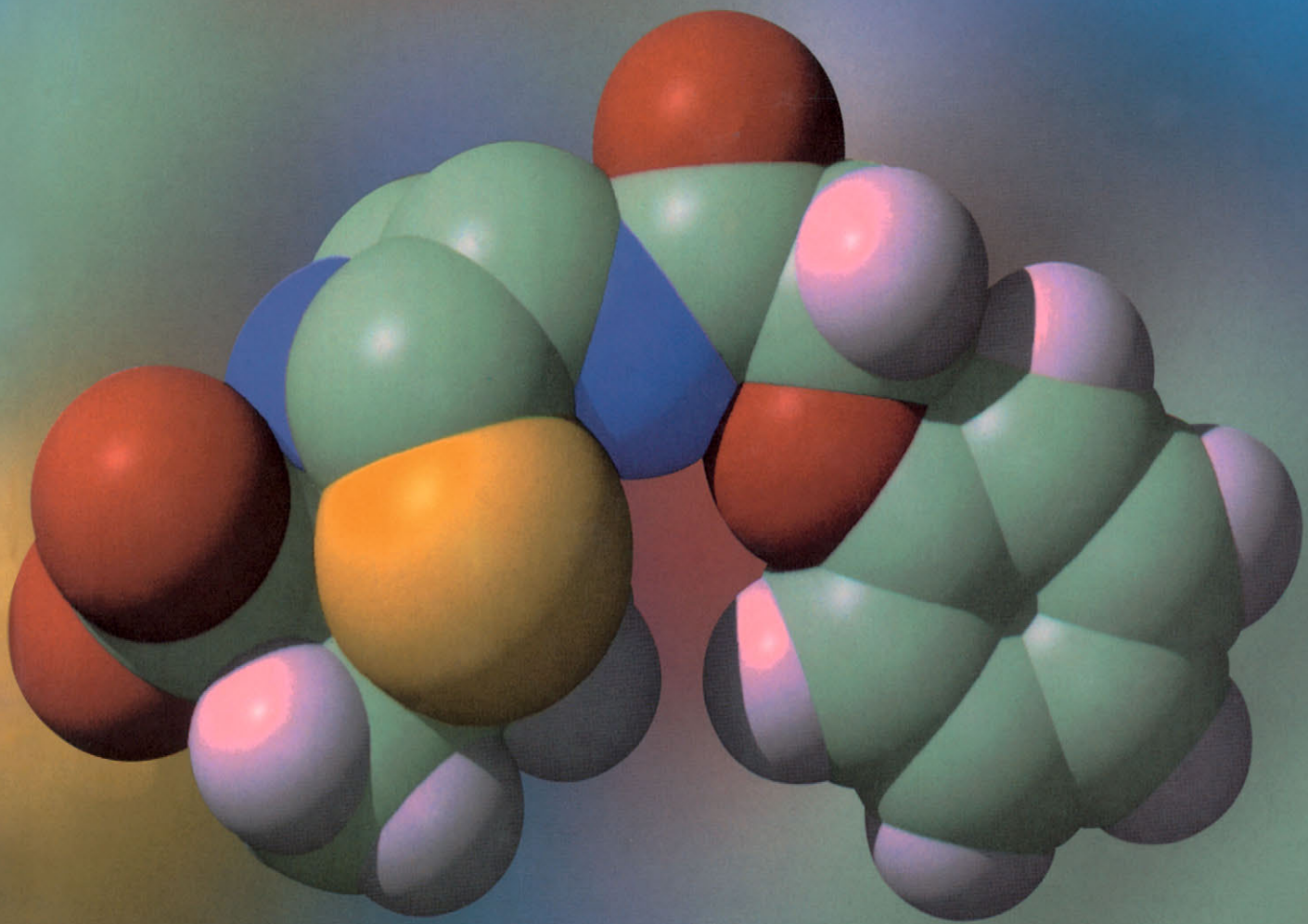
Fifth edition

Biochemistry and Molecular Biology of Antimicrobial Drug Action

T.J. Franklin and G.A. Snow



SPRINGER-SCIENCE+BUSINESS MEDIA, B.V.

