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Streptomycin: background, isolation, properties, and utilization

Nobel Lecture, December 12, 1952

*The Lord hath created medicines out of the earth;
and he that is wise will not abhor them.*

Ecclesiasticus, xxxviii, 4

The highest scientific award and honor presented to me the day before yesterday gives me the opportunity to summarize briefly the discovery and utilization of streptomycin for disease control, notably in the treatment of tuberculosis, the "Great White Plague" of man.

Historical background

Streptomycin belongs to a group of compounds, known as antibiotics, which are produced by microorganisms and which possess the property of inhibiting the growth and even of destroying other microorganisms. Antibiotics vary greatly in their chemical nature, mode of action upon different organisms, and effect upon the animal body. The selective action of antibiotics upon bacteria and other microorganisms is known as the antibiotic spectrum. Some antibiotics are characterized by a very narrow spectrum, whereas others possess a wide range of activity. Some are active only against certain bacteria and not upon others, whereas some are active against fungi, and some against viruses. There is not only considerable qualitative variation in the activity of different antibiotics, but also wide quantitative differences. Antibiotics are produced by bacteria, fungi, actinomycetes, and to a limited extent by other groups of microorganisms.

It has been known for more than six decades that certain fungi and bacteria are capable of producing chemical substances which have the capacity to inhibit the growth and even to destroy pathogenic organisms. Only within the last 12 or 13 years however, have antibiotics begun to find extensive application as chemotherapeutic agents. Among these, penicillin and streptomycin have occupied a prominent place. Penicillin is largely active upon gram-posi-

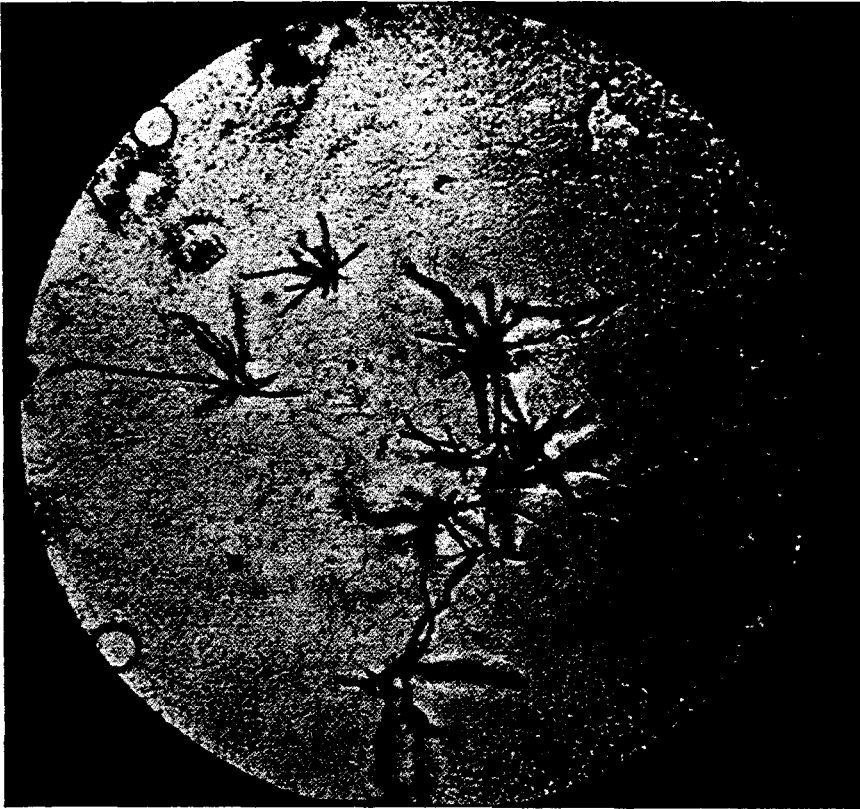


Fig. 1. *Streptomyces griseus*, streptomycin-producing strain. Vegetative and aerial mycelium.

tive bacteria, gram-negative cocci, anaerobic bacteria, spirochaetes, and actinomycetes; streptomycin is active against a variety of gram-negative and acid-fast bacteria, as well as upon gram-positive organisms which have become resistant to penicillin. Neither of these antibiotics is active upon rickettsiae, viruses, and fungi. They too differ in their physical and chemical properties and in their toxicity to animals.

Since the discovery of streptomycin, the production and clinical application of this antibiotic have had a phenomenal rise. *Streptomyces griseus*, the streptomycin-producing organism, was first isolated in September 1943, and the first public announcement of the antibiotic was made in January 1944. Before the end of that year, streptomycin was already being submitted to clinical trial. Within two years, the practical potentialities of streptomycin for disease control were definitely established.

