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Increase in Colony-Forming Units of *Candida albicans* After Treatment with Polyene Antibiotics

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Polyene antibiotics, at concentrations which do not cause detectable toxic effect, induce an increase in the number of colony-forming units of yeast cells of *Candida albicans*. This effect, which we attribute to an increase in plating efficiency, is probably caused by binding of the polyenes to fatty acids in the cell wall of fungi.

The toxic effects of polyene antibiotics on fungi are generally attributed to the binding of the polyenes to cell membrane sterols (4, 11). However, binding can also occur without toxicity (7). In this study we show that under conditions in which polyenes are not toxic to yeast cells of *Candida albicans* (low concentration of antibiotic, or low incubation temperature), they can increase the number of colony-forming units of the fungus.

C. albicans (obtained from L. Haley, Center for Disease Control, Atlanta, Ga.), was grown overnight in liquid Sabouraud medium. Cell viability was at least 97% as estimated by exclusion of trypan blue. The untreated cells, counted in a hemacytometer and then properly diluted, had a plating efficiency of 60 to 70%. That is, the number of colony-forming units on Sabouraud-dextrose-agar plates after 24 h of incubation at 37°C was 60 to 70% of the cell number counted in the hemacytometer. This plating efficiency was very reproducible in our laboratory and was also normally achieved in the Clinical Mycology Laboratory at Barnes Hospital. More prolonged incubation of the plates did not result in an increase in the number of colonies.

To measure the effects of drug, cells were transferred to fresh liquid medium and exposed to the assayed compound for 1 h at 37°C with intermittent shaking. Cells incubated in concentrations of amphotericin B (AmB) lower than that causing detectable K^+ leakage, formed more colonies in agar than did untreated cells (Fig. 1). The increase in colony-forming units was 20 to 50% (the mean from 5 experiments was 32 ± 6%), and the resulting plating efficiency was 80 to 95%.

Similar increases in colony number were also observed when *C. albicans* was exposed in liquid medium to nontoxic concentrations of nystatin or filipin. The maximal increase in colony-forming units occurred at a concentration of 0.05 μg of nystatin per ml and 1.5 μg of filipin per ml. Fifty percent K^+ leakage from *C. albicans* occurred at 0.2 μg of nystatin per ml and 8 μg/ml of filipin per ml.

We think that the increase in colony-forming units was caused by the portion of the polyene molecule which is responsible for the toxic effects. The evidence for this is that inactivation of AmB by exposure to visible light by a procedure previously described (9) or the addition of ergosterol to the cultures containing AmB decreased both of these effects proportionately.

Differences in the temperature dependence of the nontoxic and toxic effects of the polyenes made us suspect that they had different mechanisms. AmB binds comparably to yeast at 37 and 2°C (7), but toxicity is greatly diminished at the lower temperature (1, 7). In the present experiments, 30% of intracellular K^+ leaked from the cells at a concentration of 0.03 μg of AmB per ml at 37°C, but no detectable leakage occurred at concentrations up to 20 μg/ml when the cultures were incubated with antibiotic at 2°C. In contrast the increase in colony-forming units occurred at 0.01 μg of AmB per ml when the cultures were incubated at either temperature.

We know that the toxic effects of AmB are caused by its binding to ergosterol in fungal cell membranes (4). We assume that the receptors for the nontoxic binding are fatty acids located in the cell wall of *C. albicans* (5). Others have shown that polyenes can bind to fatty acids (2, 5), but it is worth noting that whereas both sterols (3) and fatty acid (6) induce a decrease of the absorbance peak of AmB at 408 nm, much higher concentrations of fatty acids are required for comparable decrease.

Further evidence that the nontoxic effect of polyenes is probably due to binding to fatty acids is provided by an experiment with miconazole. Miconazole belongs to a group of imidaz-

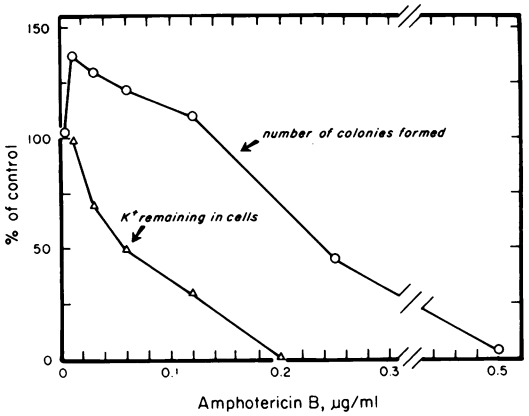

FIG. 1. *Effect of AmB on percentage of K^+ remaining in cells (△) and the number of colonies formed (○) by C. albicans on solid medium. Cells were rinsed with 0.25 M sucrose and divided into portions. The intracellular K^+ remaining was measured in one portion in a Corning flame photometer model 30. The other portion was plated on Sabouraud agar, and the number of colonies was counted after 24 h of incubation at 37°C.*

ole derivatives whose antimycotic activity is thought to be a result of binding to fatty acids in fungal cell walls (8, 12). In an experiment similar to the one described for AmB, low concentrations of miconazole induced an increase in colony-forming units comparable to that caused by the polyene antibiotics (data not shown).

We considered the possibility that the increase in colony-forming units was secondary to a stimulation of cell division. This explanation was rejected because each effect occurred at both 2 and 37°C, in phosphate-buffered saline as well as in medium, and was not accompanied by an increase in cellular protein content as measured by the procedure of Lowry et al. (10). A second possibility that AmB acted by separating cell aggregates formed in liquid cultures was also rejected because careful light microscopic evaluations of treated cultures did not show a decreased number or size of aggregates of *C. albicans* compared to controls. We also did not observe any morphological differences between untreated and antibiotic-treated cells; both cultures contained a uniform population of yeast. Therefore, we have no explanation for our observations at the present time, except to state that AmB increased the plating efficiency of *C. albicans* probably by binding to the fatty acids in the cell wall of the fungus.

The effect of AmB on plating efficiency of *C. albicans* is the earliest change induced by polyene antibiotics on fungi that we have been able to detect and may represent the biological effect of binding to cell wall that others have described (5).

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