Biochemistry and Molecular Biology of Antimicrobial Drug Action

Biochemistry and Molecular Biology of Antimicrobial Drug Action

Fifth edition

Formerly

Biochemistry of Antimicrobial Action

T. J. Franklin and G. A. Snow

Zeneca Pharmaceuticals
Alderley Park, Macclesfield
Cheshire

SPRINGER-SCIENCE+BUSINESS MEDIA, B.V.

Library of Congress Cataloging in Publication Card Number: 98-70539

ISBN 978-1-4757-4601-3 ISBN 978-1-4757-4599-3 (eBook)
DOI 10.1007/978-1-4757-4599-3

This edition is dedicated to the memory of Dr G. Alan Snow, 1915–1995, my co-author, colleague and mentor.

The cover illustration shows a computer-generated graphic of Penicillin V
**phenoxymethyl penicillin) kindly provided by Mr A.M. Slater*

Contents

Preface

The rapid advances made in the study of the synthesis, structure and function of biological macromolecules in the last fifteen years have enabled scientists concerned with antimicrobial agents to achieve a considerable measure of understanding of how these substances inhibit cell growth and division. The use of antimicrobial agents as highly specific inhibitors has in turn substantially assisted the investigation of complex biochemical processes. The literature in this field is so extensive, however, that we considered an attempt should be made to draw together in an introductory book the more significant studies of recent years. This book, which is in fact based on lecture courses given by us to undergraduates at Liverpool and Manchester Universities, is therefore intended as an introduction to the biochemistry of antimicrobial action for advanced students in many disciplines. We hope that it may also be useful to established scientists who are new to this area of research.

The book is concerned with a discussion of medically important antimicrobial compounds and also a number of agents that, although having no medical uses, have proved invaluable as research tools in biochemistry. Our aim has been to present the available information in a simple and readable way, emphasizing the established facts rather than more controversial material. Whenever possible, however, we have indicated the gaps in the present knowledge of the subject where further information is required. We have avoided the use of literature references in the text; instead we have included short lists of key articles and books for further reading at the end of each chapter.

We have drawn on the work of many scientists and we are especially pleased to express our thanks to those who have given us permission to reproduce their original diagrams and photographs. We are also grateful to the Pharmaceuticals Division of Imperial Chemicals Industries Ltd, for providing the necessary facilities for the preparation of this book.

Abbreviations used without definition for common biochemical substances are those recommended by the Biochemical Journal (1970).

June 1970

T. J. FRANKLIN
G. A. SNOW

Preface to the fifth edition

Since the previous edition of this book there have been major developments in medicine and its underpinning basic sciences. The problems posed by infectious diseases afflicting humans and their domestic animals have attracted increasing publicity and concern throughout this period. The menace of AIDS continues unabated and has reached epidemic proportions in parts of the developing world. The spread of multidrug-resistant bacteria is seemingly unstoppable, sporadic outbreaks of meningitis, bacterially mediated food poisoning and lethal viral infections out of Africa regularly alarm the public. Fortunately, against this somewhat gloomy picture can be set some notable advances. The impact of rapidly advancing technologies in molecular biology (that have prompted the expansion of the title of this book) on our understanding of the mechanisms of antimicrobial action and drug resistance has been remarkable. Valuable new antimicrobial drugs have emerged in all areas of infectious disease, perhaps most notably in the treatment of AIDS, where combinations of several new anti-HIV compounds have brought new hope to victims of this appalling disease.

In this new edition attention concentrates largely on the action of compounds in clinical use against micro-organisms; earlier accounts of anticancer compounds have been omitted since these now form a large subject in its own right. Rapidly expanding knowledge of the molecular genetics and biochemistry of antimicrobial drug resistance has required separate chapters on each of these topics. A separate chapter has also been devoted to drugs with biochemical activities that could not be included within the main mechanisms described in Chapters 2, 3, 4 and 5.

