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# Streptocin, Antibiotic Isolated from Mycelium of *Streptomyces griseus*, Active Against *Trichomonas vaginalis*, and Certain Bacteria.*

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Schatz and Waksman[1] first reported that *Streptomyces griseus* produces, in addition to streptomycin, another antimicrobial substance. The latter was found to a small extent in the culture fluid but was present to a large extent in the mycelium, from which it was extracted with ether and chloroform. This crude substance was more active against the avian strain of *Mycobacterium tuberculosis* than against the human strain, the reverse being true for streptomycin. Further studies[2] showed that the material was soluble in various organic solvents, and was active largely against certain gram-positive bacteria, and only to a limited extent against the gram-negative organisms; it was later found to be active also against fungi, including both parasitic and saprophytic forms. One of the fractions of a similar extract isolated from a different strain of *S. griseus* was found to be trichomonadicidal. This substance can be designated as *streptocin*.

Whiffen, Bohonos and Emerson[3] reported the isolation from the culture medium of *S. griseus* of an antibiotic substance which is active against yeasts and fungi, and which is not active against bacteria. This antibiotic, designated as actidione, was soluble in organic solvents and in water, and was thermostable; it is distinctly different, however, from the trichomonadicidal substance which is reported here.

*Isolation and antibacterial properties of crude preparation.* Ether extracts obtained from the culture filtrate or from the mycelium of *S. griseus* gave the same antimicrobial spectra. The yields varied from 35 to 55 mg per liter of culture filtrate, and from 55 to 75 mg for the mycelium produced in one liter of medium. There was considerable variation, however, in the potency of the crude preparations depending on the strain of the organisms and on the conditions of growth. Much smaller amounts of the crude preparation were obtained from the submerged mycelium of two streptomycin-producing strains of *S. griseus* than from stationary pellicles, as shown in Table I. The stationary preparations were also more active than those obtained from the submerged mycelium (Table II).

By the turbidimetric method, it was established that 60 $\mu$g of the crude substance was sufficient to inhibit the growth of the pathogenic *M. tuberculosis* H37, including both the streptomycin-sensitive and resistant strains (Table III).

*Isolation and purification of streptocin for trichomonadostatic and trichomonadicidal studies.* For trichomonadostatic studies, a strain of *Trichomonas vaginalis* (No. 2) was used;[4] the organism was grown on a simplified trypticase-serum medium.[5] The method of inoculation and growth and the procedure employed in measuring growth are described

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1 Schatz, A., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 244.

2 Waksman, S. A., Schatz, A., and Reilly, H. C., *J. Bact.*, 1946, **51**, 753.

3 Whiffen, A. J., Bohonos, N., and Emerson, R. L., *J. Bact.*, 1946, **52**, 640; Leach, B. E., and Ford, J. H., *J. Am. Chem. Soc.*, 1948, **70**, 1223; Whiffen, A. J., *J. Bact.*, 1948, **56**, 283.

4 Johnson, G., Trussell, M., and Jahn, F., *Science*, 1945, **102**, 126.

5 Kupferberg, A. B., Johnson, G., and Sprince, H., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 304.

TABLE I.
Production of Ether-soluble Antimicrobial Substances by 2 Strains of *S. griseus* Grown in Stationary and Submerged Cultures.

| Strain of *S. griseus* | Culture | Yield,* mg | Activity, in dilution units, per 1 mg | | |
|---|---|---|---|---|---|
| | | | *S. aureus* | *B. subtilis* | *B. mycoides* |
| 3498 | Stationary | 131 | 65 | 114 | 114 |
| 3498 | Submerged | 31 | 9 | 16 | 16 |
| 3463-4 | Stationary | 318 | 27 | 47 | 47 |
| 3463-4 | Submerged | 38 | 13 | 13 | 13 |

* Per 5 liters of medium.

TABLE II.
Antimicrobial Activity of Ether-soluble Extract of *S. griseus*.
Dilution units per 1 mg of crude extract.

| Organism | Activity |
|---|---|
| *Staphylococcus aureus* | 120 |
| *Bacillus subtilis* | 120 |
| *B. mycoides* | 120 |
| *Mycobacterium phlei* | 15 |
| *Mycobacterium* 607 | 4 |
| *M. tuberculosis* H37Rv | 2.4 |
| *M. tuberculosis* H37RvR | 2.4 |
| *M. avium* | 10.7 |
| *Escherichia coli* | 1 |
| *Proteus vulgaris* | 1 |
| *Serratia marcescens* | 1 |
| *Candida albicans* | 1 |
| *Trichophyton mentagrophytes* | 1.4 |

TABLE III.
Bacteriostatic Activity of Ether Extracts of *S. griseus* on *M. tuberculosis var. hominis*.

