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Effect of Streptomycin and Other Antibiotic Substances upon *Mycobacterium tuberculosis* and Related Organisms.*†

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The ability of certain saprophytic organisms to inhibit the growth of *Mycobacterium tuberculosis* has long been recognized. As far back as 1885, Cantani[1] obtained favorable effects from the treatment of a tuberculous patient with a culture of a common bacterium. Later, Vaudremer,[2] using extracts of *Aspergillus fumigatus* for the treatment of patients suffering from tuberculosis, reported satisfactory results. Similar observations were reported[3] concerning the effects of extracts of fungi belonging to the *Penicillium* group. The recent progress made in our knowledge of antibiotic substances and their action upon various disease-producing bacteria suggested the advisability of studying the relation of some of these substances to the causative agent of tuberculosis and other related organisms.

Some of the antibiotic substances, like penicillin,[4] have already been shown to have no effect upon *M. tuberculosis*. Other substances, however, such as streptomycin were found[5] to inhibit the growth of both *M. tuberculosis* and *M. phlei*, the degree of sensitivity of these organisms to streptomycin being greater even than that of *Escherichia coli*. The limited toxicity of streptomycin to animals is of particular interest in connection with this problem.

A detailed comparison of the antibacterial spectra of streptomycin and of streptothricin,[6] on the one hand, and of a number of other antibiotic agents, on the other, is presented in Table I. The results illustrate the high degree of selectivity of the various antibiotic substances upon different bacteria. Of the 2 gram-negative organisms used in these experiments, *E. coli* is sensitive only to streptomycin, streptothricin, clavacin, and gliotoxin, and is not affected at all or only to a very limited extent by the other three agents. The second gram-negative organism *Pseudomonas aeruginosa* is sensitive only to streptomycin and to clavacin; it showed no sensitivity at all to the other substances, at least in the concentrations used. Of the 3 strains of mycobacteria used in these experiments, *M. phlei* is most sensitive to all compounds, whereas the avian TB is more resistant than the human strain to some of the substances, especially streptomycin and streptothricin, but not to others.

When the *E. coli* units are taken as a basis for comparison, streptomycin is found to be more than 50 times as active as streptothricin against the human strain of *M. tuberculosis* and about 5 times as active against the saprophytic *M. phlei;* it is also more active against avian TB. It may be of interest to note here that *Actinomyces griseus*, the organism that produces streptomycin, contains a second antibiotic factor. This factor is ether-soluble and is present largely in the mycelium of the organism; it is more active against the avian than against the human strain of *M.*

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1 Cantani, A., *Zentrbl. Med. Wiss.*, 1885, **23**, 513.

2 Vaudremer, A., *C. R. Soc. Biol.*, 1913, **74**, 278, 752.

3 Smith, M. I., and Emmart, E. W., *Pub. Health Repts.*, 1944, **59**, 417; Miller, D. K., and Rekate, A. C., *Science*, 1944, **100**, 172.

4 Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, A. D., Heatley, N. G., Jennings, M. A., and Florey, H. W., *The Lancet*, 1941, **241**, 177.

5 Schatz, A., Bugie, E., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 66.

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TABLE I.
Antibacterial Activities of Different Antibiotic Substances.

Substance	Activity, units per gram of dry, ash-free materials*						
	Ps. aeruginosa	*E. coli*	*B. mycoides*	*B. subtilis*	*M. phlei*	Human TB (H37)	Avian TB
Streptomycin	9,500	38,000	63,000	380,000	320,000	250,000	3,800
Streptothricin	< 6,700	100,000	< 6,700	330,000	170,000	13,000	< 6,700
Chaetomin	<10,000	< 10,000	50,000,000	100,000,000	50,000,000	100,000	100,000
Fumigacin	< 3,000	3,000	300,000	590,000	150,000	22,000	15,000
Clavacin	2,000	20,000	20,000	50,000	67,000	3,300	6,700
Actinomycin	<20,000	< 20,000	>20,000,000	> 20,000,000	2,000,000	6,000,000	600,000
Gliotoxin	<20,000	30,000	1,500,000	2,000,000	1,000,000	200,000	200,000

* A unit is that amount of material that will just inhibit the growth of the organism in 1 ml of culture medium.

tuberculosis.