The most spectacular of all the clinical applications followed recognition that streptomycin was highly effective against the tuberculosis organism, not only *in vitro* but also *in vivo*. Several clinical centers undertook to test the sensitivity of different freshly isolated strains of *Mycobacterium tuberculosis* to streptomycin, its practical evaluation in control of tuberculosis in experimental animals, and finally its use in the control of tuberculosis in the human body. Following the lead of Dr. William H. Feldman and Dr. H. Corwin Hinshaw at the Mayo Clinic, the American Trudeau Society and the National Tuberculosis Association took an active part in these investigations. The first conference arranged for the evaluation of the clinical results of the use of streptomycin was held in 1945, and others soon followed.

Within three years after the announcement of the isolation of streptomycin came the almost complete elucidation of its chemistry, its first derivative (dihydrostreptomycin) was obtained, and the first 1,000 clinical cases were reported. From a laboratory curiosity, streptomycin production soon grew into a large industry, with a monthly output of more than 25 thousand kilograms in the United States alone, and with many plants being established abroad for its manufacture.

This marked rise in the development of streptomycin was due partly to the fact that the spectacular increase, between 1941 and 1943, in the use of penicillin for disease control suggested the possibility that there were other antibiotics which could be utilized for the treatment of diseases not sensitive to penicillin. It was the knowledge of the great abundance and wide distribution of actinomycetes, which dated back nearly three decades, and the recognition of the marked activity of this group of organisms against other organisms that led me in 1939 to undertake a systematic study of their ability to produce antibiotics. Between 1940 and 1952 we isolated in our laboratories 10 different chemical substances from cultures of different actinomycetes, beginning with *actinomycin* and ending with *candicidin*. Of these, *streptomycin* proved to be by far the most important chemotherapeutic agent.

Production of antibiotics by actinomycetes

The isolation of streptomycin was the culminating point of a painstaking search for antimicrobial agents produced by actinomycetes, a group of organisms closely related to the bacteria. This was preceded by long and continuous research, dating back to 1915, on actinomycetes, their occurrence

and abundance in nature, their systematic or taxonomic position, their role in soil processes, notably in the decomposition of plant and animal residues and in the formation of humus, and finally their associative and antagonistic effects upon bacteria and fungi. It was finally established that as many as 20 to 50 per cent of all the actinomycetes found in the soil and in other natural substrates had the capacity to inhibit the growth of other microorganisms.

Streptomyces griseus, the organism which comprised the streptomycin-producing strain, was known in our laboratories from the beginning of our work on actinomycetes, although it was not tested at that time for its antibiotic-producing properties. The ability of actinomycetes to exert injurious effects upon bacteria and fungi has been known for many years. Lieske

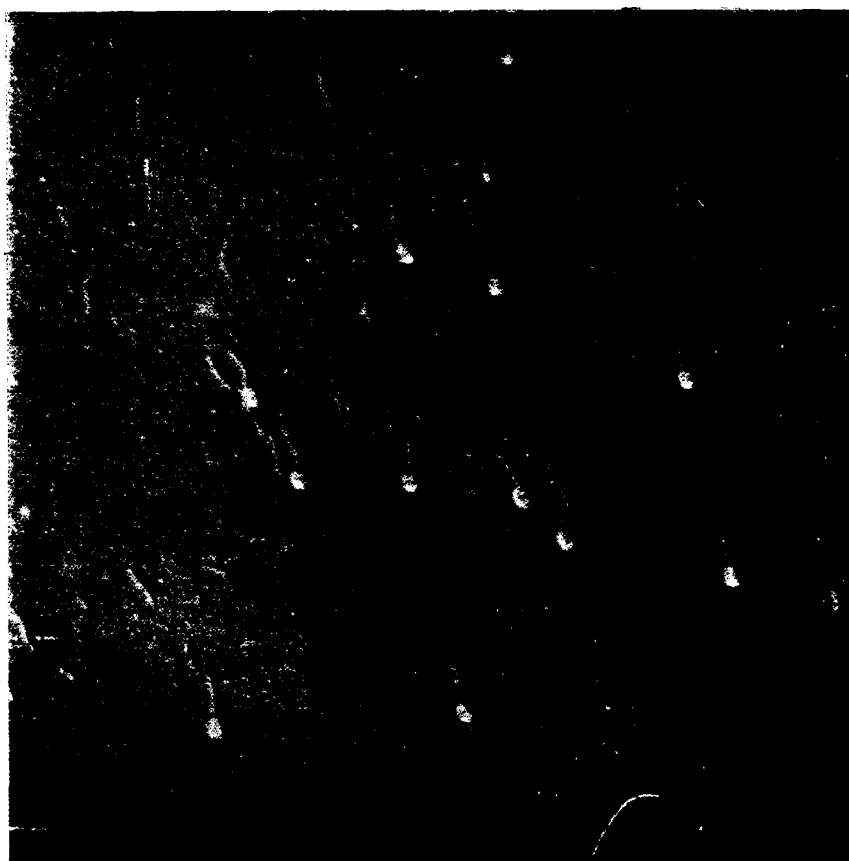


Fig. 2. Electron micrograph of actinophage, type I, of streptomycin-producing *Streptomyces griseus*, x 31,000. (Courtesy of Squibb Institute for Medical Research.)