| μg/ml of medium | Days of incubation: 4 | 7 | 11 | 23 |
|---|---|---|---|---|
| | Turbidimetric readings (in logs) | | | |
| Streptomycin-sensitive strain H37Rv | | | | |
| 200 | —* | — | — | — |
| 100 | —* | — | — | — |
| 60 | —* | — | — | — |
| 20 | 0 | 0 | 2 | 14 |
| 10 | 0 | 3 | 11 | 90 |
| 6 | 0 | 5 | 14 | 83 |
| 2 | 0 | 4 | 14 | 90 |
| 0 | 1 | 5 | 17 | 80 |
| Streptomycin-resistant strain H37RvR | | | | |
| 200 | —* | — | — | — |
| 100 | —* | — | — | — |
| 60 | —* | — | — | — |
| 20 | 0 | 0 | 2 | 14 |
| 10 | 0 | 0 | 5 | 20 |
| 6 | 0 | 2 | 6 | 25 |
| 2 | 0 | 0 | 4 | 20 |
| 0 | 0 | 3 | 7 | 25 |

* A heavy precipitate formed but no growth occurred, as determined by plating procedures.

elsewhere.[6] To prevent bacterial contamination, 250 units per ml of medium of both streptomycin[7] and penicillin[4] were added to the suspension of the trichomonads, since these antibiotics had no effect either upon cell multiplication of the trichomonads or upon the activity of streptocin.

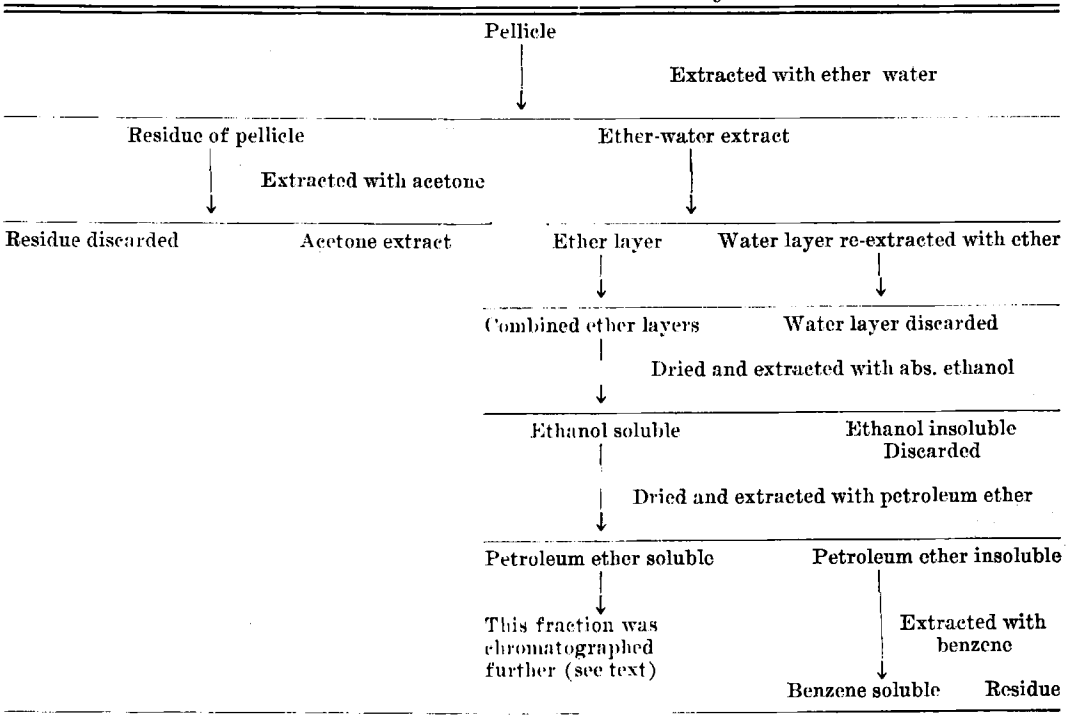
The streptocin used in the preliminary trichomonadostatic and trichomonadicidal studies was first isolated from a strain of *S. griseus* (No. 3533) which produced antibiotics active largely against gram-positive bacteria and trichomonads and was, therefore, neither streptomycin nor grisein. The culture was grown on a glucose-peptone-meat extract-NaCl-tap water medium in static or shaken condition. The mycelial growth was extracted in a Soxhlet with redistilled diethyl ether, some distilled water being added twice daily to the extraction chamber. The extraction was continued 24 hours after the ether became colorless. The ether-water mixture was removed, and redistilled acetone used for further extraction of the residual mycelium. This extraction was carried out as above. Only the fractionation of the ether-water extract is discussed in this paper.

