# Neomycin, a New Antibiotic Active against Streptomycin-Resistant Bacteria, including Tuberculosis Organisms[1]

Selman A. Waksman and Hubert A. Lechevalier[2]

*New Jersey Agricultural Experiment Station*
*Rutgers University*

THE ISOLATION OF STREPTOMYCIN from two strains of an actinomyces identified as *Streptomyces griseus,* was reported (*8*) from this laboratory five years ago. This was later followed by announcements that this antibiotic is highly effective against the tuberculosis organism (*9*), that it is active *in vivo* against a variety of pathogenic bacteria, and that it is not very toxic to animals (*7*). The ability of streptomycin to control experimental tuberculosis was soon established (*2*). Within less than a year after the isolation of streptomycin had been reported, its effectiveness upon certain forms of human tuberculosis was demonstrated (*3*). Thus, for the first time in the history of human tuberculosis, a drug was found which could be used in the treatment of tuberculosis by means of chemotherapeutic agents and which pointed to the possibility of finally eradicating the "white plague" of man.

Streptomycin has, however, certain important limitations, chief among which are (a) its neurotoxic potentialities, and (b) the development of resistance among the infectious organisms upon its prolonged administration. Recent experiments (*4*) seem to indicate that the toxic reactions can apparently be overcome, at least partly, by the use of dihydrostreptomycin, a reduced form of streptomycin. The problem of development of resistance remains, therefore, as the major limitation of this antibiotic, especially in the treatment of tuberculosis.

The possibility of isolating or synthesizing new compounds that would be effective against the streptomycin-resistant strains of bacteria suggested itself. Various reports indicated that certain synthetic compounds such as the sulfones and para-aminosalicylic acid, and various antibiotics such as different forms of streptothricin (*5*) and aureomycin (*1*) might prove to possess such properties. Although the results obtained thus far in some of the preliminary investigations with these substances are highly encouraging, they still appear to be inconclusive. We do not yet know whether or not any of these would serve to replace streptomycin, completely or partly, or to supplement it on more than a very limited scale. They may not be sufficiently active against the bacteria, or they may be toxic to animals and man, or they may lack stability, or have some other limitations.

In connection with the studies on the production of antibiotic substances by microorganisms, carried out in our own laboratories since 1939, many thousands of cultures, mostly actinomycetes belonging to the genus *Streptomyces,* were isolated from soils, composts, peats, and other natural substrates and tested for their activity against different bacteria; emphasis has been laid, in recent years, upon the mycobacteria. Surprisingly enough, a large number of the organisms thus isolated were found to possess considerable activity against *Mycobacterium tuberculosis.* Unfortunately, only a very few of the antibiotics produced by these organisms proved to be suitable chemotherapeutic agents, because of various limitations, some of which were just mentioned. The fact that numerous strains and species of *Streptomyces* possess the capacity to inhibit the growth of mycobacteria, however, and even to produce active antituberculosis compounds, and the fact that one of these, streptomycin, already had found practical application as a chemotherapeutic agent, offered the hope that sooner or later other substances would be found that might be even better. In search for new compounds, particular attention was paid to those that would be effective against streptomycin-resistant bacteria, notably against the streptomycin-resistant strains of *M. tuberculosis.* The discovery of such an agent, designated as *neomycin,* is reported here.

The organism producing neomycin was isolated from the soil. It is somewhat related to a culture isolated from the soil in 1915 by Waksman and Curtis (*10*) and designated as *Actinomyces fradii* (now listed in Bergey's Manual as *Streptomyces fradiae*).

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When the newly isolated culture was grown in various media containing a source of nitrogen (peptone, meat extract), a carbohydrate (glucose), and salt (NaCl), it was found to produce neomycin under both stationary and submerged conditions of culture. The antibiotic can easily be removed from the culture medium and concentrated by the methods of adsorption and elution which have been developed for the isolation of streptothricin and streptomycin from their respective culture media.

