1945

Streptomycin: its present status

STREPTOMYCIN: ITS PRESENT STATUS

December, 1945

by

Hyman L. Osher

B.U.S.M. III

Submitted to the
Department of Medicine
Boston University School of Medicine

December 17, 1945

Table of Contents

Table of Contents (Cont'd) Page

I. Introduction: Antagonists and Antibiotics

The recent widely publicized and epoch-making developments in the field of bacterial chemotherapy have been largely based on three important discoveries made during the past two decades. The first of these was the discovery of penicillin by Fleming (17) in 1929; the second was the early work on synthetic chemotherapeutic agents by Domagk (8) in 1935 which provided the ground work for the subsequent synthesis of the sulfonamides; and the third was the isolation of a therapeutically effective antibiotic agent from a soil bacillus by Dubos (9) in 1939.

As a result of the dramatic success attending attempts to apply the above discoveries to the treatment of human infectious diseases, unprecedented interest and research efforts are now being directed at the problem of producing new and even more effective chemotherapeutic agents. That this renewed activity on the part of the microbiologists, the chemist, the physiologist and the clinical investigator is warranted in this connection was well expressed by Sir Alexander Fleming in the Lister Memorial Lecture (1944), when he stated:

> "Penicillin may be making some small revolution in surgery, and I am glad to have had some part in it. It is, however, not the end. There is still plenty of scope for the chemist to synthesize penicillin, and then tinker with the molecule so that the present imperfections of penicillin can be remedied. Then there are

thousands of micro-organisms other than Penicillium notatum which can manufacture in their growth complicated substances, and some of these may be better than penicillin, or they may have a constitution which will give a clue to fresh chemical linkages which are destructive to bacteria."

While penicillin is to date the most effective antibiotic agent known, it should not be assumed that it was the first antibiotic to be discovered, for this is distinctly not the case. It was in the earliest years of the science of bacteriology that microbial antagonisms were first observed, and there was little delay in attempting to employ these, crudely and ineffectively to be sure, in the treatment of infections.

Pasteur and Joubert (47) in 1877 noted that certain contaminating organisms exerted an antagonistic effect on the growth of Bacillus anthracis; and that simultaneous inoculation of susceptible animals with certain other bacteria along with the anthrax bacillus repressed the development of anthrax.

Since the time of Pasteur, numerous investigations have been carried out and a vast amount of information has been accumulated with respect to microbial antagonisms. In fact, antibiotic substances have been detected, and in some cases isolated, from almost all types of living organisms.

Bacteria were investigated early and many bacterial antagonisms were discovered in vitro and in vivo. For instance, it was noted that accompanying flora could either inhibit or favor the growth of an organism; that the growth of an antagonistic organism was favored by the presence of organisms which it inhibited; that different strains varied

in their ability to inhibit or be inhibited; that environmental conditions affected the production and persistence of activity of antibiotic substances; that filtrates from cultures of antagonistic organisms inhibited only certain susceptible organisms; that certain organisms could destroy bacterial toxins; that some filtrates were active against fungi; that the antagonistic action varied from inhibition of growth to actual bacteriolysis of susceptible organisms; and that antibiotic agents varied widely in their toxicity to animals and in their chemical constitution. The most prominent antibiotic agents to be derived from bacteria thus far are listed on Table I. It is worth noting here that pyocyanase was the first antibiotic agent to be used for the treatment of infection in the year 1899 (12).

Various fungi have been demonstrated to possess antagonistic activity against bacteria, other fungi, viruses, insects and other animal forms. Active antibiotic substances have been isolated from many species of fungi, and their therapeutic application has ranged from the treatment of plant disease to that of animal and human diseases. The more important antibiotics of fungal origin are listed in Table II; of these only penicillin has achieved widespread clinical use.

Although there has been but little investigation of the antagonistic properties of viruses, it is known that they may be destroyed by other micro-organisms, and that some viruses can antagonize certain other viruses. No therapeutic application of this knowledge has yet been

reported although it has been demonstrated that a murine mutant strain of poliomyelitis virus counteracted large paralytic doses of the parent strain, presumably by means of a chemical substance produced by the former (69).

Complex antagonistic and associative relationships have been discovered between bacteria and fungi on the one hand, and animal forms such as protozoa, insects, insect larvae, worms and higher animals on the other hand. Antagonistic relations have long been known to exist between various animal forms, even including the so-called "higher animals". Examples of the production of antibacterial substances in higher animals are afforded by the presence of lysozyme in mammalian tissues and secretions, and by the inhibin contained in fresh human urine. The immune bodies produced by higher animal forms are analogous in some respects to some antibiotic substances of microbial origin (69).

While antagonistic activity of certain actinomycetes was described as early as 1890, a systematic study of the group with the object of isolating therapeutically useful antibiotic agents was not undertaken until 1939 (85,77,87 70). Table III lists the more important antibiotic agents of actinomycetes origin; the last agent listed, streptomycin, is the most promising of the group, and forms the subject of this paper.

II. Events Leading up to the Isolation of Streptomycin

Although certain of the sulfonamides have been demonstrated to have some therapeutic effect on various gram-negative infections, their relative inefficacy and rather high incidence of toxic reactions make the development of superior agents likely as well as desirable. Tyrothricin, a rather toxic substance isolated by Dubos in 1939 from a soil bacterium, Bacillus brevis, has also been found to have little or no effect on gram negative organisms (9). Even before the efficacy and exact chemotherapeutic potentialities of penicillin were fully explored it was evident that this agent was not capable of controlling infections due to most gram-negative organisms. Thus it was that in 1939 Waksman and his co-workers at the New Jersey Agricultural Experiment Station of Rutgers University undertook the investigation of various groups of micro-organisms with the aim of isolating substances which could be utilized for the treatment of gram-negative infections (83). Several substances of mold origin--clavacin, fumigacin, and chaetomin, and of actinomycetes origin--actinomycin and micromonosporin, were isolated, but testing soon revealed that these were unsuited for chemotherapeutic use by reason of excessive toxicity or limited potency or both.

Preliminary studies on the group of actinomycetes revealed that many of these possessed striking antibacterial properties (85,77,87,70). Thousands of cultures isolated from normal and enriched soils, composts, manures

and other natural sources, and from type collections were exhaustively tested and analyzed with the result that several promising organisms were selected for further study. From one of these, Actinomyces lavendulae, a substance designated as streptothricin was isolated (85,84,71); this was found to be active in vitro against many gram-positive and gram-negative organisms(84,71,20,58,39), against pathogenic fungi (59) and against mycobacteria(89). It was demonstrated also to be effective in vivo against experimental infections by gram-negative organisms (42,55,58,57). However, more recent studies have revealed that certain gram-negative and gram-positive organisms are naturally resistant to streptothricin in fairly high concentrations (61,81). In addition, pharmacologic studies indicate that streptothricin has a delayed toxic effect in experimental animals, with production of necrosis and even gangrene of the small intestine, arteritis, myocarditis, renal tubular lesions, and occasionally hepatic and central nervous system lesions following intravenous administration; with subcutaneous administration only renal lesions occur. These untoward effects of systemic administration strongly contraindicate the use of streptothricin in human beings, and Herrell (31) has suggested that the use of the substance be restricted to local treatment of infected wounds and burns and of infected body cavities. Oral administration for intestinal infections as suggested by Robinson et al (57) would seem to be a hazardous procedure, especially in view of the fact that equally effective and less

toxic agents are available for this purpose.

When it became evident that streptothricin did not fulfill the requirements of *in vivo* potency and pharmacologic freedom from toxicity, the search continued. In January, 1944, Waksman and his students, Schatz and Bugie (61), isolated an actinomycetes which was similar in morphological and cultural characteristics to an organism isolated 28 years previously by Waksman, and designated as *Actinomyces griseus* (72). The antibiotic substance produced by this organism was found to be similar to streptothricin with respect to many of its physical, chemical, and antibacterial properties, and in the method of its isolation from cultures; it differed with respect to the cultural requirements for its optimal production, its antibacterial spectrum and its quantitative activity against certain organisms. It was found early to possess many desirable antibiotic and pharmacological properties and was designated streptomycin, in accordance with the generic name *Streptomyces* proposed by Waksman and Henrici (76) for the sporulating and aerial-mycelium producing group of actinomycetes. Two active strains of *S. griseus* were obtained, one (No. 18-16) from heavily manured soil, and the other (No. D-1) somewhat less active, from a smear plate of a chicken's throat. The organism is not believed to be a normal inhabitant of the animal system.

Table IV summarizes the comparative chemical and biological properties of streptothricin and streptomycin.

III. Production

The production of antibiotic substances from micro-organisms involves several steps. The first of these is the stimulation of growth of the micro-organisms in question by introducing into the soil specific bacteria against which it is antagonistic; this step may be omitted if the soil already contains the specific antagonistic organisms. The soil is then plated upon agar media containing washed living cells of bacteria as the sole source of carbon and nitrogen for the growth of organisms capable of destroying the bacteria. After determining the specific antibacterial spectra (e.g. by the agar streak method), favorable cultural conditions are selected for the production of the specific antibiotic substance, which is then concentrated and purified. As a final preliminary step to clinical application, the antibiotic agent is studied for in vivo activity and pharmacologic properties. This procedure was employed for the isolation of streptomycin as well as streptothricin.

In contrast to streptothricin, which is produced on a variety of simple or complex media, such as glucose-tryptone, starch-tryptone or simple synthetic media, streptomycin production requires the presence of complex growth-promoting substances such as those provided by meat extract or corn steep liquor.

Growth of S. griseus was demonstrated at the outset (61) to be more rapid in shaken submerged cultures (68,88) than in stationary cultures. Both methods, however, yield

considerable streptomycin production, maximum activity being reached in two or three days with the submerged technic and in 9 to 12 days with the stationary method. The organism grows in the form of very fine colonies which impart some degree of turbidity and light brown color to the medium. Optimal antibiotic activity is achieved by adding 2.5 to 5.5 grams of meat extract per liter of medium; corn steep liquor may replace the meat extract, but this complicates the subsequent processes of purification. The nitrogen requirements of the organism may be supplied by many common nitrogen sources--peptone, casein, tryptone, amino acids or even sodium nitrate. While glucose favors the production of streptomycin and is the best carbon source for use in stationary cultures, it may safely be replaced by starch and glycerol, and is actually inferior to dextrin and lactose for use in submerged cultures. The carbohydrate is presumed to function either as a source of energy or of acid for the neutralization of the alkalinity produced by the growth of the organism. This latter factor is important, since the reaction of the medium usually reaches pH 7.7 to 8.6 or more, and excessive alkalinity destroys the active substance; attempts to limit the rise in pH by adding buffers have not been successful (75).

The basic medium adopted by Waksman and co-workers is:

Glucose	10 Gm.
Peptone	5 Gm.
Meat Extract	5 Gm.
NaCl	5 Gm.
Tap Water	1000 Ml.
Final pH	6.5-7.0

Early studies (75) showed that, contrary to what might be expected, there is no definite correlation between streptomycin production and either growth of S. griseus, glucose consumption or ammonia production.

More recent investigations (71,63,81) have demonstrated that the strains of S. griseus and A. lavendulae which produce streptomycin and streptothricin can undergo variation to form inactive strains which have lost their ability to produce these antibiotic substances. The variants of S. griseus differ from the mother culture in certain respects as summarized in Table V; the characteristics of the variants are those of the genus Nocardia and suggest that naturally occurring members of this genus may be degenerate forms or accidental variants of the Streptomyces. From a practical point of view, it should be emphasized that the active strains of S. griseus seem to be fairly stable in antibiotic potency provided the media and cultural conditions are kept constant. Thus the possibility of formation of inactive variants does not constitute a serious threat to streptomycin production, since industrial conditions are widely different from those necessary for obtaining inactive variants. Nevertheless inactive lots may occasionally result from the use of vegetative growth for inoculation purposes.

Although crude cultures containing streptomycin gradually lose activity on standing--presumably due to the adverse affect of an enzyme in the culture--streptomycin is resistant to contaminating organisms as well as to

S. griseus itself. Concentrated preparations are stable for many months, particularly when refrigerated. Streptomycin loses considerable activity, however, on exposure to acids or to glucose, but resists temperatures as high as 100 degrees centigrade with only slight loss in activity.

IV. ISOLATION; PHYSICAL, CHEMICAL AND BIOLOGICAL PROPERTIES

The isolation and concentration of streptomycin from culture media make use of the methods developed for streptothricin (84, 71, 40, 82). Several distinct steps are involved, as follows:

1. Termination of incubation when maximum antibiotic activity has been reached.
2. Separation of broth from the mass of growth of the organism by centrifugation or filtration, using a filter which does not absorb streptomycin.
3. Adjustment of cell-free alkaline filtrate to neutrality.
4. Adsorption of streptomycin on active charcoal (Norite A) which is then filtered or centrifuged off.
5. Washing of charcoal adsorbate with alcohol to remove certain impurities, and then with dilute acid-alcohol to dissolve the streptomycin.
6. Neutralization of the acid-alcohol washings which have been separated from the charcoal, and filtration to remove precipitated impurities.
7. Concentration of the streptomycin solution by removal of the alcohol with ten volumes of ether, leaving a yellow, brown or red aqueous streptomycin concentrate.
8. Preparation of a solid streptomycin concentrate by precipitation with acetone or dessication *in vacuo*.

Using crude concentrates obtained by the above procedures, the bacteriostatic spectrum of streptomycin was determined, its pharmacologic properties fairly well established, and its physico-chemical nature revealed as a water-soluble, fat-solvent insoluble, thermostable nitrogenous organic base. However, more refined preparations were sought as a means of eliminating toxic reactions due to impurities, and of better standardization of dosage with preparations of constant high potency.

The first crystalline salt of streptomycin to be obtained (22) was the reineckate, prepared by Fried and Wintersteiner by the following method:

1. Adsorption of the active principles from cultures onto charcoal.
2. Elution with dilute mineral acid.
3. Precipitation by phosphotungstic acid.
4. Conversion of the bases liberated from the phosphotungstate into a crude picrate and fractionation of the latter by chromatographic methods.
5. Isolation of the water-soluble amorphous products of high activity after removal of the picric acid from one or more of the above fractions.
6. Formation of crystalline precipitates by addition of Reinecke Salt, $NH_4\left[Cr(SCN)_4(NH_3)_2\right]$ to an aqueous solution of active amorphous products.
7. Fractional crystallization to produce pure streptomycin reineckate with a potency of 370-410 units per mg.