showed in 1921 that certain strains are able to bring about lysis of many bacteria and antagonize their growth. This process is selective in nature: some of the bacteria are affected, and others are not. Other investigators, notably Gratia and Dath and Rosenthal, demonstrated in 1925 that cultures of organisms designated as *Streptothrix*, and now known to be actinomycetes, are capable of dissolving living and dead bacterial cells. Nakhimovskaia in 1937 made the first survey of the occurrence of antagonistic actinomycetes in the soil: 80 cultures were isolated, of which 47 were able to repress bacterial growth, but only 27 liberated into the medium substances which had the capacity to inhibit the growth of gram-positive bacteria, but not of gram-negative bacteria or fungi.

When we began our investigation on the production of antibiotics by actinomycetes in 1939, only two preparations were known to possess antimicrobial properties. These were not true antibiotics, or at least were not recognized as such. One was obtained by Gratia and had the capacity to lyse dead typhoid cells and certain living bacteria; it was later designated by Welsch as actinomycetin. The other was believed to be a lysozyme, which had lytic principles, and was studied by Krassilnikov and Koreniako in 1939.

The first true antibiotic to be derived from a culture of an actinomyces was isolated in our department in 1940. The organism, *Actinomyces antibioticus*, yielded a substance which was designated as actinomycin. It was soon crystallized, and its chemical and biological properties were established. This antibiotic proved to be a quinone-like pigment with an approximate molecular formula of $C_{41}H_{56}N_5O_{11}$. It was highly active against various gram-positive bacteria but to a much lesser degree upon the gram-negative organisms. It proved to be extremely toxic to experimental animals.

This practical failure was followed by a comprehensive program of screening actinomycetes for their ability to produce different antibiotics. The fact was revealed that these antibiotics vary greatly in their chemical nature, toxicity to animals, and antimicrobial activities. Some were active against bacteria and actinomycetes, but not upon fungi; some were largely active against certain bacteria, especially the gram-positive types, but not, or to only a limited extent, against the gram-negative forms, whereas others were active alike upon various gram-positive and gram-negative bacteria. Some had a very narrow spectrum, being active only or largely upon one group of organisms, such as the mycobacteria, or only upon certain fungi, such as yeast-like forms, or upon certain viruses, such as the influenza-B types; others

had a much wider spectrum, being active upon both bacteria and fungi, or upon bacteria, rickettsiae, and the so-called larger viruses.

It was further found in our laboratory and in others that one antibiotic, such as actinomycin and streptothricin, could be produced by several different species. Some organisms give rise to different modifications of the same antibiotic, as actinomycin A, B, and C. This is true also of streptomycin, various modifications of which are produced by different species of *Streptomyces*, notably *S. griseus*, *S. bikiniensis*, and *S. griseocarneus* (streptomycin, mannosidostreptomycin, hydroxystreptomycin). Some organisms produce a mixture of different antibiotics, as in the case of different strains of *S. griseus*, which give rise not only to streptomycin, but also to the antifungal agents actidone and candicin.

Isolation of streptothricin and streptomycin

The isolation of actinomycin, the first true antibiotic from a culture of an actinomyces, pointed a way to the formation and isolation from species belonging to this group of organisms of other chemical substances possessing antimicrobial properties. A new type of substance, designated as *streptothricin*, was soon isolated, in 1942. It showed distinct promise as a chemotherapeutic agent. It was active against both gram-positive and gram-negative bacteria and was not toxic to animals. The organism producing it was identical with *Actinomyces lavendulae*, a culture found in the soil 25 years previously. This new substance possessed highly desirable physical, chemical, and antimicrobial properties and gave promise of filling the gap left by penicillin in the treatment of infectious diseases due to gram-negative bacteria. It was a basic compound soluble in water, with an optimum activity at a slight alkalinity. It was active against a number of bacteria, not only *in vitro*; but also *in vivo*, as well as against various fungi. It was resistant to heat and to the action of different microorganisms and enzymes. A study of its pharmacology, however, brought out the fact that streptothricin exerted a residual toxic effect upon the animal body; its use in the treatment of infectious diseases was, therefore, limited.