The ether and water layers were separated, and the latter was reextracted with ether. The water then contained no trichomonadicidal activity and was discarded. The two ether fractions were combined, and the ether was removed under vacuum. The brown, oily

[6] Johnson, G., and Kupferberg, A. B., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 390.

[7] Trussell, R. E., *Trichomonas vaginalis and Trichomoniasis*. C. C. Thomas, Springfield, Ill., 1947, 61.

FIG. 1.
Extraction and Fractionation of Pellicle of *S. griseus* 3533.




residue was extracted with absolute ethanol. The ethanol-insoluble material was found to have no activity. The ethanol solution was distilled *in vacuo* and the resulting dry, yellow solid extracted with petroleum ether (b.p. 39-42°). The solvent was removed and the material dried over calcium chloride. The petroleum-ether-insoluble material was extracted with benzene; the benzene-soluble and -insoluble portions were dried *in vacuo*. The various steps used in this procedure are illustrated in Fig. 1.

The 3 fractions were chromatographed on columns of Brockman's Alumina (Merck). Since the general nature of the eluants of these columns was similar, only one of these, the petroleum-ether-soluble material, is discussed. This material was redissolved in dry petroleum ether and placed on the column in this solvent. The chromatogram was developed with increasing concentrations of benzene up to the pure solvent and then successively with acetone, ethanol, and methanol, until a 50-50 mixture of methanol-ethanol was reached. The material eluted at this point yielded, on evaporation of the solvent, an apparently crystalline material which possessed trichomonadicidal activity. Increasing concentrations of methanol were then employed for further development of the chromatogram. When 1% glacial acetic acid in methanol was used, more material was eluted, which on evaporation of the solvent, yielded a needle-like crystalline material which appeared in the form of rosettes. This preparation of streptocin had a high *in vitro* trichomonadicidal activity; its antibacterial spectrum is shown in Table IV. In contrast to the crude mixture isolated from the streptomycin-

TABLE IV.
Antimicrobial Activity of Crystalline Streptocin
Dilution Units per 1 mg of Streptocin.

| Organism | Activity |
|---|---|
| *E. coli* | <3 |
| *B. mycoides* | 3 |
| *S. aureus* | 3 |
| *B. subtilis* | 75 |
| *M. ranae* | <8 |
| *Mycobacterium* 607 | 13 |
| *Mycobacterium* 607R | 13 |
| *M. avium* | 8 |

TABLE V.
Comparative Effects of Crystalline Streptocin and Actidione on Various Trichomonads.
Effects of 50 μg/ml of antibiotics upon the trichomonad population after 48 hours incubation at 37°C, numbers* expressed as cells/mm³.

| Antibiotic | *T. foetus Br.* | *T. gallinae* | *T. vaginalis* |
|---|---|---|---|
| None | 5,140 | 1,460 | 1,600 |
| Streptocin | 182 | 1,620 | 0 |
| Actidione | 1,490 | 0 | 10 |

* These figures represent the average of duplicate determinations.

TABLE VI.
Effect of Crystalline Streptocin Upon Cell Multiplication of *T. vaginalis*.

| Conc. in units/ml in final medium | Population after 48 hr cells/mm³ |
|---|---|
| 1.00* | 0 |
| 0.66 | 10 |
| 0.14 | 1,010 |
| 0 | 1,600 |

* At this concentration, the agent is trichomonadicidal.

TABLE VII.
Effect of Sublimation Upon Yield of Streptocin from Crude Extract of *S. griseus* 3533.

| | Trichomonadicidal units/mg |
|---|---|
| Crude ext. | 0.96 |
| Crude ext. sublimed | 153 |
| Residue from sublimation | 0.77 |

producing strain of *S. griseus*[1,2] streptocin has a greater effect against the avirulent strain of *Mycobacterium* 607 than against the avian strain. It can be crystallized from 1% glacial acetic acid-methanol or from acetone but not from amyl acetate.

A trichomonadicidal unit is defined as that amount of material which when present in 1 ml of STS medium will kill the 75,000 seeded organisms in 48 hours' incubation at 37°C. A comparative study of streptocin and actidione upon different species of trichomonads is presented in Table V. The effect of different concentrations of *streptocin* upon *T. vaginalis* is shown in Table VI.