It was found that the crystalline reineckate crystallized from water in thin plates which decomposed at 162-164 degrees C. (Corr.). Although the empirical formula could not be determined due to inconsistencies in some of the analytical figures, the formula $(C_{14}H_{26}O_7N_9S_4Cr)_n$ or $(C_{14}H_{26}O_2N_9S_4Cr)_n$, corresponding to $(C_{16}H_{19}O_{7-8}N_3)_n$ for the basic component were proposed as being in reasonable accord with the analytic data, with the objective of providing a basis for calculating the potency of free streptomycin. Since two preparations of streptomycin reineckate were found to have potencies of 370 and 410 units per mg, it was concluded that the pure base probably has a potency of approximately 800-910 units per mg. Streptomycin sulfate, obtained by decomposition of the reineckate with silver sulfate (Ag_2SO_4), had an activity of 850 units per mg.

Another crystalline salt of streptomycin, the helianthate, has recently been obtained (40), and it seems to be superior to the reineckate for preparative work. This compound was obtained by treating highly purified concentrates of streptomycin, such as the acidic eluate of the charcoal adsorbate, with methyl orange (the sodium salt of helianthine). This resulted in the crystallization of water-insoluble streptomycin helianthate, which was then recrystallized from aqueous methanol and the anhydrous form obtained by heating at 100 degrees C. <u>in vacuo</u>. Assay using the cup method with <u>B. subtilis</u> as test organism indicated that the

compound had an activity of about 350 units per mg. It was found to darken at 205 degrees C. and melt with decomposition at 220-226 deg C.

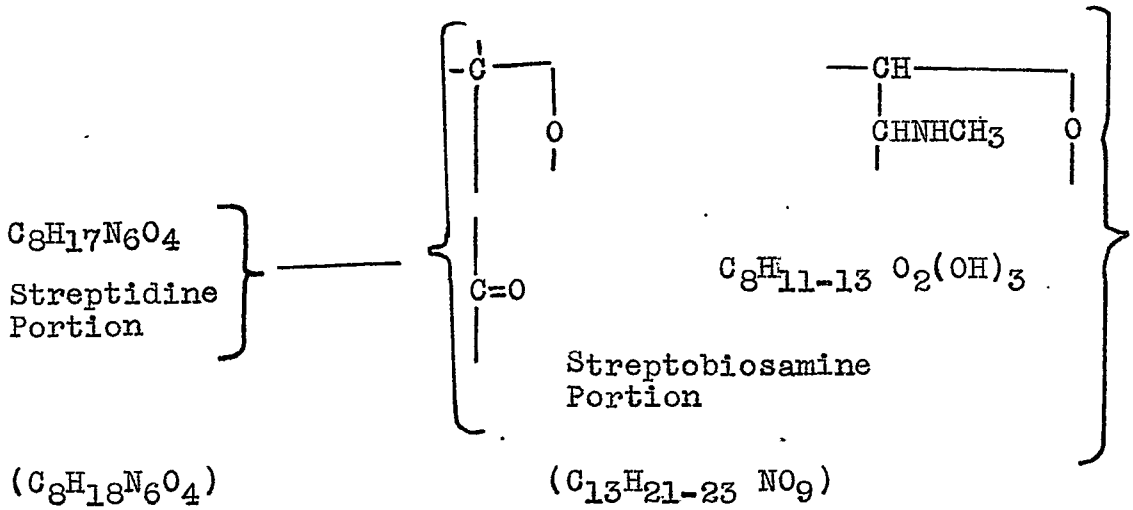
The hydrocholoride can be produced by heating the helianthate with a methanol-hydrochloric acid mixture, removing the liberated helianthine, and adding ether to precipitate the amorphous white hydrochloride from the filtrate. After drying over phosphorous pentoxide at 25 deg. in vacuo, this salt has a specific rotation of $(\alpha)_D = -84^\circ$ (C,0.5% in water) and an activity of about 800 units per mg. Microanalytical data were insufficient to assign a definite empirical formula, but indicated that there was no sulfur or phosphorus in the molecule. The ultraviolet adsorption spectra of streptomycin in phosphate buffer at pH 7, in borate buffer at pH 9 and in glycine buffer at pH 2 indicated only end absorption below about 2,300 Å. Other streptomycin salts prepared from the helianthate showed activity ranging from about 300 to 520 units per mg.

Additional chemical studies (48) resulted in the preparation of a crystalline double salt of streptomycin trihydrochloride and calcium chloride from the helianthate, by adding a methanol solution of calcium chloride acidified with hydrochloric acid to a methanol suspension of streptomycin helianthate. After removal of the insoluble calcium helianthate by filtration and concentration of the filtrate in vacuo, colorless crystals of the double salt were obtained. After drying in vacuo at 100 deg C., it

was found that the compound had an activity of about 750 units per mg. and $(\alpha)_D^{25} = -76^\circ$ (C,1% in water). Decomposition occurred at between 200 and 230 deg C. on the microblock. This compound is considered to be superior to the amorphous hydrochloride in certain respects, since the biological, chemical and physical properties of the crystalline double salt are more constant. Analytical data indicate that the formula of streptomycin is $C_{21}H_{37-39}N_7O_{12}$, rather than $(C_{10}H_{19}N_3O_{7-8})_n$ as proposed by Fried and Wintersteiner. A cryoscopic determination of the molecular weight of streptomycin trihydrochloride in water gave approximately 800 for the free base, and corrections for experimental errors seemed to account for the deviation from the calculated value of 580.

The most recent studies indicate that streptomycin has the general structure of a hydroxylated base, designated as streptidine, attached by means of a glycosidic linkage to a nitrogen containing disaccharide-like molecule, a derivative of a parent compound designated as streptobiosamine. The streptobiosamine derivative has been shown to contain a free or potential carbonyl group and a methyl-amino group (48).

Further experimental data indicate that the structure of streptomycin may be graphically represented as follows:



$(C_8H_{18}N_6O_4)$ $(C_{13}H_{21-23}NO_9)$

Hydrolysis of streptomycin to form streptidine and the streptobiosamine derivative may be represented by the equation:

$$C_{21}H_{37-39}N_7O_{12} + H_2O \longrightarrow C_8H_{18}N_6O_4 + C_{13}H_{21-23}NO_9$$

Streptomycin preparations are sufficiently stable as not to require refrigeration, but it is recommended that they be kept at a temperature not greater than 25° C. At this temperature, the material is stable for at least nine months provided the moisture content is not in excess of three per cent and the pH is from 6.0 to 7.5. Solid preparations are hydroscopic. Dilute sterile solutions may be kept at 37 deg C. for long periods without any loss of potency, and at 100 degrees C. for ten minutes with less than fifty per cent loss.

Streptomycin, in contrast to penicillin, pyocyanase and certain other antibiotics, is neither destroyed nor absorbed by resistant or sensitive organisms (78). It has been demonstrated that incubation of aqueous and nutrient broth solutions of streptomycin with Aspergillus niger or a mixed culture of fungi and bacteria for seven days at twenty-eight degrees C. resulted in no greater loss in activity than occurred in sterile controls (82). In another study, no significant loss of potency occurred after incubation with E. coli for seven days at thirty-seven degrees C., but considerable loss was noted after fourteen days (91).

V. Standardization and Assay Methods

Standardization: For purposes of investigation on a quantitative basis, it was necessary at the outset to define a unit of measurement for streptomycin. Since the substance had not yet been isolated in pure crystalline form, and since quantitative chemical methods for its measurement had not yet (and still have not) been developed, the original unit was of necessity based upon antibacterial action as determined by biological assay methods. This unit, designated the "S" or E. coli unit, was arbitrarily defined as the amount of the material which, when present in one ml. of nutrient broth or other suitable medium, will just inhibit the growth of a particular strain of E. coli (38,73).

While this unit served as a convenient quantitative standard for early experimental studies, it was soon found to be too small for pharmacological and clinical purposes. Also, comparison with penicillin (Oxford) unitage led to confusion since the latter is based upon ihhibitory action in 50 ml. of medium, and thus represents much greater in vitro antibacterial activity, although the unit of penicillin weighs 0.6 micrograms as compared with one microgram for the streptomycin unit. It should be borne in mind also, that the activity of the penicillin is determined against the relatively sensitive gram-positive organism while that of streptomycin is determined against the more resistant gram-negative bacteria (82). When both antibiotics are assayed against S. aureus SM, penicillin is found to be twelve times as potent, unit for unit, as streptomycin for that particular

test organism. However, S. aureus is many times more susceptible to penicillin than the gram-negative E. coli, and the gram-positive B. subtilis is almost ten times more sensitive to streptomycin than E. coli. Thus any comparison of the relative activity of penicillin and streptomycin must take into account the exact conditions of assay and the test organism used.

For the reasons given above, larger units of streptomycin were proposed (73) as follows:

1. An "L" unit, equal to 1000 S units and defined as the amount of streptomycin which just inhibits the growth of a standard strain of E. coli in one liter of medium.

2. A "G" unit, equal to 1 gram of crystalline material. Since one S unit weighs approximately one microgram, an L unit is equal to one milligram and a G unit, defined as one gram, turns out to be equal to one million S units. The hydrochloride salt has been recommended as the reference standard, with potency stated in terms of the pure base.

Throughout this paper, unitage will be expressed in terms of the original E. coli or S unit.

Assay: Assay methods in general may be used qualitatively for the characterization of antibiotic agents (i.e. determination of antibacterial spectra), and quantitatively for determining the amount of antibiotic activity in a given specimen (e.g. body fluid, or crude or purified preparations of the substance in question) against specific test organisms.

Many methods are available for the biological assay of antibiotic substances. The results of these assays vary considerably and depend on many factors, among which are the strain of test organism used, the exact conditions of incubation, the reaction and composition of the medium, and the mechanism and peculiar characteristics of the antibacterial activity of the agent in question (79, 69). For instance, in the case of streptomycin, the optimal pH for its activity is 9.0, and greater acidity markedly reduces its antibacterial action. In addition, streptomycin is appreciably inhibited by glucose and certain salts such as phosphates (82). Thus it is evident that assay methods must be strictly standardized with respect to materials and procedures, and that suitable specific methods be devised or adapted to fit each purpose for which assay is required. Further, interpretation of assay results should take into consideration the above factors as well as additional pertinent data to be presented in the section of this paper dealing with the characteristics of antibacterial action in vitro.

Agar Dilution Streak Method: The agar streak method, notwithstanding its shortcomings, with respect to precision and speed, is a valuable method of assaying antibiotic substances, particularly useful for screening tests with numerous organisms, for isolation procedures, for determining the antibacterial spectra of antibiotic agents, and for approximate quantitative estimations. It has the advantages of not requiring a sterile sample, and allowing the testing of unknown materials, even in non-aqueous solutions, against several organisms simultaneously. In the case of streptomycin, this method has been of invaluable service for the preliminary isolation and characterization of the substance, and is probably the most convenient method for its identification at present.

The test organisms usually employed in this method are E. coli (ATCC 9637), representing the gram-negative group; B. subtilis (ATCC 6633), representing the gram-positive bacilli; S. aureus (ATCC 6538), representing the coccus group; and B. mycoides (ATCC 6462), included for purposes of differentiating certain antibiotics from one another (e.g. streptomycin from streptothricin). Occasionally certain other organisms are included for specific purposes.

Suspensions of all of the test organisms are streaked on each of several agar plates containing various dilutions of the solution to be tested. After suitable (standard) incubationary periods, the growth of each organism on each plate is compared with the growths on control plates. The highest dilution at which growth is completely or almost completely inhibited is taken as the end point. The activity of the solution tested is expressed in "dilution units", calculated by dividing the volume of agar in the

plate by the amount of antibiotic substance added to the plate showing the end point, giving the concentration active substance in units per ml. of the preparation(79,51).

Slide Cell Method: The use of a technique modified from Fleming's slide cell method for the assay of penicillin in body fluids was found by Heilman (25) to be satisfactory for the estimation of streptomycin levels in normal serum, spinal fluid, and bile. The results of urinary assays, however, were "irregular", and specimens of bile required dilution with an equal volume of 0.84% NaCl to avoid the hemolytic effect of undiluted bile.

The procedure is in general similar to Fleming's modification of the Wright slide cell technic, differing chiefly by the substitution of B. megatherium for Streptococcus pyogenes as the test organism. Previously heated sterile defibrinated human blood is inoculated with a twenty-four hour broth culture of the test organism, and portions are quantitatively added to each of several test tubes containing appropriate dilutions of standard streptomycin solutions and of the unknown solutions. Portions of the contents of each tube are added to slide cells and incubated for 18 hours at 37^0 C. The end point is taken as the highest dilution of standard or unknown solution which completely prevents hemolysis. From this, the concentration of streptomycin in the undiluted unknown specimen is calculated by multiplying the dilution factor by the number of units of the standard causing inhibition of hemolysis in the same test.

While the slide cell method has the advantages of being easier to perform and requiring fewer materials than the cup method, it is inferior to the cup method for accurate determinations of small concentrations of streptomycin and for urinary assays. The slide cell method is not interfered with by the presence of therapeutic levels of the sulfonamides, but the effect of penicillin varies with its relative concentration as compared with the streptomycin concentration. Within certain strict limitations, each drug can be assayed in the presence of the other. Perhaps the use of an as yet undiscovered specific inactivator of streptomycin together with penicillinase will facilitate the assay of penicillin in the presence of streptomycin and vice versa.

Agar Cup (Cylinder Plate) Method: Another procedure for penicillin assay, the Oxford cup method, has been modified by Foster and Woodruff (21) for the determination of streptothricin, and by Stebbins and Robinson (67) for the determinination of streptomycin. The second modification is largely based upon the first, the chief difference being the substitution of S. aureus SM for B. subtilis as the test organism, since normal blood inhibits the latter organism and not the former (11). In addition, the pH of the medium has been increased to 7.5 - 8.0, and the salt concentration decreased considerably.

Beveled glass or porcelain cylinders are sealed to the surface of agar plates containing a 10^{-5} dilution of

a six hour broth culture of the test organism. Suitable normal serum dilutions of an aliquot of the streptomycin solution being administered to the patient and of the unknown test solution are placed in the cylinders, incubated for 16-18 hours at 30° C., and the resulting zones of inhibition then measured. A standard curve of reference is constructed from the values for the known solutions by plotting the diameters in millimeters of the zones of inhibition against the concentrations of streptomycin in units per ml. The concentration of the unknown solution is determined by reference to the standard curve with corrections applied for dilution. In assaying urine, feces, or tissue extracts, distilled water is used as the diluent instead of normal serum. Horse serum may be substituted for the patient's serum or normal human serum for diluting standard solutions without altering the results obtained(2). The cup method using the above procedure has been demonstrated to be sufficiently accurate for satisfactory biological assay of streptomycin, particularly for concentrations between one and twenty units per ml. It has the further advantages of simplicity and convenience, in that sterile preparations are not essential and several preparations or duplicates can be run on the same plate. It has the disadvantages of requiring a standard preparation for reference, and of being of little or no value for the comparison of different substances, due to differences in water-solubility and rate of diffusion. The method is limited to

the assay of water-soluble diffusible substances, and even with these, difficulty is occasionally encountered in obtaining regular zones of diffusion (2,69,79).