The experience gained in the study of streptothricin made possible effective planning of a more comprehensive study of the production of a definite type of antibiotic by actinomycetes. Particular attention was paid to substances that possessed chemical and biological properties similar to those of

streptothricin but that would be less toxic to the animal body. The method of cultivation of the organism (notably the use of submerged cultures), the method of isolation of the active substance (notably its adsorption on charcoal and its removal with dilute acid), the method of evaluation of its antimicrobial properties (notably its activity against gram-negative bacteria), were now well understood. It was, of course, desirable that any new substance should possess a spectrum which would be broader than that of streptothricin, that it be particularly active against a greater variety of bacteria which were resistant to penicillin, and, if possible, that it be active against tuberculosis. (Streptothricin was later found also to possess this property.) Less than six months after many freshly isolated cultures of actinomycetes were screened, an organism was obtained which appeared to produce the long looked-for antibiotic.

Since actinomycin was named after the organism that gave the name to the group of actinomycetales (*Actinomyces* by Harz in 1877,) and since the first promising antibiotic, streptothricin, was derived from the first name given to a member of this group (*Streptothrix* by F. Cohn in 1872), it was logical that the first chemotherapeutically active substance should be designated by the newly coined generic name of actinomycetes (*Streptomyces* by Waksman and Henrici in 1943). Thus *streptomycin* was born.

The organism producing streptomycin was identical with a culture isolated in our laboratory in 1916 and described at that time as *Actinomyces griseus* changed, according to the 1943 classification, to *Streptomyces griseus*. Although numerous additional cultures of streptomycin-producing strains of *S. griseus* have since been isolated, it is one of the 1943 cultures that has been utilized for the commercial production of streptomycin all over the world.

In addition to streptomycin, *S. griseus* was later found to produce mannosidostreptomycin, as well as certain other antibiotics, such as actidione and streptocin. Other strains of this organism produce no antibiotics at all or are able to form other substances, such as grisein and candicidin.

The medium used for the production of streptomycin contained organic sources of nitrogen and carbon, mineral salts, and certain growth-promoting substances. The culture had to be well aerated and incubated at 25 to 30°C for 3 to 5 days. The activity of streptomycin reached 100 to 200 $\mu\text{g}/\text{ml}$; in commercial production, much higher yields are usually obtained.

Recovery of the streptomycin was accomplished in a series of operations, involving removal of the mycelium by filtration, adsorption of the streptomycin on activated carbon or on some other adsorbent, elution by di-

lute acid, neutralization of the eluate, concentration by evaporation and dehydration or by solvent precipitation, and filtration and drying. Various methods have been used for further purification and crystallization of the antibiotic.

Streptomycin was found to be soluble in water and insoluble in organic solvents, such as ether, chloroform, and acetone. The chloride was completely soluble in methanol, less soluble in ethanol, and virtually insoluble in butyl alcohol, acetic acid, and pyridine. The sulfate was only slightly soluble in methanol and virtually insoluble in the other solvents.

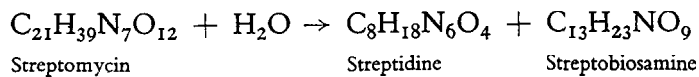
A unit of streptomycin was designated as that amount of material which was just sufficient to inhibit the growth of a standard strain of *E. coli* in 1 millilitre of culture medium. When streptomycin was later isolated in a crystalline state, it was found that 1 unit was comparable to 1 microgram of the pure base. The United States Food and Drug Administration, in consultation with the different manufacturers, decided to establish the potency of streptomycin on the basis of the weight of the active material.

The activity of streptomycin against sensitive organisms was found to be influenced by the presence of certain chemical compounds. Glucose reduced its activity appreciably; hydroxylamine, thioglycollate, and cysteine largely inactivated it. This phenomenon was utilized in testing for sterility of streptomycin preparations or for determining the admixture of other antibiotics. The activity of streptomycin was found to be influenced but little by body fluids, pus, or normal tissue juices. The optimum reaction was pH 8.0; increasing acidity resulted in a decreased bacteriostatic potency, the greatest reduction taking place between pH 6.6 and pH 5.9.

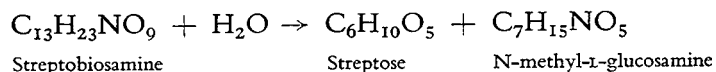
Chemical nature of streptomycin

Streptomycin belongs to the glucosides in which a diguanido-group is linked to a nitrogen-containing disaccharide-like compound. It is a strong base, with three basic functional groups. The molecular weight determination on the trihydrochloride in water gave about 800 for the free base after the necessary corrections for the chloride ion.

