had no inhibitory effect on the growth of *H. influenzæ* Type A, *Ps. pyocyaneus* (2 strains), *E. coli* (2 strains), and *B. proteus*.

Summary. A method for extracting an antibiotic substance from *Tillandsia usneoides* (Spanish Moss) is described. The crude extract is effective against all the gram-positive bacteria tested and against *Cryptococcus hominis*.

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Antifungal Properties of Clavacin.*†

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The value of certain antibiotic substances, such as penicillin and tyrothricin, for the treatment of many bacterial infections is well established, although the vast majority of such agents are considered too toxic for therapeutic use. It is of especial interest to note, however, that an agent such as tyrothricin, which is toxic on parenteral injection, is nontoxic when given orally or applied externally. Thus, in the treatment of certain types of local infections, tyrothricin has met with remarkable success.[1]

The majority of fungous infections are both local and superficial in character. It follows, therefore, that toxicity upon injection does not necessarily disqualify a possible therapeutic agent. Since many fungi are known to be susceptible to the action of antibiotic substances, and since there is a paucity of satisfactory therapeutic agents for the control of mycoses, it appears that the effects of antibiotic agents on the pathogenic fungi should be investigated.

The following report deals with the effect of clavacin on 3 representative species of fungi, viz., *Monilia albicans, Oidium asteroides,* and *Trichophyton gypseum.*‡

In the early part of this work the clavacin was produced in this laboratory according to the methods of Waksman, Horning, and Spencer,[2] employing a strain of *Aspergillus clavatus* known to produce a high yield of clavacin. During the course of the investigation, crystalline clavacin became available and the tests were repeated using 4 samples of this material obtained from other sources.§

Fungistatic Effects. The ability of clavacin to inhibit the growth of fungi was determined by growing the test organisms in the presence of graded concentrations of this agent. Measured amounts of a liquid medium‖ were placed in flasks and sterilized in the autoclave, after which the portions of medium were restored to volume by the addition of sterile distilled water. To each flask was then added, aseptically, the desired quantity of an aqueous solution of clavacin. The 2% stock solution of this compound was self-sterilizing. After thorough agitation of the mixture of the medium and clavacin solution, 25 ml amounts were aseptically dispensed into sterile flasks.

* Clavacin is also known as clavatin, clavaformin and patulin.

† These investigations were supported in part by a grant supplied by Parke, Davis and Company, Detroit, Michigan.

1 Hoogerheide, J. C., *Bot. Rev.*, 1944, **10**, 599.

‡ The culture of *T. gypseum* (granular type) was obtained through the courtesy of Dr. C. W. Emmons, U. S. Public Health Service. The others were taken from the culture collection of this laboratory.

2 Waksman, S. A., Horning, E. S., and Spencer, E. L., *J. Bact.*, 1943, **45**, 233.

§ One sample of crystalline clavacin was kindly supplied by Parke, Davis and Company and 3 samples by the Mallinckrodt Chemical Works, St. Louis, Mo.

‖ Constituents: $NaNO_3$ 2.0 g, KH_2PO_4 1.0 g, KCl 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $MnSO_4 \cdot 4H_2O$ 0.01 g, glucose 20.0 g; peptone 5.0 g, and distilled water to make 1 liter.

Heavy suspensions of each of the test organisms were prepared by washing agar slant cultures with 5 ml amounts of sterile water. One drop of the resulting suspensions was used as an inoculum. After 4 to 7 days of incubation at room temperature, the relative amounts of growth were estimated. The tests were repeated at least twice, using each of the 5 above-mentioned samples of clavacin. It was shown that concentrations of 0.04% and 0.02% clavacin had only a slight inhibitory effect on the growth of *M. albicans* and *O. asteroides*. More dilute solutions produced no visible effect on either of these two organisms. The growth of *T. gypseum*, in contrast, was completely inhibited by these concentrations of clavacin. In the presence of 0.01% of the agent, the response varied from no growth, in the case of two samples, to slight growth in the case of the other samples. A slight, but visible, inhibition occurred in a medium containing only 0.001% clavacin.

Fungicidal Effects. To determine the fungicidal effect of clavacin, the test organisms were exposed to several concentrations of the substance for various periods of time, transferred to a liquid medium and incubated to determine their viability.

Suspensions of the organisms¶ were prepared as before, filtered through glass wool, centrifuged and the supernatant fluids decanted. The sedimented cells were then suspended in previously prepared aqueous solutions of clavacin. One loopful of each suspension was transferred to 25 ml of a liquid medium** at intervals of 15 minutes during the first hour, then at hourly intervals for the next seven hours, and finally after 24 hours. This method is essentially that proposed by McCrea.††[3]

Two, or in some cases 3, experiments were performed testing each of the 5 samples of clavacin against each of the 3 fungi. There was no evidence of variation in the fungicidal power of the several samples. The 1.0% and the 0.5% solutions were found to be fungicidal for all of the organisms. A solution containing 0.1% clavacin did not kill suspensions of *M. albicans* and *O. asteroides*, even after 24 hours, but was lethal to spores of *T. gypseum* in 2 to 5 hours. For the sake of clarity, the shortest periods of exposure found to be fungicidal are presented in Table I. Inasmuch as 15 minutes was the minimum time interval used, the time involved in the killing action may actually have been less where this figure is given.

TABLE I.
Fungicidal Action of Clavacin on *M. albicans*, *O. asteroides* and *T. gypseum*.

Test organism	Concentration of clavacin		
	1.0%	0.5%	0.1%
M. albicans	½*	¾-3†	no effect in 24 hr
O. asteroides	¼-1	1-3	no effect in 24 hr
T. gypseum	¼	¼-¾	2-5

* Minimum time of exposure, in hours, found to be fungicidal.

† Two figures represent the range as observed in the several experiments.

Summary. Using 3 species of fungi, the fungistatic and fungicidal actions of clavacin were investigated. The presence of 0.02%, and with some samples 0.01%, of clavacin in the culture medium completely inhibited the growth of *Trichophyton gypseum*. A concentration of 0.001% of clavacin caused partial inhibition while 0.0001% caused no detectable effect. In contrast, the presence of 0.04% of clavacin had but a slight inhibitory effect on *Monilia albicans* and *Oidium asteroides*.

A 1% solution of clavacin was fungicidal for *T. gypseum* in 15 minutes, the shortest exposure time employed, *M. albicans* in 30 minutes, and *O. asteroides* in 15 minutes to 1 hour. Solutions containing 0.5% clavacin were fungicidal for *T. gypseum* in 15 to 45 minutes, to *M. albicans* in 45 minutes to 3 hours, and for *O. asteroides* in 1 to 3 hours. The 0.1% solutions killed *T. gypseum* in 2 to 5 hours but failed to be fungicidal for the other two organisms even after 24 hours.

¶ Vegetative cells of *M. albicans;* oidia of *O. asteroides;* principally microconidia of *T. gypseum.*

** See footnote ‖.

†† In an earlier article (Herrick, J. A., and Kempf, J. E., *J. Bact.*, 1944, **48**, 331) attention was directed to the large amount of sodium azide transferred with the inoculum and the consequent unsuitability of the procedure in that study.

[3] McCrea, A., *J. Lab. Clin. Med.*, 1931, **17**, 72.