Paper Disc-Plate Method: The recently described paper disc-plate method (41) employs the agar diffusion principle for the assay of streptomycin prepared in phosphate buffer solution at pH 7.9 or in culture broth. Agar containing B. subtilis spores is poured upon the surface of solidified, sterile agar plates, which can be stored safely for several days at 2-4° C. until needed. The streptomycin solutions to be assayed are added rapidly (within five seconds) in 0.08 ml. portions to dry filter paper disks on the surface of the agar. The diameters of the zones of inhibition are measured after 15 hours incubation at 30° C. or 4-6 hours at 37° C. The expected standard error of this method is said to be 8.5% when eight discs are used for each solution tested(41).

Test Tube Agar Diffusion Method: This method has recently been proposed by Asheshov and Strelitz (3) as suitable for use in small laboratories lacking elaborate facilities and highly trained personnel, and for situations where large numbers of assays are carried out and where some degree of precision may be sacrificed in favor of simplicity and ease of execution.

Agar stabs are prepared by allowing soft nutrient agar inoculated with the test organism to solidify in tubes

placed in an upright position. Portions of suitable dilutions of standard and unknown antibiotic solutions are placed on top of the agar stab, and the amount of activity is estimated by the depth of the zone of inhibition extending downward from the surface of the stab after overnight incubation. As in the cup plate method, the concentration of the unknown solution is determined by reference to a standard curve constructed from the values obtained with known solutions.

Choice of Assay Method: Perhaps, as suggested by Heilman (25), it is advantageous to be able to employ more than one method for the assay of streptomycin. However, this is by no means always practicable, and usually a single method is chosen after consideration of such factors as the number of assays to be run, the accuracy desired and the facilities available. At present, it appears that the cup plate method of Stebbins and Robinson has been the most widely adopted technic for quantitative estimation of streptomycin concentrations in body fluids and tissue extracts. Possibly more precise biological or even chemical assay methods will be developed in the future.

Inactivation of Streptomycin: For purposes of determining the sterility of streptomycin preparations, and for detection of admixture of other antibacterial agents with streptomycin, it would be advantageous to have a specific inactivator of this antibiotic. Thus far, no

such substance has been reported.

However, certain sulfur containing organic compounds have been demonstrated to have the ability to inactivate streptomycin along with penicillin, pyocyanine and numerous other antibiotic substances.(5,7) The most important of these inactivators is the amino acid cysteine, although 2-amino-ethanol is only slightly less effective and thioglycollic acid also has some inactivating effect. Since cysteine has no effect on streptothricin, it can be used to differentiate the latter from streptomycin and to determine the amounts of each in mixtures. However, since cysteine itself has the power of inhibiting certain organisms including B. subtilis (79,52), selection of the proper test organism is of importance in obtaining accurate results. Table VI illustrates the the effect of cysteine on the activity of streptomycin.

While the inactivation of most antibiotic agents by cysteine is irreversible, the activity of streptomycin is quantitatively restored by the addition of iodine. This is accomplished by shaking the cysteine-inactivated streptomycin solution with small amounts of a carbon tetrachloride solution of iodine until decolorization no longer occurs.

The mechanism of inactivation of streptomycin by cysteine is not known; perhaps it involves a competitive effect on bacterial metabolic processes or a reversible chemical reaction between streptomycin and cysteine. At any rate, the inhibition of streptomycin does not depend on the sulfhydryl group alone (7).

VI. Pharmacology and Toxicology

In experimental animals: In general, streptomycin closely resembles penicillin with respect to pharmacologic and toxicologic properties. Results of animal experiments by Robinson et al (59,56) indicatedthat parenteral administration is capable of producing therapeutically effective blood levels of streptomycin. Multiple divided doses given intravenously or subcutaneously every six hours for 24 hours are equally effective as single doses of the same total amount of drug in protecting mice against lethal infections by streptomycin sensitive organisms. Oral administration requires much higher dosage and is far less effective therapeutically; however, examination of the feces of mice so treated shows that the gastro-intestinal tract is sterilized insofar as lactose-fermenting organisms are concerned, and that the total bacterial content of the feces is considerably decreased. (Temporarily)

Following parenteral administration, streptomycin seems to be widely distributed in the body fluids and tissues, and Murphy and Ravdin (45) have recently demonstrated that the drug reaches the peritoneal cavity in animals with peritonitis as well as normal animals. About 70% of the drug is excreted in the urine during the first five or six hours after parenteral administration, so that frequent intramuscular injection or continuous intravenous infusion is necessary for maintenance of adequate blood levels.

In most cases, streptomycin is well tolerated by mice, rats, guinea pigs, and monkeys when given in rather large doses and over extended periods of time. However, certain impure preparations of the drug produce acute toxic reactions similar to those previously pbserved with streptothricin. These reactions manifested themselves in mice as increased activity, severe dyspnea, and in some cases death from respiratory failure. The use of purified preparations eliminated all of these untoward effects in mice, together with the blood pressure lowering effect sometimes seen in rabbits. It is thought that these toxic reactions are caused by a histamine-like impurity present in some batches of streptomycin. No delayed lethal effect similar to that of streptothricin has been observed.

Molitor and Mushett (43) have described a second type of toxic reaction which followed streptomycin administration to monkeys. This consisted of a fatty infiltration of the liver and kidneys and which gradually disappeared after administration of the drug was stopped. The results of kidney and liver function tests and of hematologic examinations indicated only minor and transient deviations from normal values, and all of the animals seemed to remain in good health. No corresponding untoward effect could be detected in rats.

Tests for cytotoxicity of streptomycin have been performed by Dr. D.H. Heilman, using the technic of Herrell, Heilman and Gage; concentrations of 1:4000 were found to

have no effect, while a 1:2000 preparation was interpreted as causing "slight decrease in migration of macrophages or a moderate inhibition of fibroblastic growth or both". Thus it appears that streptomycin has the same low degree of cytotoxicity as penicillin (15).

Absorption in Man: Oral administration of streptomycin on dosage as high as four million units per day rarely produces appreciable blood levels, although levels of one to six units per ml. have been reported following oral administration of one million units per day (2,26,94,53,10). Since active streptomycin can be recovered almost quantitatively in the stool, this failure of appreciable absorption is not due to destruction of the drug in the gastrointestinal tract. In contrast to penicillin, streptomycin has been shown to withstand the action of gastric juice containing HCl with no appreciable loss in activity (2).

A single intravenous injection produces an immediate high serum level of streptomycin which decreases rather rapidly during the first hour after injection, and more gradually during the succeeding hours. The exact levels encountered are illustrated in Figure I, taken from the recent paper by Anderson and Jewell (2). It is instructive to note that 11-12 hours after single intravenous doses of 200,000 mg and 600,000 mg units, serum levels of 1 to 2 and 3 units per ml. respectively were found (1,2).

Intermittent intravenous administration every three hours results in somewhat higher serum levels than corresponding single doses as illustrated in Figure 2. This can be attributed to accumulation of streptomycin in the body (2). In one case, when 100,000 mg units were given every three hours for three doses, serum levels of 3, 1.5, 3, 3, and 1.5 units per ml. respectively were found 1, 3, 6, 9, and 12 hours after the first dose (26).

As might be expected, continuous intravenous infusion produces constant high serum concentrations, the administration of three million grams units per day yielding levels of 20-60 units per ml(94). On theoretical grounds, this seems to be the most satisfactory method of administering streptomycin (26).

Intramuscular injection of single doses produces a slower rise in serum concentration than intravenous injection, the maximum level being reached in 2-3 hours. After the peak level has been reached, persistence of the drug is similar to that following intravenous administration (see Figure 3). In some cases, drop in serum concentration following intramuscular injection is appreciably slower than after equal dosages administered intravenously (1).

Intermittent intramuscular administration produces blood levels comparable to those achieved by intermittent intravenous administration, (Table 7), and is probably the method of choice at present. In one case, injection of 100,000 mg units every three hours for three doses gave serum levels of 1.5, 1.5, 3, 3, and 1.5 units per ml.

respectively 1, 3, 6, 9, and 12 hours after the first dose, while in another instance, the levels 9 and 12 hours after injection were 1.5 and 0 units respectively.

Subcutaneous injection of streptomycin in single doses or intermittently produces results similar to those obtained by the intravenous and intramuscular routes. As illustrated in table 8, blood levels are comparable to or even higher than those found with the latter methods. Adequate antibacterial blood levels can be obtained by the subcutaneous administration of 100,~~000~~ mg units every three hours.

Intrathecal administration of streptomycin in single doses of 5,~~000~~ mg to 20,~~000~~ mg units along with intramuscular injection of 300,~~000~~ mg units every 12 hours has been found to produce spinal fluid levels of from 5 to 14 units per ml. and serum levels of 1 to 4 units per ml. at the end of 24 hours(2). Single doses of 50,~~000~~ mg to 100,~~000~~ mg units intrathecally yield concentrations of up to 50 units per ml. after 12 hours. It appears that antibacterial concentrations of streptomycin are maintained in the spinal fluid for at least 24 hours after intrathecal administration of therapeutic doses(26).

Nebulization of streptomycin into the tracheobronchial tree produces no appreciable absorption, as illustrated by the finding that the drug was not detectable in the blood serum after administration of up to one-half ~~million~~ gram units per day in this manner (26).

Investigators at the Beth Israel Hospital in Boston have administered streptomycin via the intraperitoneal route in cases of peritonitis with what appear to be satisfactory therapeutic results. Serum levels were not determined.

Diffusion: Following parenteral administration, streptomycin is widely distributed in the various body fluids and tissues.

Repeated intramuscular or subcutaneous administration of 50,~~000~~ mg to 60,~~000~~ mg ~~units~~ of streptomycin or subcutaneous administration of a single dose of 200,~~000~~ mg ~~units~~ has been found to produce no detectable levels of streptomycin in the spinal fluid(26). With larger doses of one to three ~~million units~~ grams intramuscularly, levels of one to five units per ml. have been obtained. In general, large parenteral dosage of streptomycin in the presence of meningitis produces spinal fluid levels about one-fifth those found in the serum at the same time (26). Diffusion of streptomycin in either direction across the blood brain barrier is normally minimal, and only slightly greater in the presence of meningitis. From these data, it is apparent that effective therapy of meningeal infections requires the intrathecal administration of streptomycin (along with intramuscular or intravenous therapy, in most cases).

Following single intravenous doses of 600,~~000~~ mg ~~units~~, streptomycin first appears in the peritoneal fluid in one-half hour, and then increases in amount while the blood

level was decreasing. With doses of 125,000 units intramuscularly every third hour, the peritoneal fluid level was found to be 23 units per ml. and the blood level 15 units per ml. at the end of 24 hours. After larger doses, blood and peritoneal fluid levels were found to be about equal (94).

Relatively high concentrations of streptomycin, even higher than the blood levels in some cases, are produced in the pleural fluid, particularly after intramuscular administration (94).

According to Leopold, streptomycin penetrates both aqueous and vitreous humors after intravenous, intramuscular, and local administration (94).

After intravenous or intramuscular administration of streptomycin to the mother, the drug has been found to traverse the placenta and enter the fetal circulation and amniotic fluid in appreciable concentrations (94,26).

Upon autopsy of two treated patients, Adcock found traces to several units of streptomycin in the heart muscle, brain and liver (1). His results indicate that streptomycin is concentrated in the kidney, and that smaller amounts are found in the lung and heart while the brain and liver contain practically none.

Urinary Excretion: After parenteral administration of streptomycin, excretion proceeds largely via the kidney, less than 2 % being present in the feces. Usually maximum excretion in the urine occurs during the first four hours after single doses, one-fifth to one-third being excreted between the fourth and twelfth hours, and only small amounts after 12 hours. Traces of the drug are detectable,

however, for 24 to 30 hours after single injections, and up to 72 hours after prolonged dosage of streptomycin with storage of the drug in body tissues (2,10). During the first two hours after injection, 20 to 35 per cent of the drug can usually be recovered from the urine, and by the end of twelve hours, 50 to 87 per cent has been excreted (1,2,53). The total amount of streptomycin recovered from the urine during the first 24 hours after single parenteral doses ranges from 15 to 89 per cent, averaging 53 to 66 per cent, and varying directly with the urine volume. (1,94). Since a total of approximately 55 to 68 per cent of the drug is excreted (66 per cent in the urine and 2 per cent in the feces) during the first 24 hours after single parenteral doses, and only traces are present in the urine after this time, it appears that some of the streptomycin is either stored or destroyed in the body.

Following oral doses of streptomycin as high as four million units daily, only traces of the drug (0.2-0.5 %) are found in the urine (1,53).

As might be inferred from the above data, high concentrations of the drug are present in the urine following parenteral administration. Zintel and co-workers (94) have reported urinary levels of 353 and 16 units per ml. respectively 3 and 24 hours after a single injection of 600,000 units. Helmholz (30) found concentrations of 1,131 to 1,330 units per ml. in the urine of a patient who was receiving two million units per day for several weeks.

Fecal Excretion: The lack of appreciable blood levels of streptomycin following oral administration, together with the scanty fecal excretion of the drug following parenteral injection indicates that diffusion through the wall of the gastro-intestinal tract is limited in either direction. Concentrations of only 100 to 130 units per gram of feces have been reported following intravenous administration of four million units per day (10); this amount was sufficient, nevertheless, to free the feces of typhoid fever patients from E. typhosa.

Administration of streptomycin by the oral route results in almost total excretion in the stool, producing concentrations as high as 21,000 units per gram of fresh feces with a daily dose of four million units. As might be expected, E. typhosa was completely eradicated from the stools of typhoid fever patients, E. coli and the rest of the bacterial population greatly reduced, and the fecal odor eliminated (53,10).

Biliary Excretion: The studies of Heilman and coworkers (26) indicate that streptomycin is concentrated and excreted in the bile. They found that two hours after starting intermittent subcutaneous administration of the drug, the bile contained about twice as much streptomycin as was found in the blood serum. On the other hand, Zintel et al (94) found maximum levels of 3 to 7.5 units per ml. in the bile of patients with blood levels of 18 units per ml. after a single intravenous dose of 600,000 units.

