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Streptothricin, a New Selective Bacteriostatic and Bactericidal Agent, Particularly Active Against Gram-Negative Bacteria.*

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Most of the bacteriostatic and bactericidal substances of microbial origin that have been so far isolated act primarily against gram-positive bacteria, and only to a limited extent upon gram-negative organisms. Even those preparations, like pyocyanase and actinomycin, which act upon gram-negative bacteria are still more active against the gram-positive forms. The action of the different substances is selective both qualitatively with respect to the specific kinds of organisms affected, and quantitatively in regard to the relative concentration of the active substance required to inhibit the growth of various bacteria or to destroy them. This selective action also expresses itself in the relative toxicity of the various materials to animals and their activity *in vivo*.

A new substance can now be added to this list of antibiotic agents. This substance has been obtained from a soil *Actinomyces*, and is designated as *streptothricin*, derived from the early generic designation *Streptothrix*, given to this group of organisms. This is the fourth type of antibiotic substance produced by actinomycetes. Of the three other compounds, only one has so far been isolated in a crystalline form, namely *actinomycin*.[1] The other two are *actinomycetin*,[2, 3] a water-soluble, thermostable, protein-like preparation, which causes lysis of certain specific bacteria, the presence of the living mycelium of the antagonist being favorable to its antagonistic action, and *lysozyme*,[4, 5] a water-soluble, alcohol-insoluble, thermostable preparation, stated to be similar to the lysozyme of egg white.

The new organism was isolated† from the soil by the use of the

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

2 Gratia, A., et Dath, S., *Compt. rend. Soc. Biol.*, 1924, **91**, 1442; 1925, **92**, 1125; **93**, 451; 1926, **94**, 1267.

3 Welsch, M., *Compt. rend. Soc. Biol.*, 1937, **124**, 573.

4 Krassilnikov, N. A., and Koreniako, A. I., *Microbiologia*, 1939, **8**, 673.

5 Kriss, A. E., *Microbiologia*, 1940, **9**, 32.

† The isolation of this organism was carried out by Dr. W. Kocholaty of the University of Pennsylvania, while working in our laboratory.

bacteria-enriched agar-plate.[1] It is in many respects similar to a species described a quarter of a century previously as *Actinomyces lavendulæ*,[6] although it differs somewhat from it in certain respects. Streptothricin is produced by growing the organism in a tap-water medium containing 1% glucose or starch, 0.5% tryptone, 0.2% K_2HPO_4, 0.2% NaCl, 0.001% $FeSo_4$, and 0.25% agar. It is also formed when the organism is grown on a variety of simple nitrogenous compounds, such as glycocoll, alanine, aspartic acid, asparagine and glutamic acid, with small amounts of glucose, and even in the complete absence of the carbohydrate, with some of the amino acids. No growth of the organism is obtained on tryptophane, phenyl alanine and certain simple inorganic salts of nitrogen; with ammonium sulfate, growth is obtained but no active substance produced. The formation of the active substance is thus associated with the nitrogen nutrition of the organism. Iron appears to play an essential rôle in the production of the substance. An increase in growth as a result of an increase in carbohydrate concentration does not result in an increase in streptothricin content; however, an increase in growth as a result of an increase in the amino-acid concentration, with the same amount of carbohydrate, causes an increase in the concentration of the active substance. When streptothricin is produced upon an amino acid alone, in the absence of carbohydrate, there is a gradual increase in alkalinity, which results in the destruction of the substance.

The *A. lavendulæ* is grown upon one of the above media, for 8 to 10 days, at 28°C, and the streptothricin isolated. It is readily soluble in water and in dilute mineral acids, but is destroyed by concentrated acids. It is insoluble in ether, petrol ether, and chloroform. It is precipitated from the tryptone medium by protein-precipitants, but, upon isolation, it ceases to give proteic characteristics. This is due to the fact that it is bound in the culture to a protein, becoming inactivated upon the coagulation of the latter. In the crude culture-filtrate and in the alcohol-precipitated form, streptothricin is thermolabile, whereas, in the purified state, it is thermostable, withstanding 100°C for 15 minutes. Treatment with proteolytic enzymes does not reduce its activity. On adjusting the reaction of the medium, upon the completion of growth, to pH 3.5 with acid, a precipitate is produced, the filtrate containing virtually all the activity. Streptothricin is completely adsorbed, at neutrality, on charcoal (norit A), from which it can be removed by treatment with dilute mineral acid for 8 to 12 hours. The acid-extract is

[6] Waksman, S. A., and Curtis, R. E., *Soil Sci.*, 1916, **1**, 99.

neutralized and concentrated *in vacuo*, at 50°C, just to dryness. The residue is extracted with absolute alcohol, filtered, evaporated, and taken up in water. This particular type of preparation usually contains 40 to 50% inorganic matter and 2 to 3% nitrogen, on an ash-free basis. Further concentration and reduction in ash-content were obtained by subsequent treatments. On electrodialysis, the active substance moves to the cathode at pH 7.0. In general, the properties of this substance appear to be those of an organic base.

