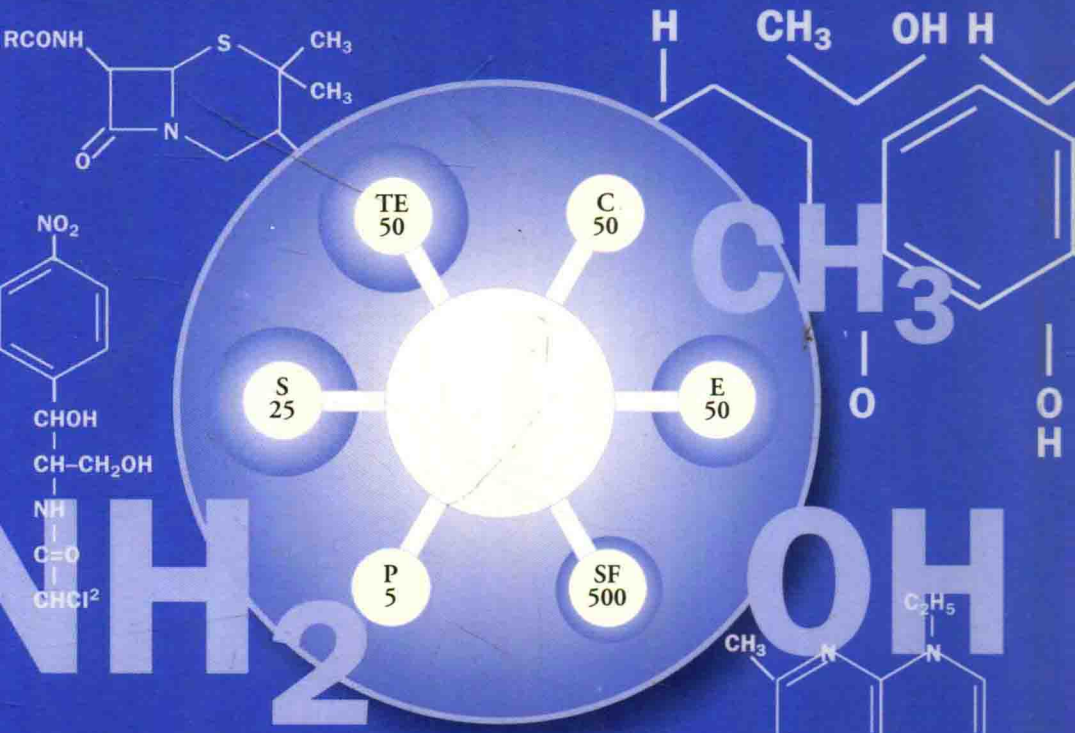
Antimicrobial Drug Action

R.A.D. Williams, P.A. Lambert & P. Singleton



A *Medical Perspectives* Book

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BIOS SCIENTIFIC PUBLISHERS

First published 1996

A CIP catalogue record for this book is available from the British Library.

ISBN 1 872748 81 3

BIOS Scientific Publishers Ltd
9 Newtec Place, Magdalen Road, Oxford OX4 1RE, UK
Tel. +44 (0)1865 726286. Fax. +44 (0)1865 246823
World Wide Web home page: http://www.Bookshop.co.uk/BIOS/

DISTRIBUTORS

Australia and New Zealand
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Victoria 3132

India
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4325/3 Ansari Road, Daryaganj
New Delhi 110 002

Singapore and South East Asia
Toppan Company (S)PTE Ltd
38 Liu Fang Road, Jurong
Singapore 2262

USA and Canada
BIOS Scientific Publishers
PO Box 605, Herndon, VA 22070

Typeset by Florencetype Ltd, Stoodleigh, Devon, U.K.
Printed by Information Press Ltd, Oxford, U.K.