Of one million units given intramuscularly to one patient in eight doses over a period of one day, the 24 hour output of streptomycin in the bile was 3,500 units. Further study seems necessary before the quantitative aspects of biliary excretion of streptomycin can be established.

Zaslow and co-workers have shown that streptomycin enters the gallbladder in large amounts when the cystic duct is patent, provided hepatic excretion is normal. Streptomycin appears to be neither concentrated by nor absorbed through the wall of the gallbladder. These data indicate that streptomycin is probably of no value in acute cholecystitis and empyema, since the cystic duct is usually obstructed in these cases.

Toxicity: In the vast majority of cases, streptomycin seems to be free of serious untoward effects even when administered in fairly large doses over extended periods of time. As yet, no case has been reported where toxic reactions were severe enough to warrant the discontinuation of treatment.

The study of the toxicity of streptomycin as well as its therapeutic administration is complicated by the impossibility of predicting the toxicological properties of various lots of the drug in man from either their antibacterial potency or their acute toxicity to experimental animals. This lack of correlation is apparently due to the fact that the morbid and lethal toxicities of streptomycin do not arise from the same causes, and that only the morbid type of toxicity occurs in man(44).

With certain impure preparations, intramuscular or subcutaneous administration occasionally produces local pain or a burning sensation of short duration at the site of injection. These symptoms usually disappear within 15- to 30 minutes, but are sometimes followed by soreness which persists up to several hours(2); they do not occur when crystalline preparations of streptomycin are used. When the intravenous route is employed, local venous irritation is occasionally observed, but thrombosis is rare.

According to Hinshaw and Feldman (34) streptomycin may have a neurotoxic effect after prolonged administration of large doses. This neurotoxic effect seems to involve the eighth nerve, and manifests itself as transient deafness or disturbance of vestibular function with marked vertigo.

The commonest group of untoward reactions occurs when a histamine-like substance is present as an impurity. This consists of a throbbing headache and a flushing of the face, and sometimes nausea, malaise, vomiting, skin rash, and muscle and joint pains. Drug fever may occur two or three days after treatment. All of these effects may be largely avoided by slow intravenous infusion of the drug(2, 82, 26).

Chills and fever, described as similiar to the reaction following administration of pyrogen-containing penicillin preparations, have been noted (26).

Cutaneous toxic reactions vary from urticarial eruptions and toxic erythema to severe dermatitis (26).

Repeated intrathecal injections of streptomycin in doses up to 100,000 units per day have been reported with no evidence of meningeal irritation or other untoward reactions (86). However in one case there occurred an immediate reaction consisting of generalized spactieity and trembling, and reduction of the respiratoryvrate to four per minute. The patient was said to have the appearance of "impending disaster", and respirations did not return to normal for 15-20 minutes. Although intramuscular imjection of the same preparation of streptomycin caused no untoward effects, the reaction was attributed to it since a control intrathecal injection of sterile physiological saline solution had no effect. Apparently this represents either a toxic response to an impure preparation of streptomycin

or a case of indimidual idiosyncrasy on the part of the patient(16).

Another unique toxic reaction has been observed in two cases following the intraperitoneal administration of 1,000,000, units of streptomycin. This consisted of dyspnea, cyanosis, Cheyne-Stokes respiration, tachycardia of over 200 per minute, and rapid fall in blood pressure to shock level, approximately 15 minutes after the drug was injected. Intravenous injection of adrenaline dramatically restored both patients to their pre-streptomycin condition, with no discernible after effects. This reaction may be attributed to the aforementioned histamine-like impurity, which is known to have been present in the preparation of streptomycin used (60).

Carefully conducted renal function tests and blood chemistry studies during and following the administration of streptomycin preparations of varying degrees of purity have consistently failed to reveal any conclusive evidence of significant renal function impairment. Although renal irritation may occur, as indicated by the occasional finding of proteinuria and microscopic hematuria, permanent renal damage was not detected even with amounts of streptomycin greater than the contemplated average therapeutic dose (26,94).

Although fatty metamorphosis of the liver of monkeys treated with streptomycin has been reported, there is no

reason to believe that this occurs in man, or that it leads to permanent liver damage when it does occur. The usual clinical and laboratory tests of hepatic function give values within normal limits during and after treatment with streptomycin (26,94,34)

No evidence of damage to the hemopoietic system was detected on the basis of erythrocyte, leucocyte, platelet and differential white counts, hemoglobin determinations, and blood smear examinations (26,94)

Thus far, no serious or uncontrollable toxic reactions to streptomycin have been reported; it appears that pure crystalline preparations of the substance are of the same order of toxicity as penicillin,

Dosage and Methods of Administration: Insufficient clinical data are available at this time for dogmatic statements concerning the optimal dosage and modes of administration of streptomycin tp be made. Certain generalizations and preliminary recommendations are, however, warranted.

The optimal dosage and route of administration depend, among other things, on the location and accessibility of the infection, the presence of possible streptomycin-inactivating factors in the body, and the susceptibility of the etiological agent. As a guide to therapy, it appears that occasional determinations of the sensitivity of the pathogenic organism should be made before, during, and after treatment.

At present, the preparations of streptomycin in general use are the hydrochloride and sulfate salts, dispensed as fine, dry powders in ampules containing one million or more S units. It is probable that future chemical studies and synthetic procedures will yield pharmacologically superior streptomycin derivatives, especially with respect to activity and rate of excretion.

For intermittent intramuscular administration, it is recommended that one or two cc. of physiologic saline solution containing 125,~~000 units~~ to 175 mg per cc. be injected every three or four hours. In some cases, injection of two to four cc. of a similar solution may be made every six hours, with satisfactory clinical results (32). If the continuous intramuscular technic is employed, the proposed daily dose,

usually one to two million units, may be given over a period of 24 hours in 500 to 1000 cc. of physiologic saline solution (32).

Satisfactory results may be obtained by the subcutaneous administration of streptomycin in accordance with the method recommended for the intramuscular route. Solutions of ~~100,000 units~~ 100 to 175 mg per cc. may be used (32). The writer believes that this method has certain advantages over the intramuscular route from the point of view of absorption and ease of administration, and recommends that it be used more widely. The disadvantage of local pain at the site of injection can be eliminated by the use of highly purified preparations. (or by addition of procaine).

The intermittent intravenous method of administration is not recommended, since it has no advantage over the intermittent intramuscular and subcutaneous methods. For continuous intravenous intravenous infusion, the proposed daily dose of from one to four million units may be dissolved in two liters of physiologic saline solution and given at a rate of about 25 drops per minute (32).

For the treatment of meningitis, 25,000 to 100,000 units of streptomycin in 5 to 10 cc. of physiologic saline solution may be given intrathecally at intervals of 24 to 48 hours (32,86). For best results intermittent intramuscular therapy should be given along with intrathecal therapy.

Streptomycin may be administered intraperitoneally for the treatment of peritonitis by instillation of one million units in 1000 cc. of physiologic saline solution.(60).

Intramuscular administration seems to be effective in the treatment of peritonitis also, as might be expected from its diffusion into the peritoneal fluid. (32).

Oral administration would appear to be of value as a preoperative measure before surgery of the gastro-intestinal tract, and in conjunction with parenteral administration for the treatment of combined intestinal and systemic infections such as typhoid fever. Amounts as high as ½ to four million units daily may be given in four or more divided doses (32,53).

For treatment of infections of the larynx and tracheo-bronchial tree, physiologic saline solutions of streptomycin containing 25,000 to 50,000 units per cc. may be nebulized and inhaled in total doses of up to 500,000 units daily (32).

Streptomycin in concentrations of 250 to ~~500~~ 10,000 units per cc. may be used for topical applications.

Intrapleural instillation is the route of choice for infections of the pleura, although high levels are obtained in the pleural fluid following intramuscular administration.

VII. Activity in Vitro

The chief objective of *in vitro* testing of an antibiotic agent is the characterization of the substance from the point of view of selective antibacterial activity, as one of the preliminary steps in evaluating the agent before clinical application. The limitations of this method are well illustrated by the fact that penicillin has been found to be therapeutically effective in infections by certain organisms against which *in vitro* tests indicated that it was ineffectual or only slightly active (31).

The exact mechanism of action of streptomycin is not yet known, but several interesting characteristics of the action of antibiotics in general and of streptomycin in particular have been discovered. Antibiotic substances act by interfering with certain vital metabolic processes of bacteria; this may consist of a chemical reaction with an essential substrate or enzyme system, an effect upon the surface tension of the organism, or interference with the processes of respiration or bacterial cell division (74).

The fact that streptomycin is inactivated by certain sulfur-containing organic compounds, together with the demonstration that the sulfhydryl group is necessary for cell proliferation (Hammett, quoted by Cavallito (5)) suggests that streptomycin may act by interfering with the sulfur metabolism of bacteria, or more specifically, with the process of cell division. This hypothesis is supported by the finding that the majority of cells exposed even to dilutions of streptomycin greater than the minimal inhibitory dilution undergo elongation,

indicating interference with the process of cell division (59). However, since streptomycin has a definite bactericidal action, and even a marked bacteriolytic effect against certain organisms, it is probable that other mechanisms are also involved. Furthermore, it seems likely that the precise mode of action is different for different bacterial strains, and varies with the relative concentrations of the antibiotic substance and the bacterial inoculums, as well as with certain experimental conditions. This is well illustrated by the finding that streptomycin causes lysis of living B. subtilis cells, and has no effect upon dead B. subtilis cells or living S. aureus (78).

The presence of resistant organisms does not alter the antibacterial action of streptomycin on susceptible organisms, and the substance is not destroyed or absorbed by either resistant or sensitive bacteria(78).

The gram staining reaction appears to be only one of the properties of specific bacteria which point to differences in susceptibility of the bacterial cell to streptomycin (and other antibiotics). Further study of antibiotic action on bacteria will undoubtedly contribute to a better understanding of the biochemical constitution of the bacterial cell.

The development of resistance to streptomycin on the part of previously susceptible organisms exposed to the substance has occasionally been observed (80, 32). This process of adaptation has been attributed to an actual modification of the individual bacterial cells, together with a process of natural selection (49,80), and may be permanent of reversible (Hinshelwood, quoted by Waksman 80). Table 9 illustrates the marked bactericidal action of streptomycin on susceptible

strains (W1,W2) of Pr. vulgaris, as well as the pronounced reduction in its activity against a resistant strain (R) of the same species. Investigation of the morphological and physiological changes associated with the development of resistance may throw light upon the fundamental mechanisms of action of streptomycin.

It is interesting that while a strain which has developed resistance to one antibiotic agent may also be resistant to a closely related agent, it is not resistant to a different type of antibiotic (80).

To what extent the factor of resistance will complicate the problem of therapy is not yet clear.

The original report of Schatz et al (61) announcing the discovery of streptomycin characterized the substance as having considerable selective antibiotic activity against gram-negative as well as gram-positive bacteria. These observations were soon confirmed and extended by Robinson and co-workers (59), who emphasized the activity of streptomycin against organisms of the colon-typhoid and Salmonella groups. Simultaneously, it was demonstrated that streptomycin was the most effective antibiotic agent of a group tested against M. tuberculosis by virtue of its highly bactericidal action in vitro, its in vivo activity, and its relatively low toxicity (62). Subsequent in vitro studies have revealed that streptomycin has an amazingly wide range of antibacterial activity; among the organisms which are notably sensitive to it are the entire group of Enterobacteriaceae, including Aerobacter, Escherichia, Klebsiella, Salmonella, Eberthella, and Shigella; the Proteus

group; Ps. aeruginosa; Hemophilus pertussis and H. influenzae; the Pasteurella group; the Brucella group; C. diphtheriae; B. anthracis; the B. subtilis group; and the Mycobacteria, including the human strain of M. tuberculosis. In addition, streptomycin has been found to be active against a mixed culture of Pr. vulgaris and an anaerobic streptococcus(75). The effect of streptomycin is limited against anaerobic spore-formers and fungi (59), and it has no influence on the toxicity of tetanus toxin (46,95). No information has yet been reported to indicate that it is effective against viruses.

The currently accepted antibacterial spectrum of streptomycin is presented in table 10, taken from a recent paper by Waksman (82). Several explanatory remarks and comments are helpful in interpreting this table. The relative values for the activity of streptomycin against the organisms listed can be taken as only approximately correct, since the determinations were made by several investigators working more or less independently and using different assay methods. In addition, it should be borne in mind that different strains of the same bacterial species have been found to vary as much as a hundred-fold or more in their sensitivity to a given preparation of streptomycin (80,82).

It is of interest to note that the list of organisms susceptible to streptomycin includes most of the organisms which are resistant to penicillin. Evaluation of the significance of this observation with respect to therapy must await the results of clinical trial of streptomycin against each of these organisms.

VIII. Activity in Vivo

Animal experimentation has been repeatedly demonstrated to be an excellent proving ground for the evaluation of agents proposed for the treatment of human infectious disease. In general, results of animal experimentation have correlated rather closely with clinical results in man, insofar as acute infectious diseases of bacterial origin are concerned. With diseases of a chronic nature and those in which the experimental disease differs from the naturally occurring human disease, (such as tuberculosis), correlation has been less close. All things considered, well executed animal experimentation is the best method available for selection of new drugs for clinical trial in man.

Early in vivo studies (38) indicated that streptomycin was capable of protecting experimental animals against lethal doses of various pathogenic organisms. Using crude preparations of streptomycin containing 30 units of the active substance per mg., it was found that mice weighing 18-20 grams could be completely protected against lethal infections due to Salmonella schottmülleri with 6.4 mg (190 units) of the material. For protection against Ps. aeruginosa, 6.4 to 12.8 mg were required. In doses of 150-300 units, streptomycin was found to afford complete protection to 11-day-old chick embryos infected with fowl typhoid (Shigella gallinarum) and B. abortus. The same preparation was also effective in protecting experimental animals against Br. vulgaris (38).

Later animal studies have demonstrated that streptomycin is capable of controlling experimental infections by the more resistant gram-positive organisms D. pneumoniae and S. aureus (59). Also, complete protection of experimental animals against lethal infections by a mixed culture of Pr. vulgaris and an anaerobic streptococcus was obtained with adequate doses of a crude streptomycin preparation (75).

