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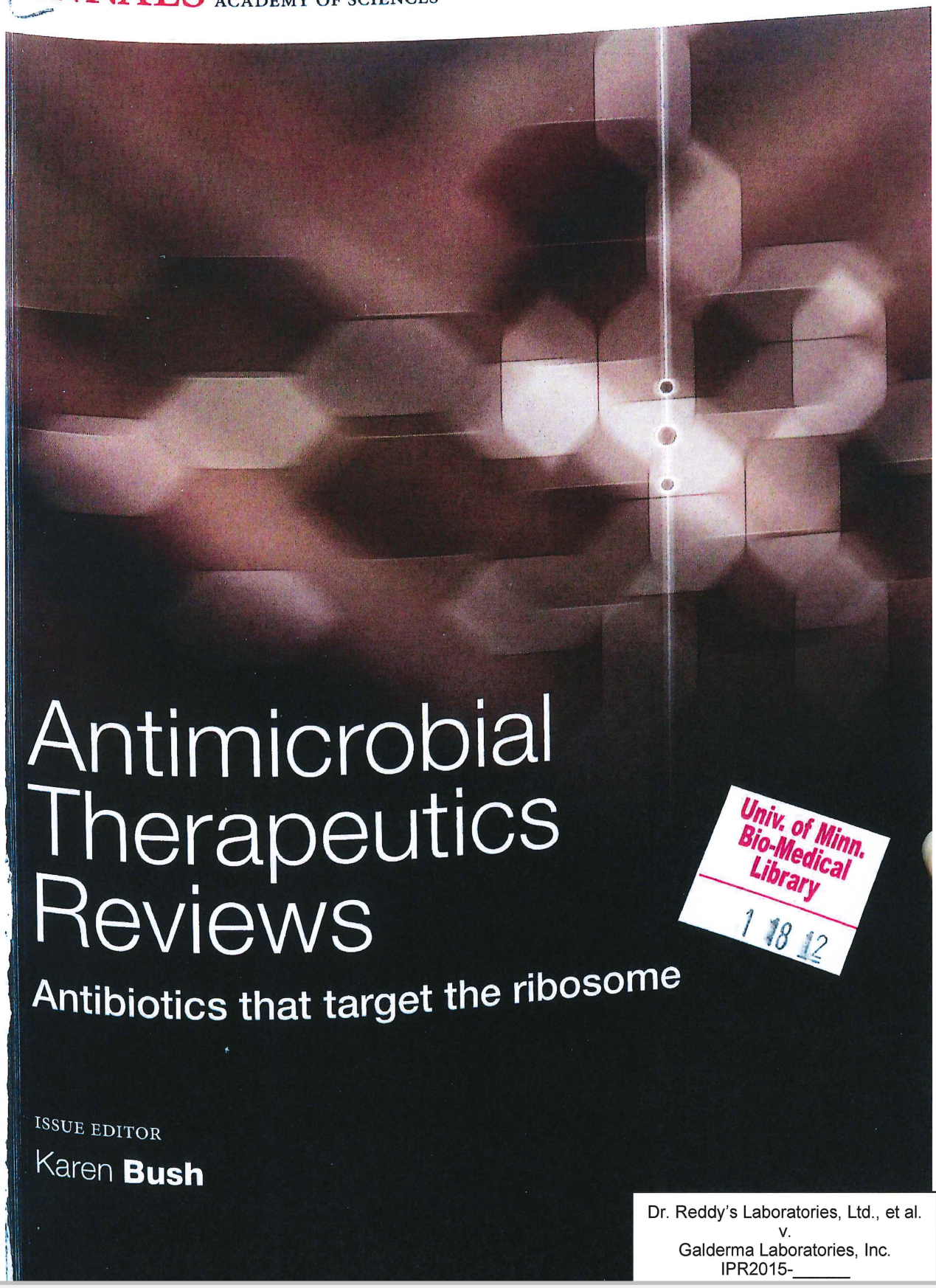

Antimicrobial Therapeutics Reviews

Antibiotics that target the ribosome

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The history of the tetracyclines