Upon hydrolysis, it splits into two compounds:

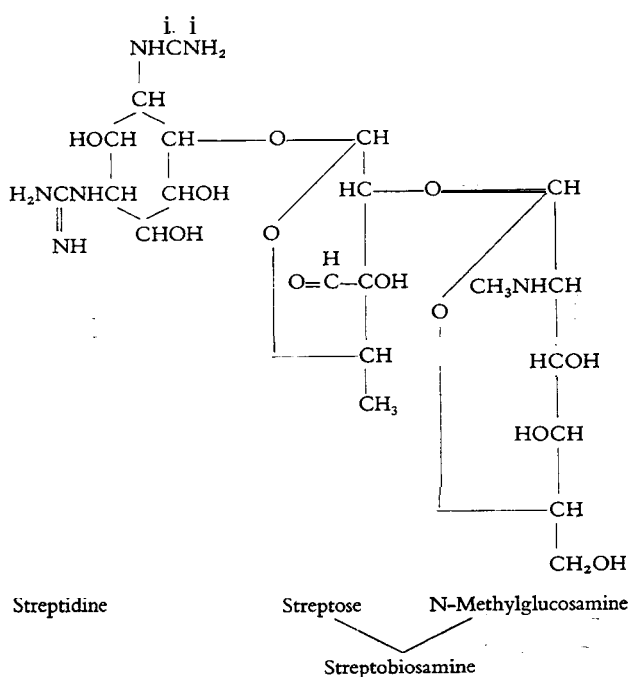


On further hydrolysis with strong mineral acids, the streptobiosamine gives a 6-carbon sugar and glucosamine:



The basic nitrogen atom in the streptobiosamine is not present as a primary amino group.

The structure of the streptomycin molecule is usually given as follows :



Antibacterial properties of streptomycin

Streptomycin is active against a large number of bacteria found among the gram-negative, gram-positive, and acid-fast groups and among the spirochaetes; it has relatively little activity against anaerobic bacteria, fungi, protozoa, and viruses. No absolute value can be given for the sensitivity of an organism to streptomycin; this depends not only upon the particular species, but also upon the strain of the organism and upon the composition of the

medium in which it is tested. Thus any given figure for a single organism is quite arbitrary, since such values can vary greatly.

Streptomycin is more active upon bacteria in young, actively growing cultures than in older cultures, although this difference does not appear to be so great as that for penicillin. When added to a 3-hour-old culture of *E. coli*, 2 μg streptomycin reduced the numbers by 75 per cent, and 5 μg by 95 per cent; the corresponding reduction for 24- to 48-hour-old cultures were 19 and 42 per cent. The size of the inoculum influences the bacteriostatic concentration of streptomycin: for instance, an inoculum of 30,000 cells of *Brucella abortus* required 1 $\mu\text{g}/\text{ml}$ whereas an inoculum of 30,000,000 cells required 4 $\mu\text{g}/\text{ml}$ for inhibition of growth. Less streptomycin was needed to inhibit growth of various strains of *Br. abortus* and *Br. suis* under aerobic conditions than in the presence of 10 per cent CO_2 .

Since streptomycin is not active against viruses, it can be utilized in their isolation and purification from bacterial infections. It is also used in the preservation of bull semen, in the purification of cultures of *Trichomonas vaginalis* and *T. foetus*, and in the development of media for the isolation of pathogenic fungi. Because of its marked antibacterial properties, streptomycin can be used in preventing wound infections and in surgical operations.

Among the antimicrobial properties of streptomycin, its bacteriostatic and bactericidal action upon different strains of *Mycobacterium tuberculosis* is particularly significant. In concentration of 0.05 $\mu\text{g}/\text{ml}$, it has been found capable of inhibiting growth of the human pathogen to a limited extent, 0.2 $\mu\text{g}/\text{ml}$ inhibits growth to a marked extent, and 0.4 $\mu\text{g}/\text{ml}$ stops growth completely, after an incubation time of 16 to 20 days. In other experiments, streptomycin gave complete inhibition of growth of *M. tuberculosis* in concentrations of 0.3 $\mu\text{g}/\text{ml}$, although occasional colonies occurred even with 0.5 $\mu\text{g}/\text{ml}$. The bactericidal action of streptomycin also varies with its concentration and the length of contact with the organism, 0.3 $\mu\text{g}/\text{ml}$ exerting a marked effect in 48 hours, and 20 $\mu\text{g}/\text{ml}$ in 6 hours.

Resistance of bacteria to streptomycin

The problem of variation in sensitivity of different strains of the same organism to streptomycin and the increasing resistance of the bacteria on prolonged contact with it are of considerable theoretical and practical importance. Variation in sensitivity may differ from day to day. This is believed to

be due, at least in part, to variations in the number of viable organisms, age and density of the culture, and the particular species involved.