TABLE 1

ANTIBACTERIAL ACTIVITY OF CULTURE FILTRATE CONTAINING NEOMYCIN

| Test organism | Units of activity in 1 ml of broth | |
|---|---|---|
| | Incubation* 3 days | Incubation* 5 days |
| *Escherichia coli* | 60 | 100 |
| *Bacillus subtilis* | 350 | 1,000 |
| *B. mycoides* | 330 | 400 |
| *Staphylococcus aureus* | 330 | 330 |
| *Mycobacterium* 607 | 330 | 1,000 |
| *M. ranae* | 330 | 1,000 |
| *M. avium* | 200 | 600 |
| *M. phlei* | 330 | 1,000 |

* Shaken cultures incubated at 28° C.

Neomycin is a basic compound, most active at an alkaline reaction. It is soluble in water and insoluble in organic solvents. It is thermostable. It is active against numerous Gram-positive and Gram-negative bacteria, especially mycobacteria, but not against fungi. This is brought out in Tables 1 and 2. The antibiotic spectrum of the crude neomycin is quite distinct from that of streptomycin, on the one hand, and of streptothricin, on the other.

TABLE 3

EFFECT OF NEOMYCIN UPON THE GROWTH OF DIFFERENT STRAINS OF *M. tuberculosis* IN DUBOS MEDIUM*

| Test organism | Growth inhibition units/ml |
|---|---|
| *M. avium*† | 4.0 |
| *M. avium* R†‡ | 4.0 |
| *M. tuberculosis* H37Rv† | 0.1 |
| *M. tuberculosis* H37RvR† | 0.2 |
| *M. tuberculosis* H37Rv | 0.5–1.0 |
| *M. tuberculosis* H37RvR | 1.0 |
| *Mycrobacterium* 607 | 0.1 |
| *Mycrobacterium* 607R | 0.25 |

* Incubation at 37°/C for 14 days.
† This particular test was made in another laboratory.
‡ R = streptomycin-resistant strain.

Neomycin preparations were found to possess several desirable properties, which fully justified a more detailed study of this antibiotic: (1) similar activity against both streptomycin-sensitive and streptomycin-resistant bacteria; (2) considerable activity (in some cases greater activity than streptomycin) against various forms of *M. tuberculosis* and other mycobacteria (see Table 3); (3) limited toxicity to animals or none; (4) activity against various bacteria *in vivo,* including Gram-positive and Gram-negative organisms and against both streptomycin-sensitive and streptomycin-resistant organisms (see Table 4); (5) lack of

TABLE 2

ANTIBIOTIC SPECTRUM OF CRUDE NEOMYCIN, AS COMPARED TO THAT OF CRUDE STREPTOMYCIN AND STREPTOTHRICIN

| Test organism | Dilution units per gm required to inhibit growth of test organisms | | |
|---|---|---|---|
| | Neomycin | Streptomycin* | Streptothricin* |
| *B. subtilis* | 150,000–750,000 | 125,000 | 125,000 |
| *B. mycoides* | 20,000–150,000 | 20,000 | 1,000 |
| *B. cereus* | 20,000– 60,000 | 30,000 | 1,000 |
| *S. aureus* | 100,000–250,000 | 15,000 | 50,000 |
| *S. lutea* | 10,000 | 100,000 | 37,500 |
| *E. coli* SS† | 25,000 | 25,000 | 25,000 |
| *E. coli* RS‡ | 20,000 | 0 | Active§ |
| *Ps. aeruginosa* | 2,500 | 1,000 | 1,000 |
| *Pr. vulgaris* | 25,000 | 10,000 | 12,500 |
| Bodenheimer's organism | 15,000 | 0 | Active§ |
| *Serratia marcescens* | 20,000 | 25,000 | 1,200 |
| *Mycobacterium* 607 | 80,000–250,000 | Active§ | Active§ |
| *Mycobacterium* 607RS | 50,000–150,000 | Inactive | Active§ |
| *Trichophyton mentagrophytes* | < 300 | < 300 | Active§ |
| *Candida albicans* | < 300 | < 300 | Active§ |

* From Shatz, Bugie and Waksman (8).
† SS = streptomycin-sensitive.
‡ RS = streptomycin-resistant.
§ Active = activity established in other reports.

resistance against neomycin among the organisms sensitive to it, or only limited development of such resistance.

