Findlay's RECENT ADVANCES in CHEMOTHERAPY

Volume III

Antibiotics

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PREFACE

At the time of his sudden death in the spring of 1952 the late Dr. G. M. Findlay had made much progress with a further volume of the series *Recent Advances in Chemotherapy.* In that and a subsequent one it was proposed to describe the sulphonamides and antibiotics and their use in the treatment of bacterial, rickettsial and viral infections. We would like to express our thanks to Mrs. Findlay for allowing us to use the extensive material he had accumulated, including abstracts of many papers.

Rapid progress is being made in chemotherapy, and when the present authors were asked to take this work over in the autumn of 1952 it seemed preferable to complete this edition with a single volume devoted to antibiotics. In this book we have therefore tried to describe the progress which has been made in recent years in the treatment of bacterial, rickettsial and viral infections, and it has been our aim throughout to provide the clinician with a reliable and up to date summary of modern methods of treatment, while outlining the fundamental principles which should govern the use of antibiotics in practice.

Our indebtedness to previous writers on the subject and to our colleagues will be obvious. In particular we would like to acknowledge the help we have derived from "Antibiotics" (Florey, Chain, Heatley, Jennings, Sanders, Abraham and Florey, 1949) and from Lady Florey's companion volume, "The Clinical Application of Antibiotics—Penicillin." To Professor L. P. Garrod we are greatly indebted for advice on design of this book and for reading much of it in manuscript. We have received much help from Professor C. F. Barwell, Dr. G. W. Hayward, Dr. E. J. L. Lowbury, Dr. H. B. May, Dr. C. S. Nicol, Dr. C. Ogilvie, and Dr. Kenneth Perry who have also read chapters in preparation. One

of us (R.A.S.) would like to thank Mr. J. L. Thornton, Librarian, St. Bartholomew's Hospital Medical College, for his invaluable help with references.

We are also grateful to the Editor of *Medicine Illustrated* for permission to reproduce Figs. 2, 3, 4 and 5, which have appeared in that journal.

Any book of this nature runs the risk of being out of date before it is in print, and our thanks are due to the Publishers not only for their courtesy and assistance, but for their efforts to avoid this danger.

F.C.O.V.
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CHAPTER I

PENICILLIN

In 1929 Fleming reported the accidental contamination of a culture plate in his laboratory with a mould from which a substance diffused lysing adjoining staphylococcal colonies, and from this chance observation there has developed a huge industry devoted to the manufacture of what is still the least toxic and in many ways the most effective antibiotic. The story of penicillin is so well known that it need receive only the most brief mention. Fleming suggested that penicillin might be useful as a local application to infected wounds, and attempts were made by Raistrick and his colleagues to isolate an active principle, but were eventually abandoned (Clutterbuck *et al.*, 1932). Little more was done until the problem was taken up again by a group of workers at Oxford in the course of a systematic investigation of natural substances with an antibacterial action. Their brilliant researches culminated in the publication by Abraham, Chain, Fletcher, Florey, Gardner, Heatley and Jennings (1941) of a full account of the methods of preparation and of the bacteriological and pharmacological properties of penicillin, together with a description of the results following its use in the treatment of six patients.

This work which was conceived before the war, received a very considerable impetus when the potential value of penicillin in the treatment of war injuries became apparent, and it was soon clear that under wartime conditions the manufacture of the amount of penicillin required would be beyond the capacity of British firms. It was for this reason that two members of the Oxford team visited the United States of America to enlist aid in the manufacture of penicillin, and from that date the initiative in the manufacture of penicillin

and other antibiotics has largely remained in the hands of American pharmaceutical firms. A great deal has been written about the early days of penicillin and the reader is referred in particular to the monograph Antibiotics, published by the Oxford team in 1949 (Florey *et al.*, 1949).

It was soon evident that penicillin was not a single substance, but a mixture of several, and for a period some confusion arose from the different terminology adopted on the two sides of the Atlantic. This difficulty has now been resolved, and the nomenclature in present use is shown in Table I.

Table I

VARIETIES OF PENICILLIN

Original designation		*Systematic name*
British	*American*	
I	F	Pent-2-enylpenicillin
—	dihydropenicillin F	*n*-amylpenicillin
II	G	Benzylpenicillin
III	X	*p*-hydroxybenzylpenicillin
IV	K	*n*-heptylpenicillin

These different penicillins vary in their activity against bacteria, and in the ease with which they are made. Benzylpenicillin has been found to produce the most satisfactory results in practice, and modern commercial penicillin consists of benzylpenicillin (penicillin G) in the form of its sodium or potassium salt.