Freshly isolated cultures of tubercle bacilli from patients with pulmonary tuberculosis are uniformly sensitive to streptomycin. When the cultures are exposed to relatively low concentrations of this antibiotic, growth of multiplying cells but not that of the non-multiplying cells is inhibited. The organism develops resistance to streptomycin *in vitro* at a rapid rate, and this resistance persists for a considerable time; it is not accompanied by any diminution in virulence. In a growing culture of tubercle bacilli, there was found to be, however, a decrease in the proportion of streptomycin-resistant cells with an increase in the age of the culture. The principal effects of streptomycin on the morphology of the organism were a loss of acid-fastness, an increase in granulation, and, in highly bacteriostatic concentration, a shortening of the rods.

Successive transfers of bacteria commonly occurring in infections of the urinary tract resulted in an increase in resistance to more than 1,000 $\mu\text{g}/\text{ml}$ of streptomycin. After 29 subsequent daily transfers on common media, the bacteria lost none of their resistance. It was suggested, therefore, that streptomycin-fastness may be a major factor in the failure of streptomycin therapy in certain infections. When the disease condition presents physical barriers to the penetration of the streptomycin, as in cases of abscesses and pyelonephritis, the organisms are exposed to sublethal concentrations, thus resulting in increased resistance. In infections with *Aerobacter cloacae*, an increase of more than 1,000 times was obtained after 36 days of treatment.

Streptomycin in experimental infections

Streptomycin proved to be highly effective in the treatment of a large number of infectious diseases brought about in experimental animals by various bacteria. This was true of *Salmonella schottmülleri*, *Shigella gallinarum*, *Brucella abortus*, *Pseudomonas aeruginosa*, *Klebsiella* or Friedländer's bacillus, *Diplococcus pneumoniae*, and a number of organisms commonly found in urinary tract infections.

Subcutaneous doses of 100 to 200 μg of streptomycin were sufficient to protect mice against lethal infections with *S. schottmülleri*. Oral doses of about 3 mg were required to protect mice infected by the intraperitoneal injection of 10 lethal doses. Following oral administration, streptomycin remained in

the intestinal tract and gave very low blood concentrations. Urine recoveries in the animals ranged from 70 to 80 per cent when streptomycin was given parenterally and less than 10 per cent when given orally.

The effectiveness of streptomycin against tularemia, caused by *Pasteurella tularensis*, has been most striking. Complete protection was also obtained in experimental infections due to *Proteus vulgaris*: in chick embryos, 1,000 μg of streptomycin were required to arrest 18-hour infections; to protect 50 per cent of the embryos, 250 μg in a single dose or 150 μg in three daily doses were required. Excellent protection was obtained by the use of 150 μg streptomycin against fowl typhoid caused by *Sh. gallinarum* in 11-day-old chick embryos.

Streptomycin was also found to have a marked effect upon experimental plague, *Pasteurella pestis* being inhibited by 1 $\mu\text{g}/\text{ml}$. A series of mice infected with the organism were treated with 400 μg streptomycin daily, beginning 2 days after inoculation and continuing for 6 days. Nine of the ten mice survived 14 days, as compared with the survival of four out of eleven for sulfadiazine-treated mice and one out of nine for the controls. Experimental infections caused by *D. pneumoniae* and *Staphylococcus aureus* were readily controlled when adequate doses of streptomycin were employed.

Of particular importance was pioneering work done by Feldman and Hinshaw on the effectiveness of streptomycin in experimental tuberculosis in guinea pigs. On the basis of an arbitrarily established index of infection, microscopically determined, 100 represented the maximum possible amount of tuberculosis. The control animals, sacrificed after 61 days, exhibited an index of 67 as contrasted to 5.8 for those which had received streptomycin. In another experiment, the corresponding values were 81.9 for the untreated and 2.8 for the treated animals. The daily administration of streptomycin per guinea pig varied from 1,387 to 6,000 μg . Two different strains of the human tubercle bacillus were equally sensitive of streptomycin. *M. tuberculosis* was recovered from the spleens of only one of the guinea pigs treated with streptomycin, the animal having received 1,387 μg streptomycin daily for 54 days.

The conclusion was reached that streptomycin is the most effective tuberculo-chemotherapeutic agent so far studied. Its relatively low toxicity for guinea pigs, its high efficacy in resolving and suppressing what would otherwise be lethal tuberculosis, established streptomycin as a drug worthy of serious consideration for the treatment of tuberculosis.

Toxicity of streptomycin

To produce toxic manifestations of streptomycin in animals, it was necessary to administer either extraordinarily large doses of the pure material or smaller amounts of certain impure preparations. No correlation was obtained between the acute toxicity of different lots to experimental animals and clinical tolerance by human beings. The behavior of the material in man could not, therefore, be foretold if antibacterial potency alone was assumed to be the criterion of purity; highly concentrated material had on occasion been unsatisfactory clinically, whereas considerably less active preparations had often produced no undesirable effects.

