STREPTOMYCIN

Nature and Practical Applications

EDITED BY

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PREFACE

Probably no other drug in the history of medical science has had such a phenomenal rise as streptomycin. The sulfa compounds, followed by penicillin, pointed to the great potentialities of chemotherapeutic agents, produced either by chemical synthesis or by certain microorganisms, in combating infections caused by bacteria and other microscopic and ultramicroscopic forms of life. These discoveries almost coincided with the reawakening of the general interest in antibiotics, or those substances which are produced by microorganisms and which have the capacity of inhibiting the growth and even of destroying other microorganisms, notably disease-producing bacteria.

Neither the synthetic sulfa drugs nor the antibiotics tyrothricin and penicillin, however, had sufficient activity upon some of the most important gram-negative bacteria or upon the acid-fast, notably the tuberculosis, organisms. Agents to combat these diseases were badly needed. The fact that the world was in the midst of a great catastrophe made the need for new chemotherapeutic agents more imperative. The isolation of streptothricin in 1942 demonstrated that such agents could be found and that the actinomycetes are probably the logical microorganisms which should be considered as potential producers of such agents. Streptomycin appeared to fill the gap.

In the five years since the isolation of streptomycin in 1943, considerable progress has been made, as evidenced by the fact that a literature of nearly 1,800 references, covering reports of investigations in many countries and languages, has accumulated. This is ample justification for an attempt to summarize the present status of the subject. In the following chapters outstanding authorities, many of whom have pioneered in the development and utilization of streptomycin, notably its isolation and clinical applications, have collaborated to present summaries of their work as well as that of others in the field. Each chapter is accompanied by only very few pertinent references. For the more complete literature the reader is referred to "The Literature on Streptomycin, 1944–1948," published by the Rutgers University Press.

It is sincerely hoped that this information will prove useful to all those who are interested in streptomycin, especially in its use in combating bacterial infections.

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MISCELLANEOUS USES OF STREPTOMYCIN

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CHAPTER 1

HISTORICAL INTRODUCTION

The organism producing streptomycin was first isolated in the laboratories of the Department of Microbiology of the New Jersey Agricultural Experiment Station, Rutgers University, in September 1943. The first public announcement of the isolation of the antibiotic was made by Schatz, Bugie, and Waksman (1) in January 1944. Its *in vivo* activity was soon established (2). Before the end of the year, its activity against the tuberculosis organism, both *in vitro* (3) and *in vivo* (4), had been demonstrated, and although available in only very small amounts, streptomycin was being submitted to clinical trials. In less than two years from the date of its isolation, extensive investigational work, comprising bacteriological, chemical, pharmacological, and clinical studies, had been accomplished, and the practical potentialities of streptomycin as a chemotherapeutic agent were definitely established.

In presenting a comprehensive summary of the clinical uses of streptomycin before the conference on Antibiotics, held by the New York Academy of Sciences, in January 1946, Hinshaw and Feldman (5) of the Mayo Clinic, dedicated their address to the second anniversary of the announcement of the isolation of streptomycin. The following year saw the inauguration of a series of intensive clinical applications of streptomycin in the treatment of numerous diseases, mostly those caused by gram-negative bacteria or bacteria resistant to penicillin and to sulfa drugs. Hope was aroused, too, that possibly an agent had finally been found which was also effective against tuberculosis. Several centers were established, during 1946, for testing the sensitivity of different freshly isolated strains of *M. tuberculosis* to streptomycin, and before that year came to an end, observations on the first hundred cases of tuberculosis treated with streptomycin were reported (6). The first observations were then made of the development of bacterial resistance to the drug and of the practical evaluation of this phenomenon in experimental animals. On February 17, 1947, in New York, a Conference was arranged by the American Trudeau Society and the National Tuberculosis Association to discuss the application of streptomycin to clinical tuberculosis, the methods of administration, and possible

toxic effects. This event took place exactly three years after the public announcement of streptomycin reached the scientific reader.

The third year also brought about the almost complete elucidation of the chemistry of the streptomycin molecule, the preparation of the first chemical derivative of streptomycin, and the report of the first thousand clinical cases treated with this drug. Within four years streptomycin production had grown from a laboratory curiosity into a large industry, with a monthly output of more than 3,000 kg of the pure base.

