# REVIEW ARTICLE

## SOME CHEMICAL AND MEDICAL ASPECTS OF THE ANTIBIOTICS*

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There is no uniformity in the use of the word antibiotic, but we propose here to accept Waksman's definition that an antibiotic is a substance produced by one micro-organism which is capable of interfering with the growth of others[1]. This definition excludes synthetic drugs, such as the sulphonamides, isoniazid, and *p*-aminosalicylic acid, and also the antibacterial substances produced by higher plants.

The use of moulds for the local treatment of infected wounds has a long history in folk medicine, but these early remedies seem to have had little rational basis. Scientific observations on the inhibition of the growth of one micro-organism by the products of another were first made in the second half of the nineteenth century[2], and at that time there began the growth of the idea that such inhibitory products might be used, by injection into the blood stream, to combat systemic infections in man. A substance which is used in this way, as a systemic chemotherapeutic agent, must clearly be much more toxic to the infecting organism than to the cells of the human body, and it must retain its antimicrobial activity in the presence of the blood and body fluids.

During the first thirty years of the present century no chemotherapeutic agent which could safely be injected into the blood stream was obtained from a micro-organism. Ehrlich[3] and his school had striking success in the synthesis of drugs that were effective against diseases caused by protozoa, spirochaetes, or the malaria parasite, but their synthetic approach did not yield any compound which could be used to deal with bacterial infections. The view therefore arose that attempts to find substances with antibacterial activity but no significant toxicity to animals had little hope of success. This view was held when Fleming[4] discovered penicillin in 1929, and it may account for the fact that he did not try to establish whether the antibiotic could be used systemically in medicine. Penicillin thus remained scarcely more than a curiosity for ten years, but, during this time, the advent of the sulphonamides showed that the chemotherapy of systemic bacterial infections was indeed possible.

The investigations of Dubos and others in 1939 on tyrothricin[5], an antibacterial product of *Bacillus brevis*, were probably the first in which both the biological and the chemical properties of an antibiotic were carefully studied[6], but unfortunately tyrothricin was too toxic for systemic use. Soon afterwards, however, the work of Florey and Chain and their

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colleagues at Oxford[7,8] led to the discovery of the remarkable chemotherapeutic properties of penicillin. This discovery stimulated an intensive search by the pharmaceutical industry, especially by firms in America, for other antibiotics of therapeutic value. The American efforts have been rewarded, for most of the antibiotics now used in medicine, other than penicillin, have been isolated and characterised in the United States of America.

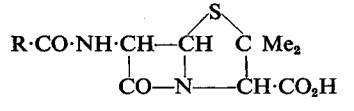
The present article will be mainly concerned with antibiotics of medical importance, but it should not be forgotten that this group of substances represents only a very small proportion of the vast number of antibiotics which have been detected, and that many of the antibiotics which do not have the exacting properties required of a chemotherapeutic agent are nevertheless of interest from a biochemical point of view.

Apart from their antimicrobial activity the antibiotics as a whole have little in common, but they can be classified, to some extent, on the basis of their biological or chemical properties, or the type of organism from which they come. We shall group them here according to their chemical structures, and it will be evident that some of the groups consist of families of closely related substances. In general, the order in which the various groups have been placed is the order in which they were discovered.

## Structural Features of some Antibiotics of Medical Importance

### The Penicillins and Polypeptide Antibiotics

The penicillins (I) are derivatives of a condensed dipeptide structure and may thus be grouped with other peptide antibiotics. The side-chain (R) of the penicillin molecule is variable, being $\Delta^2$-pentenyl in penicillin F, benzyl in penicillin G, *p*-hydroxybenzyl in penicillin X and *n*-heptyl in penicillin K. A large number of different biosynthetic penicillins, in which R·CO is derived from a substituted acetic acid, have been obtained by adding suitable precursors to the fermentation medium of *Penicillium chrysogenum* or *Penicillium notatum*[9].



