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J. Bacteriol. 1955, 70(4):435.

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PATHOGENESIS OF *CANDIDA ALBICANS* INFECTION FOLLOWING ANTIBIOTIC THERAPY

II. FURTHER STUDIES OF THE EFFECT OF ANTIBIOTICS ON THE *IN VITRO* GROWTH OF *CANDIDA ALBICANS*¹

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Received for publication April 21, 1955

One of the complications which may be encountered during the course of therapy with antibiotics is the development of secondary infections superimposed upon the condition being treated. These superimposed infections are caused by microorganisms resistant to the original treatment and, when a fungus is responsible for the new infection, the problem becomes particularly distressing because of the general lack of satisfactory therapeutic measures for management of mycotic disease. It is obvious that an understanding of the biologic phenomena responsible for the development of these superinfections would lead toward a more rational approach to antibiotic therapy.

One of the theories offered in explanation of this problem states that the antibiotics directly stimulate the fungus. Since candidiasis is one of the more frequent mycotic complications encountered, much of the reported work in this area of interest is concerned with the effect of the antibiotics on *Candida albicans*, and here the evidence is conflicting. The studies of some investigators would indicate that at least one of the more common antibiotics does stimulate the growth and virulence of *C. albicans*, while others have been unable to demonstrate this effect (Foley and Winter, 1949; Hazen *et al.*, 1953; Kligman, 1952; Lipnik *et al.*, 1952; Moore, 1951; Pappenfort and Schnall, 1951; Seligmann, 1952, 1953; Woods *et al.*, 1951). In an attempt to resolve these differences, Huppert *et al.*

(1953) studied the effect of various antibiotics on the *in vitro* growth of *C. albicans* using quantitative techniques. Their results indicated that aureomycin appeared capable of stimulating the growth of this fungus, while penicillin, streptomycin, chloramphenicol and terramycin did not. The material presented here is concerned with similar studies on antibiotics not reported in the earlier paper.

EXPERIMENTAL METHODS

Stock cultures of *Candida albicans* were maintained on Sabouraud's glucose agar, incubated continuously at 37 C, and transferred to fresh media at weekly intervals. For all other purposes the medium used was the same semisynthetic broth of Casamino Acids (Difco) which had been used in the earlier studies. The initial inoculum of cells and the final amount of growth obtained were measured in terms of total cell nitrogen as determined by the micro-Kjeldahl method.

Several flasks of the broth medium were inoculated from stock cultures and incubated for 48 hours, at which time the cells were separated and washed repeatedly. Quantitative dilutions were made from an initial suspension of these washed cells and the turbidity of the suspension measured. From a previously calibrated curve for turbidity-total cell nitrogen relationship, the concentration of cell nitrogen in the initial suspension was interpolated, and the original dilution was readjusted with sterile saline solution to contain 0.1 mg nitrogen per ml (mg N/ml). One-half ml of this final suspension, or 0.05 mg nitrogen, was used for inoculating each flask of broth in the experiment. The antibiotics were dissolved in the broth to make a series of tenfold dilutions. The highest concentration was selected at approximately 100 times the maximum blood level commonly attained. For orally

¹ This investigation was supported by funds received from the Office of Naval Research on Contract NONR 855(02). Reproduction in whole or in part is permitted for any purpose of the United States Government.

