ANTIBIOTICS

A Survey of their Properties and Uses

ANTIBIOTICS:

A SURVEY OF THEIR PROPERTIES AND USES

Published by direction of the Council of The Pharmaceutical Society of Great Britain

LONDON:

The Pharmaceutical Press, 17 Bloomsbury Square, W.C.1

1952

ANTIBIOTICS:

A SURVEY OF THEIR PROPERTIES AND USES

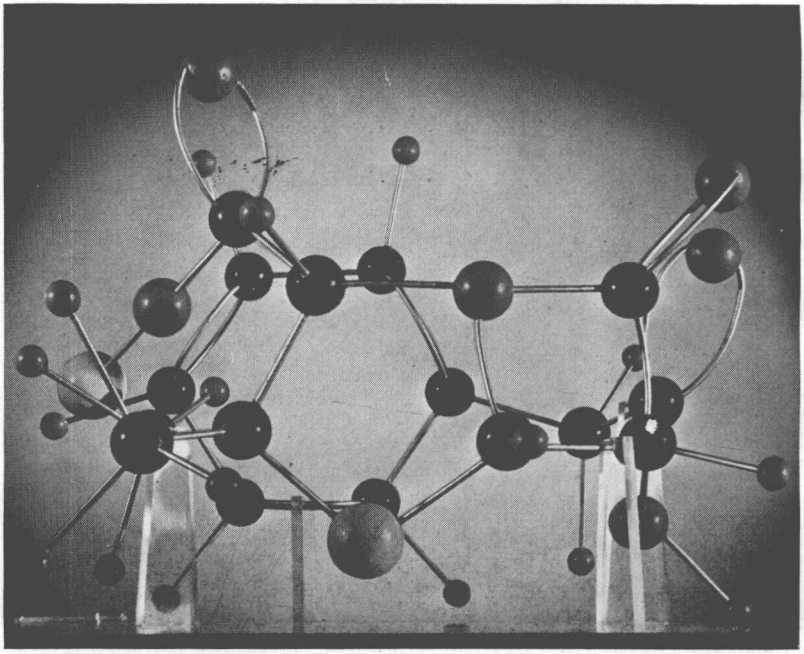

FIG. 1

Molecular Model of Sodium Benzylpenicillin

(Glaxo

Printed in Great Britain
by T. and A. Constable Ltd., Hopetoun Street,
Printers to the University of Edinburgh

PREFACE TO SECOND EDITION

The first edition of this book, issued under the title 'Penicillin: Its Properties, Uses and Preparations' in 1946, has been out of print for some months. A book on penicillin published soon after the antibiotic had become generally available must obviously be out of date some five or six years later and it was therefore undesirable to meet the continuing demand by reprinting. It was decided to rewrite the whole book so as to bring it up to date and to enlarge its scope by the inclusion of antibiotics other than penicillin.

In reaching this decision it was not overlooked that many books on antibiotics have been published. The first edition of this book was one of the earliest and a second edition has therefore, if only by reason of seniority, a claim to a place in the pharmacist's and medical practitioner's library. The real basis of such a claim is, however, that virtually none of the other books in this field covers quite the same ground. Few seem to be written for the non-specialist medical practitioner and hardly any for the pharmacist.

The welcome given to the first edition by doctors and pharmacists, and by reviewers in their professional journals, showed the need for a book written primarily to meet the requirements of those engaged in the general practice of each of the two professions. While practical aspects must be given priority, both types of reader need sufficient of the experimental and other background to enable them to prescribe and dispense antibiotics with full appreciation of what they are doing. This calls for the maintenance of a fair balance between theory and practice in regard to both medicine and pharmacy which it is the special aim of this book to give. The general availability of one or two of the newer antibiotics and of penicillin in particular has led to their extensive use in veterinary practice, and in this edition the requirements of veterinary surgeons and practitioners have been borne in mind as well as those of doctors and pharmacists.