As the output of penicillin has increased, so has it increased in purity, and the original dark brown and then yellow preparations have been replaced by the present white crystalline preparation. This, according to some authors, has not been all gain, and there is a certain amount of evidence that some impurities had themselves antibacterial properties. It is now in retrospect a tangled story in which the activities of the various forms of penicillin, of their impurities, and of the buffering effect of impurities on the stability of penicillin

are all involved. Wherever the truth may lie there is no clear clinical proof that the activity of modern commercial preparations would be enhanced by the addition of substances removed during their manufacture.

The activity of later antibiotics has been defined in terms of weight, but for penicillin the unit was initially adopted, and despite some attempts to displace it with the microgramme, it has so far remained in general use. According to Florey *et al.* (1949), it was originally described as: "that amount of penicillin which when dissolved in 1 ccm. of water gives the same inhibition as . . . (a certain partly purified) standard (solution) (Abraham *et al.*, 1941) or, "the amount of penicillin contained in 1 ml. of a certain phosphate buffer solution containing ether" (Garrod and Heatley, 1944). The present International Unit of penicillin is defined as the specific penicillin activity contained in 0·6 microgramme of the International Penicillin Standard.

The stability of penicillin depends more on its moisture content than on any other single factor, and when the moisture content is less than 0·5 per cent, crystalline sodium penicillin can be stored at room temperature for two to three years without any appreciable loss of potency. It is much less stable in solution. Deterioration occurs slowly at 4° but is accelerated by a rise in temperature, and solutions kept at room temperature suffer a variable but considerable loss of potency after twenty-four hours. This loss is affected by a number of factors amongst which the reaction of the solution is important, penicillin being most stable at a *p*H of 6 to 6·5, and it may be partially prevented by dissolving penicillin in a buffer solution. A similar effect was noticed with yellow penicillin in which some of the impurities were thought to exert a buffer action. Weak solutions are more stable than concentrated ones, and traces of copper, zinc, and mercury are harmful. High concentrations of alcohol cause loss of activity, but dilute solutions are ineffective, and no harm is likely to follow the use of alcohol for cleaning the tops of containers holding penicillin for injection, or for cleaning the

skin. Various chemical substances such as oxidizing agents, glycerine, propylene glycol and cysteine may inactivate penicillin, and this has been shown to be true for some samples of rubber tubing (Antibiotics, 1952). If a preservative is needed 0·5 per cent w/v of phenol is most commonly employed. It must *not* be used in preparations for intrathecal injection.

Distribution and excretion of penicillin

Following the injection of a soluble preparation the penicillin content of the blood increases rapidly and then falls more slowly. The rate of this fall is chiefly determined by the rate at which penicillin is removed from the blood via the kidneys, and as the capacity of the normal kidney for this purpose is practically unlimited, increasing the size of the dose results in an increase of the peak level reached in the blood, but only a relatively slight increase in the time during which penicillin remains there. Doubling the dose does not double the duration. Fig. 1 presents diagrammatically the average result obtained with a variety of doses.

Once in the blood some penicillin is bound to protein and inactivated, but most remains active, and in this active form diffuses through the body and is finally excreted in the urine. Diffusion continues as long as the blood concentration exceeds that of the tissues, and there is evidence that penicillin remains in the tissues after it can no longer be detected in the blood. Not all parts of the body are equally accessible, and very little of the drug penetrates into brain or nervous tissue, bone, the chambers of the eye or reaches sweat, tears or maternal milk (Welch and Lewis, 1951). In the absence of inflammation very little penicillin enters the cerebro-spinal fluid or pleural fluid, and, although inflammatory change increases the permeability of the lining membranes, the concentration needed for the treatment of meningeal infection, even with a sensitive organism, may only be obtained with massive intramuscular dosage. Peritoneal exudates and synovial effusions may contain relatively large amounts of

penicillin (Florey, 1952). Unlike the sulphonamides, penicillin is not inactivated by pus.

The remarkable rapidity with which penicillin is excreted by the kidneys is one of its most serious disadvantages, and from the start ways have been sought to reduce the frequent injections which are required if a constant level of penicillin in the blood is to be maintained. A number of possibilities have been explored, and these include the use of large doses of penicillin, repository preparations, attempts to raise the blood level by blocking the renal excretion of penicillin and the administration of penicillin by mouth.

The use of large injections of penicillin

In the early days of penicillin treatment the amount of penicillin prescribed was conditioned by the shortage of the drug. Now, although extravagance is to be deprecated, this restriction has been removed, and there has been a move towards larger doses at longer intervals. As will be seen from Fig. 1 doubling the size of a dose is not, unfortunately, accompanied by a doubling of the time during which penicillin can be found in the blood, and it is impracticable to provide detectable amounts of penicillin in the blood throughout the twenty-four hours of the day from a single injection of crystalline penicillin. This may be done, however, for the greater part of the twenty-four hours, by the twice daily injection of 500,000 units or more: how far it is desirable to keep penicillin present during the whole course of treatment will be discussed in a later section. An advantage of this method is that for a short time following injection very high levels of penicillin are present in the blood, and may provide the necessary head to enable penicillin to penetrate to the heart of diseased areas.