This rapid progress in the development of streptomycin was due largely to two factors: the spectacular rise of penicillin between 1941 and 1943 which suggested the possibility of finding other antibiotics that could be utilized as chemotherapeutic agents for treatment of diseases not affected by penicillin; and the investigations carried out previously in the laboratories of the Department of Microbiology on streptothricin, which is similar chemically and biologically to streptomycin. The work on streptothricin laid the foundation for the subsequent rapid progress in the isolation and use of streptomycin, the latter having a broader antibacterial spectrum and being less toxic to animals than the former.

The Committee on Chemotherapeutics of the National Research Council contributed much to the coordination of the clinical work done on streptomycin (8). At first, all the streptomycin produced was reported to the Civilian Production Administration for allocation. First consideration was given to the needs of the Army, Navy, U. S. Public Health Service, Veterans Administration, and the National Research Council, and the available supply was adjusted to those needs. No other agency was allowed to purchase streptomycin. No patient who was treated with this drug paid for it, and no physician was charged for it. The program of clinical research was thus conducted by the concerted efforts of the government, the producers of streptomycin, the National Research Council, and civilian medical scientists, with the sole purpose of obtaining in the shortest possible time the necessary information concerning this antibiotic. The program of the National Research Council was supported by grants in aid from eleven pharmaceutical and chemical companies (9).

This program constituted the first privately financed, nationally coordinated clinical evaluation in history. It was made possible by a joint contribution of nearly $1,000,000 from pharmaceutical and chemical manufacturers of streptomycin. It was estimated that, by October 1946, the manufacturing laboratories had invested $20,000,000 in production facilities for a drug of very recent origin.

Wide-scale distribution of streptomycin began in September 1946, when about 1,600 general hospitals were designated as depots for the drug. Any nondepot hospital or any physician could call upon a depot hospital for the

purchase of streptomycin when its use was indicated. The requests of other institutions were given careful consideration, since each depot hospital was limited to a monthly quota. By the beginning of 1947, the Committee on Chemotherapy had completed its work. Enough streptomycin was then being produced to make possible wide domestic sales and even export. The various companies manufacturing streptomycin have thus contributed in many ways to the elucidation of this drug as a chemotherapeutic agent.

Finally, record must be made of the Conference on Tuberculosis held by the Interim Commission of the World Health Organization, on July 30–31, 1948, in New York, attended by representatives from various countries and at which definite recommendations were drafted concerning the use and value of streptomycin in tuberculosis.

Antagonistic Properties of Actinomycetes

The isolation of streptomycin and its utilization in the treatment of numerous infections in man and in animals which previously had not lent themselves to therapy, was a high point in a long and painstaking search for antibiotics, a search which is continuing unabated. The microbes which produce streptomycin belong to the actinomycetes, a group of organisms occurring abundantly in soils, manures, composts, fresh water basins, and dust. They are filamentous and branching organisms that the bacteriologist has been accustomed to consider as bacteria and that the mycologist was inclined to classify with the fungi.

In attempts to elucidate the nature of the complex microbiological population of the soil and other natural substrates, the paramount fact that impresses itself upon investigators is that those microbes which occur in natural substrates exert a variety of associative and antagonistic influences upon one another. Among the various groups of microorganisms, the actinomycetes appear to persist longest in the soil, especially under conditions unfavorable to the growth of other organisms, such as would result from drying, treatment with antiseptics, or after extensive and prolonged decomposition of organic materials.

The ability of a large number of actinomycetes to inhibit the growth of bacteria, fungi, and other actinomycetes had been established by many investigators studying this group of organisms. Gasperini first reported in 1890 (10) that certain actinomycetes, designated as *Streptothrix*, have the capacity to develop upon the surfaces of bacteria and fungi and to digest the membranes of these organisms. At a much later date, in 1921, Lieske demonstrated that various actinomycetes were able to bring about lysis of many dead and living bacterial cells, and that the antibacterial activities of the actinomycetes were selective in nature, affecting only certain organisms, such as *S. aureus*, and not others. Soon afterwards, in 1924, Gratia

also observed that cultures of *Streptothrix* were able to lyse bacterial cells; he used such lysed cells, designated as *mycolysates*, for immunizing purposes.

Other investigations soon followed. These brought out the fact that certain species, or perhaps strains, of actinomycetes have the capacity to inhibit the growth of various bacteria, as shown by Rosenthal for the diphtheria organism. Some of these actinomycetes were found capable of producing a thermostable active substance. This substance was also strongly bactericidal, but its activity was limited to certain bacteria; it had little effect upon others.

