THE OHIO JOURNAL OF SCIENCE

VOL. XLV MARCH, 1945 No. 2

EFFECTS OF GLIOTOXIN ON TRICHOPHYTON GYPSEUM[1]

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The need for better therapeutic agents for the control of tenia pedis (athlete's foot), a disease most frequently caused by *Trichophyton gypseum*, is appreciated by the layman as well as by the dermatologist. Within the past few years, great strides have been made in the use of antibiotics in the treatment of bacterial infections. The possibility that an agent of this kind may prove valuable in the therapy of fungous infections is deserving of serious consideration.

Weindling (6, 7, 8 and 9), in his discovery, isolation and subsequent study of gliotoxin, has done significant pioneer work on the action of antibiotics upon fungi. Much additional chemical and biological data concerning gliotoxin have been contributed by Bruce and his colleagues (1, 2, 3, 4 and 5). Not only was this one of the first antibiotic substances to be studied in great detail; it is the only one which has been extensively investigated as a fungicide. Because of the circumstances involved in the discovery of this substance, its effect on phytopathogenic rather than human pathogenic fungi has received the major emphasis.

With the above facts in mind, experiments were carried out to determine the effects of gliotoxin on the dermatophyte, *T. gypseum*. Small amounts of gliotoxin were obtained on several occasions by culturing *Gliocladium fimbriatum* on a liquid medium. Except as noted, the methods used in the culture of the *Gliocladium* and in the extraction of the toxic agent were as described by Weindling (7). About 200 ml. of medium were placed in each of six 32-oz. prescription bottles. After the medium had been sterilized and inocluated, the cultures were incubated on a shaking machine operating at a rate of about 100 oscillations per minute. After three days of incubation, the gliotoxin was extracted.

The ability of gliotoxin to inhibit the growth of *T. gypseum* was next determined. Suitable amounts of gliotoxin were added to sterile culture media. Both Sabouraud's agar plates and flasks containing Chapek-Dox broth were inoculated with spores and incubated at room temperature. A concentration of 0.001% gliotoxin in the above media was found to prevent completely the growth of the test organism. The next concentration in the series, i. e., 0.0001%, had no visible inhibitory effect.

To determine the fungicidal powers of this substance, washed spores of *T. gypseum* were suspended in gliotoxin solutions in which the gliotoxin had been dissolved in 0.1 M KH_2PO_4. At hourly intervals, 5-ml. samples of the spore suspensions were centrifuged and the supernatant fluid removed. The spores were then washed in sterile water, and a loopful of the concentrated spores transferred

[1]The writer wishes to express his appreciation to Dr. Richard Weindling for a culture of *Gliocladium fimbriatum*, to Dr. C. W. Emmons for a culture of *Trichophyton gypseum* (U. S. Public Health Service culture No. 601, *T. mentagrophytes*), and to Dr. W. F. Bruce for a sample of crystalline gliotoxin of known purity, all of which were essential to the execution of this project.

to an agar slant to test their viability. A concentration of 0.01% of gliotoxin was found to kill from 99 to 100% of the spores in two hours. No actual spore counts were made, but there was no growth except in an occasional tube where a few isolated mycelia developed. The next dilution tested, i. e., 0.001%, was not fungicidal even after 24 hours.

The above experiments were repeated using gliotoxin of known purity. The results were essentially the same as those obtained with the material prepared in this laboratory.

The ultimate question of therapeutic value of gliotoxin in the treatment of human infections can be answered, of course, only by clinical tests.

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