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CHEMOTHERAPEUTIC AGENTS FOR BACTERIAL INFECTIONS

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SERIES EDITOR'S FOREWORD

In the early 1940s, the study of antibiotics became an idea whose time had come. Older microbiologists remember the enormous excitement that their discovery and practical utilization generated. World War II had provided a need and the facilities for their study. Today antibiotics are the therapeutic miracles that are commonplace. We do not treat them with contempt, but we expect to have and to be able to use agents of high cellular specificity. In the 1940s we were surprised that specific chemotherapy was possible. Today we take it for granted. Earlier there were many indications of antibiotic activity, and indeed several had been isolated, but there was no serious attempt to use these agents, and there were no reasonable methods available for their study or evaluation. All of the methods for the detection, the isolation, and the evaluation of antibiotics developed over a very short period. Literally thousands of people worked on some phase of antibiotics during the 1940s and 1950s and many hundreds of papers were published. From these, Lynn and Solotorovsky have presented in *Chemotherapeutic Agents for Bacterial Infections* a group of papers that constitute the foundations of the field of antibiotics and that permit readers to rediscover the major clinically valuable antibiotics or, indeed, to find new ones of clinical importance.

The scientific literature on antibiotics is so vast and so dispersed that much of it becomes inaccessible to readers who are not located close to major libraries or do not have the time to look for papers other than those on their own specialty. It is good to have the fundamental papers of a vast field of knowledge together in one place. Some of the early work is somewhat unsophisticated, but it remains a valuable and sound contribution to our knowledge of how antibiotics were obtained and evaluated.

WAYNE W. UMBREIT

PREFACE

The advent of antibiotics and other chemotherapeutic agents marks the most successful attempt to treat diseases. These agents are the fulfillment of Paul Ehrlich's prediction of "magic bullets" that would specifically kill pathogens while not harming the host. Antibiotics have so dominated medical treatment that it would be difficult to find an adult in the Unitd States who has not received such treatment. Antibiotic treatment has been largely successful, but resistance factors are limiting their effectiveness. A complete understanding of dosage required and spectrum of activity is necessary for effective chemotherapy.

In addition to their uses in medicine and industry to control microbes, antibiotics have allowed researchers to study in detail molecular genetics, protein synthesis, and bacterial cell wall formation. Antibiotics permit the termination of biosynthetic pathways at specific points so as to allow study of metabolic intermediates. Our understanding of nucleic acid replication and ribosome function would have been greatly limited without antibiotics. Such information has permitted the formulation of effective combinations of antibiotics to treat infectious diseases not controlled by single agents.

In this book, we have assembled papers dealing with discovery, mechanism of action, and application of the more medically important antibiotics and synthetic agents. We selected papers often because of their originality, brevity, completeness of information presented, and relative importance in advancing knowledge into a new area.

MELVYN LYNN
MORRIS SOLOTOROVSKY

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INTRODUCTION

The introduction of synthetic chemotherapeutic agents and antibiotics has been one of the major advances in medicine and biology during the twentieth century. These agents have reduced the ravages of infectious disease and have served as indispensable reagents for revealing and clarifying steps in pathways of cellular metabolism and biosynthesis. Prior to the twentieth century, quinine, extracted from cinchona bark, was used for the treatment of malaria, and an extract of ipecac root was used for the treatment of amebic dysentery. Cinchona bark first was used by the natives of Peru and ipecac root by the natives of Brazil. Quinine was extracted and identified by two young French pharmacists, Pelletier and Caventou (1820), working in the back room of a Parisian apothecary. There was no further significant progress in the treatment of infectious diseases with defined chemical agents until Paul Ehrlich, the founder of scientific chemotherapy, began his studies in 1886.

Ehrlich's earliest studies, based on observing the high degree of selective affinity of specific dyes for specific tissues, led to testing methylene blue for activity against malaria. Methylene blue stained the malaria parasite with avidity. Fortunately it also could be administered to humans. In 1891 he reported a therapeutic effect, but the level of activity did not justify replacement of quinine. His study of dyes also led to the demonstration of efficacy of trypan red for experimental trypanosomal infection in mice. Mesnil and Nicolle soon showed that trypan blue and afridol violet were active against *Trypanosoma brucei*, the agent of nagana, a cattle disease that rendered large areas of Africa uninhabitable.

Ehrlich's most important contribution to human chemotherapy emerged from the screening of organic arsenical compounds. The use of arsenicals for the treatment of infectious diseases was introduced by Thomas and Breinl (1905), who reported the successful treatment of trypanosomiasis with atoxyl (p-amino-

phenylarsonic acid). Ehrlich and Hata (1911) reported that arsphenamine cured experimental syphilis in rabbits, and it was found effective in human syphilis. Neoarsphenamine, a soluble form of arsphenamine, was introduced in 1912 (Work and Work 1948, p. 16) and remained the major drug for the treatment of syphilis until the introduction of penicillin in 1941.