The wide distribution of antagonistic actinomycetes in nature was first established by Nakhimovskaia in 1937. Out of eighty cultures isolated from a variety of soils, forty-seven possessed antibacterial properties, but only twenty-seven liberated active substances into the medium. The activity of these substances consisted largely in inhibiting the growth of gram-positive bacteria; they had no effect upon gram-negative bacteria or fungi.

In a series of detailed surveys of the distribution of antagonistic properties among actinomycetes, Waksman *et al.* reported, in 1942, the isolation of 244 cultures from various soils. Of these, 106, or 43.4 per cent, possessed some antagonistic properties, and forty-nine, or 20 per cent, were highly antagonistic. The nature of the test organism, the composition of medium, and the method of testing greatly influenced the results obtained. In another survey of 187 cultures, freshly isolated from different substrates, only 3 per cent gave a high activity against *E. coli* when tested on nutrient agar and 6 per cent on dextrose-asparagine agar; the corresponding figures for the cultures active against *B. subtilis* were 16 and 44 per cent. This pointed to the limited activity of the antagonistic actinomycetes upon gram-negative bacteria, as compared to the gram-positive forms.

The ability of actinomycetes to antagonize fungi has also been established. A survey of the antagonistic properties of eighty cultures made by Alexopoulos, using the fungus *Colletotrichum* as the test organism, gave 17.5 per cent as strong inhibitors, 38.8 per cent as weak inhibitors, the others having no effect. The inhibiting action of certain soil actinomycetes upon plant pathogenic forms was also established; it has even been proposed that this phenomenon be utilized for the control of potato scab, a disease of potatoes caused by *S. scabies*.

Thus by 1942, it was definitely established that a large proportion of the actinomycetes possessed remarkable properties of inhibiting the growth of various bacteria and of other microorganisms, and even of causing their destruction.

Production of Antibiotics by Actinomycetes

Previous to 1940, most of the investigations on the antibacterial activities of actinomycetes were limited to the living organisms. At that time, only one preparation which had antibacterial effects was known. This preparation, designated by Welsch as ACTINOMYCETIN, had the properties of a proteolytic enzyme, and was active primarily on dead bacterial cells.

The first experiments, begun in 1939, in the Department of Microbiology of the New Jersey Agricultural Experiment Station, on the production of antibacterial substances by microorganisms, led to the isolation of a culture of an actinomyces, which was later described as *A.* (*S.*) *antibioticus*. This culture produced a highly potent antibiotic, which was named ACTINOMYCIN. This antibiotic was soon crystallized, and its chemical and antibacterial properties were determined. Although interesting from a chemical and biological point of view, it did not offer any remarkable chemotherapeutic potentialities, since it was extremely toxic to the experimental animals.

Other substances were soon isolated, notably PROACTINOMYCIN and MICROMONOSPORIN. Neither of these appeared to offer any distinct possibilities as a chemotherapeutic agent. Since penicillin appeared on the horizon in 1941 and since it promised to fill the need for the treatment of diseases caused by gram-positive bacteria, it was decided to concentrate upon the isolation of antibiotics which would be active against the gram-negative bacteria and upon the acid-fast group of bacteria, including the tuberculosis organism. These studies in the New Jersey laboratories, and in others, resulted in isolation of a large number of antibiotic substances from actinomycetes. These substances are active not only against various bacteria, but also upon fungi and viruses. Special procedures had to be developed for the growth of each specific organism and for the production and isolation of each specific antibiotic. More than thirty antibiotics have now been isolated or at least are known to be produced by actinomycetes.

A comparison of the antibacterial spectra of some of these antibiotics with those produced by fungi is given in table 1. Antibiotic spectra for STREPTOTHRICIN and STREPTOMYCIN are shown in table 2. The four antibiotics of the fungi possess a high activity against gram-positive bacteria and relatively little action on the gram-negative forms, the acid-fast organisms falling between. Streptothricin and streptomycin are highly active gainst the last two groups of organisms. These two antibiotics show certain similarities in their general antibiotic spectra, but they also exhibit marked differences, as shown in their specific effects upon *B. mycoides* and *B. cereus*, on the one hand, and upon certain gram-negative bacteria, as *S.*

marcescens, on the other. Streptothricin is more active against fungi than is streptomycin. It is also more toxic.

