experiments. It has the disadvantage of requiring anesthesia and surgery early and of initiating thrombosis and edema late. There is, however, a period frequently lasting several days during which the animal is bright and active and in good general condition. Observations during this period seem reasonably reliable.

Blood pressure observations employing this technic confirm the impression that there is a normal range rather than a normal blood pressure level for the dog varying with the degree and nature of activity.

14461

Streptomycin, a Substance Exhibiting Antibiotic Activity Against Gram-Positive and Gram-Negative Bacteria.*†

ALBERT SCHATZ, ELIZABETH BUGIE, AND SELMAN A. WAKSMAN.
From the New Jersey Agricultural Experiment Station, New Brunswick, N.J.

With the exception of streptothricin,[1] most of the antibiotic substances known at the present time, including penicillin and other mold products as well as gramicidin and actinomycin, act largely upon gram-positive bacteria. The activity of these substances upon gram-negative organisms is highly selective, as in the case of penicillin, which affects the *Neisseria* group and has little activity upon *Escherichia coli* and other gram-negative bacteria,[2] or else much larger quantities are required to bring about the inhibition of these bacteria, as in the case of actinomycin.[3] Among the antibiotic agents that act selectively alike against both gram-positive and gram-negative bacteria, streptothricin occupies a prominent place; since this substance is water-soluble and possesses limited toxicity to animals, it is of particular interest from a chemotherapeutic point of view. Unfortunately, streptothricin has very little activity against a number of bacteria found among both the gram-negative (*Pseudomonas fluorescens, Ps. aeruginosa*) and the gram-positive (*Bacillus mycoides* and *B. cereus*) groups.

In a search for antagonistic organisms that are active against gram-negative bacteria, and from which antibiotic substances could be isolated, the actinomycetes were found[4] to offer extensive potentialities. Although most of the antibacterial agents produced by these organisms are also active against gram-positive bacteria, certain few of them exert a marked selective activity against many of the gram-negative types of bacteria. *Actinomyces lavendulæ,* which produces streptothricin, is such an organism. After detailed examination of a large number of cultures, either isolated at random from different natural and enriched soils and composts, or selected from the culture collection, another organism was found that produces an antibiotic substance which apparently combines many of the desirable antibacterial properties. This organism is similar, in most of its cultural characteristics as well as in its morphology,

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1 Waksman, S. A., and Woodruff, H. B., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 207; *J. Bact.*, 1943, **46**, 299.

2 Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A., Heatley, D., Jennings, M. A., and Florey, H. W., *Lancet*, 1941, **241**, 177; *Nature*, 1942, **148**, 758; **149**, 356.

3 Waksman, S. A., and Woodruff, H. B., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 609; *J. Bact.*, 1941, **42**, 231.

4 Waksman, S. A., Horning, E. S., Welsch, M., and Woodruff, H. B., *Soil Sci.*, 1942, **54**, 281; Welsch, M., *J. Bact.*, 1942, **44**, 571.

TABLE I.
Production of Antibiotic Substances by 5 Actinomycetes.*

Organism	Nature of substance	Medium	Dilution units†			Diffusion units‡
			B. subtilis	*S. aureus*	*E. coli*	
A. antibioticus	Actinomycin	Tryptone-starch	750	500	0	—
" "	"	Nutrient broth	500	200	0	123
A. lavendulæ	Streptothricin	Tryptone-starch	500	150	75	115
" "	"	Nutrient broth	20	<30	<30	10
A. griseus (18-16)	Streptomycin	Tryptone-starch	150	30	75	123
" " "	"	Nutrient broth	500	30	75	190
A. griseus (D-1)	Streptomycin	Tryptone-starch	75	<30	<30	30
" " "	"	Nutrient broth	100	30	<30	70
Micromonospora sp.	Micromonosporin	Tryptone-starch	30	—	0	6
" "	"	Nutrient broth	100	—	0	5

* The cultures were grown in a submerged and agitated state, at 28°C for 6 days.
† Plate method used.
‡ Calculated against a streptothricin standard, using *B. subtilis* spores as the test organism.