Drugs were developed for the treatment of trypanosomiasis (African sleeping sickness and Chaga's disease), leishmaniasis (kala azar and oriental sore), malaria, and amebiasis. Plimmer and Bateman (1908) reported that the antimonial, tartar emetic, was effective against animal trypanosomiasis, and a year later Manson demonstrated its efficacy in human trypanosomiasis. Leishmaniasis also responded to tartar emetic. Improved drugs for the treatment of trypanosomiasis appeared by 1920. These were tryparsamide, a pentavalent arsenical named atoxyl to suggest reduced toxicity, and suramin (Bayer 205), a nonmetallic colorless derivative of trypan red.

The first successful synthetic antimalarial, plasmoquine, was produced in 1924 (Schulemann, Schonhoffer, and Wingler 1932). The testing of derivatives retaining the side chain of plasmoquine but altering the heterocyclic nucleus led in 1933 to Atabrine (Mauss and Mietzach 1933). Atabrine was used effectively as a suppressant during World War II. A dye, it colored the user's skin yellow. Continued screening that retained the plasmoquine side chain but further altered the heterocyclic nucleus led in 1939 to chloroquine (Coatney 1963). It was evaluated in 1943 and found more effective than Atabrine and free of pigmenting effect (Loeb et al. 1946). Pyrimidines also are effective as antimalarials. Of these, pyrimethamine, reported in 1951 (Rollo 1951), is recognized as highly effective.

Amebiasis for centuries was treated successfully with extracts of the ipecacuanha plant. In 1817 the active principle, now designated emetine, was isolated and identified by Pelletier and Magendie (Work and Work 1948, p.2). It is an alkaloid, the methyl ether of cephaeline. Emetine with bismuth iodide is used for the elimination of cysts. Of the arsenicals, carbasone and carbamidophenylarsonic acid have had limited use. Of the 8-hydroxyquinolines, an iodinated derivative, chinioform, and an amino derivative, chloroquine, have been used.

The bacterial diseases did not yield readily to therapy. During the same year that Ehrlich and Hata announced arsphenamine, Morgenroth and Levy (1911) reported that ethylhydrocuprein was effective against pneumococcal infection in mice, but it failed against pneumonia in humans.

Persistent study in Germany of dyestuffs, notably the azo dyes, for activity against bacterial infections led to the discovery of prontosil, 4'sulfonamide-2:4 diaminoazobenezene. In 1935, Gerhard Domagk reported activity against experimental streptococcal and staphylococcal infection in mice. The results of successful clinical trial were reported soon after (Colebrook and Kenny 1936). The announcement of prontosil stimulated the interest in chemotherapy in many Western countries. A French team led by Trefouel (Trefouel et al. 1935) soon found that p-aminobenzenesulfonamide, a metabolite of prontosil, was the active constituent. This observation opened the way for the preparation of derivatives superior to the original compound in properties as to decrease toxicity, extend spectrum, or alter absorption, distribution, and excretion. The first useful derivative, sulfapyridine, was announced in 1938 by Whitby. Others meriting consideration were sulfathiozole, sulfadiazine, and sulfaguanidine, which were described in the next three years. The sulfones, a different category of derivatives, are important because they achieved significantly altered spectra. The diaminodiphenylsulfones as reported in 1942 exhibited a low degree of activity against tuberculosis in guinea pigs and possibly in humans (Feldman, Mann, and Hinshaw 1942). They have been used more successfully for treatment of human leprosy (Erickson 1950). The experience with the diaminodiphenylsulfones encouraged the search for antituberculosis agents and provided the opportunity to establish a reliable *in vivo* testing procedure.

The extended study of sulfanilamides led to the discovery of the thiosemicarbazones in 1950 by Benisch and Domagk (Karlson, Gainer, and Feldman 1950). Within this series were derivatives active against tuberculosis *in vitro* and *in vivo*. The use in humans was limited by toxicity and the superiority of other antituberculosis agents that had become available. In turn, the study of possible derivatives from the thiosemicarbazones led to the discovery of isonicotinic acid hydrazide (Paper 41). Isonicotinic acid hydrazide (isoniazid) also could have emerged from the study of derivatives of nicotinamide, which had been shown to possess a low level of activity against tuberculosis in the experimental animal.

The nitrofurans are also recognized as chemotherapeutic agents but of minor importance. They are used in both human and veterinary medicine (Dodd and Stillman 1944). In humans they have been used primarily to treat infections of the urinary tract. Derivatives selected for administration by oral route and for topical application have been described. Nalidixic acid, discovered in 1962 (Buchbinder et al. 1963), similarly has been used for the treatment of genito-urinary infections.

