NEOMYCIN

Its Nature and Practical Application

SELMAN A. WAKSMAN, *Editor*

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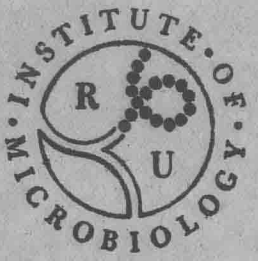

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PREFACE

The isolation of streptomycin in 1943 and its rapid and extensive adoption for the treatment of various infectious diseases marked a definite milestone in the history of medical science and practice. Although penicillin was already well established at that time and was rapidly becoming an indispensable chemotherapeutic agent, it possessed certain limitations. Chief among these were its ineffectiveness against most gram-negative bacteria and the tuberculosis organism, and the gradual development of resistance among some of the organisms originally sensitive to it. The introduction of streptomycin tended to fill some of these gaps.

But streptomycin also had serious limitations. It favored rapid development of resistance among various sensitive bacteria; it tended to cause certain serious reactions, notably vestibular disturbance and injury to the auditory system. This suggested the desirability of continuing the search for other antibiotics with properties similar to those of streptomycin but without its disadvantages.

The screening program initiated in our laboratory in 1939 for the isolation of antibiotics from cultures of actinomycetes, which yielded actinomycin in 1940, streptothricin in 1942, and streptomycin in 1943, was again resorted to. As a result, several new antibiotics were soon isolated, some of which were active against bacteria, such as grisein, and others against fungi. None of these, however, seemed to be of sufficient interest to deserve clinical consideration. Finally, in 1949, the isolation of neomycin was announced from our laboratory (657).

This antibiotic at first appeared to be an important new chemotherapeutic agent that had all the desirable properties of streptomycin, without its disadvantages. Certain data were presented in 1950 on the effectiveness of neomycin in experimental tuberculosis. The hope was expressed that the clinical results soon to be forthcoming would establish neomycin as an ideal chemotherapeutic agent in the treatment of tuberculosis. The new antibiotic was similar in many respects to streptomycin: it was a basic compound; it was water-soluble and heat-stable; it was effective against a large number of gram-positive, gram-negative, and acid-fast bacteria. Resistance seemed to develop more slowly among sensitive bacteria, and its antibiotic spectrum appeared to be wider.

Unfortunately, the clinical evidence, especially in the treatment of tuberculosis, was disappointing. When administered parenterally, neomycin had an even more injurious effect on the auditory system than did streptomycin; virtually every patient who was saved from tuberculosis became deaf.

Gradually, however, it became established that, despite this serious limitation, neomycin would find an important place in human and animal therapy. Since it was not readily absorbed from the intestine, it proved

to be an ideal intestinal antiseptic when administered orally. It was highly effective against skin and other surface infections caused by various bacteria. The very fact that it was at first considered to occupy an unimportant place among such major antibiotics as penicillin and streptomycin suggested its use in the treatment of certain infections where avoidance of sensitization of the patient to one of the other chemotherapeutic agents was essential. Its highly desirable properties were soon recognized; these established neomycin among the important antibiotics at the disposal of the physician in the treatment of numerous infectious diseases.

In 1953, a preliminary monograph (650) was published summarizing information concerning the formation, isolation, properties, and practical utilization of neomycin. Since then, neomycin has found ever wider use throughout the world. It has proved to be an ideal agent in combination with other antibiotics and hormones in the treatment of many infections. New organisms capable of producing neomycin or neomycin-like substances were soon isolated, and new methods of production and isolation were developed.