TABLE 1

Antibacterial spectra of several antibiotics (12)

Minimum inhibitory concentration of antibacterial substances in micrograms per milliliter

	GLIOTOXIN	PENICILLIC ACID	PENICILLIN G	PENICILLIN X	STREPTOMYCIN	STREPTOTHRICIN
B. mycoides	0.25	32	30.00	30.00	0.13	100.0
B. subtilis	0.25	8	0.03	0.06	0.25	0.8
S. aureus	0.15	16	0.016	0.03	0.03	0.1
E. coli	25.00	64	14.00	14.00	0.25	0.3
Kl. pneumoniae	6.00	64	110.00	240.00	0.13	0.1
Ps. aeruginosa	500.00	1,000	500.00	500.00	4.00	2.0
M. phlei	4.00	64	14.00	29.00	0.25	7.0
M. smegmatis	4.00	32	450.00	470.00	1.00	14.0

TABLE 2

Comparative bacteriostatic spectra of streptomycin and streptothricin

On basis of crude, ash-free dry material

ORGANISM	GRAM STAIN	UNITS OF ACTIVITY PER MILLIGRAM ASH-FREE DRY MATERIAL	
		Streptomycin	Streptothricin
B. subtilis	+	500	500
B. mycoides	+	1,000	<3
B. cereus	+	120	<3
B. megatherium	+	400	150
S. aureus	+	60	200
S. lutea	+	400	150
M. phlei	+	400	50
M. tuberculosis	+	120	—
Ph. pruni	−	400	400
E. coli	−	100	100
S. marcescens	−	100	5
A. aerogenes	−	40	50
Pr. vulgaris	−	40	50
Ps. fluorescens	−	8	<3
Ps. aeruginosa	−	4	<3
Cl. butylicum	−	12	<3

Actinomycetes were thus found to be active producers of antibiotics that vary greatly in their antimicrobial spectra. Some of these spectra are very narrow, as in the case of the so-called ANTISMEGMATIS factor, which is active

only against *M. smegmatis*, *M. phlei*, and the nonpathogenic strain 607 of *M. tuberculosis* and NOCARDIN, which is active against *M. tuberculosis*. Other antibiotics, such as streptothricin, streptomycin, CHLOROMYCETIN, and AUREOMYCIN, have very wide antibiotic spectra, those of the last two affecting rickettseae and some of the larger viruses.

Some antibiotics are now known to be produced by more than one species of actinomyces. This was found to hold true for actinomycin, which is formed by *S. antibioticus* and by a number of other species belonging to the genus *Streptomyces*, and for streptomycin, which is produced by *S. griseus* and by *S. bikiniensis*. Some antibiotics, such as members of the streptothricin group, vary greatly in their chemical make-up, in their selective activity upon different microorganisms, and in their toxicity to animals. Certain actinomycetes produce more than one antibiotic. This is true, for example, of *Streptomyces* F, which produces streptomycin and streptothricin, and of *S. griseus*, which produces, in addition to streptomycin and mannosidostreptomycin, actidione and streptocin.

ISOLATION OF STREPTOTHRICIN

With the isolation of streptothricin in 1942 by Waksman and Woodruff (11), an antibiotic was obtained which showed distinct promise in activity against gram-negative bacteria, accompanied by a limited toxicity to animals. This substance was produced by a culture of an actinomyces, known as *A.* (*S.*) *lavendulae*, which was obtained from the soil, in the laboratories of the Microbiology Department of the New Jersey Agricultural Experiment Station in 1941. The name streptothricin was derived from *Streptothrix* given to the actinomycetes by Ferdinand Cohn, in 1875. Other strains of this organism or of closely related organisms were later isolated and found capable of producing this antibiotic.

Streptothricin was found to possess highly desirable physical, chemical, and antibacterial properties and offered promise as a chemotherapeutic agent. Streptothricin is water-soluble, fairly resistant to heat, and is active over a wide pH range, with an optimum at a slight alkalinity. It is active against numerous gram-negative and certain gram-positive bacteria, both *in vitro* and *in vivo*. It is active also against fungi, but not against viruses. It is highly resistant to the action of different organisms; it cannot be destroyed by fungi, by bacteria, or by enzymes.

Streptothricin is formed also by certain actinomycetes in admixture with other antibiotics, as for example, with streptomycin. Its production takes place under both stationary and submerged conditions of culture. Actinomycetes capable of producing streptothricin or related compounds are widely distributed in nature; this explains the large number of compounds already isolated which have properties similar to those of this antibiotic.

It is sufficient to mention STREPTIN, LAVENDULIN, ACTINORUBIN, STREPTOLIN, and ANTIBIOTIC 136.