Drs Bob Nolan and Keith Barret-Bee, who were major contributors to the fourth edition, have since moved on to new fields of endeavour and, sadly, Alan Snow died in 1995. Although I must therefore take sole responsibility for the content of this new edition, I have been greatly helped by the incisive comments of my colleagues Drs Terry Hennessey and Wright Nichols on certain sections of the book. Over the years many helpful comments and criticisms from our readers have been invaluable in planning future editions. I hope that they will continue to let me have their views.

Finally I would like to express my thanks to Zeneca Pharmaceuticals for the provision of facilities which have made this new edition possible.

TREVOR J. FRANKLIN

Chapter one

The development of antimicrobial agents past, present and future

1.1 The social and economic importance of antimicrobial agents

Few developments in the history of medicine have had such a profound effect upon human life and society as the development of the power to control infections due to micro-organisms. In 1969 the Surgeon General of the United States stated that it was time 'to close the book on infectious diseases'. His optimism, which was shared by many, seemed justified at the time. In the fight against infectious disease several factors had combined to produce remarkable achievements. The first advances were mainly the result of improved sanitation and housing. These removed some of the worst foci of infectious disease and limited the spread of infection through vermin and insect parasites or by contaminated water and food. The earliest effective direct control of infectious diseases was achieved through vaccination and similar immunological methods which still play an important part in the control of infection today. The use of antimicrobial drugs for the control of infection is almost entirely a development of this century, and the most dramatic developments had taken place only since the 1930s. No longer was surgery the desperate gamble with human life it had been in the early nineteenth century. The perils of childbirth had been greatly lessened with the control of puerperal fever. The death of children and young adults from meningitis, tuberculosis and septicaemia, once commonplace, was, by the late 1960s, unusual in the developed world.

Unfortunately, since the heady optimism of those days we have learned to our cost that microbial pathogens still have the capacity to spring unpleasant surprises on the world. The problem of acquired bacterial resistance to drugs, recognized since the early days of penicillin use in the 1940s, has become ever more menacing. Infections caused by the tubercle bacillus and *Staphylococcus aureus,* which were once readily cured by drug therapy, are now increasingly difficult or even impossible to treat because of widespread bacterial resistance to the available drugs. Nor is resistance confined to these organisms, many other species of bacteria, as well as viruses and protozoa, are also becoming drug-resistant. The ability of micro-organisms to kill or disable the more vulnerable members of society, especially the very young and old and patients with weakened immune defences, is reported in the media almost daily. Alarming reports of lethal enteric infections, meningitis and 'flesh-eating' bacteria have become depressingly familiar. If this were not enough, the spectre of the virus (HIV)

infection which leads to AIDS (acquired immune deficiency syndrome) threatens human populations around the world, in nations both rich and poor. While drug therapy for AIDS is increasingly effective, other terrifying viral infections such as Ebola and Lassa fever, for which there are no treatments, make their appearance from time to time. Throughout much of the tropical and subtropical world malaria continues to exact a dreadful toll on the health and lives of the inhabitants. Although mass movements of populations and the failure to control the insect vector, the anopheline mosquito, are major factors in the prevalence of malaria, the increasing resistance of the malarial protozoal parasite to drug treatment is perhaps the most worrying feature.

Another area of concern is the increasing incidence of serious infections caused by fungi. Thirty years ago such infections were relatively rare. More common infections like thrush and ringworm were more of an unpleasant nuisance than a serious threat to health. Today, however, many patients with impaired immunity caused by HIV infection, cytotoxic chemotherapy for malignant disease, or the immunosuppressive treatment associated with organ graft surgery, are at risk from dangerous fungal pathogens such as *Pneumocystis carinii* and *Cryptococcus neoformans*.

Fortunately, despite the threats posed by drug-resistant bacteria, viruses, protozoal parasites and fungal pathogens, the current scene is not one of unrelieved gloom. Most bacterial and fungal infections can still be treated successfully with the remarkable array of drugs available to the medical (and veterinary) professions. Work continues to develop drugs effective against resistant pathogens and significant progress can be reported against HIV and herpes infections. Vaccines have been remarkably successful in preventing some bacterial and viral infections. Indeed, outstanding amongst the medical achievements of the twentieth century have been the eradication of smallpox and the dramatic reduction in the incidence of poliomyelitis by mass vaccination programmes.