It was obvious from the first that penicillin would not long remain the only antibiotic of practical value in therapeutics. Its success prompted research in a vast number of laboratories, both academic and industrial, throughout the world. The discovery of streptomycin, produced by a soil organism, led to the examination of many thousands of samples of soil from every corner of the globe and this work has resulted in the commercial production of chloramphenicol (now obtained synthetically), aureomycin, terramycin, neomycin and several others. The search has been carried out much more intensively in America than in the United Kingdom, and has been rewarded by

corresponding success. It is indeed fortunate that other useful antibiotics should continue to be isolated, thus giving grounds for confidence that even if resistance does develop in previously sensitive bacteria, the physician will still have a few arrows in his quiver with which to fight infections.

Unfortunately economic conditions in the world have prevented the general use in this country of several of the newer antibiotics of American origin. No doubt dollars would be provided if any of them were regarded as essential for the saving of life, but at present (May, 1952) penicillin, streptomycin, chloramphenicol and aureomycin are the only antibiotics available in more than experimental quantities in the United Kingdom. This book is therefore concerned almost entirely with these four substances. Some hundreds of substances with antibiotic properties have so far been described in the literature. A few are likely to come into general use and several have been considered worthy of clinical trial; notes have been included on some of these substances which the reader is likely to come across in the literature. As we go to press terramycin has become available for the treatment of a small number of diseases but experience of its use in the United Kingdom is as yet very limited.

Several hundred patents have been granted in the field covered by the book. While patent specifications often provide a great deal of useful information—indeed that is the *quid pro quo* given to the community by the inventor in return for his monopoly—in the case of antibiotics and particularly penicillin the number of patents is so large that it is extremely difficult, even for the specialist, to separate the good from the bad, and to know which claims are useful and which are not. Selection from the mass of material available has been essential but it is not claimed that the particular patents referred to in the book are necessarily the best in their various fields either in respect of the usefulness of the invention described or their strength as patents. One of the alterations in the law introduced by the Patents Act, 1949, in connection with the publication of specifications filed under the International Convention, has had the effect that information which used to be available within eighteen months of the filing of the application in a foreign country is now not available until considerably later. While therefore there is a surfeit of information on suggested procedures in the manufacture of penicillin, there is very little available about the manufacture of its newer competitors.

In the first edition some indication was given of the standards and assay procedures adopted by the Food and Drug Administration of the United States of America. The control exercised in America over the quality of antibiotics and their innumerable preparations is now very extensive and is specified in great detail. While it is interesting to compare the tests and standards used on the two sides of the Atlantic, the

information would be of little or no practical value to most of those for whom this book is intended, and as it would need far more space than is available, even to summarise the requirements, practically no reference to American standards is included.

In the Preface to the previous edition it was pointed out that no one person could hope to follow more than a fraction of all the work published on penicillin. This is still true today, more than five years later. There is still much to be learned from the study of penicillin and its derivatives, and the output of research papers on that subject is continuing at a high level. The position is the same with streptomycin, chloramphenicol and aureomycin. The task of preparing this edition has therefore been divided and several experts on different aspects of antibiotics have contributed the chapters dealing with their respective specialities. Nevertheless an attempt has been made to reduce as far as possible the overlapping of subject matter, the sometimes conflicting opinions and the changes of style which tend to irritate the readers of most books having heterogeneous authorship. Thanks are due in particular to S. J. Edwards, D.Sc., F.R.C.V.S., who contributed the chapter on antibiotics in veterinary practice, to A. Kekwick, M.A., F.R.C.P., who contributed the account of the clinical uses of the antibiotics, to F. A. Robinson, M.Sc., Ll.B., F.R.I.C., who contributed the chapter on their chemistry, to G. Sykes, M.Sc., F.R.I.C., who wrote the chapter on standards and assay, and to B. J. Thomas, M.P.S., who dealt with the pharmacy and pharmaceutical preparations of antibiotics. Thanks are also due to Glaxo Laboratories Ltd., who have regularly provided abstracts of patent specifications relating to antibiotics, and to a number of manufacturers for the loan of photographs.