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Abbreviations

ABC	ATP-binding cassette
AC	acetyl transferase
AD	adenyl transferase
ANS	6-anilino-1-naphthalenesulfonic acid
CAT	chloramphenicol acetyltransferase
CHO	Chinese hamster ovary
CNS	central nervous system
CSF	cerebrospinal fluid
DAB	diaminobutyric acid
DFMO	DL-α-difluoromethylornithine
DHF	dihydrofolic acid
DHFR	dihydrofolate reductase
DHPS	dihydropteroate synthetase
DNA	deoxyribonucleic acid
DON	diazo-oxo-norleucine
dsDNA	double-stranded deoxyribonucleic acid
dTMP	deoxythymidine monophosphate
EDTA	ethylenediaminetetracetic acid
EF	elongation factor
EP	enzyme–product complex
ES	enzyme–substrate complex
FP	ferriprotoporphyrin IX
G6PD	glucose 6-phosphate dehydrogenase
K_i	binding affinity of an inhibitor
Km	Michaelis constant
LTA	lipoteichoic acid
MDR	multidrug resistance
MIC	minimum inhibitory concentration
M_r	molecular mass
mRNA	messenger RNA
MRSA	methicillin-resistant *Staphylococcus aureus*

NADPH	nicotinamide adenine dinucleotide phosphate
$NADPH^+$	oxidized form of NADPH
NAG	N-acetylglucosamine
NAM	N-acetylmuramic acid
NPN	1-N-phenylnaphthylamine
ODC	ornithine decarboxylase
pABA	p-aminobenzoic acid
pAS	p-aminosalicylic acid
PBP	penicillin binding protein
PEP	phosphoenolpyruvate
poly (U)	poly-uridine
PT	phosphotransferase
rbs	ribosome binding site
RNA	ribonucleic acid
rRNA	ribosomal RNA
SDS	sodium dodecylsulfate (sodium lauryl sulfate)
SDS–PAGE	sodium dodecylsulfate–polyacrylamide gel electrophoresis
THF	tetrahydrofolic acid
tRNA	transfer RNA
UDP	uridine diphosphate
Und-P	undecaprenyl phosphate
Und-P-P	undecaprenyl pyrophosphate
UV	ultraviolet
V_{max}	maximum velocity

Preface

The chemical and intellectual background to the era of effective antibacterial therapy was established at around the turn of the 20th century. The early chemotherapeutic drugs were toxic and had to be used with great care. The discovery of the sulfa drugs was a revelation and a stimulus in the 1930s and 1940s but then the naturally occuring antibiotics were discovered and hailed as wonder drugs. Since then many classes of antibiotics have been exploited and significant new families of chemotherapeutic synthetic drugs, especially the quinolones, have been developed. The mechanisms of action of most of these compounds have been worked out in some detail.

The progressive development of resistance to these drugs has been noticed, often soon after the first use of particular antibiotics. The rate of increase in resistance varies from compound to compound, and in some cases strategies have been developed to combat this problem. This has included production of modified forms of the original drug. However the progress of resistance has, at best, been slowed down.

There is now considerable concern that the stock of natural antibiotics that remain to be discovered is running out. These circumstances raise the prospect that infectious diseases may reassert themselves as major causes of sickness and death even in affluent countries.

In such a climate it seems critically important that students understand how antibiotics produce their lifesaving effects, and how the microorganisms may defeat them.

R.A.D. Williams

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Chapter 1

Chemotherapy of microbial infections

1.1 Introduction: infections and the history of chemotherapy

1.1.1 Microbial infections

The tissues, body fluids and surfaces of humans and animals provide suitable environments in which micro-organisms may grow. These include the prokaryotic bacteria (formerly known as eubacteria), but not the prokaryotic archaea (formerly known as archaebacteria). Pathogenicity is the ability of a micro-organism to cause disease in its host. In addition to the bacteria, pathogens also include a wide range of eukaryotes, such as fungi, yeasts (Chapter 6) and protozoa (Chapter 7) as well as noncellular 'organisms', the viruses. These last are infectious particles composed of either DNA or RNA, enclosed in protective coats called capsids. The viruses need the materials, enzymes and the synthetic apparatus of a cellular host in order to complete their life cycles.