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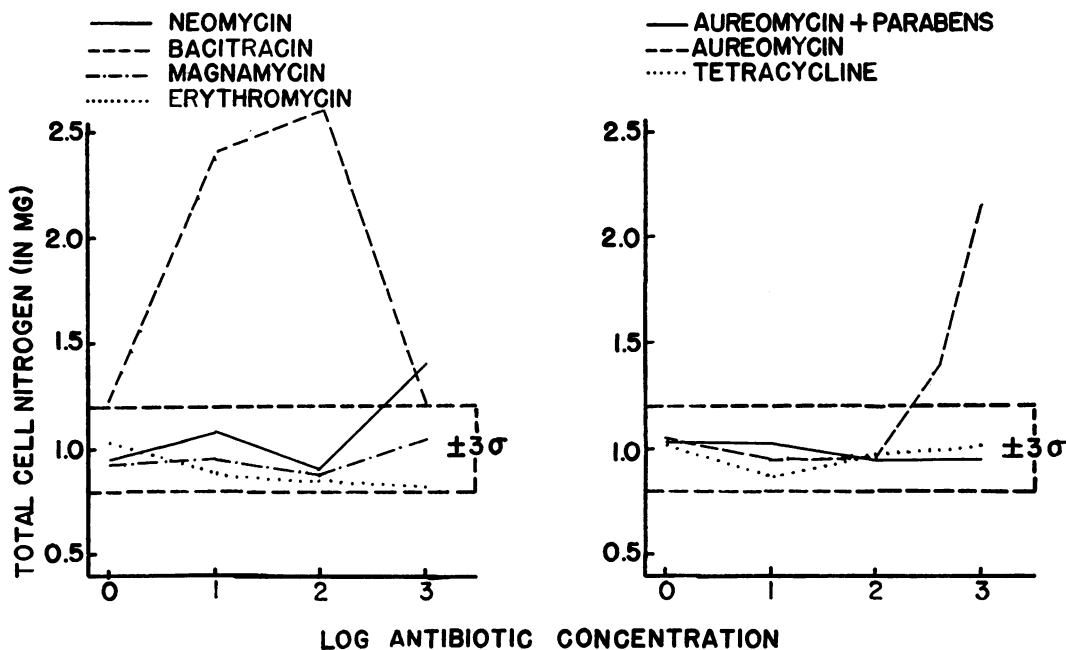


Figure 1. The effect of various antibiotics on the growth of *Candida albicans*. The following values represent the highest concentration tested of each antibiotic: neomycin, 5 mg/ml; bacitracin, 2500 units/ml; magnamycin, 0.5 mg/ml; erythromycin, 0.5 mg/ml; aureomycin (both preparations), 0.5 mg/ml; tetracycline, 0.5 mg/ml.

administered antibiotics this approximates the level attained in the stool. Control flasks were run with each series of experiments and every experiment was run in duplicate. All the cultures were incubated at 37 C for 48 hours, and at the termination of the incubation period the flasks were placed in a boiling water bath for 15 minutes, the cells were separated and washed repeatedly, and then transferred quantitatively to a 50-ml volumetric flask and made up to volume. Aliquots were removed for nitrogen determinations.

RESULTS

The combined results are plotted in figure 1, in which the extreme limits of a large series of control flasks are indicated by the horizontal lines of $\pm 3\sigma$. With the methods used in these experiments it was possible to show that neomycin and bacitracin, as well as aureomycin, stimulated the growth of *C. albicans*. The increased growth caused by neomycin was not remarkable but it was statistically significant. With bacitracin, on the other hand, all concentrations of the antibiotic gave an increased yield of the fungus cells. The amount of fungus growth with this antibiotic reached a peak at approxi-

mately 250 units/ml, and then fell off rather sharply. It might be of interest to determine whether the curve for bacitracin declines only as far as the control level or whether it continues to fall indicating a positive inhibition of the growth of *C. albicans*.

Two additional points should be noted here. The addition of parabens (methyl and propyl esters of parahydroxybenzoic acid) to the aureomycin preparation completely removed all evidence of the stimulation of fungus growth obtained with pure aureomycin. With the aureomycin-parabens preparation, however, there was no evidence of any direct inhibition of the yeast growth. The second point of note is that tetracycline, which so closely resembles aureomycin, gave no evidence of stimulation at all.

DISCUSSION

The stimulation of *C. albicans* by neomycin may be significant when one considers the clinical experiences of Livingood and his colleagues (1952). They report a series of 264 cases in which neomycin was used for the topical treatment of a variety of cutaneous pyogenic infections. Reactions to treatment were encountered in seven

instances, but all of these were represented by the development of cutaneous candidiasis. Furthermore, they state that, "In approximately one-third of the patients with secondarily infected burns, the prolonged local application of neomycin, . . . resulted in the replacement of the bacterial flora with a fungus organism, including *Candida albicans* and *Aspergillus*." While this phenomenon might possibly be an expression of direct stimulation of fungi by the antibiotic, one wonders whether or not the same results might have been experienced with an antibiotic which gave no evidence of stimulation.