The abbreviations used for weights and measures are in accordance with those of the British Pharmacopœia, 1948; i.u. has been used as an abbreviation for international unit. Temperatures are expressed in degrees centigrade.

May, 1952

PREFACE TO FIRST EDITION

No drug so innocuous and yet so potent as penicillin has hitherto been available to the medical profession. Yet the circumstances of its introduction were such as to surround it with numerous taboos which it was more easy to create than it has been to dispel. Perhaps the most extraordinary of many suggestions which were made was that the dispensing of the new drug should be undertaken by bacteriologists rather than by those trained in such work. The use of penicillin by the medical profession was limited to the favoured few; shortage of supplies made it necessary to distribute it at first to the Services only, then to hospitals only, and in order to avoid wastage it had to be used only for infections proved by bacteriological tests to be sensitive. These restrictions are no longer operative but the evil they have done, unavoidable at the time, lives after them. Many of the techniques developed, such as continuous drip infusion, are suitable only for hospital practice and cannot usually be employed by the general practitioner. The majority of published papers on penicillin relate to conditions normally treated in hospitals; and, from the pharmaceutical angle the belief is widely held that the dispensing of penicillin calls for some entirely new technique, an erroneous view which it is hoped this book will help to dispel.

Since the time when doctors were allowed to obtain penicillin for civilian patients only through local hospitals a continuous stream of enquiries has reached the Editor of *The Pharmaceutical Journal* seeking information on all aspects of the manufacture and use of the new drug and its preparations. To meet this demand material for a book was prepared a year ago, but before it could be sent to the printer it became evident that the successful development of the deep culture process would make it possible to remove the restrictions imposed by shortage of supplies and rapidly lead to such fundamental changes in all aspects of the medicine and pharmacy of penicillin as to render the proposed book out of date.

The general release of penicillin is a sign that supplies are now adequate for all civilian needs. Dosage schemes can be based on the needs of the patient and it is no longer necessary to use the drug only for those cases which are liable to prove fatal without it. The Pharmacopœia has given official recognition to the pharmaceutically obvious fact that aseptic technique is only necessary in making those preparations which are to be used aseptically. In consequence both doctors and pharmacists must now regard as obsolete much of the advice they have received from clinical publications based on work done before 1946.

Developments in the knowledge and application of penicillin will undoubtedly occur continuously and rapidly, but early changes of a fundamental nature, such as have resulted from the availability of ample supplies, are less likely to take place, with the possible exception of the greater use of oral administration. The time seemed therefore opportune for the publication of an up-to-date summary of our knowledge based on the needs of pharmacists and doctors. A great deal more is known about the chemistry of penicillin than can at present be disclosed. A possible explanation of this perpetuation of wartime secrecy is given in Chapter III, and a lifting of the ban may confidently be expected in the near future. Nevertheless it was clearly undesirable to postpone the issue of a publication dealing primarily with the practical medical and pharmaceutical aspects of penicillin until fuller chemical information became available.

In the interests of the patients whom both exist to serve, pharmacists and general practitioners need guidance now as to the best methods of preparing and using this remarkable new drug. It is the aim of this book to provide it. Practical considerations have throughout been foremost and therefore details of manufacturing procedures have been kept to the minimum necessary to enable the reader to take an intelligent interest in the materials he is called upon to handle. For the convenience of those who wish to go more deeply into any of the matters mentioned, references have been given for most of the statements made. The bibliography on penicillin is enormous; no one person can follow more than a fraction of all that is being published on the drug. Further, medicine is not an exact science and individual preferences exist for particular techniques. It is not claimed therefore that each reference quoted is invariably the most authoritative pronouncement on a subject. In the chapter on "Clinical Use" no attempt has been made to provide an account of surgical procedures which would not normally be undertaken by a general practitioner, but his attention has been drawn to the possibility often afforded by the proper use of penicillin of avoiding recourse to surgery.