1.2 An outline of the historical development of antimicrobial agents

1.2.1 Early remedies

Among many traditional and folk remedies three sources of antimicrobial compounds have survived to the present day. These are cinchona bark and quinghaosu for the treatment of malaria and ipecacuanha root for amoebic dysentery. Cinchona bark was used by the Indians of Peru for treating malaria and was introduced into European medicine by the Spanish in the early seventeenth century. The active principle, quinine, was isolated in 1820. Quinine remained the only treatment for malaria until well into the twentieth century and still has a place in chemotherapy. The isolation of artemisinin, the active compound in the traditional Chinese remedy, quinghaosu, is much more recent and only in recent years has its therapeutic potential against malaria been fully appreciated. Ipecacuanha root was known in Brazil and probably in Asia for its curative action in diarrhoeas and dysentery. Emetine was isolated as the active constituent in 1817 and was shown in 1891 to have a specific action against amoebic dysentery. In combination with other drugs it is still used for treating this disease. These early remedies were used without any understanding of the nature of the diseases. Malaria, for example, was thought to be caused by 'bad air' (mal'aria) arising from marshy places; the significance of the blood-borne parasite was not recognized until 1880 and only in 1897 was the anopheline mosquito proved to be the specific insect vector when the developing parasite was observed in the intestine of the mosquito.

1.2.2 Antiseptics and disinfectants

The use of disinfectants and antiseptics also preceded an understanding of their action, and seems to have arisen from the observation that certain substances stopped the putrefaction of meat or rotting of wood. The term 'antiseptic' itself was apparently first used by Pringle in 1750 to describe

substances that prevent putrefaction. The idea was eventually applied to the treatment of suppurating wounds. Mercuric chloride was used by Arabian physicians in the Middle Ages for preventing sepsis in open wounds. However, it was not until the nineteenth century that antiseptics came into general use in medicine. Chlorinated soda, essentially hypochlorite, was introduced in 1825 by Labarraque for the treatment of infected wounds, and tincture of iodine was first used in 1839. One of the earliest examples of disinfection used in preventing the spread of infectious disease was recorded by Oliver Wendel Holmes in 1835. He regularly washed his hands in a solution of chloride of lime when dealing with cases of puerperal fever and thereby greatly reduced the incidence of fresh infections, as did Ignaz Semmelweiss in Vienna a few years later. These pioneer attempts at antisepsis were not generally accepted until Pasteur's publication in 1863 of the microbial origin of putrefaction. This led to an understanding of the origin of infection and suggested the rationale for its prevention. As so often in the history of medicine, a change of practice depended upon the personality and persistence of one man. In antiseptics this man was Lister. He chose phenol, the antiseptic that had been introduced by Lemaire in 1860, and applied it vigorously in surgery. A 2.5% solution was used for dressing wounds and twice this concentration for sterilizing instruments. Later he used a spray of phenol solution to produce an essentially sterile environment for carrying out surgical operations. The previous state of surgery had been deplorable; wounds usually became infected and the mortality was appalling. The effect of Lister's measures was revolutionary and the antiseptic technique opened the way to great surgical advances. Even at this time, about 1870, the use of antiseptics was still empirical. An understanding of their function began with the work of Koch, who from 1881 onwards introduced the techniques on which modern bacteriology has been built. He perfected methods of obtaining pure cultures of bacteria and of growing them on solid media and he demonstrated practical methods of sterile working. Once it became possible to handle bacteria in a controlled environment the action of disinfectants and antiseptics could be studied. The pioneer work on the scientific approach to this subject was published by Kronig and Paul in 1897.

