The Tetracyclines

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Preface

The history of antibiotics may well have begun with the ancient Sudanese-Nubian civilization (see Chapter 1, "Historical Introduction"), but this volume reflects a more contemporary appraisal of the antibiotic era. We have compiled a comprehensive review of the tetracyclines which includes all the major subdivisions of these chemically important and clinically useful antibiotics.

There can be little doubt about the contribution of antibiotics to both the increase in human life span and the alleviation of much human suffering. The tetracyclines are still playing an important role in these areas and will continue to do so in the foreseeable future.

We hope this volume will be an important contribution to a better understanding of the chemistry, biochemistry, and medical aspects of tetracycline antibiotics.

We are indebted to the individual authors who have given so much of their time and effort in the preparation of the chapters.

Pearl River, NY — Joseph J. Hlavka
Ocean Gate, NJ — James H. Boothe

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CHAPTER 1

Historical Introduction

J. H. BOOTHE and J. J. HLAVKA

The tetracyclines have been an important component in the treatment of bacterial infections since their discovery in 1948. During this time the literature relating to these antibiotics has become quite voluminous, complex, and scattered among publications in many different scientific disciplines and languages. Numerous reviews of specific areas have been written in the past, such as the recent comprehensive review by MITSCHER (1978), but the present work is, to our knowledge, the first attempt to combine reviews of all the major subdivisions from chemistry to clinical applications of the tetracyclines in one volume. It should be understood, however, that no review could (or should) contain every literature reference to the subject being reviewed, but rather that important and pertinent literature be selected to illustrate the various chapters.

The history of antibiotics began with the often-told events related to the observations of penicillin activity by Fleming in 1928, the rediscovery and purification by Florey and Chain in 1938, and the large-scale development and clinical use of penicillin during World War II. By 1946–1947, the problems related to penicillin production had essentially been solved. The material was readily available at a reasonable price, and in fact the world's needs could be satisfied by a few large efficient pharmaceutical producers. Stimulated by the tremendous successes of penicillin, many of the drug companies and academic institutions now began to search for new antibiotics by screening soil samples for antibiotic-producing microorganisms. Such a screening program was established at Lederle Laboratories and surprisingly quickly the producer of what became known as chlortetracycline or Aureomycin was discovered (DUGGAR 1948). In retrospect, the early discovery of the penicillin and the tetracycline families of antibiotics seems especially fortuitous since large screening programs in the succeeding 30–40 years have discovered relatively few, new and useful families of antibiotics even though about 6,000 antibiotics have now been described in the literature.

Chlortetracycline was introduced to the medical profession in 1948 and was enthusiastically accepted on the basis of its merits. It first of all inhibited a much wider spectrum of microorganisms than the then available penicillins, covering not only the gram-positive organisms but many of the gram-negative ones as well. Secondly, it was orally active, being very well absorbed from the stomach and upper small intestine. The second member of the family, oxytetracycline, was discovered independently by the Charles Pfizer Company and introduced in 1950 (FINDLAY et al. 1950). In 1953, the nomenclature parent of the series, tetracycline, was discovered (BOOTHE et al. 1953; CONOVER et al. 1953) and introduced, and rather quickly became the favorite of the medical profession. Tetracycline was

discovered almost simultaneously as a product of reductive dechlorination of chlortetracycline and as a fermentation product of a *Streptomyces* species (Minieri et al. 1953, 1954). The study of mutant strains of *Streptomyces*, both natural and induced, undertaken in attempts to improve fermentation yields, provided the next medically useful tetracycline, 6-demethylchlortetracycline (McCormick et al. 1957). This compound provided clinically useful antibiotic blood levels for longer periods, thus permitting the use of lower dose levels to produce the same therapeutic effect.

The remaining medically useful members of the tetracycline family, rondomycin (Blackwood et al. 1963), doxycycline (Stephens et al. 1963), and minocycline (Martell and Boothe 1967) were all the results of lengthy programs of chemical modification of members of the naturally occurring tetracyclines. These semisynthetic tetracyclines will be reviewed in some detail later in this book, but briefly these compounds offer improved therapeutic efficacy in the areas of potency, serum half-life, and, in some cases, broadened spectrum of activity.

Having reviewed the relatively brief, 50-year history of antibiotics in medicine, and the even shorter history of the tetracyclines, it should be noted that these compounds probably played a role in ancient history that has not yet been fully appreciated or studied. In 1980, a group of anthropologists from the University of Massachusetts observed fluorescent areas in bones obtained from an ancient (350 A.D.) Sudanese-Nubian civilization. These fluorescent areas were identical both as to location in the bone and fluorescent characteristics to modern bone from patients treated with tetracycline antibiotics (Bassett et al. 1980). This observation has been at least partially confirmed by fluorescence spectrum measurements and by microbiological inhibition studies of the old bone (Boothe 1984). The deposition of tetracycline antibiotics in developing bones and teeth as a very stable calcium chelate complex was observed early in biological studies. In fact, it is the basis for the recommendation that children below the age of 8 years should not be treated with tetracyclines because of occasional teeth staining.

