ANTIBIOTICS

Preface

The purpose of this book is to present in a succinct, integrated plan the facts and principles of fundamental and permanent value relating to antibiotics. Much of this information is now widely scattered in thousands of publications embracing the vast scientific literature of the subject. Some of it is derived from the authors' own researches in this field published over a period of years.

The object has been, first, to give an understanding of the fact that, in developing chemical therapy by means of antibiotics, man has merely adapted to his own ends the capacity that certain microorganisms possess to wage a war of extermination against other microorganisms, and, second, to present a general survey of the principles involved in the industrial, commercial, pharmaceutical, and medical aspects of the field of antibiotics.

The advent, about 1942, of the modern era of antibiotic chemotherapy for the treatment of systemic infections unquestionably marks one of the greatest advances in man's continuing battle against infectious diseases.

In terms of human suffering that has been alleviated, the discovery of practical means of applying microbial antagonisms for curative purposes easily ranks with the great contribution of the nineteenth century, i. e., the introduction of anesthesia in surgery one hundred years earlier.

In terms of volume, the industrial production of antibiotics outranks all other medicinals. It has been estimated that in 1948 penicillin and streptomycin alone accounted for more than one half of manufacturers' income from the sale of synthetic drugs. This figure is especially impressive when it is realized that penicillin first became available commercially in 1943 and that streptomycin was not available com-

mercially until two or three years later. Within the last year three additional antibiotics, bacitracin, aureomycin, and chloromycetin have become available. More will undoubtedly follow.

Penicillin proved to be of immense value in World War II, not only in humanitarian terms, but also in military terms, because its use returned to active service many men who otherwise would have succumbed to infection or would have been permanently disabled. In the same way, on a larger scale, many other antibiotics are proving of immense economic as well as humanitarian value in the postwar era, by reducing periods of hospitalization or of quarantine, and by rehabilitating many chronic cases. Thus, proper use of antibiotics lightens the financial burden of public health and welfare agencies.

For this reason, countries not self-sufficient with respect to production of antibiotics, find it economically advantageous to spend their limited supplies of dollars for importation of these agents in preference to a number of other "essential" commodities. Drug exports from the United States for 1948 totaled 191 million dollars: approximately 40 per cent of this sum was spent for antibiotics (mostly penicillin and streptomycin).

Antibiotics have been used largely for treatment of human patients, but with increasing production, the cost of penicillin has dropped so markedly that its wide application in veterinary medicine has become practicable, resulting in large savings for cattle, dairy, poultry, and other livestock industries. With time, similar developments may be expected for other antibiotics.

There is a genuine need for a treatment of the broad principles of antibiotics and antibiotic chemotherapy sufficiently comprehensive to satisfy those whose business is concerned with the health sciences but not so technical as to discourage the interested individual whose major activities lie in other fields.

The authors modestly hope that the present volume will help to satisfy this need by filling the gap between the necessarily brief popular accounts on the one hand and the technical accounts intended for specialists on the other hand.

Since the aim of this book has been to achieve an integration of pertinent facts and principles in a coherent picture, no attempt has been made to cite exhaustively all of the extensive literature pertaining to the several aspects of the subject, but all important critical review papers are listed, as are the principal scientific articles of historical interest.

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It is with deep appreciation that we acknowledge the services of Louise Noack Gray, who designed the cover of this book, and the ceaseless secretarial and proofreading labors of my wife, Jean, and my mother. R. P.

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Among the lower beings, even more than among the higher animals and plant species, life destroys life. —L. PASTEUR AND J. JOUBERT (1877)

CHAPTER

1

The Concept of Antibiosis

The component members of any society, whether it be an aggregation of human beings or of other living organisms, are continuously influenced by their environment. Furthermore, the neighboring beings or organisms or cells constitute an important part of that environment. Several organisms or cells, whether of the same or of different kinds, cannot long exist in a limited space without affecting and (or) being affected by their neighbors. This fact is fundamental in all studies of growth and development, whether one is concerned with an individual organism or with the growth, development, and decline of mixed populations of plants or of animals. The same principle applies even in the waxing and waning of human civilizations.* This book treats of various ways in which some of these relationships among micro-organisms can be applied for practical clinical control or cure of pathogenic disorders of man and his domestic animals and plants and, so far as is possible with present knowledge, of the mechanisms by which the end result is accomplished. This is the subject matter of the comparatively new field of antibiotics.