Streptothricin is highly active, even in crude preparations, against various gram-negative and gram-positive bacteria, in concentrations

TABLE I.
Inhibitory Effect of Streptothricin upon Growth of Various Bacteria.

Organism	Crude streptothricin added per 10 cc agar, mg					
	3	1	0.3	0.1	0.03	0.01
Bac. subtilis	0‖	0	0	0	0	1
Bac. mycoides	2	2	2	2	2	2
Bac. macerans	2	2	2	2	2	2
Bac. megatherium	0	0	0	0	1	2
Bac. polymyxa	0	0	2	2	2	2
Bac. cereus	2	2	2	2	2	2
Micrococcus lysodeikticus	0	0	0	1	2	2
Staphylococcus musicæ	0	0	0	1	2	2
Sarcina lutea	0	0	0	0	1	2
*Aërobacter aërogenes**	0	0	1	2	2	2
Aërobacter aërogenes	0	0	0	Tr	2	2
Escherichia coli†	0	0	0	0	2	2
Escherichia coli 4348	0	0	Tr	1	2	2
Serratia marcescens	0	1	2	2	2	2
Serratia marcescens	1	1	2	2	2	2
Pseudomonas fluorescens‡	2	2	2	2	2	2
Shigella gallinarum	0	0	0	0	1	2
Pasteurella pseudotuberculosis	0	0	0	Tr	2	2
Salmonella cholerasuis	0	0	0	Tr	2	2
Salmonella schottmülleri	0	0	0	1	2	2
Salmonella abortivoequina	0	0	0	Tr	2	2
Salmonella typhimurium	0	0	0	2	2	2
Hemophilus suis	0	0	0	2	2	2
Hemophilus influenzæ	0	0	0	0	0	1
Brucella abortus	0	0	0	0	2	2
Azotobacter agile	0	0	0	0	0	2
Azotobacter vinelandii	0	0	0	0	0	2
Azotobacter chroococcum	0	0	0	Tr	2	2
Azotobacter indicum	0	0	0	2	2	2
Mycobacterium phlei	0	0	0	1	2	2
Clostridium butylicum§	2	2	2	2	2	2
Lactobacillus casei§	0	0	0	2	2	2
Actinomyces albus	0	0	0	1	2	2
Actinomyces violaceus-ruber	0	0	—	—	—	—
Actinomyces lavendulæ	0	1	2	2	2	2

*Representing 3 distinct strains.
†Representing 5 strains of *E. coli* obtained from different sources.
‡Representing 4 strains.
§Cultured anaërobically.
‖0 = no growth, 1 = limited growth, 2 = good growth, Tr = trace of growth.

of 0.1 mg crude material per 10 cc agar, as shown in Table I. There is very little difference in the degree of sensitivity between *E. coli* and *S. lutea; B. subtilis* is more sensitive than either of those, whereas *Bac. mycoides* is not sensitive at all. Various spore-forming bacteria are thus found to show marked differences in the degree of sensitivity to this substance.

Streptothricin also possesses strong bactericidal properties, especially for various gram-negative bacteria (Table II). However, higher concentrations are required than for its bacteriostatic action.

Summary. A new type of antagonistic substance, designated as streptothricin, has been obtained from a soil *Actinomyces*. The substance appears to be formed from certain amino acids in the medium. Streptothricin is insoluble in ether, petrol ether or chloroform, and is carried down by precipitating the medium with ethyl alcohol. It is best separated from the medium by adsorption on norit-A, followed by elution with dilute mineral acid. Further concentration is accomplished by neutralization of the acid and concentration *in vacuo*. The active substance is low in nitrogen, is not affected by proteolytic enzymes and can withstand 100°C, for 15 minutes. Its properties are those of an organic base.

The bacteriostatic action of streptothricin is unique. The addition of 0.1 mg of crude material to 10 cc of nutrient agar inhibits the growth of *E. coli* and various other gram-negative bacteria; among the gram-positive bacteria, some, like *Bac. subtilis* and micrococci, are more sensitive, whereas others, like *Bac. mycoides,* are far more resistant. Streptothricin possesses also marked bactericidal properties, especially upon certain gram-negative bacteria.

TABLE II.
Bactericidal Action of Streptothricin upon *E. coli* and *B. subtilis*.
Washed suspension of bacteria in 10 ml sterile water.

Bacteria at start, per 1 cc	*E. coli* 500,000,000		*M. lysodeikticus* 17,600,000	
	Number of viable cells per 1 cc left after			
Streptothricin, mg	17 hr × 1000	65 hr × 1000	17 hr × 1000	65 hr × 1000
0	450,000	413,000	1,900	393
0.01	430,000	362,000	800	82
0.1	290,000	213,000	500	10
1.0	2,400	18	100	5
5.0	1,000	7	—	—