Many micro-organisms are harmless commensals, including much of the normal microbial flora of the genitalia, the gastrointestinal tract and the skin. The density of micro-organisms varies, feces containing the highest density, at 10^{11} cells g^{-1} (this includes 400 identified species). The total number of micro-organisms carried by man probably exceeds the number of cells in the human body. Some of the normal microbiota of man may be beneficial, if only because they occupy niches and so deny these to more dangerous micro-organisms. However, some members of the normal flora may cause damage to the host over a relatively long period of time, as in the case of dental caries and periodontal disease. Some individuals may carry micro-organisms of proven pathogenic potential for many years without showing any symptoms of disease. The AIDS crisis has dramatically demonstrated

the role of the immune system in controlling infections by micro-organisms that are not usually pathogenic in the healthy individual, but which become pathogenic when the immune system is suppressed.

There are a number of features that protect humans and animals from infection of the tissues and body fluids. The immune system normally responds to invasion of the tissues, and copes adequately with many infections. The enzyme lysozyme occurs in tissue fluids and oronasal secretions, where it hydrolyzes the peptidoglycan (Section 3.2) of the bacterial cell wall, and so helps to control the colonization of the body by lysozyme-sensitive bacteria. Sensitivity to this enzyme is governed by whether or not it has access to the peptidoglycan. Other features that minimize the microbial colonization of human tissues include the maintenance of rather low concentrations of the essential nutrients for micro-organisms in human tissue fluids. For example, iron is avidly bound to proteins such as lactoferrin and transferrins [1], and is not available to most bacteria in this form. Some pathogens have developed the ability to procure iron from the iron-binding complexes of the body. Thus, neisseria have receptor proteins for the iron transporters, and hemolytic bacteria such as the hemophili acquire iron from hemoglobin, and also from heme complexed with haptoglobin and hemopexin [2].

1.1.2 Virulence

Virulence is defined differently in different systems [3], but generally comprises infectivity (the capacity to colonize the host) and also the severity of the disease caused in the host. Highly virulent strains cause high morbidity and perhaps high mortality. Virulence depends on the interaction between the virulence determinants of the parasite and the defensive properties of the host. As the host develops its antibodies against the infectious agent by clonal selection, the parasite may simultaneously evolve in order to avoid the immune response. Trypanosomes, *Neisseria meningitidis*, *Salmonella typhimurium* and *Streptococcus pyogenes* are all adept at evolving within the host in this way [4].

1.1.3 The prevalence of infectious diseases and the importance of infections in the past, at present and in the future

The infectious diseases have been major causes of human death throughout history. Major epidemics have occurred periodically and have sometimes caused substantial reductions in population. For example, the great plague of 1664 claimed 15–21% of the population of London. Family sizes were large in Western Europe within living memory, and it was not uncommon for several children in a family to die of infection in one year. The breakdown of societies as a result of wars, famines, economic failure and political upheaval has often been

associated with epidemics of infectious diseases, and this continues to be the case.

Improvements to the supply of drinking water and the disposal of sewage have probably made the greatest contribution to the reduction of infections. The era of freedom from the fear of death caused by infection is both recent and brief, as is the ability to prevent and cure infections, at least in affluent societies. In a short period of time the belief that death due to infection is a thing of the past has become widespread. Unfortunately, this happy era may not last. About half of the major companies in Japan and the USA reduced or stopped their antibacterial drug development programs in the late 1980s, and as a result there are few new drugs that can be used in practice in the forseeable future [5]. The resurgence of infectious diseases due to the development of resistance to the available drugs is a real threat to the health of even the most affluent societies.