The use of repository forms of penicillin

Methods of delaying the absorption of penicillin from the injection site and so prolonging the time during which penicillin is discharged into the blood, date from the introduction

by Romansky and Rittman (1944) of a suspension of penicillin in oil and beeswax. This preparation provided detectable amounts of penicillin in the blood for up to twenty-four hours after a single injection of 300,000 units, and it was widely used, particularly for the treatment of venereal diseases. It was, however, difficult to give and was responsible for un-

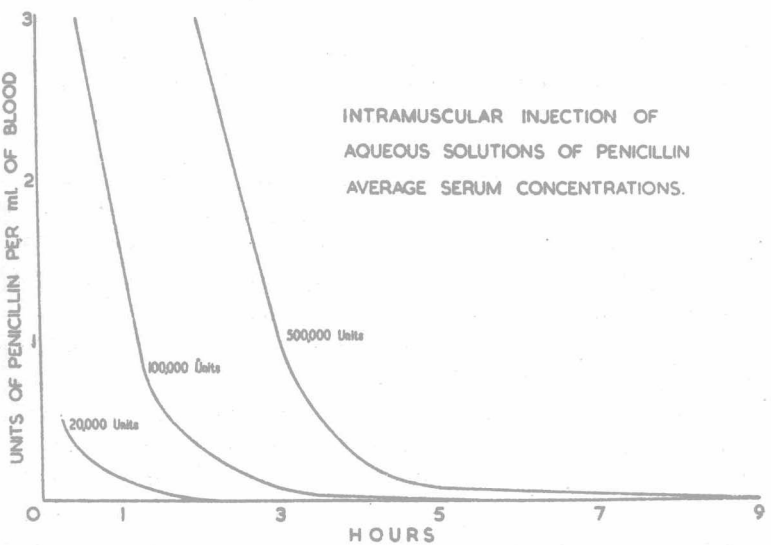

FIG. 1.

desirable foreign body reactions, and has now been almost completely superseded by procaine penicillin.

Procaine penicillin, first described by Salivar *et al.* (1948), and Sullivan *et al.* (1948), is a true salt formed by the equimolecular combination of its components. It has a solubility in water of only about 0·67 per cent and may be suspended in an oil or water vehicle, watery preparations being the easier to inject. Since the demonstration by Hobby *et al.* (1948) that procaine penicillin in oil produced long sustained serum levels in animals, numerous authors have shown that a comparable effect occurs in man. There are almost certainly

differences between the various commercial preparations, both in methods of manufacture and length of action, but in every case a single injection of 300,000 units should maintain some penicillin in the blood for the greater part of the twenty-four hours. The addition of aluminium monostearate to the oily preparation, as suggested by Buckwalter and Dickison (1948) will prolong absorption still further, and the same object may be achieved by increasing the amount of procaine penicillin administered.

The popularity of procaine penicillin increased rapidly, and at one time it appeared that for most purposes it would replace other forms. The likelihood of its doing so is now less certain. Persistence of penicillin in the blood can only be obtained by a sacrifice of blood concentration, and the levels which follow injections of procaine penicillin are always relatively low, and are not initiated by the high peaks seen when crystalline penicillin is used. When dealing with susceptible organisms in accessible situations this is probably immaterial, but organisms whether susceptible or not, in an inaccessible situation provide a somewhat different problem, and there is some evidence that in this circumstance the blood concentration following the use of procaine penicillin with aluminium monostearate is insufficient (Griffiths *et al.*, 1949). The production of these low levels has been countered by the addition of soluble penicillin to procaine penicillin and aluminium monostearate, thus providing an initial peak following injection, and in the treatment of staphylococcal sepsis a preparation of this kind has been shown to be more satisfactory than one of procaine penicillin and aluminium monostearate alone (Griffiths *et al.*, 1950). For these infections preparations of procaine penicillin alone are widely used, and appear to be satisfactory. An alternative method of dealing with this problem consists of increasing the frequency of injections. This has little to recommend it if only two or three daily injections are given, as the same number of injections of soluble penicillin will keep penicillin in the blood throughout most of the period of treatment, and in addition

will provide much higher levels immediately following injection. The suggestion of Romansky and Kelser (1952) that more frequent injections of procaine penicillin have a cumulative effect and enable really high levels of penicillin to be reached and held, may provide the most satisfactory method of treating some serious infections.