If we assume that at least some ancient civilizations were exposed to tetracycline antibiotics (and perhaps to other antibiotics which were less stable and thus decomposed in the intervening years) the question arises as to the source. The most likely answer seems to be that they were produced during the growth of an antibiotic-producing organism either in stored grain or in some fermented liquid such as a beer.

It may be instructive to make future determinations as to which competing civilizations were exposed to these antibiotics, for example, the Greeks or the Persians, the Romans or the Carthaginians, the Philistines or the Israelites.

The scientific and medical history of the tetracycline antibiotics seems to have been paralleled by a long and complicated series of legal entanglements. It often seemed that the legal minds were as numerous and as active as the scientific ones. Some of the more prominent of these legal affairs are briefly described: (1) In the late 1950s a number of cultures of tetracycline-producing streptomycetes, along with much technical information, were stolen from the Lederle Laboratories by a few employees and sold to some foreign producers. The solving of the case and the prosecution of the leader is described in some detail in *The Million Dollar Bugs* (Pearson 1969), which makes a lively detective yarn. (2) Congressional hearings

on the questions of pricing and price fixing were held by various committees over a number of years beginning in 1959 with those held by the late Senator Estes Kefauver. (3) The Federal Trade Commission held hearings and proceedings at various times from 1958 to 1968 which resulted in an order to allow open licensing of tetracycline. (4) Criminal proceedings involving conspiracy to fix prices were brought in 1961 by the US Department of Justice against the five companies who made and sold tetracycline antibiotics. The companies were first convicted of price fixing, but the US Court of Appeals later reversed the conviction, and the reversal was affirmed by the US Supreme Court. The case was remanded for a new trial, and on retrial the companies were found not guilty. (5) The original conviction for price fixing stimulated the filing of civil suits by large users of tetracycline to recover overpayments. One hundred and fifty-eight treble damage actions were filed, many of which were settled before the reversal of the conviction. After the price-fixing conviction was reversed, the civil suits were based mainly on the contention that the original patent on tetracycline had been obtained fraudulently by withholding information from the patent examiner. The most recent and final judicial decision has affirmed the validity of the 1955 tetracycline patent and the last legal action against the companies was dropped in 1982.

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CHAPTER 2

Fermentation and Mutational Development of the Tetracyclines

J. J. GOODMAN

A. Introduction

The first of the tetracycline family of antibiotics, chlortetracycline (CTC), was introduced over 3 decades ago (DUGGAR 1948) and in the following years, with the introduction of new members of the family, the tetracyclines have retained their importance in both human therapy and animal feed use. According to reports of the US Tariff Commission, annual production is in the order of 11,000–13,000 tonnes. Numerous reviews dealing with the pathways of biosynthesis and the genetics and biochemistry of the producing organisms have appeared and these will be cited in the appropriate sections. The production aspects of the fermentation and the factors leading to higher yields have been dealt with less frequently and this is understandable in view of the proprietary nature of much of the information. An excellent review by DI MARCO and PENNELLA appeared in 1959 (DI MARCO and PENNELLA 1959) and the most recent general review is by HOSTALEK et al. (1979). A great deal of the pertinent information is to be found in the patent literature, where, again for obvious reasons, only minimal disclosures are often made. A compendium giving excerpts from the United States patent literature appeared in 1968 (EVANS 1968). This review will attempt to deal with the fermentation and mutational development of the tetracyclines from the practical perspective of increasing fermentation potencies. The large number of enzymes and individual steps involved in biosynthesis and the large number of possible compounds on the pathway to the final products (see HOSTALEK et al. 1974 for a review) can all have a potential effect on ultimate yield. As products of fermentation the tetracyclines are "mature" products. If a recent report (BASSETT et al. 1980) on their detection in ancient bones holds up, their existence predates the antibiotic era by at least 1,400 years! In the modern antibiotic era as products mature, improved production strains come along more slowly and useful media combinations are more likely already to have been tested. Maintenance of high yields becomes increasingly a matter of constant attention to small details rather than through some dramatic change. As more sophisticated production technologies get locked in place it becomes more difficult to introduce changes. The gap between tank results and shaken flask results becomes wider though one may hope that the latter will still be predictive of the potential of a new strain or medium ingredient.

This review will include both older as well as the more recent literature citations. Where no specific organism is stated all reference to oxytetracycline (OTC) production will be by *Streptomyces rimosus*, and similarly unless otherwise stated in the text references to production of any of the other tetracyclines will be by