The term "antibiotic," according to its derivation, has a very broad meaning and might be used to indicate any agent or condition detrimental to life. The word "antibiosis" appears to have been coined in 1889 by Vuillemin who wrote, "The lion that springs on its prey and

* For a more comprehensive treatment of this idea, developing some of the points of similarity between microbial societies and societies of higher animals, including man himself, see Pratt (1949).

the serpent that poisons the wound before devouring its victim are not considered to be parasites. There is nothing unequivocal about it—one creature destroys the life of another in order to sustain its own, the first being entirely active, and the second entirely passive; one is in unrestricted opposition to the other. The relation is so simple that it has never been named, but instead of being examined in isolation it can be viewed as a factor in more complex phenomena. For simplicity we shall refer to it as *antibiosis;* the active participant will be the antibiote." As currently used in pharmaceutical and medical practice, however, the term antibiotic has a very definite and limited connotation. It is used to designate a metabolic product of one micro-organism that is detrimental or inimical to the life activities of other micro-organisms, usually even when present in extremely low concentrations. According to this commonly employed definition, antibacterial substances derived from higher plants or from animals (other than protozoa) are excluded from the subject matter of antibiotics.*

In some cases the antibiotic principle has been extracted from the producing micro-organism or from the liquid in which it has grown and has been purified and identified chemically, but in many cases only crude or more-or-less purified extracts have been obtained. The best-known antibiotics are penicillin and streptomycin. Structural formulas have been proved for both compounds.

Antibiotics have been known, if not in the pure form, at least by their effects for centuries; but have only recently assumed the dominating position in clinical medicine and in the pharmaceutical industry that they now occupy. Although the Chinese were aware at least 2,500 years ago of the ameliorating properties of molded curd of soy bean when applied to carbuncles, boils and like infections and used this treatment as standard procedure in such conditions, the potential significance and value of micro-organisms as curative agents or as sources of

* The major portion of this book is concerned with the antimicrobial properties of substances produced by micro-organisms. However, we shall devote a small section (part of Chap. 10) to a discussion of similarly acting agents derived from higher plants and animals.

useful drugs for the treatment of systemic infections seems to have been neglected until the latter part of the nineteenth century. This is not surprising, however, when one realizes that the subject of bacteriology, as a scientific study, was born only about the middle of the nineteenth century as the result of the work of Pasteur and his students. As a matter of fact, Pasteur and Joubert (1877) appear to have been the first to recognize the clinical potentialities of micro-organisms as therapeutic agents as well as causal agents in disease. They observed that anthrax bacilli *(Bacillus anthracis)* grow rapidly when introduced into sterile urine at an appropriate pH, but that they fail to grow and soon die if one of the "common bacteria" of the air is inoculated into the urine at the same time. They wrote "It is remarkable that the same phenomenon is seen in the body, . . . leading to the astonishing results that anthrax can be introduced in profusion into an animal, which yet does not develop the disease; it is only necessary to add some 'common bacteria' at the same time to the liquid containing the anthrax bacteria. *These facts perhaps justify the highest hopes for therapeutics.*" (The italics are ours.)

A few years later Tyndall (1881) in his "Essays on the Floating Matter of the Air" described the clearing of solutions made turbid by the growth of bacteria when species of *Penicillium* subsequently grew on the surface of the liquid, and in 1885 Cornil and Babes conceived the role of definite chemical inhibitors in these phenomena of antagonism in the microbial world. These chemical entities that are produced by one micro-organism and that, depending upon their concentration in the medium, inhibit, kill or lyse other micro-organisms, are now called antibiotics.

The therapeutic use of antibiotic agents represents merely a practical, controlled and directed application of phenomena that occur naturally and continuously in soil, sewage, water and other natural habitats of micro-organisms. During the late years of the nineteenth century and the first decade of the twentieth century, several antibiotics were tested clinically. The first crude attempts made in the 1880's involved "replacement therapy," i.e., inoculation of a

patient infected with a pathogenic organism with a nonpathogenic bacterium that previously had been demonstrated to be antagonistic to the pathogen in vitro. This technic was used with some succcess in the treatment of tuberculosis, diphtheria, anthrax, cholera, plague and other diseases. The next refinement, introduced in the 1890's, was administration of crude, or more-or-less purified, extracts of one organism to check the growth of, or even to eradicate, invading pathogens. Extracts of fungi as well as of bacteria were used successfully in this way. About 1900 pyocyanase, an extract from the blue-pus organism, *Pseudomonas pyocyanea (Ps. aeruginosa)*, was prepared commercially in Germany and was used extensively. The historical phases of the early experiments in this phase of therapy have been discussed in a competent and interesting way by Florey (1945) and by Weintraub (1943).