A number of other substances have been described which when combined with penicillin delay its absorption, but so far only one has come into general use. Szabo *et al.* (1951) showed that when N,N^1-dibenzylethylenediamine was combined with penicillin, the resulting preparation was very sparingly soluble in water. This substance was first tried in man by Elias *et al.* (1951) who found that following a dose of 2,500,000 units penicillin was present in the blood for as long as fifteen days after injection, an observation that has been supported in this country by the work of Fletcher and Knappett (1953).

Interference with the renal excretion of penicillin

Penicillin is mainly excreted through the renal tubules, and in the presence of nephritis and other lesions impairing renal function, excretion is retarded. This has been deliberately brought about by the administration of a number of drugs which are excreted through the tubules and presumably compete with penicillin. Diodrast was the first to be suggested (Rammelkamp and Bradley, 1943) and was followed by *p*-amino-hippuric acid (Beyer *et al.*, 1944). Carinamide (formerly caronamide) introduced and tested by Beyer and his colleagues (Beyer, 1947; Beyer *et al.*, 1947) was found to be a relatively non-toxic substance which could be given by mouth, and which would increase the serum concentration of penicillin between 1·5 fold and 12 fold (Florey, 1952). More recently benemid, a non-toxic proprietary compound, has been shown to be as effective in daily doses of 2 grams as daily doses of 24 grams of carinamide (Boger *et al.*, 1950). These preparations have been used in the treatment of infections due to resistant organisms, more perhaps to obtain

high penicillin levels than prolonged ones, but owing to the more abundant supplies of penicillin, the discovery of new antibiotics and the use of combinations of antibiotics, they have not been extensively employed.

Oral penicillin

When given by mouth the absorption of penicillin is irregular and unpredictable in all except infants, and has not been significantly improved by the use of a large number of different vehicles. This uncertainty of absorbtion has meant that the oral route has been used mainly for infections due to very susceptible organisms or for prophylaxis, and in these circumstances the results have been good. Success with its use has been reported in the treatment of pneumonia (Bunn *et al.*, 1945) hæmolytic streptococcal infections (Robinson *et al.*, 1948) and gonorrhoea (Meads and Finland, 1946) and it is the method of choice in the prevention of streptococcal infection of the throat, and if it is thought desirable, in the prevention of gonorrhoea (Eagle *et al.*, 1949). Binns (1953), in a review of the literature, recommends that oral penicillin should be given on an empty stomach, half an hour or more before meals. For an adult he suggests a dose of 400,000 units given four hourly, and preceded in seriously ill patients by one or more injections of sodium penicillin. Infants come in a class by themselves, as they absorb the drug more constantly and may therefore be treated with greater assurance. It is perhaps worth observing that some oral preparations are unpalatable, and that in children apparent treatment failure may be due to refusal to swallow the drug (Dixon, 1952 personal communication).

A new preparation of penicillin absorbed with regularity and capable of producing blood levels comparable with those following intramuscular injection, would be very valuable, and there is some suggestion that N,N^1-dibenzylethylenediamine di-penicillin may be such a substance. Cathie and MacFarlane (1953) found that when it was given in a dose of 300,000 units to 118 patients, 101 of whom were children, a

therapeutic blood concentration was obtained in every case after three hours, and when the dose was repeated at six hourly intervals a cumulative effect was seen. They nevertheless recommended an initial dose of intramuscular penicillin. If these results are confirmed this method of administration should save the time of nurses and doctors and spare the patient discomfort. A less satisfactory feature is that it may lead to self treatment, which so far with penicillin has been almost unknown in Great Britain.

Other preparations of penicillin

The Hydriodide of Diethylaminœthyl Ester of Penicillin G.

The properties of this substance which is marketed under a variety of trade names and was first prepared in Denmark, have been described by a number of authors (e.g. Heathcote and Nassau, 1951; Jensen, 1951; Flippin *et al.*, 1952). All were agreed that it has an affinity for lung and nervous tissue, and that higher concentrations are found in the lung and in the cerebro-spinal fluid than after comparable doses of other forms of penicillin. For this reason it has been widely recommended for the treatment of infections of the lung. The justification for doing so is so far a theoretical one and there are so far no accounts of controlled trials in which this substance has been shown to be more effective than procaine penicillin. Penicillin penetrates the lung very readily and providing the infecting organism is sensitive, the results are so good with any reasonable preparation that there may be little to be gained by increasing the concentration. Alternatively the findings of Hallas-Moller *et al.* (1952) may be relevant. These authors agreed that this ester had a considerable affinity for lungs and brain, but pointed out that esters are not usually active, and claimed that the normal methods of testing for penicillin allowed for hydrolysis of the ester in the test. If this was prevented, procaine penicillin gave a higher concentration of free penicillin. Their results are not denied by the advocates of this substance, who,