This accumulated information necessitated the preparation of a new and more comprehensive volume on neomycin. This is not to be considered as a second edition of the earlier work, although some of the information presented previously has been included. Whereas the earlier monograph summarized primarily the work done in the Department of Microbiology of Rutgers University, with but few additional outside contributions, the present volume is a result of the cooperative effort of a large number of investigators and clinicians from many different laboratories. An attempt has been made to present in this volume a comprehensive summary of the nature and utilization of neomycin, accompanied by as complete a bibliography of the literature pertaining to neomycin as could be assembled. The literature reference numbers in the following chapters refer to this bibliography at the back of the book. Certain supplementary references are included following the individual chapters. These are references that do not pertain directly to neomycin, except for a few that were verified too late to be assembled into the main bibliography. Most of these references are available in the library of the Institute of Microbiology, from which they can be borrowed, or from which photostatic or microfilm copies can be purchased.

The editors wish to express their sincere appreciation to all those who contributed the various chapters of this volume, and to the medical directors of several industrial companies manufacturing neomycin, particularly Dr. J. A. Dugger, for their valuable suggestions. They also wish to thank Herminie B. Kitchen for her careful reading of the entire manuscript and for many helpful suggestions.

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SECTION IV

MISCELLANEOUS USES

Chapter 1

HISTORICAL BACKGROUND

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Neomycin is a typical antibiotic, that is, it is a chemical substance that is produced by a microorganism, and in dilute solution exerts a growth-inhibiting effect upon other microorganisms. A basic compound, soluble in water and heat-stable, it is produced by various strains of *Streptomyces fradiae* and is active upon numerous gram-positive, gram-negative, and acid-fast bacteria. Its activity ranges from 0.01 to more than 10 μg/ml. It possesses a rather limited toxicity to animals, especially under certain prescribed conditions. It does not favor rapid development of resistance among sensitive organisms and shows little, if any, cross-resistance with other antibiotics used as chemotherapeutic agents.

The isolation of neomycin resulted from a comprehensive screening of the actinomycetes, which have yielded many important chemotherapeutic substances during the last 15 years. Although it had been demonstrated prior to 1940 that actinomycetes possess remarkable growth-inhibiting effects upon various bacteria and fungi, no pure antibiotic substance had been isolated from these microorganisms, and the true significance of this activity was but little understood. In 1940, the first true antibiotic (actinomycin) was isolated from a culture of an actinomycete (S1), thus establishing the importance of actinomycetes as producers of antibiotics.

Since 1940, more than 300 antibiotic substances have been isolated from cultures of actinomycetes. Many of them have been crystallized and their chemical natures determined. Nearly 20 of them have already found application in the chemotherapy of numerous infections of man and animals. Most of these are active only upon bacteria; some are active largely upon fungi; others are active on both bacteria and fungi; some are active also upon rickettsiae and some of the larger viruses. A few of these antibiotics also exert an effect on malignant cells; unfortunately, none can as yet be considered a true anticancer agent that can safely be used in the treatment of clinical cases, although some are showing distinct promise.

As a result of the various screening programs carried out in numerous laboratories throughout the world, it has been established that nearly 50 per cent of all actinomycetes isolated from natural substrates, especially from various soils, are able to exert a growth-inhibiting effect upon various microorganisms. The composition of the medium in which the tests are made is of great importance in this connection. Among different strains of the same organism there are great variations, both qualitative and quantitative, in antibiotic-producing capacity. Under specific conditions of culture, some organisms are able to produce only one antibiotic; others give rise to two or more antibiotics; frequently one is readily excreted into the medium and the other retained in the mycelium. Occasionally, the difference among the antibiotics produced by a single organism, or by different strains of the same organism, or even by different species, is only a matter of one or two atoms, as in the case of the tetracyclines, the streptomycins, and the neomycins.

This history of antibiotic production abounds in illustrations of the formation of more than one antibiotic by the same organism. Thus, *S. griseus* is capable of producing not only streptomycin, but also cyclohexamide; *S. rimosus* is capable of forming oxytetracycline and rimocidin. Furthermore, different strains of the same organism are capable of forming different antibiotics; this is true, for example, of the streptocin-, grisein-, and candicidin-producing strains of *S. griseus*. On the other hand, the same antibiotic can be formed by different organisms, as in the production of streptomycin by *S. griseus* and *S. bikiniensis*; this is also true of the penicillin-producing fungi, largely of the *Penicillium notatum-chrysogenum* group.