Because of the high toxicity of clavacin and its low activity against the human TB, it is automatically eliminated from practical consideration. Chaetomin, a substance that has a high degree of activity against both TB strains, is also eliminated from practical consideration, because of the fact that it has so far been found to have no activity *in vivo*, due to its neutralization by certain mechanisms in the body fluids.[7]

Streptomycin appears to be a promising antibiotic substance from the point of view of practical utilization against the human TB organism, because of its relatively greater *in vitro* activity against this strain of *M. tuberculosis* and its lower toxicity,[8] as compared with the very high toxicity of actinomycin,[9] as well as the relatively high toxicity of gliotoxin.[10]

Streptomycin was also found to exert a definite bactericidal action upon the human strain of *M. tuberculosis*, as illustrated by the results of the following experiment. An aqueous solution of streptomycin containing 3,000 units per 1 ml was heated at 70°C for 10 minutes. This solution was added, in concentrations of 300, 100, 30, 10, 3, 1, .3, .1 and .03 units per 1 ml, to a number of 5 ml portions of Long's liquid medium placed in tubes. All the tubes were inoculated with a clump of the H37 strain of the organism, and incubated at 37°C. Of the 4 tubes prepared for each dilution, 2 were removed after 3 days and the remaining 2 after 7 days. The TB clumps were carefully taken out of the tubes, washed in sterile portions of water, and streaked over the surface of a slant of Long's agar medium, to determine viability of the cells. Slides were also prepared from each clump of cells and stained by the acid-fast technic. The use of clumps of cells rather than of suspensions facilitated the removal of any streptomycin adsorbed on the bacterial cells that might have tended to inhibit the growth of the bacterium on the agar slants.

The 3-day exposure tests gave positive growth on all the slants from the tubes containing 300 to .3 units per 1 ml, but the number of TB colonies was fewer and slower in developing than those in the controls. This may be due to the fact that a few cells within the interior of the clump were not in direct contact with the bactericidal agent. The tubes receiving 0.1 and 0.03 units of streptomycin per 1 ml gave growth on the slants nearly equal to that on the controls, both in the number of colonies and in their size and development. The 7-day exposure tests showed that the slants inoculated from the tubes receiving 300 units of streptomycin solution gave no significant amount of growth of the TB organism. Slants from the 100 and 30 unit tubes showed a trace of growth in the form of a few isolated spots. Definite development of occasional colonies was observed on the slants corresponding to the

[7] Waksman, S. A., and Bugie, E., *J. Bact.*, 1944, in press.

[8] Jones, D., Metzger, H. J., Schatz, A., and Waksman, S. A., *Science*, 1944, **100**, 103.

[9] Waksman, S. A., Robinson, H., Metzger, H. J., and Woodruff, H. B., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 261.

[10] Robinson, H. J., Thesis, Rutgers University, 1943.