The antibiotics comprise a more extensive and novel range of agents for the treatment of infectious diseases than do the synthetic chemotherapeutic agents. The term *antibiotic* was introduced by Selman Waksman in 1942 for the newly discovered potent therapeutic agents (Waksman 1975, p. 28). An antibiotic is a compound produced by a microorganism that is active against other organisms at concentrations so low as not to be toxic for a host receiving the substance. The activity is specific in nature. Each antibiotic therefore has a characteristic spectrum and pharmacological activity. Antagonism between microorganisms was recognized early in the history of bacteriology. It was observed by Pasteur and Joubert (1877), and they recognized that the phenomenon could be the basis for therapy. During the years that followed, there was a continuing thread of studies associated with antagonisms of microorganisms. These studies included the isolation of pyocyanase from *Pseudomonas pyocyanea* and its clinical use by Emmerich and Low (1899) from 1899 into the first decade of the twentieth century for the treatment of diphtheria and other infectious diseases. Yet another and later interesting effort was the use by Gratia and coworkers of actinomyces-induced lysates of bacterial pathogens, mycolysates, to treat streptococcal and staphylococcal disease. The plant pathologists were the first to achieve the isolation of an antimicrobial agent produced by a fungus. Weindling and Emerson (1936) crystallized the antibiotic now known as gliotoxin from cultures of *Trichodermis lignorum* that they had carried as *Gliocladium fimbriatum*.

The report of a soluble antimicrobial product active *in vivo* was presented by Dubos in 1939. From cultures of *Bacillus brevis* was extracted an agent that inhibited the growth of *Streptococcus pneumoniae in vitro* and protected mice against infection with *S. pneumoniae*. Hitherto the antibacterial products of microbes were demonstrated only *in vitro*. The inhibiting substance, eventually called tyrothricin, was shown to consist of a mixture of two antibacterial agents, tyrocidin and gramicidin (Paper 1). Tyrothricin and its constitutents were too toxic for use in humans and did not diffuse. The agents were effective only when injected into the area where the infecting inoculum was introduced. Thus mice infected intraperitoneally could be treated effectively only when tyrothricin or its components were injected into the peritoneal cavity. Tyrocidin and gramicidin were identified as cyclic homodetic unsubstituted polypeptides consisting of six to ten amino acids arranged in characteristic sequence.

Other polypeptide antibiotics with greater though still limited degrees of clinical utility have been developed. Bacitracin, consisting of mixture of related components, in which bacitracin A, a dodecapeptide, predominated, was announced in 1945 (Paper 22). Bacitracin was active against gram-positive organisms, including clostridia, and especially active against beta hemolytic group A streptococci. Nephrotoxicity limited its use to short periods when administered parenterally. More frequently it has been applied topically or orally. In 1947 the polymyxins (aerosporins), another group of polypeptide antibiotics, were reported independently in the United States (Paper 18; Benedict and Langlykke 1947) and in England (Ainsworth, Brown, and Brownlee 1947), which were active against gram-negative organisms. The susceptible organisms did not include proteus organisms, which have presented a continuing problem in therapy. The polymyxins are strongly basic cyclic compounds containing aliphatic acids in addition to amino acids. Like the other polypeptide antibiotics, the polymyxins are nephrotoxic; the B and E forms are less toxic than the A, C, and D forms. The E form also is known as colistin.

Viomycin (Anon 1950), also known as vinacetin A, is a cyclic polypeptide that is active against *M. tuberculosis in vivo* and *in vitro*. Its use is limited by the nephrotoxicity encountered among polypeptide antibiotics.

At the time that Dubos had published his first report, the epochal investigations of the Oxford penicillin team led by Florey and Chain had been started. Penicillin was discovered by Fleming in 1929 (Paper 2). He encountered it as the product of an accidental fungal contaminant in an agar plate culture of staphylococci. A mold colony had produced a zone of bacterial lysis on a culture dish that had been left at the bench while Fleming was away on a holiday. Fleming was in search of antiseptics that would not be destructive to tissues and proposed that penicillin might be useful for the treatment of superficial infections. Raistrick attempted to isolate penicillin, but a lack of stability of the compound hampered this attempt and a later one by Reid.

Florey and Chain were interested in bacteriolytic agents and inhibitors of bacteria produced by other microorganisms. From a survey of the pertinent literature, Chain concluded that Fleming's penicillin was one of the most impressive and best described phenomena of bacterial antagonism. Work was started on penicillin in 1938 (Paper 3). The difficulties Raistrick experienced were resolved by performing the required acid extraction at refrigerated temp-