1.1.4 Development of chemotherapeutic drugs against infections

The earliest known chemotherapeutic agents were of plant origin. The Greeks employed extract of male fern to treat worm infestations, and extracts of cinchona bark were used by South American Indians to treat malaria. The first materials that were not of plant origin which were used chemotherapeutically included mercury, for the treatment of syphilis. Until the beginning of the twentieth century such preparations were the only chemotherapeutic agents available.

At the turn of the century, Ehrlich developed the concept of cell-surface receptors as an extension of the differential staining of tissues by dyes used in histology. Receptor sites on cells recognize particular chemicals, which bind to them, sometimes with great avidity. Ehrlich argued that various cells might have distinct receptors to which different chemicals would bind selectively. He reasoned that toxic chemicals which bound to receptors on pathogens, but not to the host's cells, would be ideal drugs for the treatment of infections. Ehrlich's arsphenamine (Salvarsan, an arsenical drug) was used to treat syphilis until it was superseded by penicillin in the 1940s. Research on dyestuffs as specific chemotherapeutic agents led to the development of the 'sulfa drugs' or sulfonamides (Section 2.3).

In 1928, Fleming observed that colonies of the fungus *Penicillium* inhibited the growth of certain bacteria on plates. Ten years later, Chain and Florey proved that purified penicillin inhibited bacteria infecting mice as well as in cultures *in vitro*. In 1941 the effectiveness of penicillin was demonstrated in man, and the era of antibiotic chemotherapy had begun. The search for other antibiotics was undertaken in many centers, and a great many substances with very diverse

structures and modes of action have been discovered. Some of these have been utilized in the chemotherapy of infections in humans and animals.

The introduction of drugs that combat bacterial infections has been a development without parallel in medicine, but it did not occur overnight. The sulfonamides were introduced in 1936, penicillin in 1941 (after its accidental discovery), followed by streptomycin in 1945 (after the first purposeful screening), cephalosporin in 1955, rifampicin in 1959, nalidixic acid in 1962 and cephamycins in 1972.

1.1.5 The problem of resistance

The number of antimicrobial agents available was recently so large that it seemed likely that at least one of them would be suitable for all infections. This suitability has been reduced and may be lost altogether by the development of resistance. The wide range of available choices may cause confusion and result in the prescription of drugs that are inappropriate. The tendency to overprescribe is due to the use of antibiotics to satisfy the expectation of patients that they should receive an antibiotic, the use of antimicrobial drugs without sound evidence that a bacterial infection actually exists, and the use of such preparations without proof that a bacterial infection is sensitive to the drug being prescribed, as well as their prescription for viral infections in order to 'cover against secondary bacterial infections'.

The major problem with overprescription, apart from the waste of money involved, is that the more the microbial population is exposed to any inhibitory drug, the more likely it is that resistance will develop. Therefore, unnecessary prescribing should be reduced so that established drugs may retain their effectiveness. A more complex issue is the nonmedical use of antibiotics. Huge amounts of drugs have been included in the diet of farm animals in order to improve the conversion rate (of food into animal products, i.e. eggs, milk or meat). The improved food production may benefit mankind generally, and it has certainly benefited the agriculture industry, but if there is a major resurgence of infectious disease it may prove to have been a profound mistake.

The cost of drugs is a major problem in counteracting resistance. Tuberculosis is ideally treated with three or four drugs over a 2-month period. This is not financially possible in many countries, where treatment may take place over a shorter period, involving fewer drugs and without the support of microbiology laboratory facilities to determine sensitivity [5].

Related to these concerns about the continued effectiveness of drugs is the worry that many of the available antimicrobial drugs that exist in nature may already have been discovered. The more recently discovered

drugs may also be more expensive, and yet not necessarily more effective, than were the earlier drugs before resistance to them developed. It has been suggested that the pharmaceutical industry and governments should make more funds available for research in this area, and that they should publicize drugs under development, but not yet under license, that might be used in the event of an emergency [6].