The active component can be sublimed at 100°C in a vacuum of $1 \times 10^{-5}$ mm. This sublimation can be accomplished from the initial extract as shown in Table VII. Ac-

TABLE VIII.
Comparative Chemical and Biological Properties of Streptocin and Actidione.

| Streptocin | Actidione (3) |
|---|---|
| Marked antibacterial activities | No antibacterial activity |
| Limited antifungal activities | Marked activity against fungi |
| Trichomonadicidal activity 1:29,000 against *T. vaginalis* | Trichomonadicidal activity <1:10,000 against *T. vaginalis* |
| No effect against *T. gallinae* at 50 μg/ml | Trichomonadicidal against *T. gallinae* at 50 μg/ml |
| Extremely low toxicity I.V. in mice | $MLD_{50}$ I.V. in mice 150 mg/kg |
| Insoluble in $CHCl_3$ | Soluble in $CHCl_3$ |
| Forms gel with amyl acetate | Recrystallizes from amyl acetate |
| Stable at pH 11.0 | Unstable to alkali |
| Sublimable | Nonsublimable |

TABLE IX.
Antimicrobial Activity of Certain Chromotographic Fractions Other Than Streptocin.

| Fraction | Dilution units per 1 mg | | | |
|---|---|---|---|---|
| | *E. coli* | *B. mycoides* | *S. aureus* | *B. subtilis* |
| 10% acetone-benzene to 25% ethanol-acetone | 1 | 24 | 75 | 100 |
| Absolute ethanol | 3 | 3 | 8 | 24 |
| 50% methanol-ethanol | 3 | 3 | 3 | 100 |

tidione could not be sublimed under the same conditions. The crystalline material is soluble in water, methanol, and ethanol but not in chloroform. On standing, the material loses its crystalline structure. It is stable in a pH range of 2.0 to 11.0. It is stable to heat at 100°C for at least 60 minutes. The dry material is stable at 5°C for at least 4 months. Spectrophotometric measurements indicated no absorption maxima between 2200 and 6750 Å.

The comparative chemical and biological properties of streptocin and actidione are shown in Table VIII. These results show that streptocin is distinct from actidione. The antimicrobial spectrum of streptocin is similar to that of crude material isolated from the different strains of *S. griseus*, in that it acts largely upon gram-positive bacteria. However, other chromatographic fractions obtained from the crude material possess similar antimicrobial properties but are not trichomonadicidal. The antibiotic properties of several such fractions are shown in Table IX.

*Course of production of streptocin by S. griseus 3533.* The course of production of streptocin can be measured by its trichomonadicidal activities. Most of the material containing the trichomonadicidal activity is produced in the pellicles, and stationary cultures give much higher yields than submerged cultures. The results of typical experiments are shown in Fig. 2. Maximum production of the antibiotic is obtained in static cultures on the fifth day, when 76% of the activity is found in the pellicle and 24% in the culture filtrate.

The *Trichomonas foetus* used in these experiments was received from Dr. B. B. Morgan, University of Wisconsin. The authors of the New Brunswick group wish to acknowledge the assistance of Miss Dorothy Smith in testing the activity of the ether extract of pellicle of streptomycin-producing

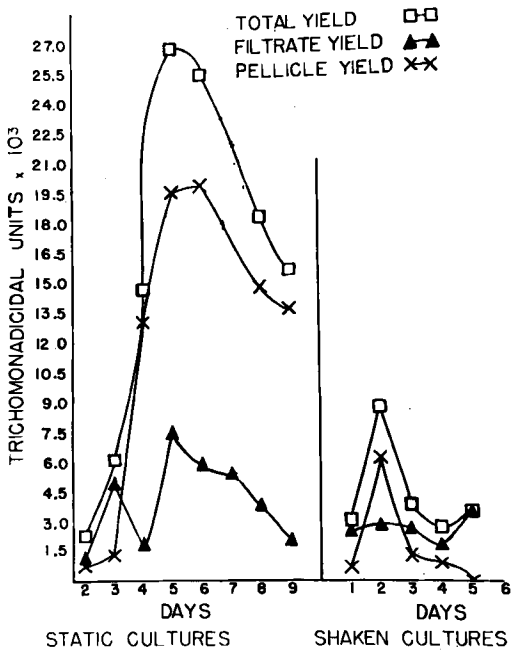



FIG. 2.
Comparison of relative yields of trichomonadicidal activity from the pellicle and filtrate of *S. griseus* No. 3533 grown in static and shaken cultures.

*S. griseus* against the various strains of *M. tuberculosis*. The authors of the Ortho group wish to acknowledge with appreciation the technical assistance of Mrs. Mary Williams.

*Summary.* Several antibiotic substances were found in the ether-soluble extract of the mycelium of various cultures of *S. griseus*, which differed in their chemical nature and antimicrobial activities. One of these fractions from *S. griseus* No. 3533, designated as *streptocin*, possesses strong trichomonadicidal properties. It is active against gram-positive bacteria. Small amounts of streptocin are also present in the culture filtrate of the organism.

Streptocin is distinct from actidione in its physical and chemical properties and in the nature of its antimicrobial spectrum.