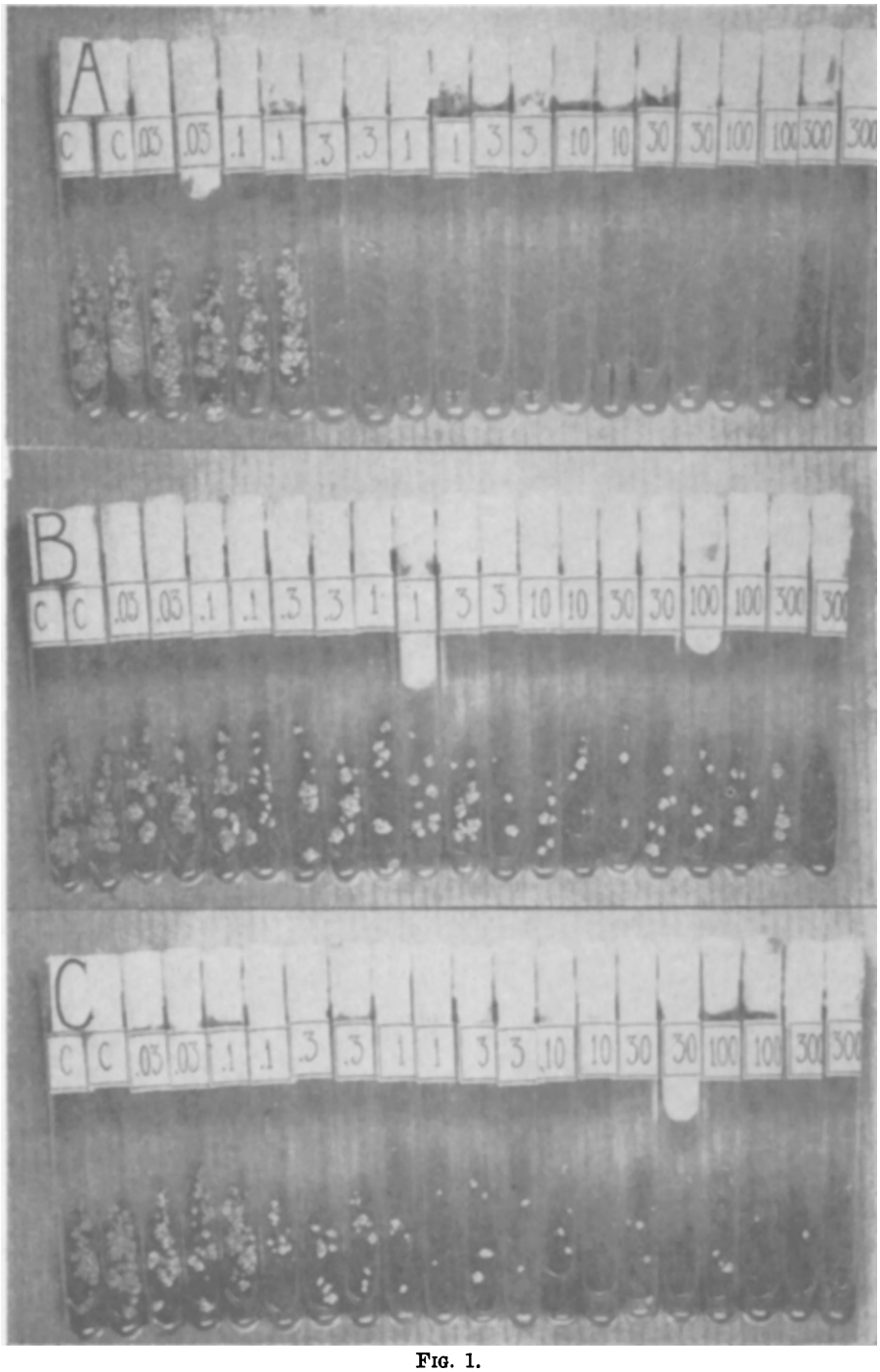

FIG. 1.

The bacteriostaic and bactericidal action of streptomycin upon a human strain (H37) of *M. tuberculosis;* the figures on the tubes give the units of streptomycin, C being the control.

A. Bacteriostatic action.
B. Bactericidal action, three days.
C. Bactericidal action, seven days.

tubes receiving 10 to 1 unit of streptomycin, whereas those slants that were prepared from the tubes with .3 to .03 units of streptomycin gave almost as good growth as the controls.

In each series of agar slants, corresponding to the two periods of exposure of the TB cells to the different concentrations of streptomycin, there was an increase in the number and size of the colonies as the dilution of the antibiotic agent, to which the clumps had originally been exposed, was increased. Approximately the same sized clumps were selected for inoculation, mashed thoroughly, and smeared over the entire surface of the slant.

A microscopic examination of the acid-fast stained slides corresponding to the 3- and 7-day exposures revealed the fact that whereas the control cultures contained cells normal in size, shape, and in staining reaction, the tubes receiving 300 units streptomycin solution showed that a few cells were somewhat longer, more slender and less intensely acid-fast; occasionally a clump appeared as a mass of cocci (round, acid-fast granular bodies), imbedded in a mass of blue substance; some of the cells appeared blue (non-acid-fast). The tubes receiving 100 to 10 units of the streptomycin solution showed that all cells were normal but some blue substance appeared in an occasional clump. The tubes with 3 to .03 units of streptomycin solution showed all the cells to be normal, without any blue substance. It has been suggested[11] that this "cyanophile" substance is indicative of subsequent distintegration or autolysis of the cells. The bacteriostatic and bactericidal effects of streptomycin upon *M. tuberculosis* are illustrated graphically in Fig. 1.

