age and growth under continuous light and total darkness might have on the virulence of type A and B yeasts for mice.

Preparation of yeast cells, strains 217A (albino) and 217B (brown), was as previously described (2) except that the growth medium was modified Sabouraud broth. Growth conditions under continuous fluorescent illumination and total darkness have also been described (1). For each experimental condition, white male mice weighing 13 to 15 g in groups of 10 were inoculated intravenously with 0.2 ml of a critically standardized suspension of yeasts in 0.85% NaCl containing 200×10^6 living yeasts per ml (2). Ten control mice each received 0.2 ml of modified Sabouraud broth. Animals infected with the A and B phenotypes were housed in separate rooms to reduce the risk of cross-infection (2). The number of deaths was recorded daily.

The experiments were carried out twice in their entirety. Thus, 20 mice were tested for each condition. The results are shown in Table 1. Statistical analysis of the total data by analysis of variance indicates the following. The B phenotype was distinctly more virulent than A. This confirms the findings of Tewari and Berkhout (3).

For the A phenotype, there were minimal statistical differences in the lethality of 48- and 72-h-old inocula. The 96-h inoculum, however, was considerably less virulent even though the total number of viable cells was the same in all. The differences in virulence of the A phenotype inoculating suspensions, whether grown in light or darkness, were not statistically significant.

For the B phenotype, age of inoculum as related to virulence was also highly significant. In this instance, the 96-h inoculum was the most virulent. In addition, mice infected with yeast grown continuously in the light lived significantly longer than mice inoculated with yeasts grown continuously in the dark, even though it was established in an earlier study that a light or dark environment did not affect the growth rate or viability of yeasts of this strain (1).

Since there are no morphological differences between the A and B mycelial phenotypes in the yeast phase and biochemical differences demonstrated thus far are minimal and poorly understood in this dimorphic organism, we cannot speculate on the mechanism of virulence as affected by light. These findings do emphasize, however, the need to control growth conditions as well as age and viability in standardizing inocula for virulence and possibly other types of experiments with A and B yeasts.

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TABLE 1. *Cumulative numbers of dead mice after infection with Histoplasma capsulatum strain 217A and 217B yeasts grown under various experimental conditions*

Time of death after infection (days)	No. of dead mice postinfection											
	48 h[a]				72 h[a]				96 h[a]			
	Light grown		Dark grown		Light grown		Dark grown		Light grown		Dark grown	
	A[b]	B[b]	A	B	A	B[c]	A	B	A	B[d]	A	B
5							1					
6	2		5				2	1	2		1	2
7	10	4	12	12	4	1	4	8	3		2	3
8	15	8	19	18	9	3	8	10	8		7	10
9	18	13	20	20	11	6	10	12	12	2	12	14
10	19	17			14	8	17	16	16	7	16	19
11	20	18			17	16	20	19	19	17	20	20
12		20			18	18		20	20	18		
13					20							

[a] Age of inoculum.
[b] A, Strain 217A; B, strain 217B.
[c] Two mice died on day 15.
[d] One mouse died on day 18; one mouse survived the 21st day of observation period.

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LITERATURE CITED

1. Berliner, M. D., and N. Biundo. 1973. *Histoplasma capsulatum*: effects of continuous light and total darkness on the mycelial and yeast phases *in vitro*. Sabouraudia **11**:48-51.
2. Daniels, L. S., M. D. Berliner, and C. C. Campbell. 1968. Varying virulence in rabbits infected with different filamentous types of *Histoplasma capsulatum*. J. Bacteriol. **96**:1535-1539.
3. Tewari, R. P., and F. J. Berkhout. 1972. Comparative pathogenicity of albino and brown types of *Histoplasma capsulatum* for mice. J. Infect. Dis. **125**:504-508.