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The history of the tetracyclines involves the collective contributions of thousands of dedicated researchers, scientists, clinicians, and business executives over the course of more than 60 years. Discovered as natural products from actinomycetes soil bacteria, the tetracyclines were first reported in the scientific literature in 1948. They were noted for their broad spectrum antibacterial activity and were commercialized with clinical success beginning in the late 1940s to the early 1950s. The second-generation semisynthetic analogs and more recent third-generation compounds show the continued evolution of the tetracycline scaffold toward derivatives with increased potency as well as efficacy against tetracycline-resistant bacteria, with improved pharmacokinetic and chemical properties. Their biologic activity against a wide spectrum of microbial pathogens and their uses in mammalian models of inflammation, neurodegeneration, and other biological systems indicate that the tetracyclines will continue to be successful therapeutics in infectious diseases and as potential therapeutics against inflammation-based mammalian cell diseases.

Keywords: tetracyclines; history; bioactivity; mechanism; resistance; uses

The antibiotic era

In early 1948, five-year-old Toby Hockett (Fig. 1) was rushed by his parents to Johns Hopkins Children's Hospital in Washington, DC, with severe abdominal pain, and diagnosed with a ruptured appendix. Although emergency surgery was successful, a serious infection and complications set in, and the few antibiotics clinically useful at the time proved ineffective, leaving him facing imminent death.[1]

However, a new experimental antibiotic had recently arrived on campus for clinical use, a compound that had hardly been used in humans and was still being evaluated as a new chemotherapeutic agent. In this post–WWII era, antibiotics were considered novel therapeutics, with penicillin being heralded as a "wonder drug" having saved countless lives on the battlefields. It was also a period of chemical discovery, where the scientific methods of microbiology and organic chemistry were merging, and the promises of infectious disease chemotherapy became a major drive of medical research in academia and the chemical industry.

Since the antibiotics of the day failed Toby Hockett, his parents in desperation consented for him to be treated with the yellow-colored compound recently sent by the Lederle Laboratories Division of American Cyanamid, under the name Aureomycin™. It was a risk without choice; "I remember being put on the operating table, screaming and crying, and seeing the gas mask coming down on my face and being in that hospital for a very long time after that," he recalled. Within months he fully recovered and was one of the first of many people whose lives were saved by Aureomycin.[1]

Aureomycin had been discovered almost five years earlier in the early 1940s by Lederle, whose mission to generate new compounds and drugs, particularly antibiotics, had begun even earlier in the late 1930s.[2] Industrial chemical producers in this era were aware of the discovery and commercial value of penicillin, and it changed the course of their business. Normally they were resigned to producing consumer products, but now they began hiring scientists from many medical disciplines and started screening chemicals, biologics, immune-sera, and

Figure 1. Five-year-old Tobey Hockett, one of the first patients treated with Aureomycin.

other promising and potential molecules against a host of diseases in a spirit of optimism, growth, and medical discovery that was unprecedented in the history of the emerging pharmaceutical industry.

In 1938, Cyanamid president William B. Bell unveiled to his executives a new mission statement, "You may come up with nothing, but you may discover a single drug that may conquer even one major disease, then the public will be well served and our company will prosper,"[2] thus formalizing the company's entry into the area of antibiotic discovery.

The discovery of the tetracyclines

In the early 1940s antibiotic discovery was progressing rapidly, best exemplified through the work and methods of microbiologist René Dubos[3] and the chemical diversity derived from the soil actinomycetes shown by Selman Waksman and colleagues.[4] It was evident that the microbial world produced a wealth of natural products and antibiotic compounds capable of fighting microbial diseases. But it was their medical and financial potential that drove the expansion of many pharmaceutical companies within the United States, with American Cyanamid as one of the first to commit to antibiotic research and development.

Cyanamid built new laboratories in Pearl River, NJ under the direction of general manager Wilbur Malcolm and their head of research, Yellapragada Subbarow. They then began a search for an antibiotic they felt should rival Waksman's streptomycin, enlisting as consultant 71-year-old Benjamin Minge Duggar (Fig. 2), a retired professor of plant physiology and economic botany from the University of Wisconsin, to head their soil screening department.