These and other results not reported here showed that the addition of 200-300 units of streptomycin per 1 ml of medium, in which living cells of the TB organism were suspended, was sufficient to kill the cells within a period of a few days; the addition of smaller amounts of streptomycin brought about the killing of the cells if a long enough period of incubation was allowed, namely, 10 or more days. The death of the cells was not accompanied by their visible disintegration, although certain atypical forms occasionally appeared. The mechanism of the action of streptomycin upon the TB cells must, therefore, be considered as still a matter for further investigation.§

It may be of interest to report here briefly the relative activity of some of the antibiotic substances against a bacterium that has frequently been classified with the Actinomycetales and that is said to be closely related to the Mycobacteria, namely, *Erysipelothrix*. *E. muriseptica* was found‡ (Table II) to be more sensitive to streptomycin than to streptothricin on the basis of their respective *E. coli* units. The practical advantage of streptomycin over clavacin and chaetomin lies again in its lower toxicity, as compared with clavacin, and in its *in vivo* activity, as compared with chaetomin.

TABLE II.
Effect of Antibiotic Substances upon *E. muriseptica*.

Substance	Activity units	Activity against *E. muriseptica*
Clavacin	100,000*	30,000
Chaetomin	1,000,000†	>100,000
Streptothricin	100,000*	15,000
Streptomycin	30,000*	12,000

* *E. coli* units.
† *B. subtilis* units.

The results of a study of the action of streptomycin upon several typical actinomycetes are presented in Table III. The organisms selected for this experiment comprise both soil organisms and animal pathogens. The results obtained show that some actinomycetes are highly sensitive to streptomycin. The pathogenic *Streptomyces* strain (*A. albus*) was inhibited by less than 1 unit of the substances, whereas the 2 pathogenic *Nocardia* strains (*A. asteroides* and *A. gypsoides*) were more resistant. There was even a greater variation among the saprophytic strains; *A. griseus*, the organism producing streptomycin, was most resistant to its action,

[11] LaPorte, R., *Ann. Inst. Past.*, 1943, **69**, 262.

§ At the suggestion of the authors the effect of *in vivo* tests of streptomycin upon *M. tuberculosis* is now being studied by Drs. Feldman, Hinshaw, and Heilman of the Mayo Foundation, Rochester, Minn.

‡ These tests were made by Miss H. C. Reilly of this laboratory.

TABLE III.
Effect of Streptomycin upon Growth of Different Actinomycetes.

Organism	Units of streptomycin required to inhibit growth in 1 ml of medium	
	2 days	3 days
*A. albus**	0.4-1.25	0.4-1.25
A. asteroides	—	12.5
A. gypsoides	—	4.0-12.5
A. antibioticus	< 0.4	< 0.4
A. lavendulae	1.25	1.25
A. griseus	>12.5	>12.5
Actinomyces 3462	4-12.5	4-12.5

* Said to be causative agent of ear infection.

whereas *A. antibioticus*, the organism that produces the powerful actinomycin, was most sensitive to it.

Summary. *Mycobacterium tuberculosis* is subject to the bacteriostatic action of a variety of antibiotic substances. There is considerable variation in this respect, both in the sensitivity of the same organism to different substances and of different species or even strains of the same species of *Mycobacterium* to the same substance. Streptomycin is also highly effective against various related organisms, namely, *Erysipelothrix* and actinomycetes, comprising both saprophytic and parasitic strains, with considerable variation among different species.

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Identification of the Serum Fraction Carrying Syphilitic Reagin by Electrophoresis.*

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In earlier reports[1,2] it was shown that the serum proteins in all stages of syphilis show characteristic and significant alterations from the normal values. An increase in all of the globulin components was found with a decrease in the concentration of the albumin component which maintained the total protein concentration in the normal range. Since no correlation could be established between the concentration of any globulin component or components and the strength of the quantitative serological reactions, the fraction containing the reagin responsible for the positive serological reactions could not be determined. The present investigations were undertaken to establish the identity of the syphilitic reagin.

Soon after the Wassermann reaction was introduced and other flocculation and complement fixation tests appeared, a number of studies were made on syphilitic sera in an effort to determine in which fraction or fractions the reagin occurred.[3-7 and others] In general all workers were in agreement on the fact that the reagin was present in the globulin fraction and was not present in the albumin fraction. Further work on fractionation of the globulins, however, brought forth disagreement among the investigators on the globulin fraction carrying the reagin. The earlier fractionation technics involved separating the globulin into pseudoglobulin and euglobulin and the lack of exact characteriza-

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4 Mackie, T. J., *J. Hygiene*, 1926, **25**, 176.

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7 Eagle, H., *Laboratory Diagnosis of Syphilis*, C. V. Mosby Co., St. Louis, 1937, Chap. XVI.