Among the various toxic reactions resulting from the administration of streptomycin, the otic complications received particular attention. In one experiment, 81 patients treated with streptomycin were examined for evidence of aural toxicity. Two months after cessation of therapy, three of these patients showed an absence of vestibular response. During the third month after therapy, one of these three showed increasing recovery of response. All three complained spontaneously of dizziness, which decreased steadily during treatment. One patient showed a high tone loss, but the audiogram was normal in these three patients. Another patient showed a severe loss of hearing 2 weeks after treatment; 2 weeks later he had shown decided improvement in hearing; he had a normal vestibular response throughout. It was not certain that the low tone and high tone losses were due to streptomycin.

Diseases responding to streptomycin

A variety of human and animal diseases caused by various bacteria respond readily to streptomycin treatment. This was brought out in the first comprehensive study of a group of diseases which could be considered to be definitely controlled by streptomycin or to give promise that they would respond favorably. These included tularemia, urinary tract infections, especially those resistant to sulfa drugs and to penicillin, *Klebsiella* and *Hemophilus* infections, bacteremia due to penicillin-resistant organisms, various forms of meningitis, and whooping cough. Streptomycin was also found to be helpful in treatment of a variety of other diseases, such as leprosy, typhoid fever, brucellosis, certain forms of tuberculosis, and probably also bacillary dysentery, cholera, and bubonic plague.

The Committee on Chemotherapy, originally organized by the Committee on Medical Research of the OSRD, undertook the supervision and coordination of the first large-scale series of investigations on the use of streptomycin in the treatment of bacterial infections. The various infectious diseases have been divided, in their relation to streptomycin, as follows:

Diseases definitely indicated for streptomycin treatment

1. All cases of tularemia.
2. All cases of *H. influenza* infections:
 - Meningitis
 - Endocarditis
 - Laryngotracheitis
 - Urinary tract infections
 - Pulmonary infections
3. All cases of meningitis due to :
 - E. coli*
 - Pr. vulgaris*
 - K. pneumoniae*
 - B. lactis-aerogenes*
 - Ps. aeruginosa*
 - S. paratyphi*
4. All cases of bacteremia due to gram-negative organisms:
 - E. coli*
 - Pr. vulgaris*
 - A. aerogenes*
 - Ps. aeruginosa*
 - K. pneumoniae*
5. Urinary tract infections due to:
 - E. coli*
 - Pr. vulgaris*
 - K. pneumoniae*
 - B. lactis-aerogenes*
 - H. influenzae*
 - Ps. aeruginosa*

Streptomycin found to be a helpful agent but position not yet definitely defined

- (1) Peritonitis due to gram-negative bacteria.
- (2) B. Friedländer's pneumonia.
- (3) Liver abscesses due to gram-negative bacteria.
- (4) Cholangitis due to gram-negative bacteria.
- (5) Penicillin-resistant but streptomycin-sensitive organisms infecting heart valves.
- (6) Tuberculosis.
- (7) Chronic pulmonary infections due to mixed gram-negative flora.
- (8) Empyema due to gram-negative infections.

The Committee evaluated the first 1,000 cases treated with streptomycin. In 409 urinary tract infections, the general recovery rate was 42 per cent. Of 100 *H. influenzae* meningitis infections, recovery was obtained in 79 per cent; in 17 of the fatal cases, treatment was too late to be effective. In 14 cases of meningitis caused by other gram-negative organisms, only 4 died. In 91 bacteremia cases, 49 recovered, 12 improved, 4 showed no response and 26 died; in this group as well, the fatal cases were treated too late. Striking results were obtained in tularemia (63 recoveries out of 67 cases), and in otitis media (7 immediate recoveries out of 8 cases). In 44 cases of pulmonary infections, recovery or improvement was obtained in 61 per cent. Streptomycin was extremely effective against acute Friedlander bacillus infections.

In the evaluation of streptomycin in tuberculosis, 75 cases were used. These included miliary, meningial, larynx, skin, and renal tract forms. It was recommended that a minimum period of treatment should be 3 to 6 months with daily doses of 1.5 to 3.0 g. Hinshaw, Feldman, and Pfuetze, on the basis of treatment of 100 tuberculosis cases, came to the rather optimistic conclusion, that streptomycin is an antibacterial agent which possesses the unique ability to inhibit the growth of *M. tuberculosis* both experimentally and clinically. It was recommended that treatment of tuberculosis consist of large doses of streptomycin for prolonged periods.