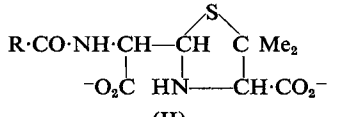
(I)

The collaborative Anglo-American work on the chemistry of penicillin during the last war[10] was followed by a period in which relatively little further progress was made. In recent years, however, there have been some new developments in this field. One of the biosynthetic penicillins (penicillin V) has been found to be much more stable than penicillin G in dilute acid solution and to be useful, in consequence, for oral administration[11]; a rational synthesis of penicillin V and certain other penicillins has now been developed[12]; a penicillin with a new type of side-chain and antibacterial activity, known as cephalosporin N[13] or synnematin B[14], has been discovered among the metabolic products of certain species of

*Cephalosporium*; and some light has been thrown on the way in which the molecule of penicillin G is formed by *Penicillium chrysogenum*[15].

*The Penicillin Structure*

Essential features of the structure of the penicillins are illustrated by the chemical behaviour of the penicilloates (II)[10], the products obtained when the penicillins are inactivated, by hydrolysis under mild alkaline conditions or in the presence of the enzyme penicillinase. When a solution of a penicilloate is treated with mercuric chloride a mercaptide is precipitated and one mole of carbon dioxide is liberated. The mercaptide can be decomposed with hydrogen sulphide to give the characteristic amino acid penicillamine. Penicillamine contains the sulphur atom of the penicillin molecule and belongs to the "un-natural" D-series.



(II)

The solution remaining after removal of the mercaptide contains an aldehyde, called penilloaldehyde, which is characteristic of the penicillin being studied. In general, the penilloaldehyde can be precipitated as its 2:4-dinitrophenylhydrazone. Oxidation of the penilloaldehyde with silver oxide yields an acid which can be hydrolysed with strong acid to give glycine, a product obtained from all penicillins, and substituted acetic acid which varies from one penicillin to another.

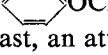
Consideration of these degradation reactions led to the proposal of a thiazolidine-4-carboxylate structure (II) for sodium penicilloate. Thiazolidine-4-carboxylic acids are readily formed by condensation of an aldehyde group with the nitrogen and sulphur atoms of an α-amino β-thiol acid such as penicillamine. Four stereoisomers of II, derived from D-penicillamine, are possible and by 1945 three of these isomers including one which is identical with the product obtained from penicillin, had been synthesised.

When these facts had been established it remained to formulate the structure of the penicillins by removal of the elements of water from the corresponding penicilloates. A number of physico-chemical arguments could be adduced in favour of the general structure I. The common penicillins have one carboxyl group but no basic group, while the penicilloates have two carboxyl groups and one weak basic group. The simplest way of accounting for the absence of a basic nitrogen atom in the antibiotic is to assume that a carboxyl group of penicilloic acid is condensed with the NH of the thiazolidine ring to give a four membered β-lactam ring. Today this may seem a plausible assumption, but when the β-lactam-thiazolidine structure for penicillin was first proposed in October, 1943[16], it was not generally accepted and other possibilities were given much attention. The question was only finally settled by the crystallographic X-ray studies of Crowfoot and Low[17], which showed

that the relative positions of the atoms in the molecule was consistent only with structure I.

*Synthesis of Penicillins*

In spite of the relative simplicity of the penicillin molecule, a satisfactory synthesis of a natural penicillin was not announced until the spring of 1957[12]. Workers both at Oxford and in America had undoubtedly synthesized benzylpenicillin in minute yields (0·1 per cent) during 1944[10], but a great deal of effort on both sides of the Atlantic failed to increase the efficiency of the process used. The main cause of the failure of attempts to form a $\beta$-lactam thiazolidine ring system by cyclisation of penicilloic acids lay in the fact that the latter readily underwent an alternative type of cyclisation characteristic of *N*-acylated $\alpha$-amino acids: a carboxyl of penicilloic acid condensed with the C=O group of the side chain to give a five-membered azlactone (oxazolone) ring.

