Streptomycin Sensitivity Testing

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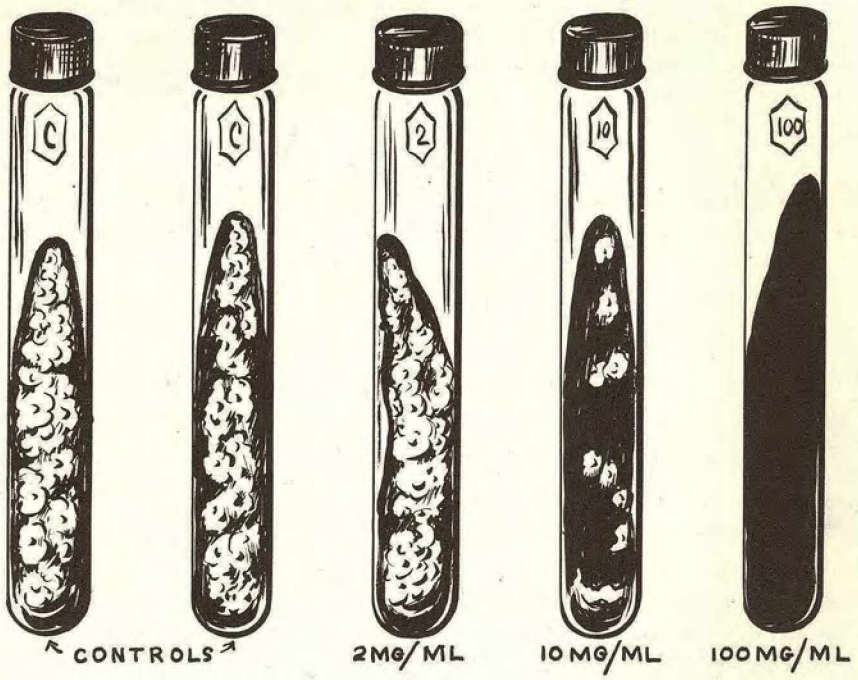
Streptomycin has had a relatively short but nonetheless dramatic history. Within a period of 5 years, extensive laboratory and clinical tests have firmly established the drug as the most promising agent available today for the treatment of tuberculosis. Since the discovery of the tubercle bacillus by Koch in 1882, a variety of chemical compounds of different types had been examined for therapeutic qualities but with discouraging results. Until the discovery of streptomycin there was, in fact, little hope that a successful therapeutic agent ever would be available.

Streptomycin was first described by Waksman and coworkers in January 1944. It was isolated from a soil micro-organism, *Streptomyces griseus*, from which it derives its name. Early experiments indicated that the substance would suppress the growth of a wide variety of bacteria in the test tube, among them the human tubercle bacillus. Subsequent studies at the Mayo Clinic, begun in early 1944, indicated a definite therapeutic effect of streptomycin on experimental tuberculous infections in guinea pigs. Results were so encouraging that large-scale production of the drug was begun almost immediately. The following year, Feldman and Hinshaw of the Mayo Clinic first reported on the possible value of streptomycin in the treatment of human tuberculosis, and extensive clinical trials since that time have fully substantiated these early impressions.

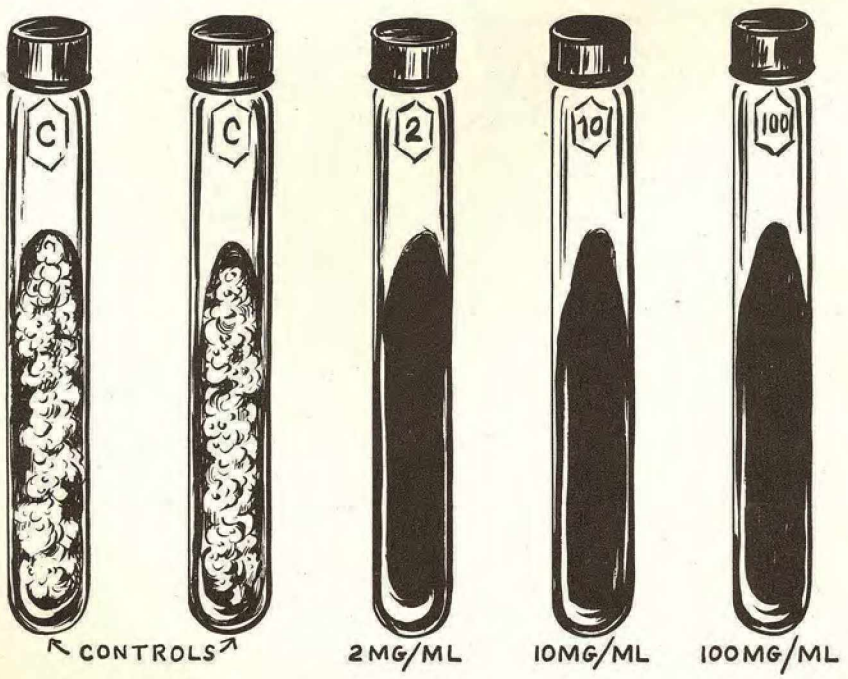
In certain types of the human disease, the drug has been found to exert a marked suppressive effect upon the course of the disease but none would consider it a cure. As clinical trials were extended, it was observed that certain toxic manifestations (for example, rash and deafness) appeared, as did definite resistance of the tubercle bacilli to the drug, a phenomenon which has recently served to reemphasize the need for close collaboration between bacteriologist and physician. Youmans and his coworkers found, for example, that organisms isolated from patients after prolonged treatment with streptomycin often exhibited an increase in resistance to the drug of from 500 to 1,000 fold as compared to tests performed prior to treatment. Subsequent studies in other laboratories have confirmed these findings. It is evident that nearly half of all tubercle bacilli have some resistance to streptomycin, varying from very little to complete.