to *A. griseus* isolated from the soil some 28 years ago.[5] The active substance is in many respects similar to streptothricin, although it differs from it in its greater activity against various gram-negative bacteria, notably the *Ps. aeruginosa* group, as well as against those aerobic spore-forming bacteria, such as *B. mycoides,* which are resistant to streptothricin. Because of its similarity to streptothricin, this substance may be designated as *streptomycin,* derived from the generic name that has recently been given to the aerial-mycelium producing and sporulating group of actinomycetes, namely, *Streptomyces.*[6] Two strains of the organism producing streptomycin were obtained, one (No. 18-16) from a heavily manured field soil and the other (D-1), a somewhat less active form, from a smear plate of the throat of a chicken; it is doubtful, however, whether this organism is a normal inhabitant of the animal system.

In order to compare the antibacterial properties of the culture filtrate of the two strains of this organism with those of other actinomycetes producing antibiotic substances, the results of a typical experiment are reported in Table I. The filtrates of *A. antibioticus* and of *Micromonospora* sp. gave no activity against *E. coli;* however, *A. lavendulæ* and the 2 strains of *A. griseus* exerted an appreciable effect upon this bacterium. Results obtained by the agar diffusion method were found to be comparable to the dilution units for *B. subtilis* and *Staphylococcus aureus.* The nature of the medium in which the organisms were grown is of considerable importance in the production of the different antibiotic agents: streptothricin is produced most abundantly in a tryptone-starch medium, and streptomycin in ordinary nutrient broth, namely, a peptone-meat extract medium.

A further study of the influence of the composition of the medium upon the production of the active agent by *A. griseus* brought out the fact that, whereas streptothricin is formed abundantly in a simple medium, streptomycin requires the presence of a specific growth-promoting substance supplied by meat extract. Corn steep liquor can take the place of the meat extract. Addition of glucose further increases the yield of the substance. The nature of the protein hydrolyzate is apparently immaterial, since tryptone gave about the same degree of activity as peptone. A medium was finally adopted, consisting of 1% glucose, 0.5% peptone, 0.3% meat extract or 1.2% corn steep, and 0.5% NaCl.

The course of production of streptomycin under submerged and stationary conditions is brought out in Table II. The organism did not form any acid either in the submerged or

[5] Waksman, S. A., *Soil Sci.,* 1919, **8**, 71.

[6] Waksman, S. A., and Henrici, A. T., *J. Bact.,* 1943, **46**, 337.

TABLE II.
Metabolism of *A. griseus* and Course of Production of Streptomycin.

Shaken cultures						Stationary cultures					
	Dilution units						Dilution units				
Days	*B. subtilis*	*E. coli*	Diffusion units	pH	Growth mg	Days	*B. subtilis*	*E. coli*	Diffusion units	pH	Growth mg
2	150	40	10	7.8	270	3	40	5	6	7.7	73
3	250	50	70	7.7	185	5	150	20	12	7.8	171
4	200	40	60	7.8	—	7	225	20	53	7.9	163
7	500	125	70	8.2	—	9	275	75	—	8.3	264
						12	>300	100	55	—	—

TABLE III.
Comparative Bacteriostatic Spectra of Streptomycin and Streptothricin.
On basis of crude, ash-free dry material.

Organism	Gram stain	Units of activity per gram ash-free dry material	
		Streptomycin × 1000	Streptothricin* × 1000
B. subtilis 0	+	125	500
B. mycoides 0	+	250	<3
B. mycoides 317-911	+	20	<3
B. cereus	+	30	<3
B. mesentericus	+	15	—
B. megatherium	+	100	150
S. aureus	+	15	200
S. lutea	+	100	150
M. phlei	+	100	50
M. tuberculosis	+	30	—
Phytomonas pruni	—	100	400
Listerella monocytogenes	—	10	—
Shigella gallinarum	—	—	150
E. coli	—	25	100
S. marcescens	—	25	5
A. aerogenes	—	10	50
P. vulgaris	—	10	50
S. aertrycke	—	2.5	—
S. schottmülleri	—	—	50
Ps. fluorescens	—	2	<3
Ps. aeruginosa	—	1	<3
Cl. butylicum	—	3	<3

* These results are partly based on data reported previously; data obtained more recently with purified preparations give the same type of spectrum. Since streptothricin represented a more purified and, therefore, more concentrated preparation than streptomycin, a better comparison would be with the activity against *E. coli* as a unit; the units for the other test organisms would, therefore, have to be multiplied by 4.

in stationary cultures, as was found to be the case of *A. lavendulæ*,[7] the reaction of the medium becoming alkaline even in the presence of glucose. Growth was much more rapid in shaken cultures, although very good activity was also obtained in a stationary condition.