When detailed studies of the pharmacology of streptothricin were undertaken it was discovered that this antibiotic leaves a residual toxic effect in the animal body. Its use for parenteral administration was, therefore, excluded. Its possible practical application may be limited to oral or topical administration.

ISOLATION OF STREPTOMYCIN

The experience gained in the study of the formation and isolation of streptothricin from cultures of actinomycetes proved to be highly important in planning a search for other antibiotic agents that would possess similar or even more desirable biological and chemical properties, such as a broader antibiotic spectrum and less toxicity to the animal body. After further extensive studies of many actinomycetes representing a great variety of species and strains, two cultures were found to yield the desirable antibiotic. These were isolated from the soil and from the throat of a chicken. They both belonged to a species described as *A. griseus*, the first representative of which was isolated in this country in 1916 from the soil. The generic name of the organism was changed by Waksman and Henrici in 1943 from *Actinomyces* to *Streptomyces*. To honor this new generic name, the new antibiotic was designated as streptomycin.

As previously noted, the two cultures of the streptomycin-producing organism were first isolated in September 1943. Because of the similarity of the new antibiotic to streptothricin, both in isolation procedures and in its antibiotic spectrum, rapid progress was made in the development of suitable methods for the growth of the organism, *S. griseus*, for the isolation of streptomycin, and for the evaluation of its antimicrobial properties. In January 1944, four months after the two fresh isolates of *S. griseus* were obtained, the isolation of streptomycin was announced.

Thus streptomycin came into being. Recent plans of the National Tuberculosis Association for publication of its monograph *Streptomycin in the Treatment of Tuberculosis in Man* and the appearance of several volumes, in foreign languages, dealing with the same subject, emphasized the fact that finally a chemical compound has been discovered which has remarkable chemotherapeutic properties against the "white plague" of mankind, and that the eradication of this dreadful disease may be at hand.

As this is being written, several papers have appeared in the November issue of the American Review of Tuberculosis in which it is reported that dihydrostreptomycin, a derivative of streptomycin, is much less toxic than streptomycin when given in comparable doses and for similar periods, and may, therefore, be preferable in the treatment of some types of clin cal

tuberculosis; its biological properties are similar, however, to the parent streptomycin, as shown in table 3.

TABLE 3

In vitro activities of pure streptomycins against various organisms
(Modified from paper 13)

TEST ORGANISM	MINIMAL INHIBITING CONCENTRATION*			
	Streptomycin	Dihydrostreptomycin	Mannosidostreptomycin	Dihydromannosidostreptomycin
	μg/ml	μg/ml	μg/ml	μg/ml
Kl. pneumoniae (ATCC 9997)	1.76	1.76	6.39	6.59
A. aerogenes (ATCC 129)	2.71	3.27	10.80	11.10
E. coli (D 56)	6.05	6.79	24.80	23.80
S. schottmülleri (D 51)	10.10	36.50	14.30	14.40
S. typhosa (D 15)	12.20	51.00	12.40	12.90
S. enteriditis (D 61)	4.14	5.50	12.70	13.60
Sh. sonnei (H 1414)	7.42	8.52	30.60	30.30
Sh. dysenteriae (H 141)	6.26	5.82	27.20	27.10
Br. abortus (Huddleson 1119 avirulent)	0.816	0.738	2.93	2.53
H. influenzae type b (D 68)	2.30	1.53	8.53	5.53
M. pyogenes (*Staphylococcus*) var. *aureus* (209P)	0.828	1.39	5.64	7.77
S. pyogenes (C203)	11.70	15.90	82.90	87.90
M. tuberculosis:				
H37Rv	2.00	2.20	5.50	6.50
Ravenel	0.58	0.62	2.50	2.20
BCG	0.52	0.55	1.90	1.70
N†	0.54	0.56	2.50	2.10
T†	0.55	0.54	2.20	2.00
P†	0.62	0.85	2.30	2.20
OD†	0.63	0.75	2.30	2.60
K†	1.00	1.70	3.90	3.90

* All figures are given in terms of weight of the trihydrochlorides. On the basis of assays with *Kl. pneumoniae*, the streptomycin and dihydrostreptomycin would have an activity of 820 units per mg, the mannosidostreptomycin an activity of 236 units per mg and the dihydromannosidostreptomycin 228 units per mg.

† Strains of *M. tuberculosis* freshly isolated from human cases.

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