The administration of 1,000 units of streptomycin per day in divided doses was found by Heilman (27) to completely protect mice against lethal infections with Pasteurella tularensis. Furthermore, the organism was completely eradicated from the spleens of treated animals as determined by cultural and animal inoculation methods. These results were interpreted as suggestive that streptomycin may be of use in the treatment of tularemia in man, a widespread disease of high morbidity and for which there is no satisfactory therapy at present.

Additional studies by Heilman (28) showed streptomycin to be notably effective in protecting mice against lethal intra-abdominal injections of three different strains of Klebsiella when administered in doses of 185 to 500 units per day for 2 to 3 days. With mice intranasally infected with a type A strain of K. pneumoniae, administration of 500 units of streptomycin per day for 3 days had only a slight protective effect, but prolonged the life of infected animals considerably. When treatment was continued as above for 7 days, the mortality was reduced from 100 per cent to 13 per cent.

In a study designed to compare the relative dffects of streptomycin and penicillin in the treatment of the spirochetal infections relapsing fever and leptospirosis icterohemorrhagiae, Heilman(29) infected 100 mice intra-abdominally with citrated rat or mouse blood containing large numbers of virulent Borrelia novyi. Half of the animals were treated by subcutaneous injection of 1,000 units of streptomycin per day in divided doses, starting the day after inoculation, and the other half served as untreated controls. It was found that the blood of treated animals became free of spirochetes considerably faster than that of untreated controls. Four (8%) of the treated mice died, while of the untreated, 10 (20%) died. In a similar experiment , using hamsters infected with L. icterohemorrhagiae, 25 treated animals remained well, whereas all 25 untreated controls died. Attempts to demonstrate Leptospira in the livers and kidneys of the treated animals, by guinea pig inoculation were unsuccessful. On the basis of these and further similar experiments, it was concluded that streptomycin exerted considerable protective effect on experimental infections due to Borrelia novyi and Leptospira icterohemorrhagiae, but was not as effective, unit for unit, as penicillin under similar circumstances. Since, as has already been pointed out, the unit of penicillin represents a much larger amount of antibacterial activity than the unit of streptomycin, the latter conclusion does not indicate that streptomycin will necessarily be relegated to the position of an adjunct to penicillin in the treatment of spirochetal infections in man.

Hegarty (24) has reported that streptomycin is effective in vitro and in vivo against Hemophilus pertussis. He found that 3 units per ml. produced definite inhibition of growth, while 15 units per ml. was bactericidal. Fifty per cent of mice infected with 5,000 cells were protected by 2,000 units of streptomycin daily for 10 days, and the survival time of those that died was extended to 22 days as compared to 5½ days for untreated controls.

Seeler et al (64) studied the effect of streptomycin on on avian malaria. It was found that the frug was ineffective in reducing the percentage of parasitized erythrocytes at the peak of trophozoite-induced Plasmodium cathamerium and Pl. lophurae infections of Pekin ducklings and P. gallinaceum infections of whtie leghorn chicks.inIn single comb white leghorn chicks infected by intravenous injection of P. gallinaceum sporozoites, streptomycin in doses of 400,000 units per Kg. per day reduced the percentage of parasitized erythrocytes to one-half that in untreated controls. Sulfadiazine and quinine were found to have much more suppressive effect than streptomycin.

At the suggestion of Dr. Waksman, Feldman and Hinshaw of the Mayo Clinic set about to determine what effects, if any, streptomycin has on the pathogenesis of experimental tuberculosis in guinea pigs. Their preliminary experiments (14) demonstrated that streptomycin treatment, started at the time of inoculation of the animals with a virulent human strain of M. tuberculosis, was capable of a striking suppressive effect on the development of the disease.

Later experiments (14,15) were designed to test the ability of streptomycin to control well established tuberculosis in guinea pigs, by delaying treatment for 15 and 48 days after infection. In one study, 49 guinea pigs were used, 25 of them receiving 6,000 units of streptomycin daily in four divided doses, and the remaining 24 serving as untreated controls. Biopsy of the liver was done on all of the animals one day before treatment was started (47th day of disease) so that an estimate of the state of infection prior to treatment was available. When, after 166 days of treatment, the experiment was terminated, only 2 (8%) of the treated group had died, as compared with 17 (70%) of the untreated group. At necropsy, most of the untreated controls showed severe, widely disseminated parenchymal tuberculous infection, with grossly detectable lesions of the spleen, liver, lungs and tracheobronchial lymph nodes. On the other hand, the treated animals showed no evidence of widespread tuberculosis, except in the case of one animal which died after 98 days of treatment; only 4 of the 25 animals showed gross involvement of the organs of predilection. The average numerical index of infection as determined by an arbitrary method proposed by Feldman (13), was 80.4 for the untreated controls and only 1.24 for the treated animals. Liver biopsy revealed that the destructive necrotizing lesions present before treatment was started were resolved completely or converted into atrophic remnants. However, 15 of 23 spleens removed from treated animals were demonstrated by inoculation tests to contain virulent organisms. Tuberculin sensitivity was greatly reduced in 14 of 23 treated animals, and was absent

in the remaining nine. It appeared that streptomycin exerted bactericidal as well as bacteriostatic action against M. tuberculosis, even though the schedule of treatment was such that the treated animals were devoid of the drug during about one-half of each 24 hours. It was considered the results of these experiments were sufficiently impressive to warrant clinical trial of streptomycin in the treatment of human tuberculosis, even though experimental tuberculoses in guinea pigs differs in so many respects from naturally occurring human tuberculous infections assto constitute an essentially distinct disease.

Results similar to those of Feldman and Hinshaw were obtained by Youmans and McCarter (92,93), who studied the effect of streptomycin on tuberculous infections in mice produced by M. tuberculosis var. hominis. In view of the fact that mice are much more resistant to tuberculosis than guinea pigs, and since the infection in mice is primarily pulmonary, it was suggested that these results might be more nearly analogous to those which would be obtained in the treatment of human tuberculosis. However, to attempt to predict the latter on the basis of animal experiments is treading dangerously close to the realm of speculation.

Smith and McCloskey (66) have compared the relative effects of streptomycin and promin in the treatment os experimental guinea pig tuberculosis, and have confirmed the generally held impression that the former is far superior to the latter with respect to both efficiency and non-toxicity. More specifically, the chemotherapeutic index of streptomycin was found to be more than 10 times that of promin, which has hitherto been

considered the best chemotherapeutic agent for the treatment of experimental tuberculosis. In addition, the simultaneous administration of streptomycin and promin produced results considered to be much better than those obtained with either agent alone. Not only did the administration of streptomycin potentiate the action of promin, but it also reduced the anemia-producing tendency of the latter, and allowed higher blood levels to be obtained. The chemotherapeutic efficiency of the combined method of treatment was found to be almost three times as great as anticipated from simple summation. The authors recommend cautious application of the combined streptomycin-promin treatment in properly selected clinical cases.

IX. Clinical Reports

Largely as a consequence of the limited supplies of streptomycin available, clinical expierience with this agent has been rather meager, a total of about 100 cases having thus far been reported in the literature.

Typhoid Fever: The first report on the clinical use of streptomycin was by Reimann et al (53), who treated five cases of typhoid fever with streptomycin. In 2 patients there was striking clinical improvement after 36 hours to 7 days treatment, and it seems reasonable to ascribe the subsequent cures to streptomycin, since sudden recovery is rare in typhoid fever. In a third patient recovery was synchronous with therapy, and may have been spontaneous or induced by streptomycin. In a 4th patient oral therapy was ineffective, and inadequate continuous intravenous therapy later instituted had a questionable effect. The fifth patient failed to improve in spite of the fact that blood levels were twice the amount necessary to kill E. typhosa in the test tube. The causative organism was found not to have become resistant, and failure of the drug to sterilize the blood was attributed to inadequate dosage or possibly to unknown factors in the body which interfere with drug action on E. typhosa. The authors concluded that although the therapeutic value of streptomycin could not be definitely determined from the above five cases results were strongly suggestive that the drug contributed to or actually caused recovery.

It was recommended that one to four million units of streptomycin daily be employed as a minimal dose for the treatment of systemic or urinary tract infections with E. typhosa. Combined oral and parenteral therapy was advised during and after typhoid fever and other bacillary infections of the gastro-intestinal or urinary tract to cure the disease, prevent reinfection and to avoid the carrier state. Oral therapy was advocated as of probable value for prophylaxis of typhoid fever and similiar infections.

Anderson and Jewell(2) and Herrell and Nichols (32) have each reported single cases of typhoid fever in which results of streptomycin therapy were suggestive but difficult to evaluate. A definitive estimate of the efficacy of streptomycin in this disease, must await further study on a larger number of cases.

Gastro-intestinal infections: Flippin (18) has successfully treated Salmonella infections of the gastro-intestinal tract with streptomycin. It is probable that the drug may be of some value in the treatment of similiar bacillary infections of the gastro-intestinal tract including Shigella dysentery.

Brucellosis: Of five cases of brucellosis treated by Herrell and Nichols(32), only one was considered to have beem cured by streptomycin. In this case although the drug caused repeated sterilization of the blood stream, permanent recovery occurred only after the tremendously enlarged and infected spleen was removed. For this reason, it is suggested that the advisability of considering splenectomy in similiar cases of chronic

brucellosis be determined. In a second patient recovery occurred after two courses of streptomycin treatment, butiit is not known whether this was spontaneous or induced by the drug. Two additional cases failed to improve symptomatically although blood cultures became negative. The fifth case was not cured, but the dosage used was far below the probable minimum therapeutic dosage of one million to two million units daily. The impression of the investigators was that further clinical trials of streptomycin in acute cases of undulant fever with bacteriemia are justified, but that the drug is of doubtful value in chronic undulant fever without bacteriemia. Flippin (18) has treated acute brucellosis successfully with intra-muscular streptomycin. Reimann(54) has obtained encouraging but inconclusive results in the treatment of systemic Brucella infections with streptomycin.

Bacteremia: Two cases of E.coli (1) bacteremia originating from pyelonephritis were successfully treated by Herrell and Nichols(32) using total doses of 1,000, 000 and 20,000,000 units of streptomycin respectivelly, but bacilluria persisted in the second case. Cases of bacteremia due to A. aergenes (2), Ps. aeruginosa (3), and mixed Salmonella (4) and Proteus (5) ammoniae (6) infections of the urinary tract have been successfully treated by the same workers. Details of these cases are listed in table XI. Anderson and Jewell (2) have reported a case of GroupB. Salmonella (7) sepsis in which streptomycin treatment was of doubtful value, since the recovery may have been largely spontaneous. In this connection it should be pointed out that about one -half of cases of bacteremia due to gram- negative bacilli recover without treatment.

Urinary Infections: The experiments of Helmholz (30) led him to conclude that "streptomycin should prove to be the most useful urinary antiseptic so far developed". He found that a concentration of 100 units per ml. of streptomycin was sufficient to rapidly reduce the number of any organisms found in urinary infections, and that 235 units per ml., about one-fifth the urinary concentration found with the recommended therapeutic dosage of streptomycin produced rapid sterilization of massively infected urine.

The results of treatment of 13 patients with infections of the urinary tract are listed in table XII. It will be seen that in ten of these cases, the response to streptomycin treatment was considered good, even though several of the infections were polyvalent. The investigators found that best results were obtained in infections due to Pr. ammoniae or A. aerogenes, and that those due to Ps. aeruginosa and E. coli did not respond as satisfactorily to streptomycin treatment. Of course, the factor of pathologic changes in the urinary tract, such as obstruction with stasis must be considered, and adequate surgical measures should be instituted for best results. It appears that the optimal daily dosage of streptomycin is one to to two million units, and that alkalinization of the urine enhances the activity of streptomycin.

Greey (23) of the University of Toronto has obtained results similar to the above. He found that streptomycin was dffective for treatment of chronic urinary tract infections owing to such gram-negative organisms as Pr. vulgaris, A. aerogenes, E. coli, Ps. aeruginosa and Eberthella sp.

with a daily dose of one million units divided into 8 intramuscular injections, urinary cultures, become negative for Pr. vulgaris in 4 hours, and for coliform organisms in 8 hours. In one case, E.coli was eliminatedfrom the urine after two hours of treatment. Although infections were permanently cleared when the urinary tract was normal, damaged tracts were found to be readily susceptible to reinfection via the catheter.

Similiar satisfactory results in the treatment of heretofore resistant urinary infections have been obtained by the U.S. Army Medical Corps, and by the Pratt Diagnostic and Beth Israel Hospitals in Boston. (60)

Influenzal Meningitis; Weinstein(86) has treated seven cases of meningitis due to Hemophilus influenzae; 6 were type B and the remaining organism could not be typed with either type A or type B antiserum. Treatment consisted of intermittent intra- muscular administration of 125,000 units of streptomycin every 3 hours for seven to ten days, and intra-thecal injection of 25,000 units every 24 hours and continued for 4 to 7 days after intra-muscular administration was stopped. Sterilization of the spinal fluid occurred within 24 to 48 hours, and all 7 cases recovered without complications or relapses

Herrell and Nichols (32) have reported 4 cases of influenzal meningitis which were successfully treated with streptomycin. Three of the infecting organisms were type B and the fourth was untyped; two of the patients received

other medication in addition to streptomycin. Details of cases are given in table 13. Smith abd Birmingham (65) have obtained comparable results in the treatment of influenzal meningitis with streptomycin. They point out that the organism becomes drug fast when insufficient doses are used, and have achieved sterilzation of the blood and spinal fluid within nine hours after starting treatment with two hundred thousand units every two hours. they suggest that the treatment of choice will probably consist of combined serum and streptomycin, sulfadiazine and streptomycin, or all three; the results of Weinstein, however, indicate that no treatment in addition to streptomycin is necessary. The same workers (65) treated a single case of Salmonella meningitis with streptomycin, but it is not certain what part of the drug played in the recovery. Although there have thus far been no reports concerning the treatment of E. coli meningitis with streptomycin, it appears probable, on atheoretical basis, that the drug will be effective in this lethal disease for which there has hitherto been no adequate therapy.

In the few cases of tuberculous meningitis thus far treated, streptomycin has failed to give evidence of amy pronounced therapeutic effect (65, 16).

Pulmonary Suppurative Disease: Of five cases of pulmonary suppurative disease(bronchiectasis) due to gram-negative organisms, streptomycin treatment by nebulization and/or intramuscular injection eradicated the infection in four (32). Irreversible pathologic changes such as

bronchiectasis and fibrosis were, of course, not benefited. The details of these cases are listed in table 14.