TABLE 4

COMPARATIVE ACTIVITIES OF NEOMYCIN AND STREPTOMYCIN *in Vivo* UPON STREPTOMYCIN-SENSITIVE AND -RESISTANT BACTERIA

| No. of animals or embryos | Treatment | Dilution of bacterial culture | Surviving mice or egg embryos in days | | | Percent survival |
|---|---|---|---|---|---|---|
| | | | 2 | 5 | 10 | |
| | *Staphylococcus aureus*—streptomycin-sensitive* | | | | | |
| 5 | Control | $10^{-3}$ | 0 | 0 | 0 | 0 |
| 5 | Control | $10^{-5}$ | 1 | 1 | 1 | 20 |
| 5 | Streptomycin, 25 μg | $10^{-3}$ | 5 | 5 | 5 | 100 |
| 5 | Streptomycin, 100 μg | $10^{-3}$ | 5 | 5 | 5 | 100 |
| 3 | Neomycin, 50 units | $10^{-3}$ | 3 | 3 | 3 | 100 |
| 3 | Neomycin, 200 units | $10^{-3}$ | 3 | 3 | 3 | 100 |
| | *Salmonella schottmulleri*—streptomycin-resistant* | | | | | |
| 5 | Control | $10^{-3}$ | 0 | 0 | 0 | 0 |
| 5 | Control | $10^{-4}$ | 4 | 2 | 0 | 0 |
| 5 | Streptomycin, 100 μg | $10^{-3}$ | 2 | 0 | 0 | 0 |
| 5 | Streptomycin, 5000 μg | $10^{-3}$ | 1 | 1 | 1 | 20 |
| 5 | Neomycin, 100 units | $10^{-3}$ | 5 | 5 | 5 | 100 |
| 5 | Neomycin, 200 units | $10^{-3}$ | 5 | 5 | 5 | 100 |
| | *Salmonella pullorum*—streptomycin-sensitive | | | | | |
| 10† | Control | $10^{-2}$ | 4 | 0 | 0 | 0 |
| 10 | Control | $10^{-4}$ | 6 | 0 | 0 | 0 |
| 10 | Neomycin, 100 units | $10^{-2}$ | 5 | 5 | 5 | 50 |
| 10 | Neomycin, 100 units | $10^{-4}$ | 9 | 9 | 9 | 90 |

* Subcutaneous, single dose; mice used.
† Inoculation into allantoic cavity, single dose.

When a 20-hour-old agar culture of *E. coli* was suspended in water and plated out in nutrient agar containing 5 μ or 25 μ of neomycin per ml, no colonies of *E. coli* developed out of 22 billion cells, after 9 days' incubation at 28° C. Similar concentrations of streptomycin would usually allow the development of a dozen or more bacterial colonies per plate. When pieces of agar were removed from the plates containing neomycin and inoculated into sterile agar plates, only the 5 μ/ml plate gave growth from some of the pieces, but the 10 μ/ml and the 25 μ/ml plates gave no growth at all, thus pointing to the high bactericidal properties of neomycin. When plates containing different concentrations of this antibiotic were streaked with streptomycin-sensitive, -resistant, and -dependent strains of *E. coli* (*9*), the first two were sensitive alike to neomycin, and the last did not make any growth at all. This established further the difference in the biological and chemical nature of neomycin from streptomycin.

Broth or agar cultures containing sufficient neomycin to inhibit the growth of various bacteria were incubated for considerable periods of time. No further bacterial development occurred beyond a certain initial inhibiting concentration, thus pointing on the one hand to the stability of neomycin, as contrasted to that of aureomycin, and on the other hand to the lack of resistance developed among the sensitive bacteria, as contrasted to streptomycin and especially to grisein.

Since neomycin has not yet been obtained in crystalline form, very little can be said of its chemical nature. Preliminary results, however, point to its being distinctly different chemically from streptothricin and from streptomycin. More detailed studies will be published later.

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