The importance of performing streptomycin sensitivity tests, therefore, cannot be overemphasized, particularly since recent laboratory studies indicate that prolonged treatment of animals experimentally infected with a streptomycin-resistant bacillus actually decreases their survival time. Of even greater clinical importance is the occasional appearance of an organism whose growth is actually enhanced by streptomycin. Such an organism was recently isolated in the laboratories of the Tuberculosis Section. It is highly important, then, that streptomycin sensitivity tests be performed on tubercle bacilli isolated from patients prior to treatment and at frequent intervals during treatment. In the event that drug resistance is detected in these bacilli, specific alternate therapy may be initiated.

GROWTH OF STREPTOMYCIN-RESISTANT TUBERCLE BACILLI ON TEST MEDIA
(Growth on Media with 10 Milligrams Streptomycin per Milliliter)



RESULTS OF STREPTOMYCIN SENSITIVITY TEST WITH SUSCEPTIBLE ORGANISMS
(No Growth in Presence of Streptomycin)



Using currently available techniques, streptomycin sensitivity tests may be performed with either pathologic materials (such as sputum, urine, and pus) or pure cultures of the organism. The preferred medium for the sensitivity test is that recently recommended by the American Trudeau Society. It consists of a nutrient agar base containing glycerol and malachite green to which are added, just before tubing, a sterile, fresh, egg-yolk solution and streptomycin dilutions to give final concentrations of 2, 10, and 100 micrograms per milliliter of medium. The tubes of medium are allowed to harden in a slanted position and are then stored in the refrigerator until needed. The performance of sensitivity tests with pathologic materials necessitates preliminary chemical treatment of the specimen in order to eliminate contaminating micro-organisms, other than the tubercle bacillus, which might interfere with the test. Following decontamination and concentration (centrifugation at high speed), the resulting sediment is ready for inoculation. Pure cultures of the tubercle bacilli to be tested are prepared by grinding a clump of the bacilli in a test tube with a sterile wire loop and suspending them in a small quantity of sterile saline. Whether pure culture or other test substance be used, 0.1 milliliter of the prepared material is inoculated onto each of the culture tubes containing the graded amounts of streptomycin and on a control tube containing no drug.

After inoculation, the tubes are incubated at 37° C. and examined for growth of tubercle bacilli every week for a total of 4 weeks. Reports are made just as soon as grossly visible growth is adequate for evaluation of sensitivity. For purposes of reporting, the actual number of colonies on each culture slant is counted and recorded since any interpretation of results is based on the assumption that:

(a) each visible colony was derived from a single bacillus in the original suspension, and

(b) each tube of medium received a representative portion of the material.

Reports are based upon a comparison of the number of colonies on the streptomycin tubes with the number of colonies on the control tube. If growth appears only on the control tube, the bacilli are considered to be completely sensitive (nonresistant) to the drug concentrations tested. By the same token, if equal numbers of colonies appear on all of the streptomycin tubes and the control tube, the organisms are reported as completely resistant. Intermediate findings indicate partial resistance. For example, if the control tube has approximately 100 colonies and only 50 colonies appear on one of the streptomycin tubes (say the 2-unit tube), the organisms are considered to be partially resistant; that is, roughly 50 percent of the original bacillary population withstood the concentration of streptomycin in question.

Growth in test tubes of tubercle bacilli isolated from patients prior to treatment with streptomycin is completely inhibited by 0.5 to 1.0 micrograms of drug per milliliter of medium. By the end of the first month of therapy, resistance usually first becomes apparent and after 3 or 4 months, it is not unusual to encounter bacilli which will tolerate from 50 to over 1,000 micrograms. The clinical importance of this phenomenon has already been noted. The resultant need for the utmost cooperation between physician and bacteriologist cannot be ignored. Testing of tubercle bacilli for streptomycin sensitivity requires frequent recourse to techniques which only a well-staffed laboratory can furnish. The Tuberculosis Section, fully aware of this need, has incorporated in its training courses detailed instruction in the use of currently approved laboratory procedures.