Since that time the history of antiseptics has been one of steady but unspectacular improvement. Many of the traditional antiseptics have continued in use in refined forms. The phenols have been modified and made more acceptable for general use. Acriflavine, introduced in 1913, was the first of a number of basic antiseptics. It had many years of use but was displaced by colourless cationic antiseptics (acriflavine is bright orange). In surgery the antiseptic era gave way to the aseptic era in which the emphasis is on the avoidance of bacterial contamination rather than on killing bacteria already present. All the same, infection of surgical wounds remains a constant risk and antiseptics are still used as an extra precaution or second line of defence. Surgical staff also 'scrub up' with mild antiseptic solutions before entering the operating theatre. Disinfectants play an important part in the hygiene of milking sheds, broiler houses and other places where strict asepsis is impracticable.

1.2.3 The beginnings of chemotherapy

The publications of Pasteur and Koch firmly established that micro-organisms are the cause of infectious disease, though for some diseases the causative organism still remained to be discovered. It was also known that bacteria are killed by various antiseptics and disinfectants. Not surprisingly attempts were made to kill micro-organisms within the body and so to end the infection. Koch himself carried out some experiments with this aim. He had shown that mercuric chloride is one of the few disinfectants able to kill the particularly tough spores of the anthrax bacillus. Koch therefore tried to cure animals of anthrax infection by injecting mercuric chloride. Unfortunately the animals died

of mercury poisoning and their organs still contained infectious anthrax bacilli. A slightly more successful attempt to cure an infection with a toxic agent was made by Lindgard in 1893. He treated horses suffering from surra, a disease now known to be caused by trypanosomes, with arsenious oxide. There was some improvement of the disease, but the compound was too toxic to be generally useful.

However, chemotherapy really began with Paul Ehrlich. During the 10 years from 1902 onwards Ehrlich's work foreshadowed almost all the concepts which have governed subsequent work on synthetic antimicrobial agents. His first ideas arose from studies with 'vital stains', dyestuffs that were taken up selectively by living tissue. One such dye was methylene blue, which in the animal body is concentrated in nervous tissue. Ehrlich showed that the same dye was readily taken up by the malaria parasites in the blood so that they become deeply stained. Consequently methylene blue was tried against human malaria and showed some effect, though not sufficient to make it a useful treatment. Nevertheless this minor success started a line of thought that was to prove of the greatest significance. Ehrlich believed that antimicrobial agents must be essentially toxic compounds and that they must bind to the micro-organism in order to exert their action. The problem was to discover compounds having a selective action against the microbial cell compared with the cells of the host animal. Starting from methylene blue, Ehrlich began to search for other dyestuffs that would affect protozoal diseases. In 1904, after testing hundreds of available dyes, he eventually found one that was effective against trypanosomiasis in horses. This compound, called trypan red, was a significant landmark in the treatment of microbial infections since it was the first man-made compound that produced a curative effect.

However, it was not in the field of dyestuffs that Ehrlich achieved his greatest success. Following the early work on the treatment of trypanosomiasis with arsenious oxide, Koch tested the organic arsenical atoxyl (Figure 1.1). This compound produced the first cures of sleeping sickness, a human trypanosomal disease. Unfortunately, however, the compound produced serious side-effects, some patients developing optic atrophy. The curative effect of this compound stimulated Ehrlich to make other related arsenicals. He tested these on mice infected experimentally with trypanosomiasis and showed that curative action did not run parallel with toxicity to the mice. This suggested that if enough compounds were made some would have sufficiently low toxicity to be safe as chemotherapeutic agents. Ehrlich continued his search for compounds active against various micro-organisms and showed that arsenicals were active against the causative organism of syphilis. He began a massive search for an organoarsenical compound that could be used in the treatment of this disease and eventually in 1910 discovered the famous drug salvarsan (Figure 1.1). This drug and its derivative neo-salvarsan became the standard treatment for syphilis. Coupled with bismuth therapy they remained in use until supplanted by penicillin in 1945. This was the most spectacular practical achievement of Ehrlich's career, but scientifically he is remembered at least as much for his wealth of ideas that have inspired workers in the field of chemotherapy down to the present day. These ideas are so important that they deserve separate consideration.