An even more complicated aspect of antibiotic production is the fact that some antibiotics do not represent a single chemical entity, but occur either as mixtures of closely related compounds, or in different forms, when produced by different organisms. Streptomycin and mannosidostreptomycin, for example, are formed in the same cultures of *S. griseus*, whereas hydroxystreptomycin is formed by another organism (*S. griseocarneus*). The various forms of actinomycin are produced either by different species of *Streptomyces* or by the same organism at different stages of growth.

These observations apply equally to the neomycin-producing cultures of *S. fradiae*. Not only are different antibiotics (neomycin and fradicin) produced by a single strain of this organism, but neomycin itself is formed as two chemical entities, which have been designated as B and C. The various neomycin-producing cultures also show considerable variations in their morphological, cultural, physiological, and biochemical properties, as well as in their ability to form the various closely related antibiotics. Some of these cultures are typical strains of *S. fradiae*. Others vary in certain

minor characteristics. Still others are sufficiently different to be considered as distinct species by one not entirely familiar with the great variability among cultures of actinomycetes.

The relative concentration of these two forms of neomycin cannot be easily controlled by the selection of neomycin-producing strains and modifications of medium.

Other cultures of *S. fradiae*, and perhaps other organisms, produce other forms of neomycin, which show minor differences in chemical composition but apparently have similar biological properties. Some of these forms have been designated by a variety of names, such as streptothricin B_1 and B_2, framycetin (Soframycin), and flavomycin.

In view of the extensive antibiotic screening programs, it is no wonder that the same antibiotic frequently has been isolated simultaneously by different investigators. An antibacterial agent (novobiocin) was isolated in four different laboratories and described under different names (cathomycin, albamycin, cardelmycin, and volcanomycin).

As new and promising antibiotic-producing organisms are isolated and as new antibiotics are recognized, a number of different problems face the investigator. These may be briefly outlined as follows:

1. Development of suitable media for growth of the particular culture and for production of the antibiotic.
2. Development and isolation of strains giving higher yields than the original culture.
3. Simplification of the procedures for the isolation and purification of the active chemical substance.
4. Determination of the pharmacological properties of the new antibiotic, especially its toxicity to experimental animals.
5. Evaluation of the selective activity of the new antibiotic against different disease-producing organisms, and comparison of its effectiveness with that of known antibiotics.
6. Clinical evaluation of the new antibiotic.

All these require the closest possible collaboration of the microbiologist, the geneticist, the chemist, the pharmacologist, and the clinician, as well as the engineer who must develop suitable methods for large-scale production of the antibiotic.

Any discussion of the historical background leading to the isolation of a particular antibiotic must take cognizance of one or all of the following factors:

Biological origin of the antibiotics. This involves the problems of biosynthesis. Each of the three major groups of antibiotic-producing organisms has yielded large numbers of antibiotics, differing in chemical properties and potential therapeutic applications.

Among the bacteria, the gram-negative organisms form pyocyanase and other lipoid substances (pyo-compounds), colicines, and a variety of other substances, none of which has so far found any practical application; the gram-positive bacteria, largely the aerobic spore-formers, have yielded a variety of polypeptides, including tyrothricin, bacitracin, polymyxin, and subtilin.

The filamentous fungi have yielded the penicillins, fumagillin, and a few other compounds of considerably lesser importance; the higher or mushroom fungi have yielded a number of compounds, such as clitocybin and polyporin, none of which has found any practical application; the yeasts have so far yielded no satisfactory antibiotic.

The actinomycetes produce by far the largest number of effective chemotherapeutic agents. The genus *Streptomyces* has yielded most of these compounds, including all the important therapeutic substances that are produced by the actinomycetes. These include the streptomycins, the tetracyclines, chloramphenicol, the neomycins, erythromycin, novobiocin, and many others.