1.2 Some definitions

Chemotherapy is the study and practice of the use of drugs that inhibit or kill an invading species while causing minimum damage to the host. Since this definition, by Ehrlich, the term chemotherapy has been applied to the treatment of infection by unicellular and multicellular organisms, and also to the drug treatment of cancer and viral infections. Only those agents that are effective against micro-organisms will be considered here, but the principle of selective toxicity is the basis of all these forms of chemotherapy. This principle aims to exploit differences between the biochemistry of host tissues and that of the infectious agents, or differences between normal cells and cancer cells. Ideally, the host's biochemical processes of metabolism and biosynthesis should be unaffected by chemotherapeutic drugs. In practice, the ratio of therapeutic to toxic effects, representing the degree of discrimination, should be as high as possible.

Sterilization is the destruction of all microbial life by chemical or physical means. Disinfectants include toxic chemicals such as phenols, formaldehyde and sodium hypochlorite, which may be used in places with dense microbial populations. Ethylene oxide vapor may be used to sterilize apparatus or rooms, and β-propiolactone is a chemical sterilizing agent for solutions, which breaks down by hydrolysis to form a nontoxic acid. Dry heat (150–160°C for 1 h) and moist heat (121°C for 15 min) are commonly used to sterilize equipment, and high-intensity γ-irradiation is used to sterilize disposable plasticware and needles.

Sanitizing agents, antiseptics or germicides (these terms are used interchangeably) may not sterilize completely, but they do reduce the microbial population to acceptable levels. They are milder than the disinfectants, and so are suitable for use on living tissue. They include dilute solutions of chlorinated phenols, some ionic detergents and the flavines used in wound dressings. Antimicrobial agents are those chemotherapeutic compounds, whether synthetic or natural, that are toxic to micro-organisms. Antibiotics are chemicals produced by micro-organisms (some bacteria, fungi and actinomycetes) that are highly toxic to other micro-organisms at low concentrations. Some antibiotics can be synthesized, but most are made by culturing the producing micro-organisms on a large scale under closely controlled conditions. Antibiotics represent the majority of the antimicrobial drugs, and are

Table 1.1: The use of major antibiotics in a large teaching hospital

Antibiotic	Amount used per year (kg)
Penicillins	121.0
Erythromycin	10.1
Cephalosporins	8.1
Aminoglycosides	2.1
Fusidate	1.4
Tetracyclines	1.3
Chloramphenicol	0.6

used in very large quantities. *Table 1.1* indicates the quantities of major antibiotics described in this book that were supplied by the pharmacy of The Royal London Hospital during a single year.

Some antibacterial agents are bactericidal, (i.e. they kill the bacteria), but often it is not necessary to kill all the infective micro-organisms during the treatment of disease. Bacteriostatic compounds prevent further growth of the bacteria, allowing the host's normal defense mechanisms to clear up the infection. The two types of action may not be clearly distinguishable because the test of whether bacteria are alive is their successful growth. It is important to remember that many chemotherapeutic agents do not eradicate all the infective organisms, but they allow the body defenses to do this, and thus normal immunological and phagocytic mechanisms must be working efficiently if such drugs are to be effective. It has become more important to be aware of this difference, now, at a time when increasing numbers of patients are immunocompromised. It is therefore important that patients complete their course of drugs in order to avoid the persistence of bacteria that have an enhanced likelihood of resistance.

1.3 Targets of drug action

The sites of action against which the chemotherapeutic antimicrobial drugs have been directed are diverse (*Table 1.2*), but the most widely useful drugs have been directed against the synthesis of peptidoglycan of the bacterial cell walls (Chapter 3) and bacterial protein biosynthesis (Chapter 5). Other significant targets include intermediary metabolism involving the folate coenzymes (Chapters 2 and 7), the biosynthesis of DNA and RNA (Chapter 4) and cell membranes (Chapter 6).

1.4 Sensitivity of bacteria and acquired and infectious resistance

Bacteria differ in their sensitivities to any particular antimicrobial drug, and the determination of sensitivity is important in the reduction