Tularemia: The experiments of Foshay (19) indicate that P. tularensis is one of the most sensitive organisms to the action of streptomycin in vitro. He found that concentrations of only a few units per ml. were bactericidal in a matter of seconds or minutes. Whereas the average duration of the untreated disease is about four months, seven cases treated with relatively low doses of streptomycin were observed to recover promptly. One patient was discharged as cured on the seventeenth day of the disease, after only 9 days of streptomycin treatment. Another patient with generalized infection of the peritoneal cavity and perisplenitis was found to have noninfective peritoneal fluid after only 6 days of treatment. Normally the peritoneal fluid is infective for at least 9 months. Streptomycin therefore appears to be the most effective treatment yet developed for this widespread disease of high morbidity.

Peritonitis: Three cases of peritonitis due to gram-negative organisms have been successfully treated with streptomycin (60). In two of these, additional treatment was employed, and although the results were suggestive, no definite conclusions concerning the value of streptomycin can be drawn from these cases. In a single case of E. coli peritonitis (retrocecal abscess) following rupture of the appendix, intramuscular streptomycin therapy combined with surgical drainage produced rapid recovery. Here again, results can be considered hardly more than suggestive.

Syphilis: In three cases of early syphilis treated by Harrell and Nichols (32) with inadequate doses of streptomycin darkfield examinations were rendered temporarily negative in from 21 to 81 hours after treatment was instituted. Temporary improvements occurred in a case of gummatous syphilis during a short period of treatment with obviously insufficient doses. The value of streptomycin in syphilotherapy can not be stated until more cases are treated with adequate doses over an extended period of time.

Wound Infections: Howes (37) has sutured at least 14 wounds with a mixture of 200,000 units of streptomycin and 5% marfanil without any wound infections or untoward reactions. In a case of mixed E. coli and Pr. vulgaris sepsis following excision of a large bladder diverticulum, surgical drainage and irrigation of the wounds with a streptomycin solution (100,000 units / 50cc.) produced rapid recovery(60). Obviously, accepted surgical principles must continue to be employed even in infections by streptomycin - sensitive organisms.

Miscellaneous: Table 3 lists several miscellaneous infections treated by Harrell and Nichols (32)., together with results of streptomycin therapy. In some of these, the dosages used are now known to be entirely inadequate. In the four cases of ozena listed, symptomatic improvement followed treatment with streptomycin, but evaluation of results must wait follow-up studies.

Tuberculosis: Chemotherapy has until very recently been disappointing in the treatment of human tuberculosis. The

Sulfone group of agents have

failed to live up to their predicted efficacy on the basis of animal experiments, largely as a result of excessive toxicity in man(35,36). The greater effectiveness of streptomycin in experimental tuberculosis, coupled with its lack of appreciable toxicity, has led to the hope that this agent might be the long sought-after "cure for tuberculosis".

Preliminary studies by Hinshaw and Feldman(34) seem to indicate that streptomycin has a limited suppressive effect, particularly on the more unusual types of pulmonary and extrapulmonary tuberculosis. Present evidence points to a bacteriostatic rather than bactericidal action, since prompt reactivation of infection often follows cessation of treatment.

No rapidly curative effect was observed in 8 cases of pulmonary tuberculosis treated for 8 months. Extensive and progressive lesions of known recent origin tended to improve rapidly in a manner resembling the natural healing process. This was especially apparent in fine, widely distributed lesions of hematogenous distribution.

Striking and unmistakable improvement occurred in the X-Ray appearance of the lung lesions in two patients with early miliary tuberculosis but no parallel improvement in clinical condition was detected.

Treatment of the empyema was irritating and produced indefinite or disappointing results.

In 4 of 5 patients with solitary kidneys and tuberculosis of the genito-urinary tract treatment caused disappearance of tubercle bacilli

from the urine within 2-4 weeks of streptomycin treatment.

Three patients with draining sinuses due to suppurating tuberculous lymphadenitis showed prompt and striking response to streptomycin treatment; it is possible that recurrence of the infection may take place.

Doctors and patients should be cautioned that sanatorial care and collapse therapy and other proven methods of treatment of tuberculosis should not be abandoned in favor of the relatively untried chemotherapeutic agents. Undue optimism on the part of the laity as to the effects of newly developed antibacterial agents must be avoided, since morale, which is so important in patients with chronic debilitating diseases such as tuberculosis, is undermined by premature optimistic reports which may not be fullfilled in clinical practice.

X. Conclusions

It is still too early to assign the exact place that streptomycin will occupy in our chemotherapeutic arsenal. Certainly, it holds promise of serving as a valuable addition to our armamentarium for combatting bacterial infections, particularly those which are resistant to the action of penicillin and other established agents. It has already given evidence of outstanding therapeutic effect in some diseases, such as influenzal meningitis and tularemia; in other diseases, such as brucellosis and tuberculosis, results have been inconclusive or disappointing. To make predictions would be foolhardy as well as useless, since only by continued clinical investigation can the status of streptomycin in each disease be clarified.

The information arising from the investigative work done on streptomycin is of value even apart from its specific clinical applications. Perhaps equally important from the long range point of view is the additional light which it must inevitably throw upon the physico-chemical mechanisms of bacteriostatic and bactericidal action both *in vitro* and *in vivo*, and upon their relations to certain properties of bacterial organisms. More exact appreciation of these fundamental concepts, together with a better understanding of the ultimate nature of bodily resistance will facilitate future developments in the broad field of treatment of infectious disease.

APPENDIX

Figures and Tables

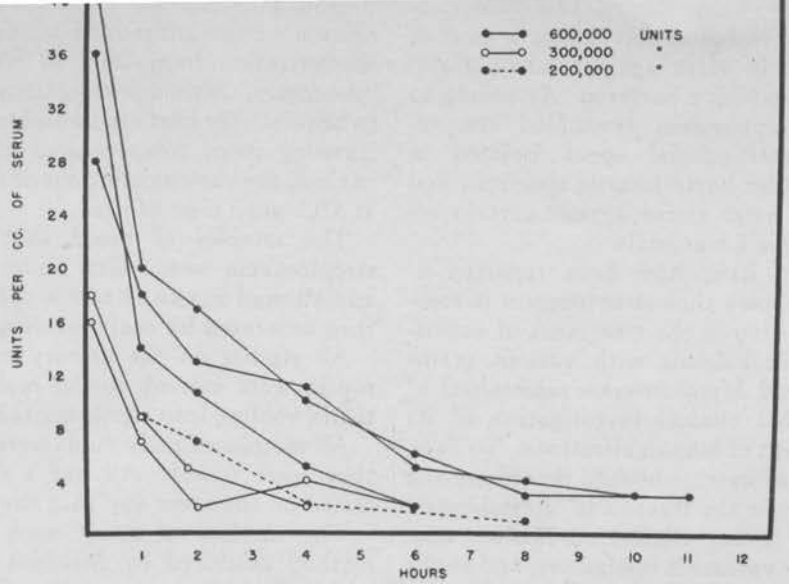


FIGURE 1. *Concentrations of Streptomycin in Serum following the Intravenous Injection of Varying Doses.*

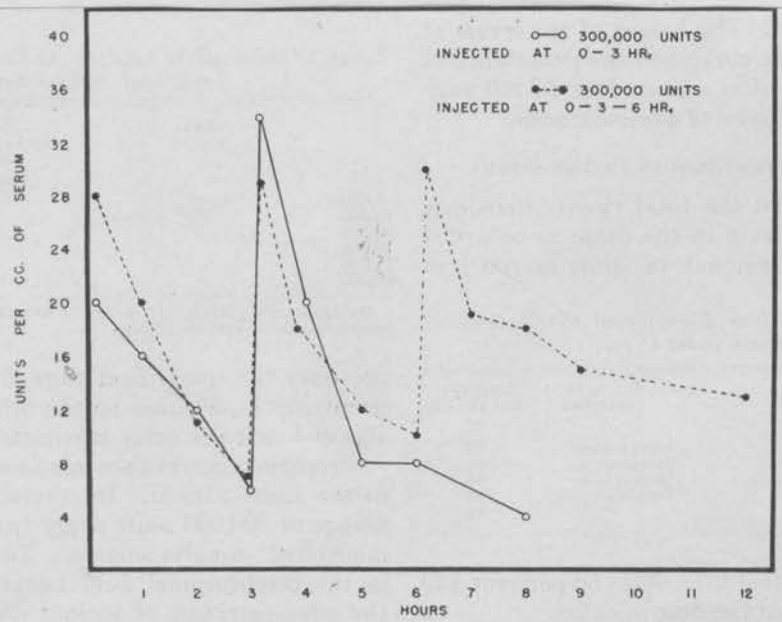


FIGURE 2. *Concentrations of Streptomycin in Serum following Multiple Intravenous Injections at Three-Hour Intervals.*

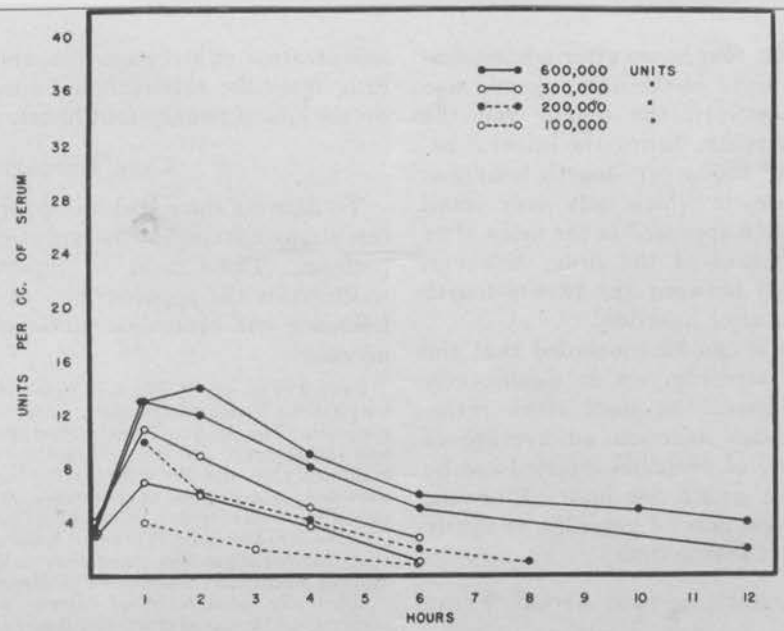


FIGURE 3. *Concentrations of Streptomycin in Serum following Intramuscular Injection of Varying Doses.*

Table II

Antibiotics from Fungi

Name	Origin	Susceptible Organisms
Penicillic acid	Penicillium puberulum	Gram-positive and gram-negative bacteria.
Penicillin	Penicillium notatum P. chrysogenum	Gram-positive bacteria, gonococci, meningococci, spirochetes, actinomycetes, clostridia, C. diphtheriae.
Citrinin	Penicillium citrinum	Gram-positive bacteria
Gliotoxin	Trichoderma lignorum	Kills gram-positive bacteria, S. paratyphi, S. dysenteriae, fungi.
Fumigatin	Aspergillus fumigatus	Chiefly gram-positive bacteria.
Claviformin*	Penicillium claviforme	Gram-positive and gram negative bacteria.
Fumigacin**	Aspergillus fumigatus	Gram-positive bacteria.
Clavacin*	Aspergillus clavatus	Gram-positive and gram-negative bacteria; some fungi.
Aspergillic Acid	Aspergillus flavus	Gram-positive and gram-negative bacteria.
Flavicin	Aspergillus flavus	Gram-positive bacteria.
Helvolic Acid**	Aspergillus fumigatus	Gram-positive bacteria.
Patulin*	Penicillium patulum	Gram-positive and gram-negative bacteria.
Gigantic Acid	Aspergillus giganteus	Gram-positive bacteria.
Flavicidin	Aspergillus flavus	Gram-positive bacteria.

*Claviformin, clavacin and patulin are similar if not identical.
**Fumigacin and helvolic acid are similar if not identical.

Table III

Antibiotics from Actinomycetes

Name	Origin	Susceptible organisms
Actinomycetin	Actinomyces	Gram-positive and gram-negative bacteria.
Actinomycin A	A. antibioticus	Gram-positive bacteria and fungi.
Actinomycin B	A. antibioticus	Gram-positive bacteria and fungi.
Proactinomycin	Proactinomyces gardneri	Gram-positive bacteria and gram-negative cocci.
Streptothricin	A. lavendulae	Gram-positive and gram-negative bacteria, fungi, Mycobacteria.
Streptomycin	A. griseus	Gram-positive and gram-negative bacteria, Mycobacteria, Actinomycetes.

Table IV

Comparative Chemical and Biological Properties Of Streptothricin and Streptomycin (75)

	STREPTOTHRICIN	STREPTOMYCIN
Source of Antibiotic substance	Actinomyces lavendulae	Actinomyces griseus
Medium for production of sustance	Tryptone starch broth	Nutrient glucose broth
Need for growth promoting substance	0	+++
Solubility	Soluble in water, acid solutions, but not in chloroform or ether	Same
Heat Stability	Thermostable	Same
Sensitivity to acids	Sensitive	Same
Effect of Glucose	Inhibitory	Same
Bacteriostatic Spectrum	Certain gram-positive & gram-negative bacteria: relatively inactive against B. mycoides & S. marcescens	More generalized action against bacteria including B. mycoides and S. marcescens
Activity against Trypanosoma equiperdum	None	None
Antifungal Activity	Active against pathogenic saprophytic fungi	None
Toxicity to Animals	Limited toxicity; 1.0-3 gm. of purified material per kg. of body weight	Less toxic; about 7.0 gm. of purified material per kg. of body weight
Activity in vivo	Active	Active

Table V

Cultural and Physiological Characteristics of the Streptomycin Producing Strain of A. griseus and its Inactive Variant.(63)

Active Strain	Inactive Variant
1. Antibiotic activity. Produces streptomycin in both shaken and stationary cultures.	1. No streptomycin formed either in shaken or stationary cultures.
2. Surface growth always heavily sporulated; grayish-green aerial mycelium.	2. No sporulating aerial mycelium; scant development of aerial hyphae with tendency to form spores in old cultures.
3. Reaction: Medium always changes to alkaline; pH 7.5-8.5.	3. Reaction of medium at first acid, pH 5.0-6.5; later becoming alkaline.
4. Glucose. Glucose completely consumed in 6-8 days in stationary cultures and in 3-4 days in shaken cultures.	4. Glucose utilized more slowly.
5. Lysis in shaken cultures. Shaken cultures produce very fine flocculant growth, tending to lyse slowly after about 15 days.	5. Cultures produce at first balls of growth which change into the turbid, flocculant type; rapid and complete lysis in 7-10 days.
6. Lysis in stationary cultures. Surface pellicles stable; any submerged, flocculant growth tends to lyse as the surface pellicle develops.	6. Stationary cultures produce no surface growth but flocculant, submerged mycelial growth which lyses slowly, only after a month or longer.
7. Viscosity. Culture filtrate does not show any viscosity.	7. Culture filtrate becomes viscous during or after lysis.
8. Reinoculation. Inoculation of cultures with lysed inactive culture induces no lysis or reduction in activity.	8. Inoculation of cultures with spores of active strain produces growth and antibiotic activity if some glucose remains.
9. Variation. Sporulating strain gives rise to non-sporulating variants.	9. Asporogenous variants may reconvert to active, sporogenous forms.
10. Sensitivity to streptomycin. Very resistant to this antibiotic.	10. Very sensitive to this antibiotic.