While scientific facts are, fortunately, the same throughout the world, standards and legislation differ widely. Details have therefore been given wherever possible of American standards and legislation relating to penicillin so that the reader may compare the different practice prevailing on the two sides of the Atlantic.

Finally, thanks are due to a number of firms both in the United Kingdom and the United States of America for the loan of photographs.

October, 1946

CONTENTS

ILLUSTRATIONS

CHAPTER 1

Historical Summary

IT has been known for many years that some bacteria and moulds interfere with the growth of other micro-organisms and as long ago as 1877 Pasteur and Joubert[1], in describing experiments on the growth of anthrax bacilli, suggested that this antagonism might be of value for therapeutic purposes. Twenty-two years later Emmerich and Loew[2] reported that experimental anthrax could be cured by the local application of a liquid containing "pyocyanase", an enzyme produced in the culture medium in which *Pseudomonas pyocyanea* had been grown. A number of further suggestions for the therapeutic use of the antibacterial products of the metabolism of bacteria and moulds have been made from time to time but penicillin is the first product to be brought to full fruition and its outstanding success has marked the opening of a new epoch in the war against disease.

PENICILLIN

In spite of the hints afforded by earlier research the discovery of penicillin owes much to an accident which Florey[3] has described as "quite one of the luckiest accidents that have occurred in medicine, for, without exception, all other mould antibiotics so far examined are poisonous". The accident was described by Professor Sir Alexander Fleming of St. Mary's Hospital, London, in 1929[4]. In the course of an investigation into the destruction of bacteria by leucocytes, an agar plate on which staphylococci were growing became contaminated by an airborne mould which was subsequently identified as *Penicillium notatum*. This is not a common species of *Penicillium*; it was first discovered on hyssop by Westling in Norway. Fleming observed that in the neighbourhood of the mould the colonies of staphylococci were undergoing lysis or dissolving.

In order to investigate this action the mould was subcultured and grown in a peptone broth medium. The filtered medium was found to inhibit the growth of certain bacteria even when diluted 800 times, and it was to this medium, after filtration, that Fleming applied the name "penicillin". At room temperature the activity was lost after ten to fourteen days but it could be preserved longer by neutralisation. Boiling for a few minutes did not destroy the activity, but boiling for an hour in alkaline solution markedly decreased it, and heating in an autoclave at 115° for twenty minutes caused complete destruction.

The antibacterial action was stated to be very marked on pyogenic cocci and on organisms of the diphtheria group (see p. 152), whereas the coli-typhoid group and the influenza bacillus were found to be insensitive to it. The medium was no more toxic to animals than the original broth and did not appear to damage leucocytes. In the very few instances in which it was applied to septic indolent wounds it was found to have a beneficial effect, Fleming reporting that "it certainly appeared to be superior to dressings containing potent chemicals". He pointed out that the substance greatly facilitated the isolation of Pfeiffer's bacillus and went on to say that "it may be an efficient antiseptic for application to, or injection into, areas infected with penicillin-sensitive microbes". At the same time it was recognised that the instability of penicillin caused difficulties both in regard to extraction and therapeutic application.

Following up Fleming's work, chemists under Raistrick at the London School of Hygiene and Tropical Medicine endeavoured to isolate the active principle[5]. They used a so-called "synthetic" medium —that is, a medium containing substances of known chemical composition and excluding proteinaceous materials which cause difficulty in extraction procedures. Their work demonstrated the instability of penicillin under the conditions they used. Concentration of the culture medium at 40° under reduced pressure resulted in complete loss of activity, and, while the active substance could be extracted by ether from the acidified culture medium, inactivation occurred when the ether was evaporated in the air. The difficulties seemed to make it unlikely that penicillin had any significant future. Fleming still continued to use media containing penicillin for the isolation of penicillin-insensitive organisms, such as *Hæmophilus pertussis*, which causes whooping-cough, and the influenza bacillus of Pfeiffer[6,7], but apart from this the substance failed to attract attention. To some extent, no doubt, this neglect was due to reports on the sulphonamides which began to appear in 1933. These compounds gave medical practitioners more potent weapons than any they had previously used against streptococcal and other infections, and it is not surprising that the existence of an even more effective, but unproved, antibacterial substance should have remained unnoticed.