The trend in the pharmaceutical and medical professions has ever been away from the preparation and administration of crude extracts and toward the goal of administering definite amounts of identified compounds. The phase of chemotherapy concerned with antibiotics is no exception. The most recent research and development in this field, resulting in the preparation of crystalline antibiotics on an industrial scale and intelligent therapeutic use of these agents according to a definite dosage schedule, has involved the co-operation and effort of many biologists, chemists, physicists, and engineers in this country and abroad, but was stimulated by, and may be said to have stemmed largely from, Fleming's observation in 1929 of the action of *Penicillium sp.* on *Staphylococcus aureus* and from the classical studies of the British school made during the following years on the metabolic products of molds, especially of the Penicillia.

In the early years of World War II the sulfonamide drugs were the only really effective chemotherapeutic agents available for treatment of bacterial infections, and these drugs had several undesirable characteristics. In many patients they produce serious untoward reactions. A number of strains of bacteria are naturally resistant to their action, and in other cases highly resistant strains of bacteria

often develop from susceptible species that have been exposed to the sulfonamide drugs, so that a considerable percentage of bacterial infections is not amenable to sulfonamide therapy. Furthermore, even in the case of susceptible pathogens, the usefulness of the sulfonamides is limited by the fact that their antibacterial activity is lessened when large numbers of bacteria are present or even when dead bacteria, pus, blood serum, or other products likely to be found in infected wounds are present. Therefore, there was an intense desire to find new antibacterial agents that might not have these undesirable properties. The available evidence suggested that penicillin might be at least a partial answer, and accordingly the governments of the United States and of Great Britain agreed to pool their research resources of man power, facilities, etc., to sponsor an intensive coordinated program of co-operative research on this antibiotic. The decision proved to be a wise one and in a period of a few years penicillin developed from a laboratory curiosity to a major item in medicine and in the pharmaceutical industry, and helped to save the lives of untold thousands of battle casualties and civilians.

Shortly before initiation of the intensive research program on penicillin the work of Dubos (1939) on antibacterial products found in filtrates from cultures of *Bacillus brevis* had reawakened interest in antibiotics as potential chemotherapeutic agents. Dubos isolated tyrothricin which was shown to be extremely active against a number of pathogens previously not amenable to chemotherapy, but unfortunately this natural product was too toxic for parenteral administration, a fact that seriously limited its usefulness. This served to stimulate a search for other less toxic antibiotics.

Subsequently, Waksman and his students (1944) discovered a new source of antibiotics in the actinomycetes, a mold-like class of organisms considered to be intermediate between bacteria and fungi in evolutionary development. The importance of Waksman's observations and his diligence in initiating the development of streptomycin on a scientific plane and on a commercial scale cannot be overemphasized, since research on this antibiotic and its commercial

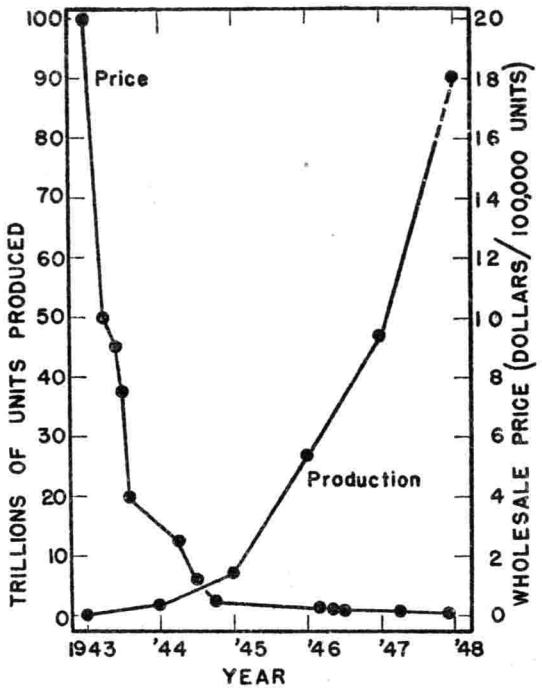

FIG. 1. Production of penicillin in the United States from 1943 through 1948 and the wholesale price of penicillin per 100,000 units during the same period. The production figure for 1948 is estimated from the actual production of the first nine months, hence the dashed line for the last three months. One unit crystalline penicillin = 0.6 microgram (6×10^{-7} gram). The first penicillin, marketed at \$20/100,000 units, was an impure amorphous product with approximately 250 to 350 units of activity per mg. The current product is a highly purified crystalline preparation assaying 1500 to 1600 units or more per mg. and costs about \$0.09 per 100,000 units (January 1949). The theoretical potency of the sodium salt of crystalline benzyl penicillin is 1667 units per mg.

development did not receive the kind of government support that did so much to further production of penicillins in the United States and Great Britain.