Activity of the antibiotics. Since in this volume we are dealing with an antibiotic produced by actinomycetes, we will limit this discussion to the products of this group of organisms:

1. Substances active upon bacteria alone—streptomycin, grisein, neomycin, viomycin, novobiocin, and a variety of others.

2. Substances active upon fungi alone—cyclohexamide, fradicin, nystatin, candicidin, ascosin, candidin, and certain others.

3. Substances active upon both bacteria and fungi—thiolutin, streptothricin, and others. Although it has been said repeatedly that streptomycin is active only on bacteria, it has recently been shown to be active also on certain fungi belonging to the Phycomycetes, some of which cause serious plant diseases.

4. Substances active upon bacteria, rickettsiae, and some of the larger viruses, and which have been dubbed "broad-spectrum" antibiotics—chloramphenicol, chlortetracycline, oxytetracycline, and tetracycline.

5. Substances active not only upon bacteria or fungi, but also upon malignant cells—the actinomycins, azaserine, sarkomycin, carzinophilin, and several others.

6. Substances active upon the small viruses. Although various preparations reputedly have such properties, their exact nature and activity are still unknown. None has found practical application.

Physical and chemical properties of the antibiotics. Antibiotics represent a great variety of compounds, differing greatly in their physical and chemical properties, composition, and biological activities. The only general

statement which can be applied to the chemistry of antibiotics is that they are organic substances.

Chemically, antibiotics can be classified by different methods, depending on the criteria that are chosen. The elementary composition is one such criterion. As such we can recognize:

1. Antibiotics containing carbon, hydrogen, and oxygen, such as clavacin, penicillic acid, and citrinin, among the products of fungi; resistomycin and fungichromin, produced by actinomycetes.

2. Antibiotics containing carbon, hydrogen, oxygen, and nitrogen, including actinomycin, streptomycin, and cycloserine.

3. Antibiotics containing sulfur, such as thiolutin, cinnamycin, and thiomycin, and, of course, penicillin and gliotoxin.

4. Antibiotics containing chlorine, such as chloramphenicol and chlortetracycline.

5. Antibiotics containing metals, such as grisein, which contains iron.

Antibiotics can also be classified on the basis of their acidity or basicity. As such we can recognize basic substances, such as streptothricin and streptomycin; neutral substances, such as chloramphenicol; amphoteric substances, such as tetracycline; and acidic substances, such as chartreusin.

Antibiotics can also be classified on the basis of specific chemical groupings which are present in their molecule. For example, candicidin and nystatin contain conjugated double bonds and are polyenic substances. Mycomycin contains both acetylenic and enic groupings. The actinomycins contain polypeptidic chains. The naphthacene nucleus is the basic group of the tetracyclines. The final chemical classification of antibiotics must await the elucidation of the structure of most of these compounds.

The great variation in physical properties of the antibiotics reflects their lack of chemical homogeneity. Some are very soluble in water, such as streptomycin; others can be put into solution only in certain organic solvents, such as pure candicidin.

Chemotherapeutic use of the antibiotics. This depends on the antimicrobial spectrum of the antibiotic, its toxicity to animals, and prior availability of other agents. It is necessary to emphasize here also the problem of the development of resistance to the various antibiotics and of cross-resistance among them, phenomena that may become of considerable practical importance in the case of agents used in therapy.

This, then, is the background that led to the isolation of neomycin, and these are some of the problems that had to be faced in the early stages of its evaluation as a potential chemotherapeutic agent. In the 17 or 18 years since the science of antibiotics originated, these substances have revolutionized medical and veterinary practice. They have come

to occupy a prominent place in animal feeding, and promise to do for plant diseases what they have done for animal diseases. As one looks back over the developments during this brief period, one wonders what the future holds in store for us.

SUPPLEMENTARY REFERENCE

S1. WAKSMAN, S. A., AND WOODRUFF, H. B. Actinomyces antibioticus, a new soil organism antagonistic to pathogenic and nonpathogenic bacteria. J. Bacteriol., **42**: 231–249, 1941.

SECTION I

Microbiological and Chemical Aspects