In 1938, Florey, the Sir William Dunn Professor of Pathology in the University of Oxford, and his colleagues were nearing completion of an investigation into the properties and mode of action of lysozyme, the natural antibacterial substance present in tears and widely distributed in nature, which was also discovered by Fleming. Their interest in the problem of natural immunity led them to draw up a plan for a comprehensive investigation of antibacterial substances produced by bacteria and fungi. Florey states[8] that the organisms known to produce antibacterial substances which it was proposed to investigate were

eventually narrowed down to two, *Ps. pyocyanea* and *P. notatum*. Three antibacterial substances which were obtained from *Ps. pyocyanea* were found to be toxic, whereas penicillin was found to have no toxic effects. In spite of the difficulties which the work of Raistrick and his colleagues had shown to surround the investigation of pencillin, the Oxford workers were attracted to this substance largely by the fact that it differed from almost all other antibacterial substances in being active against staphylococci. They quickly made one fundamental advance on Raistrick's observations. Whereas Raistrick had shown that activity was lost when an ethereal solution of penicillin was evaporated, Florey and his colleagues found that the activity passed back into aqueous solution if the ethereal solution were shaken with an aqueous solution of an alkali. This made it possible to effect appreciable purification by alternating transference to aqueous and organic solvents.

An essential step towards rapid progress was an assay process which did not take the time nor need the care of the usual serial dilution method. This step was successfully taken by Heatley (see Chapter 5, p. 78) and it was then seen that penicillin was an organic acid, soluble in organic solvents. Aqueous solutions were found to be stable only between pH 5 and 7. By extraction with ether and subsequent transference to dilute alkali, an aqueous solution containing the alkali salt of the acid was obtained, loss of activity being avoided by keeping all solutions cold. On freezing the aqueous solution and evaporating the water from it while in the frozen state, the first crude preparation of pencillin was obtained.

The preparation probably contained less than 1 per cent of penicillin, yet in a dilution of 1 in 500,000 it inhibited the growth of staphylococci and thus its activity was comparable with that of other known antibacterial substances. This fact led the Oxford team to believe that they had obtained almost pure penicillin. Yet this impure product could be injected in large doses into mice with hardly any signs of toxicity. Its chemotherapeutic effect in experimental animals was soon demonstrated, and in the first paper on penicillin from the Oxford workers[9] it was reported that the crude substance which they had so far succeeded in obtaining was effective in remarkably high dilution in protecting mice against streptococci, staphylococci, and *Clostridium septique*.

A year elapsed before the results of trials in human beings were reported[10]. A man weighs 3000 times as much as a mouse, and Florey has mentioned that months of labour were required to produce enough penicillin to treat the first human case[3]. The injection was followed by a considerable reaction, but the patient, suffering from a mixed staphylococcal and streptococcal infection, improved dramatically in a few hours. Unfortunately there was insufficient material available to continue the treatment and the patient died. Nevertheless, the potential

value of the new drug had been proved. Purification by chromatography was found to remove the pyrogenic material responsible for the reaction which occurred in the first patient, and when further supplies were available—some of which were recovered from the urine of treated patients—it was quickly demonstrated that in penicillin the medical profession had a drug more effective and far less toxic than the sulphonamides, one that was not affected by blood, pus, or the products of tissue autolysis, and moreover, one which was as effective against staphylococcal infections *in vivo* as it was *in vitro*.