Table VI

Influence of Cysteine Concentration upon the Activity of Streptomycin (52)

Mg. Cysteine per 100 Micrograms of Streptomycin	Streptomycin Activity, Units, After		
	0 hrs.	5 hrs.	24 hrs.
0.0	37.5	37.5	25.5
0.5	37.5	29.5	22.0
2.5	23.0	14.0	2.0
12.5	2.0	2.0	1
25.0*	1.7	1.7	1
50.0*	5.3	4.5	1.8

* Results obtained with 25.0 and 50.0 mg. cysteine were rather confusing; several zones of alternating inhibition and stimulation were produced. The cysteine itself at similar concentrations appeared to have a marked inhibiting effect on B.subtilis by the assay method.

Table VII

Concentrations of Streptomycin in Blood Serum Following Repeated Injections

Administration of Streptomycin			Blood Serum Levels (Units per cc.)					
			Hours after first dose					
Route	Dose*	No. Doses	1	3	6	9	12	Method of Assay
I.V.	100,000	3	3	1.5	3	3	1.5	Slide cell
			5.4	2.9	4.5	3.4	2.3	Cup plate
I.V.	100,000	3	3	1.5	1.5	1.5	1.5	Slide cell
			4.6	3	4	5.4	2.8	Cup plate
I.M.	100,000	3	1.5	1.5	1.5	1.5	0	Slide cell
			2.7	2.6	2.6	3.3	1.2	Cup plate
I.M.	100,000	3	1.5	1.5	3	3	1.5	Slide cell
			3	3.3	4.5	4.3	2.8	Cup plate
I.M.	400,000	3	3	3	6	3	3	Slide cell
			3.6	2.1	11	3.9	2.3	Cup plate
I.M.	400,000	3	3	3	3	3	0	Slide cell
			5.7	2.8	5.8	6.9	2.6	Cup plate
I.V.	400,000	3	14.5	14.9	13.2	7.5	4.1	Cup plate

From Heilman et al. (26)

* given every three hours

Table VIII

Concentrations of Streptomycin in Blood Serum Following Single or Intermittent Injections

Route of Admin.	Amount per dose (units)	Blood Serum Levels (Units per cc.) Hours After Injection ½	1	2	3	4	Method of Assay
I.V.	100,000	6	6	3	0	..	Slide cell
I.M.	100,000	3	3	3	3	..	Slide cell
Subcut.	100,000	3	3	3	1.5	..	Slide cell
Subcut.	200,000	6	6	6	6	..	Slide cell
		8.8	8.8	7.2	6.2	..	Cup plate
Subcut.	200,000	12.5	6	6	3	..	Slide cell
		10	7.7	6	..	..	Cup plate
Subcut.	200,000	12.5	25	6	6	..	Slide cell
Subcut.*	100,000	..	12.5	..	..	..	Slide cell
Subcut.*	100,000	..	12.5	25	6	..	Slide cell
Subcut.*	115,000	..	12.5	..	..	..	Slide cell
Subcut.*	115,000	..	6	..	..	..	Slide cell
Subcut.*	150,000	..	12.5	..	..	..	Slide cell
		..	16.6	..	..	..	Cup plate
Subcut.*	250,000	..	25	25	25	25	Slide cell
			31	24	16	10	Sup plate

From Heilman et al. (26)

*Intermittent injection every three hours.

Table IX

Bactericidal Action of Streptomycin Upon *Pr. vulgaris*

Streptomycin, units per ml.	Pr. vulgaris W 1	Pr. vulgaris W 2	Pr. vulgaris R
	At start		
	69.5	45.5	26.0
	After 28 hours' incubation		
0	375.0	615.0	560.0
1	0.01	0.65	190.0
5	0**	0**; ***	9.0
25	0	0**, ***	3.15

* No. of cells in millions per ml.
** One colony on a plate from 1:1000 dilution
***7-8 colonies on a plate from 1:10 dilution

Table X

Range in Sensivity of Different Bacteria and Actinomycetes to the Bacteriostatic Action of Streptomycin* (82)

Organism	Micrograms
Aerobacter aerogenes	0.5-2.5
Bacillus anthracis	0.375
B. cereus	0.83
B. megatherium	0.25-3.0
B. mesentericus	1.67
B. mycoides	0.1-3.8
B. subtilis	0.12-1.0
Brucella abortus	0.5-3.75
Br. melitensis	0.5
Br. suis	0.5
Clostridium butylicum	8.34
Cl. septicum	>105
Cl. sordellii	>105
Cl. tetani	>104
Cl. welchii	>104
Corynebacterium diphtheriae	0.375-3.75
Diplococcus pneumoniae	8.0
Eberthella typhi	1.0-37.5
Erysipelothrix muriseptica	2.5
Escherichia coli	0.3-3.75
E. communior	1.0-4.0
Hemophilus influenzae	1.56-5.0
H. pertussis	1.25-3.0
Listerella monocytogenes	2.5
Klebsiella ozenae	0.375-1.5
K. pneumoniae	0.625-8.0
Malleomyces mallei	10->10.0
Mycobacterium avium	10.0
M. phlei	0.12
M. tuberculosis, var. hominis	0.15
Neisseria gonorrohoeae	5.0
N. intracellularis	5.0
Pasteurella aviseptica	15.00
P. lepiseptica	0.5-2.5
P. pestis	0.75-1.5
P. tularensis	0.15-0.3
Phytomonas pruni	0.25
Proteus vulgaris	0.4-3.0
Pseudomonas aeruginosa**	2.5-25.0
Ps. fluorescens**	12.5
Sarcina lutea	0.25
Serratia marcescens	1.0
Salmonella aertrycke	4.0-10.0
S. enteritidis	0.5
S. schottmülleri	2.0
Salmonella sp.	60.0
Shigella paradysenteriae	0.25-3.75
Staphylococcus aureus***	0.5->16.0

Table X (continued)

Organism	Micrograms
Streptococcus faecalis	50.0
S. hemolyticus	2.0->16.0
S. lactis	4.0
S. salivarius	5.0-25.0
S. viridans	>16
Vibrio comma	6.0-37.5
Actinomyces bovis	3.75
Nocardia asteroides	12.5
N. gypaoides	4.0-12.5
Streptomyces albus	0.4-1.25
S. antibioticus	<0.4
S. lavendulae	1.25
Streptomyces sp. 3462	4-12.5

* Micrograms of streptomycin per ml. of suitable medium required to inhibit growth.
** Out of a group of Ps.aeruginosa and Ps. fluorescens (by Hirshfeld) some required over 256 micrograms per ml. to inhibit growth.
*** Some staphylococci have been repotted (by Hirshfeld) to require over 256 micrograms per ml. to inhibit growth.

Table XI

Bacteriemia Owing to Gram-negative Organisms (32)

Infecting Organism	Associated Lesion	Days Treated	Total Dose*	Route of Adm.	Result
Escherichia coli	Pyelonephritis and uremia	7	1,400,000	I.V. drip	Good. Recovery from bacteriemia but death from coronary occlusion
A. aerogenes	Severe urinary tract infection	14	19,000,000 (2 courses)	I.V. drip	Good. Recovery from bacteriemia but assoc. E. coli bacilluria remained
Ps. aeruginosa	Prostatic obstruction with severe cystitis and pyelonephritis	7	14,000,000	I.V. drip	Good. recovery from bacteriemia but E. coli bacilluria remained
E. coli	Pyelonephritis and diabetes	13	20,000,000	I.V. drip and I.M.	Good. Recovery from bacteriemia but E. coli bacilluria remained
Salmonella, Proteus ammoniae	Severe urinary tract infection	17	20,400,000	I.M.	Good. Recovery from bacteriemia but Proteus infection in urine remained.
Brucella	Undulant fever with possible endocarditis	14	14,600,000	I.V. drip and I.M.	Doubtful. Blood cultures became neg. but further observation necessary
Brucella	Undulant fever	36	43,000,000 (3 courses)	I.V. drip	Good. Ultimate recovery. See text p. 58.
Brucella	Undulant fever	7	7,000,000	I.M. and I.V. drip	Doubtful. Blood cultures became negative under treatment but symptoms persisted

* S units

Table XII

Infections of the Urinary Tract (32)

Infecting organism	Days Treated	Total Dose*	Route of Adm.	Result
Ps. aeruginosa E. coli	17	16,600,000 (3 courses)	I.M.	Good. Definite improvement following streptomycin combined with surgical treatment.
Ps. aeruginosa	8	7,200,000	I.M.	Good. Urine cultures became negative and temperature returned to normal.
Ps. aeruginosa	4	4,000,000	I.M.	Failure.
Ps. aeruginosa E. coli	7	5,600,000	I.M.	Good. Urine cultures became negative.
Ps. aeruginosa	13	14,400,000	I.M.	Good. Recovery complete.
Pr. ammoniae E. coli A. aerogenes	5	4,000,000	I.M.	Good. Sterile urine at time of dismissal.
Pr. ammoniae	5	6,000,000	I.M.	Good. Sterile cultures after third day of treatment.
Ps. aeruginosa	9	9,000,000	I.M.	Good. Urine cultures negative after 8 days of treatment.
S. faecalis S. aureus Ps. aeruginosa	6	6,000,000	I.M.	Good. Penicillin and sulfathiazole ineffective. Sterile urine following streptomycin.
Ps. aeruginosa	4	4,000,000	I.M.	Doubtful. Epididymitis subsided and temperature became normal; but cultures remained positive.
Proteus ammoniae	7	7,000,000	I.M.	Good. Cultures became negative; but much additional treatment was given.
A. aerogenes E. coli	6	4,800,000	I.M.	Failure. Temporary improvement followed by recurrence of E. coli bacilluria.
A. aerogenes	8	6,400,000	I.M.	Good. A. aerogenes infection controlled; Strep. faecalis infection developed while under treatment.

* S units

Table XIII

Treatment of Miscellaneous Infections With Streptomycin (32)

Diagnosis	Infecting Organism	Days Treated	Total Dose (units)	Adm.	Result
Osteomyelitis	Ps. aeruginosa	3	3,000,000	Local and I.V. drip	Failure. Supply of streptomycin exhausted.
Ruptured bladder with cellulitis	None identified	9	5,850,000	I.M.	Failure. Surgical drainage nedessary.
Cholangitis	E. coli	10	8,000,000	I.M.	Good. Remained afebrile after treatment began. Bile sterile.
Meningitis	H. influenzae type B	10	8,400,000	I.M. and intrathecally	Good. Patient received serum and sulfonamides in addition to streptomycin.
Meningitis	H. influenzae type B	7	5,800,000	I.M. and intrathecal	Good. Sulfadiazine and sulfamerazine in addition to streptomycin.
Meningitis	H. influenzae type B.	12	10,100,000	IM and intrathecal	Good. No other treatment except streptomycin.
Meningitis	H. influenzae	18	18,500,000	IM and intrathecal	Good. Infection controlled but died 2 months later of post-meningitis hydrocephalus (communicating).
Ozena	K. pneumoniae	10	10,000,000	IM and IV drip	Doubtful. Culture became negative. Symptomatic improvement.

(Continued)

Table XIII (continued)

Diagnosis	Infecting Organism	Days Treated	Total Dose (units)	Adm.	Result
Ozena	K. pneumoniae	14	14,000,000	I.M.	Failure. Temporary improvement but organism became resistant.
Ozena	K. pneumoniae	12	9,600,000	I.M.	Doubtful. Symptomatic improvement. Obtained negative culture.
Ozena	K. pneumoniae	10	10,000,000	I.M.	Doubtful. Culture became negative and aymptomatic improvement but no follow-up available

Table XIV

Streptomycin in Pulmonary Suppurative Disease (32)

Infecting Organism	Days Treated	Total dose (units)	Administration	Results
K. pneumoniae	7	1,120,000	I.M. and nebulization	Good. Klebsiella eliminated from sputum after treatment. Residual bronchiectasis.
K. pneumoniae	10	2,000,000	I.M. and nebulization	Good. Sputum has remained negative for 11 months.
Paracolon bacillus	30	30,000,000	Intramuscular	Good. Sputum became negative. Bronchiectasis and fibrosis persisted/
Ps. aeruginosa E. coli	4½	4,500,000	Nebulization	Good. Sputum became negative.
E. coli H. influenzae	8	1,900,000	Nebulization (1 day) and supraglottical	Failure. Treatment discontinued. Increasing dyspnea with decompensated emphysema.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Adcock: Quoted by Waksman (82)

2. Anderson, D.G.; Jewell, M.: The Absorption, Excretion, and Toxicity of Streptomysin in Man; N.E.J.M. 233: 485-91, Oct. 25, 1945

3. Asheshov, I.N.; Strelitz, F.: A Simplified Method for the Assay of Antibioties; Science 101: 621; June 15, 1945

4. Brink, N.G.; Kuehl, F.A.,Jr.; Folkers, K.; Streptomyces Antibiotics: III. Degradation of Streptomycin to Streptobiosamine Derivatives; Science 102: 506-7, Nov. 16, 14

5. Cavallito, C.J.; Bailey, J.H.: Preliminary Note on the Inactivation of Antibiotics; Science 100; 390, Oct. 27, 1944

6. Chain, E.; Florey, H.W.: Penicillin; Endeavor 3: No. 9, Jan., 194

7. Denkelwater, R.G.; Cook, M.A.; Tishler, M: The Effect of Cysteine on Streptomycin and Streptothricin; Science 102: 12, July 6, 1945

8. Domagk, G.: Ein Beitrag zur Chemotherapie Der Bakteriellen Infectionen; Deutsche Med. Wchnschr; 61: 250-253, 1935

9. Dubos, R.J.: Bactericidal Effect of an Extract of a Soil Bacillus on Gram-positive Cocci, Proc Soc. Exper. Biol.&Med. 40: 311-12, 1939; J. Exper. Med. 70: 1-10, 11-17, 1939