Duggar was world renowned for his extensive knowledge and study of soil fungi; he collected soil samples from all over the world sent to him by friends from sites he instructed would yield actinomycetes soil bacteria, or "ultra-molds" as he called them, those with ground coverings left undisturbed and natural. The samples were subjected to culture and broth dilution assays performed by his technicians, in which the microorganisms were plated, and the colonies assayed for antibiotic activity against a panel of Gram-positive and Gram-negative bacteria.[2] Although many soil organisms were known to produce antibiotics, most were toxic or had undesirable properties, and the team encountered many false leads.

One sample, however, drew their attention early on. It was marked A-377 and sent by William Albrecht, dug from Plot 23 on Sanborn field, a dormant timothy hayfield on the University of Missouri campus, outside Columbia, Missouri. It yielded an unusual yellow-colored colony that inhibited the growth of all their strains in an initial panel of bacteria, and produced remarkably large zones of growth inhibition in agar. This was an unheard-of property at this point, as compared to the few antibiotics available for comparison. They further found that even crude extracts of the colony retained remarkable antibacterial activity against lethal scrub typhus and the rickettsias, such as Rocky Mountain spotted fever, an infection for which there was no

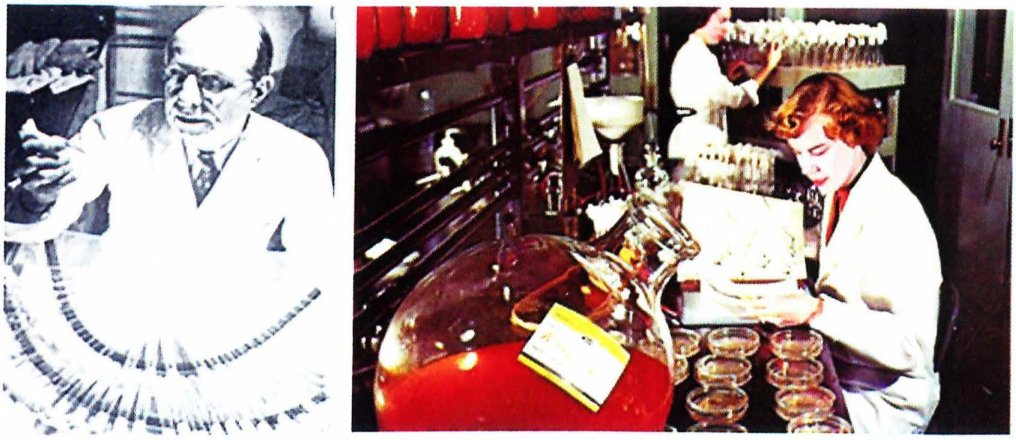

Figure 2. (Left) Septuagenarian Benjamin Minge Duggar, who discovered the "ultra-mold" *Streptomyces aureofaciens*, a soil bacterium producing Aureomycin. (Right) Technicians screening soil samples in the Lederle laboratories.

cure.[2] Soon enthusiasm about its broad range of activity and potency against lethal pathogens led to the labeling of the unknown substance as a "broad spectrum" antibiotic, becoming one of the first in medical history to attain this title. Duggar named the compound *aureomycin* in reference to its yellow color and the gold-colored *Streptomyces* strain from which it was extracted. He continued the study of the ultra-mold and its medical microbiology, taxonomy, and physiology, naming it *Streptomyces aureofaciens and* first publishing his results in 1948 in the *Annals of the New York Academy of Sciences*.[5] This established Aureomycin as a new and potent broad-spectrum antibacterial agent that was safe and effective, although its exact chemical structure had yet to be determined.