The antibiotics already occupy an eminent rank in the pharmaceutical industry and, in terms of volume of business, may soon occupy a dominant position. It has been estimated that penicillin and streptomycin together accounted for more than one half of manufacturers' income realized in 1948 from the sale of synthetic drugs, an especially impressive figure when it is realized that penicillin first became available commercially in 1943 and that the first commercial lots of streptomycin were not offered for sale until two or three years later. The tremendously rapid growth of the antibiotics industry is indicated by the data in Figure 1 which show the annual production of penicillin from 1943 through 1948.

Other antibiotics which are available for distribution through regular retail outlets are aureomycin (Duomycin), bacitracin, chloromycetin (Chloramphenicol), and tyrothricin. Several others are undergoing clinical trial. Polymyxin and subtilin appear to be the most promising of the latter group. More than one hundred antibiotics have been discovered and many of these have been fully or partially characterized chemically. Only a small percentage of the antibiotics that are discovered prove to be clinically useful, however. The reasons for this will become apparent as our story unfolds in the following pages.

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Life is a principle of growth, not of standing still, a continuous becoming, which does not permit of static conditions.

—JAWAHARLAL NEHRU (1946)

CHAPTER

2

Biologic Significance of Fields of Diffusion

MUTUAL RELATIONS AMONG ORGANISMS

It was pointed out in Chapter 1 that several organisms cannot long exist in the same environment without in some way affecting or being affected by their neighbors. Both organisms may benefit mutually from the association, each supplying a need of the other. This is the case with the algae and fungi which together form lichens. Such a relation is called symbiotic (sym = together + bios = life). The condition of living together in this fashion is called symbiosis. In other instances one organism may live at the expense of the other, as, for example, do the various mildew and rust fungi that are pathogenic on plants and the different fungi that parasitize man and other animals. Such a condition is known as parasitism (derived from Greek and meaning literally, to eat beside or at the table of another). The aggressor in such a relationship is called a parasite and the other member is called the host.

In still other cases one of the associates may inhibit or prevent altogether the growth of another without deriving any known particular direct benefit from the arrangement although it may benefit indirectly by the fact that some of its competitors for *lebensraum* and for available nutrients are eliminated from the scene. Such a relationship is known as antagonism. The active aggressor is called an antago-

nist and is comparable to the "active antibiote" in Vuillemin's definition of antibiosis. It is principally with the antagonistic relationships of micro-organisms that this book is concerned.

DISCOVERY OF ANTIBIOTICS FROM SOIL ORGANISMS

In bygone times considerable interest was evidenced in the soil as a potential reservoir of various kinds of pathogenic organisms. It was argued that since animal and human excreta containing various pathogens ultimately often find their way into the soil and since bodies of individuals who die from various diseases are buried in the earth, the soil and possibly neighboring streams and water supplies might become infected. However, following the entrance of bacteriology as a real science in the nineteenth century, soils were searched intensively for organisms known to be pathogenic for man and other animals, and it was soon found that such organisms generally do not long survive in normal soil.

When this was discovered, it was thought, at first, that perhaps the soil serves as a filter or that many pathogenic organisms fail to survive because of their peculiar physical and chemical requirements (temperature, humidity, nutrition, etc.). However, it was soon found that the chances of survival are much greater in sterilized soil than in natural soil and that some organisms which do not survive in natural soil actually grow well in sterilized soil. This gave weight to the idea that micro-organisms in normal unsterilized soil tend to prevent the growth of introduced foreign pathogens. With the exception of the micro-organisms causing anthrax *(Bacillus anthracis)*, tetanus *(Clostridium tetani)*, gas gangrene (various species of *Clostridium*), undulant fever *(Brucella abortus)*, and typhoid fever *(Eberthella typhosa)*, most micro-organisms pathogenic for man fail to survive more than a few days in soil, either because of failure to obtain proper nutrition, or because of inability to compete with other micro-organisms, or as the result of actual antibiosis.

The hypothesis that destruction of pathogens in the soil obtains through the production of antibiotic substances by other micro-