The growth of the organism was allowed to proceed for 5 to 12 days and the streptomycin isolated from the culture filtrate, using a method similar to that developed previously for the isolation of streptothricin.[1]

The antibacterial behavior of streptomycin, as compared with that of streptothricin, can best be illustrated by an examination of the respective bacteriostatic spectra for the two substances, as presented in Table III. Concentrated preparations, although not of the same degree of purification, of the materials were used. Taking the activity against *E. coli* as a standard, streptomycin was found to have

[7] Woodruff, H. B., and Foster, J. W., *Arch. Biochem.*, **2**, 301.

the same activity as streptothricin against *B. subtilis, A. aerogenes,* and *P. vulgaris;* it was less active against *S. aureus* and certain strains of *Salmonella;* it was much more active against *B. mycoides, B. cereus, Mycobacterium phlei, Serratia marcescens, Ps. aeruginosa, Ps. fluorescens,* and *Cl. butylicum.*

Streptomycin, like streptothricin, possesses strong bactericidal properties, and preliminary experiments tended to indicate that the two substances are also comparable in their low toxicity to animals and in their *in vivo* activity. The various chemical and biological properties of streptomycin tend to point to this compound as one closely related to streptothricin; the fact that it differs from the latter in the nature of its antibacterial activity may indicate a closely related but not the same type of molecule.

Summary. A new antibacterial substance, designated as streptomycin, was isolated from two strains of an actinomyces related to an organism described as *Actinomyces griseus.* This substance resembles streptothricin in its solubility in water, mode of isolation and concentration from culture medium, its selective activity against gram-negative bacteria, and its limited toxicity to animals. However, the two substances differ in the nature of their respective bacteriostatic spectra as well as in their quantitative action upon different bacteria. It is suggested that one is dealing here with two closely related chemical compounds.

14462

Production of Riboflavin Deficiency in the Monkey.*

HARRY A. WAISMAN. (Introduced by C. A. Elvehjem.)
From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

In a recent report from this laboratory[1] it was demonstrated that the rhesus monkey (*Macaca mulatta*) grows well and remains in excellent health for at least 14 months on a highly purified diet consisting of sucrose 73, purified casein† 18, salts 4, corn oil 3, cod liver oil 2, and adequate quantities of the pure vitamins together with a "folic acid" concentrate. Through the use of this diet we have attempted to produce specific vitamin deficiencies by withholding the vitamin concerned from the daily supplement. The "folic acid" concentrate (norite eluate according to the method of Hutchings *et al.*[2]) was assayed for its content of each of the B group of vitamins and found to contain between 1 and 2 γ of riboflavin per gram equivalent of original "solubilized liver extract"‡ from which the concentrate was made. The maximum riboflavin furnished by a 5% level of the concentrate was therefore 10 γ. Negligible quantities of riboflavin were contained in the purified casein and in the sucrose used in the diet.

The monkeys were given the basal diet *ad libitum* and all the vitamins except riboflavin were supplied in the daily supplement according to the procedure previously described.[3] Recently obtained monkeys placed on the deficient diet showed growth failure after 6 to 8 weeks while other monkeys

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1 Waisman, H. A., and Elvehjem, C. A., *J. Nutrition,* 1943, **26**, 361.

† Smaco vitamin-free casein, obtained from SMA Corp., Chagrin Falls, Ohio.

2 Hutchings, B. L. Bohonos, N., and Peterson, W. H., *J. Biol. Chem.,* 1941, **141**, 521.

‡ Obtained through the courtesy of Wilson Laboratories, Chicago, Illinois.

3 Waisman, H. A., Rasmussen, A. F., Jr., Elvehjem, C. A., and Clark, P. F., *J. Nutrition,* 1943, **26**, 205.