1.2.4 The debt of chemotherapy to Ehrlich

The very term chemotherapy was invented by Ehrlich and expressed his belief that infectious disease could be combated by treatment with synthetic chemicals. Successes since his day have entirely justified his faith in this possibility. He postulated that cells possess chemical receptors which are concerned with the uptake of nutrients. Drugs that affect the cell must bind to one or other of these receptors. The toxicity of a drug is determined partly by its distribution in the body. However, in the treatment of an infection the binding to the parasite relative to the host cell determines the effectiveness of the compound.

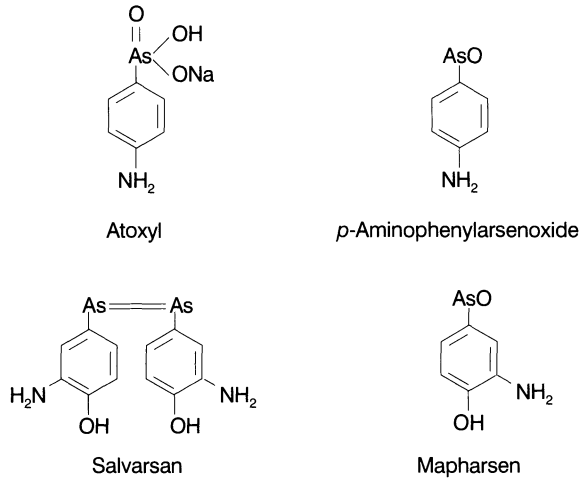


FIGURE 1.1 Arsenical compounds used in the early treatment of trypanosomiasis or syphilis.

Thus Ehrlich recognized the importance of quantitative measurement of the relationship between the dose of a compound required to produce a therapeutic effect and the dose that causes toxic reactions. Such measurements are still of prime importance in chemotherapy today.

Ehrlich pioneered methods that have since become the mainstay of the search for new drugs. One aspect of his approach was the use of screening. This is the application of a relatively simple test to large numbers of compounds in order to obtain evidence of biological activity in types of chemical structure not previously examined. The second of Ehrlich's methods was the deliberate synthesis of chemical variants of a compound known to have the required activity. The new compounds were examined for increased activity or for improvements in some other property such as reduced toxicity. Any improvement found was used as a guide to further synthesis, eventually arriving, by a series of steps, at the best possible compound. These methods are now so well accepted that their novelty in Ehrlich's day can easily be forgotten. They depend on the thesis that a useful drug possesses an ideal combination of structural features which cannot be predicted at the outset. A compound approximating to this ideal will show some degree of activity, and can therefore act as a 'lead' towards the best attainable structure.

According to Ehrlich a chemotherapeutic substance has two functional features, the 'haptophore' or binding group which enables the compound to attach itself to the cell receptors, and the 'toxophore' or toxic group that brings about an adverse effect on the cell. This idea has had a continuing influence in subsequent years. In cancer chemotherapy it has frequently been used in attempts to bring about the specific concentration of toxic agents or antimetabolites in tumour cells. In antimicrobial research it has helped to explain some features of the biochemical action of antimicrobial compounds.

Ehrlich also recognized that compounds acting on microbial infection need not necessarily kill the invading organism. It was, he suggested, sufficient to prevent substantial multiplication of the infectious agent, since the normal body defences, antibodies and phagocytes, would cope with foreign organisms provided that their numbers were not overwhelming. His views on this topic were based in part on his observation that isolated spirochaetes treated with low concentrations of salvarsan remained motile and were therefore apparently still alive. Nevertheless they were unable to produce an infection when they were injected into an animal body. It is a striking fact that several of today's important antibacterial and antifungal drugs are 'static' rather than 'cidal' in action.

Another feature of Ehrlich's work was his recognition of the possibility that drugs may be activated by metabolism in the body. This suggestion was prompted by the observation that the compound atoxyl was active against trypanosomal infections but was inactive against isolated trypanosomes. His explanation was that atoxyl was reduced in the body to the much more toxic *p*-aminophenylarsenoxide (Figure 1.1). Later work showed that atoxyl and other related arsenic acids are not in fact readily reduced to arsenoxides in the body but local reduction by the parasite remains a possibility. Ehrlich, surprisingly, did not recognize that his own compound salvarsan would undergo metabolic