The results obtained with the preparation of aureomycin which contained methyl and propyl parabens is of considerable interest. Previous experiments (Huppert *et al.*, 1953) with aureomycin had indicated that all of the commercial packages of this antibiotic which had been tested resulted in increased amounts of

growth of *C. albicans*. The incorporation of the parabens removed this evidence of stimulation suggesting some degree of inhibition of the fungus even though the total amount of growth was not reduced below that found in controls. This would indicate that the parabens should be investigated further for potential use as a prophylactic agent in conjunction with antibiotic therapy.

The results obtained in these studies of the effect of antibiotics on the *in vitro* growth of *C. albicans* indicate that direct stimulation of the fungus by these agents most probably is not the fundamental biological mechanism responsible for the superimposed infections. Judging from the literature it has been the experience of many clinicians that this phenomenon might be a complication of therapy with any of the antibiotics. The results reported by some of these investigators are listed in table 1. Up to the

TABLE 1

A list compiled from some of the pertinent literature showing the association between antibiotic therapy and the development of candidiasis

| Reference | Antibiotic(s) Used | Total No. Cases | No. Cases with Candidiasis | |
|--------------------------------|---|-----------------|----------------------------|---------------|
| | | | Before therapy | After therapy |
| Harris (1950) | Aureomycin, choramphenicol | 135 | Not reported | 9 |
| Williams (1950) | Chloramphenicol | 200 | Not reported | 12 |
| Jackson <i>et al.</i> (1951) | Terramycin | 91 | 0 | 8 |
| Pappenfort and Schnall (1951) | Aureomycin | 48 | Not reported | 16 |
| Woods <i>et al.</i> (1951) | Penicillin, aureomycin, chloramphenicol | 25 | Not reported | 25 |
| Livingood <i>et al.</i> (1952) | Neomycin | 264 | Not reported | 7 |
| McVay and Sprunt (1951) | Aureomycin | 66 | 12 | 37 |
| | Aureomycin + parabens | 82 | 10 | 16 |
| Sharp (1954) | Terramycin | | | |
| | Throat | 57 | 13 | 27 |
| | Sputum | 28 | 14 | 22 |
| | Rectal | 85 | 0 | 52 |
| | Sulfadiazine | | | |
| Throat | 46 | 14 | 18 | |
| Sputum | 33 | 20 | 22 | |
| Rectal | 69 | 2 | 14 | |

present time no reports of candidiasis as a complication of therapy with bacitracin, magnamycin, erythromycin, or tetracycline have come to our attention. The antibiotics which have been associated with this superinfection phenomenon are penicillin, streptomycin, chloramphenicol, terramycin, aureomycin and neomycin. Of these, it has been possible to demonstrate *in vitro* stimulation of *C. albicans* only with aureomycin and neomycin. There is, therefore, no apparent correlation between direct stimulation of this fungus under *in vitro* conditions and the development of superimposed candidiasis during antibiotic therapy.

The explanation for superimposed fungus infections during antibiotic therapy most probably lies in a biological mechanism other than direct stimulation of these fungi by the various antibiotics. Since this complication appears to begin with changes in the microbial flora of the gastrointestinal tract, it would seem logical to investigate the effect of administering antibiotics on the occurrence and fate of *C. albicans* in the gastrointestinal tract of experimental animals.

ACKNOWLEDGMENT

The authors are indebted to Dr. Bernard G. Greenberg and to Dr. Harry Smith, Jr., Department of Biostatistics, University of North Carolina School of Public Health, for the statistical design and analysis of experiments, and also to Dr. Michael Carlozzi, Pfizer Laboratories, for graciously supplying some of the antibiotics used.

SUMMARY

In broth cultures the presence of the antibiotics aureomycin, neomycin and bacitracin produces a total growth of *Candida albicans* greater than that found in control cultures. No similar stimulation of the growth of this fungus is found with magnamycin, erythromycin or the preparation of aureomycin which contains parabens. When methyl and propyl parabens are mixed with aureomycin, the stimulation of the growth of *C. albicans* which occurs with pure aureomycin is eliminated. The growth of the fungus in the presence of the aureomycin plus parabens preparation, however, is not significantly lower than that obtained in control cultures. It is suggested that the parabens be

investigated further for possible use as a prophylactic in conjunction with antibiotic therapy.

The role of direct stimulation of this fungus by antibiotics in the development of superimposed infections during therapy is discussed. No correlation has been found between the ability of an antibiotic to stimulate the *in vitro* growth of *C. albicans* and the association of the antibiotic with the development of these superinfections.

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