10. Elias, W.F.; Durso, J.: Blood, Urine, and Fecal Levels of streptomycin in the Treatment of Human Infections of E. Typhosa; Science 101: 589,91, June 8, 1945

11. Elias, W.F.; Merrion, H.J.; Speicher, T.: Inhibition Factors in the Determination of Penicillin in Human Sera; Science 102: 123-25, Aug. 31, 1945

12. Emmerich, R.; Loew, C.: Quoted by Chain & Florey

13. Feldman, W.H.: A Scheme for Numerical Recording of Tuberculous Changes in Experimentally Infected Guinea Pigs; Am. Rev. Tuberc. 48: 248, 1943

14. Feldman, W.H.; Hinshaw, H.C.: Effects of Streptomycin on Experimental Tuberculosis of Guinea Pigs; Proc.Staffl Meet. Mayo Clin.: 19: 593-599, Dec. 27, 1944

15. Feldman, W.H.; Hinshaw, H.C.; Mann, F.C.: Streptomycin in Experimental Tuberculosis; Am. Rev. Tuber. 52: 269-98, 1945

16. Finch: Peter Bent Brigham Hospital, Personal Communication

17. Fleming, A.: On the Antibacterial Action of Cultures of a Penicillin, with Special Reference to their Use in the Isolation of B. Influenzae; Brit.J.Exp.Path. 10: 226-236, 1929

18. Flippin, H.F.: Quoted by Waksman (82)

19. Foshay, L.: Quoted by Waksman (82)

20. Foster, J.W.; Woodruff, H.B.;: Microbiological Aspects of Streptothricin. II. Antibiotic Activity of Streptothricin Arch. Biochem. 3: 241-55, Dec. 1943

21. Foster, J.W.; Woodruff, H.B.: Quantitative Estimation of Streptothricin; J. Bact. 45: 408, April, 1943

22. Fried, J.; Wintemsteiner, O.; Crystalline Reineckates of Streptothricin and Streptomycin; Science 101: 613-15, June 15, 1945

23. Greey, : University of Toronto,: Quoted by Waksman (82)

24. Hegarty, C.P.: Quoted by Waksman (82)

25. Heilman, D.H.: A Method for Estimating the Concentration of Streptomycin in Body Fluids, Proc. Staff Meet. Mayo Clin. 20: 145-150, May 16, 1945

26. Heilman, D.H.; Heilman, F.R.; Hinshaw, H.C.; Nichols, D.R.; Herrell, W.E.: Streptomicin: Absorption, Diffusion, Excretion, and Toxicity; Am. J. Med. Sci. 210: 576-84, Nov. 1945

27. Heilman, F.R.: Streptomycin in the Treatment of Experimental Tularemia; Proc. Staff Meet. Mayo Clin. 19: 553-9, Nov. 29, 1944

28. Heilman, F.R.: Streptomycin in the Treatment of Experimental Infections with Micro-organisms of the Friedlander Group (Klebsiella). Proc. Staff Meet. Mayo Clin, 20: 33-39, Feb. 7, 1945

29. Heilman, F.R.: Streptomycin in the Treatment of Experimental Relapsing Fever and Leptospirosis Icterohemorrhagica (Weil's Dis). Proc. Staff Meet. Mayo Clin 20: 169-76 May 30, 1945

30. Helmholz, H.F.: The Effect of Streptomycin on Bacteria Commonly Found in Urinary Infections, Proc. Staff Meet. Mayo Clin. 20: 357, Oct. 3, 1945

31. Herrell, W.E.: Penicillin and Other Antiiotics, W.B.Saunders Co. Philadelphia, 1945

32. Herrell, W.E.; Nichols, D.R.: The Clinical Use of Streptomycin: a Study of Forty-five Cases; Proc. Staff Meet. Mayo Clin. 20: 449-462, Nov. 28, 1945

33. Hinshaw, H.C.: Quoted by Waksman (82)

34. Hinshaw, H.C.; Feldman, W.H.: Streptomycin in Treatment of Clinical Tbc; a preliminary report. Proc. Staff Meet. Mayo Clin. 20: 313-18, Sept. 5, 1945

35. Hinshaw, H.C.; Feldman, W.H.: Chemotherapy of Clinical and Experimental Tbc. Med. Clin. N.A. 29: 918-22, 1945

36. Hinshaw, H.C.; Feldman, W.H.; Pfuetze, K.H.: Present Status of Chemotherapy in Tuberculosis, Ann. Int. Med. 22; 696, '4

37. Howes: Quoted by Waksman (82)

38. Jones, D.; Metzger, H.J.; Schatz, A.; Waksman, S.A.: Control of Gram-negative bacteria in Experimental annuals by Streptomycin, Science, 100: 103, Aug. 4, 1944

39. Kocholaty, W.: Microbial Antagonism and Brucella Abortus, J. Bact. 44: 143, July, 1942

40. Kuehl, F.S.,Jr.; Peck, R.L.; Walti, A.; Folkers, K.: Streptomyces Antibiotics. I. Crystalline Salts of Streptomycin and Streptothricin. Science 102: 34-35, July 13, 1945

41. Loo, Y.H.; Skell, P.S.; Thornberry, H.H.; Ehrlich, J.,MCGuire, J.M.; Savage, G.M.; Sylvester, J.C.: J. Bact. 1945. (In press) Quoted by Waksman (82)

42. Metzger, H.J.; Waksman, S.A.; Pugh, L.H.: In Vivo Activity of Streptothricin Against Brucella Abortus; Proc. Soc. Exper. Biol. & Med. 51: 251-2 Oct., 1942

43. Molitor and Mushett: Quoted by Waksman (82)

44. Molitor, Robinson, Mushett: Quoted by Waksman (82)

45. Murphy, J.J.; Ravdin, R.G.: Quoted by Zintel et al (94)

46. Neter, E.: Effects of Penicillin, Clavacin, and Streptomycin Upon Tetanus Toxin; J. Infect. Dis. 76: 20-3, 1945

47. Pasteur, L.; Joubert, J.: Charbon Et Septicemie; Compt. Rend. Acac. of Sc. 85: 101-105, 1877, quoted by Chain and Florey (6) and Waksman (69)

48. Peck, R.L.; Brink, N.G.; Kuehl, F.A. Jr.; Flynn, E.N.; Walti, A.; Folkers, K.;: Streptomyces Antibiotics. II. Crystalline Streptomycin Trihydrochloride--Calcium Chloride Double Salt. J. Am. Chem. Soc. 67: 1866-7, Oct., 1945

49. Perlman, D.; McCoy, E.: Some Effects of Induced Streptothricin Resistance on Lactobacillus Cases. J. Bact. 49: 271-5, March 1945

50. Rake, G.; Hamre, D.; Kavanagh, F.; Koerber, W.L.; Donovick, R.: On the Toxicity of Streptothricin. Am. J. Med. Sci. 210: 61, July 1945

51. Reilly, H.C.; Schatz, A.; Waksman, S.A.: Antifungal Properties of Antibiotic Substances. J. Bact. 49: 585-94, 1945

52. Reilly, H.C.; Waksman, S.A.: Inhibition of Streptomycin Activity by Cysteine. To be published (Quoted by Waksman (82))

53. Reimann, H.A., Elias, W.F.; Rice, A.H.: Streptomycin for Typhoid: Pharmacologic Study. J.A.M.A. 128: 175-180, May 19, 1945

56. Robinson, H.J.: Some toxicological, bacteriological, and pharmacological properties of antimicrobial agents produced by soil micro-organisms. Thesis, Rutgers Univ., 1943 46 pp

55. Robinson, H.J.; Graessle, D.E.; Smith, D.G.: Chemotherapeutic Properties of Streptomycin. Am. J. Med Sci. 209: 128, 19

54. Reimann, H.A. Quoted by Waksman (82)

57. Robinson, H.J.; Graessle, O.E.; Smith, D.G.: Studies on the Toxicity and Activity of Streptothricin. Science 99: 540, June 30, 1944

58. Robinson, H.J.; Smith, D.G.: Streptothricin as a Chemotherapeutic Agent. J. Pharmacol. & Exper. Therap. 81: 390-401, 1944

59. Robinson, H.J.; Smith, D.G.; Graessle, D.E.: Chemotherapeutic Properties of Streptomycin. Proc. Soc. Exper. Biol. & Med. 57: 226, 1944

60. Rutenberg, A.; Beth Israel Hospital, Boston: Personal Communicati

61. Schatz, A.; Bugie, E.; Waksman, S.A.: Streptomycin, a Substance Exhibiting Antibiotic Activity Against Gram-positive and Gram-negative Bacteria. Proc. Soc. Exper. Biol. & Med 55 66-69, Jan. 1944

62. Schatz, A.; Waksman, S.A.: Effect of Streptomycin and other Anti-Biotic Substances upon Hycobacterium Tuberculosis and Related Organisms. Proc. Soc. Exper. Biol. & Med. 57: 244-8, Nov. 1944

63. Schatz, A.; Waksman, S.A.: Strain Specificity and Production of Antibiotic Substances IV. Variations Among Achnomycetes, with Special Reference to Actinomyces Griseus. Proc. Nat'l. Sci. U.S. 31: 129-137, May 15, 1945

64. Seeler, A.O.; Malanga, C.; Pierson, J.: Effect of Streptomycin on Avian Malaria. Proc. Soc. Exper. Biol. & Med. 59: 291, June 1945

65. Smith, M.; Birmingham, Baltimore: Quoted by Waksman (82)

66. Smith, M.I.; McClosky, W.T.: The Chemotherapeutic Action of Streptomycin and Promin in Experimental Tuberculosis. Public Health Reports 60: 1129-37, Sept. 28, 1945

67. Stebbins, R.B.; Robinson, H.J.: A Method for Determination of Streptomycin in Body Fluids. Proc. Soc. Exper. Biol. & Med. 59: 255, June 1945

68. Stokes, J.L.; Woodward, C.R.,Jr.: The Isolation from Soil of Spore-forming Bacteria which Produce Bactericidal Substances. J. Bact. 43: 353-263, Feb. 1942

69. Waksman, S.A.: Microbial Antagonisms and Antibiotic Substances, The Commonwealth Fund, New York, 1945

70. ———————— Antagonistic Relations of Micro-organisms. Bact. Rev. 5: 231-291, 1941

71. ———————— Production and Activity of Streptothricin. J. Bact 46: 299-310, Sept. 1943

72. ———————— Cultural Studies of Species of Actinomyces. Soil Sci. 8: 71-215, 1919

73. ———————— Standardization of Streptomycin. Science 102, 40-1 July 13, 1945

74. ———————— Nature and Mode of Action of Antibiotic Substances J. Bact. 45: 64, 1943

75. ————————; Bugie, E.; Schatz, A.: Isolation of Antibiotics from Soil Micro-organisms, with Special Referance to Streptothricin and Streptomycin. Proc. Staff Meet. Mayo Clin. 19: 537, Nov. 15, 1944

76. ————————; Henrici, A.T.: The Nomenclature and Classificatio of the Actinomycetes. J. Bact. 46: 337-41, 1943

77. ————————; Horning, E.S.; Welsch, M.; Woodruff, N.B.: Distribution of Antagonistic Actinomycetes in Nature. Soil Sci.: 54: 281-96, 1942

78. ————————; Reilly, N.C.: Bactericidal Action of Antibiotic Substances. J. Infect. Dis. 75: 150-9, 1944

79. ————————————: Agar Streak Method for Assaying Antibiotic Substances. J. Ind. & Eng. Chem, Anal. Ed. 17: 556-58, 1945

80. ————————; ————————; Schatz, A.: Strain Specificity and Production of Antibiotic Substances. V. Strain Resistance of Bacteria to Antibiotic Substances, Especially to Streptomycin. Proc. Nat'l. Acad. Sci. U.S. 31: 157-164, June 1945

81. ————————; Schatz, A.: Strain Specificity & Production of Antibiotic Substances VI. Strain Variation and Production of Streptothricin by Actinomyces lavendulae. Proc. Nat'l. Acad. Sci. 31: 208-214, 1945

82. ————————; ————————: Streptomycin--Origin, Nature, and Properties. J. Am. Pharm. Assoc. 34: 273-291, Nov. 1945

83. ————————; Woodruff, H.B.: The soil as a source of Micro-organisms Antagonistic to Disease-Producing Bacteria. J. Bact. 40: 581, 1940

84. ———————————— : Streptothricin, new selective bacteriostatic and Bactericidal Agent, particularly Active against Gram-positive and Gram-negative bacteria. Proc. Soc. Exper. Biol. & Med. 49: 207-10, Feb. 1942

85. ————————————; Horning, E.S.: The Distribution of Antagonistic Properties Among Actinomycetes. J. Bact. 42: 816, Dec. 1941

86. Weinstein, Louis: Personal Communication

87. Welsch, M.: Bacteriostatic & Bacteriolytic Properties of Actinomycetes. J.Bact. 44: 571-88, 1942

88. Woodruff, H.B.; Foster, J.W.: Cultivation of Actinomycetes under Submerged Conditions, with Special Reference to the Formation of Streptothricin. J. Bact. 45: 30, Jan. 1943

89. ————————————: In vitro Inhibition of Mycobacteria by Streptothricin. Proc. Soc. Exper. Biol. & Med. 57: 88, 1944

90. ————————————: Microbiological Aspects of Streptothricin. I. Metabolism and Streptothricin Formation in Stationary and Submerged Cultures of Actinomyces Lavendulae. Arch. Biochem. 2: 301.315, Aug. 1943

91. Youmans, G.P.: The Effect of Streptomycin in Vitro on M. Tuberculosis var. Hominis. Quart. Bull. Northwest Univ. Med. Sch. 19: 207-9, Oct. 1945

92. ————————; McCarter, J.C.: A preliminary note on the Effect of Streptomycin on Experimental Tuberculosis of White Mice. Quart. Bull. Northwest. Univ. Med. Sch. 19: 210, 1945

93. ————————————: Streptomycin in Experimental Tuberculosis. Its Effect on Tbc. Infections in Mice Produced by M. Tuberculosis var. Hominis. Am. Rev. Tuberc. 52: 432-439, Nov. 1945

94. Zintel, H.A.; Flippin, H.F.; Nichols, A.C.; Wiley, M.M.; Rhoads, J.E.: Studies on Streptomycin in man; I. Absorption, Distribution, Excretion, and Toxicity. Am.J. Med. Sci 210: 421-430, Oct. 1945

95. Zobell, C.E.; Uphaur, H.C.: A Report on the Marine Bacteria of the West Coast. J. Bact. 48: 261, 1944