The efforts at Cyanamid were expanded, bringing in R.D. McCormick to produce the compound using advanced fermentation methods. Soon the company was producing Aureomycin in commercial quantities. By December 1, 1948, the drug was approved by the FDA for clinical use and was an immediate success in the clinic, saving countless lives against a broad spectrum of infectious diseases, and generating notoriety and profits for the company. It appeared that the mission statement of Cyanamid by William Bell had come to fruition.[2]

Within a short time, other chemical companies were announcing their own discoveries of new bioprospected antibiotics, and by 1950, Alexander Finlay and colleagues at Charles Pfizer Co., Inc., Groton, CT, had gathered thousands of soil samples from around the world, and isolated the soil bacterium *Streptomyces rimosus*.[6] Their organism produced a compound with similarity in color to Aureomycin, but it was slightly more water soluble and had better bioactivity, giving it a medical and competitive edge over Aureomycin in the treatment of infectious diseases. The compound was named Terramycin in reference to *terra*, Latin for *earth*, and perhaps its origin, Terre Haute, Indiana. It was approved by the FDA in 1950, competing directly with Aureomycin while gaining success in the treatment of a broad spectrum of infectious diseases.[7]

The chemical structures of both Aureomycin and Terramycin, however, were difficult to solve and remained elusive for both companies, although they shared their respective compounds with each other in order to determine their common structural features and substructures and settle their disparate molecular identities. In this era of chemical characterization of natural products, instrumental analysis was limited to ultraviolet-visible spectroscopy (UV-Vis) and infrared spectroscopy, and structural proofs routinely relied on chemical modifications and degradation studies that few laboratories in the world were equipped to perform. Scientists at Pfizer, led by Karl Brunings (Fig. 3), and in collaboration with the legendary Harvard University chemist Robert Woodward, raced to prove the chemical structures of both compounds. By 1952 the preliminary chemical structures of both Aureomycin

Figure 3. Pfizer group members from the structure determination and tetracycline team (left to right): Frederick Pilgrim; Lloyd Conover, inventor of tetracycline; Karl Brunings, director of chemical research; Phil Gordon; and Charles Stephens, inventor of doxycycline.

and Terramycin (Fig. 4) were solved by the Pfizer-Woodward team, postulating that both compounds possessed a DCBA naphthacene core with similar functional groups with only minor differences in structure.[8] The core scaffold for this new family of antibiotics became descriptively known as the tetracyclines; however, Terramycin possessed an additional C5 position hydroxyl group and was devoid of a C7 chlorine atom, compared to Aureomycin.

The major structural features of the molecules were published by the Pfizer-Woodward group in a landmark paper in 1954 titled "The Structure of

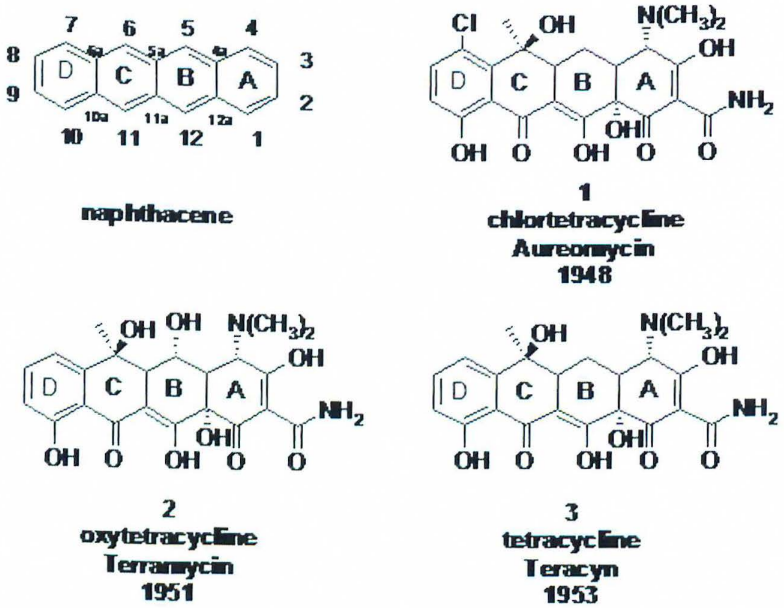

Figure 4. The naphthacene ring system and its structural locants of the first-generation antibiotics: chlortetracycline (1), oxytetracycline (2), and tetracycline (3), followed by the year approved by the FDA.

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