Streptomycin

In contrast to the accidental discovery of penicillin, streptomycin was the result of a long and painstaking search for an antibiotic which would be active against gram-negative bacteria.

The organism which produces streptomycin is *Streptomyces griseus*, a member of the widely distributed group of micro-organisms known as actinomycetes. The antibacterial activity of a large number of organisms of this group was known as long ago as 1921, when Lieske[11] showed their ability to effect the lysis of certain dead and living organisms. In 1939 an extensive investigation was begun in the Department of Microbiology of the New Jersey Agricultural Experimental Station, Rutgers University, into the production of antibacterial substances by the actinomycetes. By the time the first reports on the clinical use of penicillin were published in 1941, a number of antibiotics had already been isolated and their properties investigated, but none of them appeared suitable for chemotherapeutic use, chiefly because of their toxicity. As penicillin seemed likely to be effective only against gram-positive organisms, the search was concentrated on antibiotics likely to be active against gram-negative organisms.

In 1942, Waksman and Woodruff[12] reported the isolation of streptothricin from a culture of *Actinomyces lavendulæ*. This substance was relatively stable and quite active against numerous gram-negative and some gram-positive organisms, but its early promise of emulating penicillin was not fulfilled; it was found subsequently to possess a residual toxic effect in animals. However, in September, 1943, cultures were obtained from *Actinomyces griseus* which appeared to contain a highly promising antibiotic. Profiting from the experience gained in the isolation of streptothricin, Schatz, Bugie and Waksman[13] were able to announce in January, 1944, the isolation of a new antibiotic which they called streptomycin, a name derived from the generic name of the producing organism; a revision of the classification of the *Actinomyces* by Waksman and Henrici in 1943 had resulted in the generic name being changed to *Streptomyces*.

Streptomycin is similar to streptothricin, both chemically and anti-

bacterially, but it has a wider antibacterial spectrum and lower toxicity. Clinical trials[14] soon established that streptomycin was effective against many pathogenic bacteria, including *Mycobacterium tuberculosis*, which were resistant to penicillin and the sulphonamides.

Dihydrostreptomycin

Investigations[15,16,17] into the chemical structure of streptomycin led to the discovery in 1946 that two atoms of hydrogen could be added to the molecule by catalytic hydrogenation and clinical trials on the resulting dihydrostreptomycin suggested that it was as effective as the parent substance against *Myco. tuberculosis* and that its toxicity was lower when prolonged treatment was required. Further experience has not confirmed that the toxicity is lower (see p. 118).

Aureomycin

Following the isolation of streptomycin investigations continued into the production of antibiotics by the actinomycetes and in 1948 Duggar[18], working in the Lederle Laboratories Division of the American Cyanamid Co., announced the discovery of an antibiotic from a new species of this group of organisms. Because of the yellow colour which developed in the fungus during its growth and the golden-yellow colour of the antibiotic, the name *Streptomyces aureofaciens* was given to the organism and the antibiotic was called aureomycin.

Bacteriological and pharmacological studies soon established that a potentially valuable chemotherapeutic agent had been discovered. It was found to be effective when given orally against numerous gram-negative and gram-positive organisms, as well as against some rickettsial and viral infections, and to possess a low toxicity. It was soon being manufactured in large quantities in America, but because of currency difficulties only small quantities were available in this country for some time and its use has been restricted by the Ministry of Health to certain diseases in which it is more effective than other antibiotic and chemotherapeutic substances.

Chloramphenicol

The discovery of this antibiotic was first reported[19] in 1947 by a group of workers in the research laboratories of Parke, Davis & Co. at Detroit, U.S.A., in conjunction with a botanist from Yale University. It was obtained from a hitherto unknown species of *Streptomyces* isolated from a sample of soil from a field in Venezuela. A similar organism had been isolated[20] from a compost soil from a farm in Urbana, U.S.A., by a group of workers at the University of Illinois, and they too observed