Various forms of tuberculosis were soon found to respond promptly to streptomycin. These included ulcerating tuberculous lesions of the larynx, hypopharynx, and the tracheobronchial tree, which received combined intramuscular and aerosol treatment. Some types of extrapulmonary tuberculosis also responded well, especially chronic, long-standing draining sinus tracts, which usually closed within a few weeks. These remained closed only

if treatment was continued for several weeks after superficial healing. Encouraging results were also obtained in treatment of tuberculosis of bones and joints. Some patients with early tuberculous meningitis responded promptly to adequate intramuscular and intrathecal administration, but frequently these gains were not permanently sustained; temporary clinical remission was frequently realized after streptomycin treatment of early but acute and severe tuberculous meningitis; consciousness could be regained, sometimes within a few days, fever would decline within a few weeks, the patient appearing normal for several months; subsequent exacerbations of the diseases were likely to occur and were not likely to respond to treatment.

Disseminated hematogenous tuberculosis of the miliary type heretofore did not respond to treatment, spontaneous recoveries being extremely rare. By the use of streptomycin it was possible to bring about a complete clinical and roentgenologic remission. Tuberculosis of the alimentary tract and tuberculous peritonitis also showed striking symptomatic improvement.

The rapid progress made in the use of streptomycin in tuberculosis was thus well expressed by Walker, Hinshaw, and Barnwall: "The introduction of streptomycin into clinical medicine has been peculiarly exciting to phthisiologists. Not only have they never had an *antibiotic* which was helpful in the treatment of tuberculosis in man, they have never had a proved effective drug of any sort. So far as chemotherapy is concerned, not only was their situation comparable to that of the syphilologist prior to the discovery of penicillin, it was worse than his position before the appearance of Ehrlich and arsenic. Phthisiologists have been dependent on rest in bed, and, more recently, on collapse therapy. The relatively rapid evaluation of a new drug - rapid for tuberculosis if not for pneumococcic pneumonia - is partly the result of experience gained from the investigation of other drugs in other diseases. It is partly a result of the employment, here and in England, of cooperative methods of investigation which have yielded data, reasonably uniform, rapidly, and in large amount."

I need hardly survey the subsequent developments in the use of streptomycin for the different infectious diseases, especially tuberculosis. I need hardly dwell upon the various regimens for treatment, based on special dosages and modes of administration, and its combined use with para-aminosalicylic acid (PAS). I need not list the numerous antibiotics that have been isolated from cultures of actinomycetes, some in our own laboratories, such as neomycin, and some in numerous other laboratories throughout the world. Suffice to say that streptomycin pointed the way, both through the planned

screening programs and through its specific activity against the gram-negative bacteria and tuberculosis, to many of these antibiotics. The rest is history. Medical science and clinical practice have been revolutionized. One may look forward to further discoveries of agents that will combat diseases not now subject to therapy, to more active and less toxic agents than those now available, and to combined therapy of several antibiotics or of antibiotics and synthetic compounds which will prove to be more effective than the use of single substances.

Summary

The discovery of streptomycin as a product of a rather obscure group of microorganisms, the actinomycetes, led to the study of these organisms as potential producers of other chemotherapeutic substances. Following streptomycin, there came in rapid succession chloramphenicol, chlortetracycline, neomycin, oxytetracycline, and, more recently erythromycin and others. These antibiotics have found extensive application as chemotherapeutic agents in the treatment of numerous infectious diseases never before subjected to therapy.

In the treatment of tuberculosis, the more controlled dosage of streptomycin and the supplementary use of PAS tended to overcome some of the limitations of the antibiotic, notably its toxicity and the development of bacterial resistance. The recent introduction of isonicotinic acid hydrazide suggests the possibility that its combined use with streptomycin will tend further to control the disease and overcome undesirable complications. The conquest of the "Great White Plague", undreamt of less than 10 years ago, is now virtually within sight.

I wish to take this opportunity to thank all my collaborators, associates, and graduate students who have participated in the investigations necessary to the development of our broad antibiotic program. Special thanks are due to the following assistants and graduate students: Misses Elizabeth S. Horning, Elizabeth Bugie, Doris Jones, D. Hutchinson, H. C. Reilly and Dorothy G. Smith; Drs. M. Welsch, W. Geiger, W. Garson, E. A. Swart, and Messrs. H. B. Woodruff, Albert Schatz, H. Lechevalier, S. R. Green, D. A. Harris, W. P. Iverson, D. Reynolds, H. J. Robinson, and Otto Graessle; to the chemists, pharmacologists, bacteriologists, and engineers of Merck & Co.; to Drs.

W. H. Feldman and H. C. Hinshaw of the Mayo Clinic; to Dr. C. S. Keefer of the Committee on Medical Research; and to numerous clinical investigators who have helped to make streptomycin the important chemotherapeutic agent it is today.

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