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Streptomycin II, An Antibiotic Substance Produced by a New Species of Streptomyces.*†

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The production of streptomycin by actinomycetes is limited to certain strains of a single species of the genus *Streptomyces,* known as *S. griseus.* In addition to the first 2 streptomycin-producing strains isolated in 1943,[1] only 3 other streptomycin-producing strains have thus far been reported. Although over a hundred cultures of *S. griseus,* isolated from different substrates, have now been tested in our laboratory for their antibiotic properties, only one culture was found[2] capable of producing streptomycin; several others were capable of producing antibiotic substances such as grisein,[3] which differed, however, from streptomycin. Similar results were obtained by Carvajal,[4] who isolated 2 streptomycin-producing strains of *S. griseus.* No other organisms culturally different from *S. griseus* have been found capable of producing streptomycin.

In connection with a study of the bacterial population of the waters around Bikini and Rongelap Atolls during the recent atomic bomb experiments in the Marshall Islands,‡ a culture of an actinomycete was isolated from one of the Bikini soils. This culture showed strong inhibition of various Gram-positive and Gram-negative bacteria, as well as of acid-fast bacteria. This culture belonged to the genus *Streptomyces,* but was distinct from streptomycin-producing *S. griseus.*

This culture was very similar, both morphologically and culturally, to *S. griseolus* (Actinomyces 96) described by one of us in 1919.[5] It differed, however, in some of its cultural properties and in its ability to produce an active antibiotic substance. Because of these differences and also because of the peculiar natural substrate from which this culture was isolated, it is proposed to designate it as *Streptomyces bikiniensis.* It produces on synthetic and on organic media containing glucose, a dark gray aerial mycelium, which consists of dichotomous branches with straight chains of conidia. A thick complete pellicle is formed on the surface of stationary cultures, with protein-containing media being pigmented brown to black.

Cross streak tests of the culture were made using various test bacteria. The zones of inhibition compared favorably both with streptomycin-producing strains of *S. griseus* and the streptothricin-producing *S. lavendulae.*

The highest antibiotic potency was produced by the organism both under stationary and submerged conditions of culture on media commonly used[1] for the production of streptomycin. The antibiotic spectrum of the crude culture filtrate suggested a definite similarity of this substance to streptomycin. This similarity was confirmed when the active substance was isolated from the culture medium. The titre of the crude culture filtrate ranged in activity from 200 to 250 streptomycin units per ml. The corresponding activity against the various bacteria, such as *Escherichia coli, Bacillus subtilis, Bacillus mycoides, Staphylococcus aureus* and *Myco-*

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1 Schatz, A., Bugie, E., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 66.

2 Waksman, S. A., Reilly, H. C., and Johnstone, D. B., *J. Bact.,* 1946, **52**, 393.

3 Reynolds, D. M., Schatz, A., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 50.

4 Carvajal, F., *Mycologia,* 1946, **38**, 596.

‡ This work was done during the Operation Crossroads, in 1946, where the senior author was a civilian biological investigator.

5 Waksman, S. A., *Soil Sci.,* 1919, **8**, 121.

bacterium tuberculosis, was comparable to that of streptomycin.

Isolation of the active substance has been obtained by methods used to isolate streptomycin. The methyl alcohol formic acid procedure yielded preparations assaying 158 streptomycin units per mg.

The crude preparation thus isolated from the culture filtrate of *S. bikiniensis* had no activity against fungi. Bacteria made resistant to streptomycin were also resistant to this substance. It was inactivated by cysteine, it gave reduced activity in the presence of glucose, and it was sensitive to increased acidity to the same degree as streptomycin. These results tend to establish the fact that the new antibiotic is similar to streptomycin if not identical with it.

The toxicity of the preparation was determined by the use of chick embryos. As much as 600 units of the material, deposited upon the chorio-allantoic membrane, and concentrations greater than 1200 units injected into the yolk sac, of 11-day embryos gave 100% survival. One may thus conclude that the minimum lethal dose of the new antibiotic is relatively high, at least in the chick embryo.

As long as this preparation has not been crystallized and as long as its activity *in vivo* against various bacteria has not been determined, it is not possible to state definitely that the new antibiotic is streptomycin. Some question may also be raised concerning its absolute identity with streptomycin, since it is produced by a totally different organism and occasional quantitative differences in the degree of sensitivity of certain bacteria to this antibiotic as compared with streptomycin are obtained. For these reasons, it is proposed to designate the new preparation as Streptomycin II.

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Preparation of Hemoglobin Solutions Containing Hemoglobin Reducing Enzymes.

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Employing the following procedure we are able consistently to obtain sterile solutions, free from cellular debris, containing enzymes which bring about the consumption of the entire oxygen content of the solution, thereby converting the hemoglobin present into the extremely stable reduced form.

Procedure. Human erythrocytes are separated from the plasma by means of a bucket centrifuge. These cells are suspended in 2 volumes of a solution containing 6% glucose, 0.15% nicotinic acid amide and 0.0006% ammonia. This suspension is centrifuged at approximately 20,000 r.p.m. in a Sharples separator-type bowl that separates the cells from the wash solution and at the same time lyses them, though with little loss of hemoglobin into the wash solution. The washed, lysed cells, having a hemoglobin concentration of approximately 30%, are delivered directly into 100 cc of distilled water containing sufficient nicotinic acid amide to produce a concentration of 0.15% in the final product, which is diluted to contain approximately 7% hemoglobin. The mixture is diluted with 2 volumes of distilled water containing sufficient glucose to produce an estimated final glucose concentration of 6%. The diluted mixture is adjusted to pH 5.8 with N/10 HCl and is centrifuged through a Sharples clarifier-type bowl to remove the stromata. The centrifugate is stirred for 30 minutes with "Decalso," 30 g per liter, from which it is decanted. An amount of normal NaOH solution calculated to neutralize the HCl is added. These last 3 steps have been adopted from the work of Hamilton and Farr.[1] Am-

[1] Hamilton, P. B., and Farr, L. E., *Fed. Proc.*, 1946, **5**, 136.