Sheehan and Henery-Logan have now shown that this difficulty can be avoided by use of the penicilloic acid corresponding to phenoxymethylpenicillin (penicillin V)[12]. Phenoxymethylpenicillin, one of a number of biosynthetic penicillins first obtained in the Lilly Research Laboratories[9], was found by Brandl and Margreiter in 1954 to be unexpectedly stable to acid[11]. It would seem that the structural features of the side-chain which are responsible for this acid stability also confer on the corresponding penicilloate a resistance to azlactonisation. By the action of di*cyclo*hexylcarbodi-imide on potassium phenoxymethylpenicilloate (II, R =  —) in aqueous solution at room temperature a $\beta$-lactam ring was formed and phenoxymethylpenicillin (penicillin V) (I, R =  —) was obtained in a yield of from 15 to 20 per cent. In contrast, an attempt to synthesise benzylpenicillin by the same method gave yields of only about 0·1 per cent.

During an extensive investigation of the problems of penicillin synthesis Sheehan and his colleagues have discovered methods for making new types of penicillin which have not yet been found in nature. These synthetic penicillins contain the fused $\beta$-lactam thiazolidine ring system of the natural products, but their side-chains are not derived from a substituted acetic acid. One of the new types contains a benzylsulphonamido (I, R·CO— is replaced by R·$SO_2$) and another a carbamido group (I, R·CO— is replaced by RO·CO—) in place of the phenylacetamido group of benzylpenicillin[18].

*Cephalosporin N*

In 1953 a new type of penicillin was discovered which was named cephalosporin N[19]. This antibiotic is produced by a fungus of the genus *Cephalosporium* which had been sent to Oxford from Sardinia[20]. It is distinguished from the natural and biosynthetic penicillins hitherto encountered by its marked hydrophilic character and by its antibacterial activity. It could not be extracted into any organic solvent other than liquid phenol and it is significantly more active than benzylpenicillin

against *Salmonella typhi* but very much less active against *Staphylococcus aureus*[21].

Cephalosporin N is inactivated by penicillinase and it yields the characteristic amino acid, D-penicillamine, on hydrolysis with acid. However, another amino acid, D-α-aminoadipic acid, is also found in the acid hydrolysate. Aminoadipic acid is an uncommon aminodicarboxylic acid and, although the L-isomer was known to occur in nature, the D-isomer had not previously been found.

Cephalosporin N, like other penicillins, could be degraded to D-penicillamine, carbon dioxide, and a penilloaldehyde. The penilloaldehyde, unlike that from the common penicillins, formed a 2:4-dinitrophenylhydrazone which was water-soluble and therefore failed to precipitate from solution. It was evetually identified, however, by oxidation to an acid which was isolated in crystalline form. This acid is a dipeptide of α-aminoadipic acid and glycine and was shown by degradation and synthesis to be D-δ-amino-δ-carboxyvalerylglycine (III)[22]. The aldehyde

$$R{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO_2H$$

(III)

is therefore D-δ-amino-δ-carboxyvalerylaminoacetaldehyde and cephalosporin N is (D-4-amino-4-carboxy-*n*-butyl) penicillin

$$(\text{I, R} = \overset{+}{H_3N}\!\!\diagdown\!\!\underset{-}{O_2C}\!\!\diagup\!\!CH{\cdot}(CH_2)_2CH_2\text{—})^{23}.$$

This structure, with its zwitterionic side-chain, accounted for the hydrophilic character of the antibiotic.

While work on cephalosporin N was in progress at Oxford, an antibiotic produced by *Cephalosporium salmosynnematin*, which was called synnematin B, was being studied in the U.S.A. The chemical nature of this antibiotic had not been determined, but consideration of the published data suggested to us that the substance might well be very similar to cephalosporin N and therefore a hydrophilic penicillin[24]. In 1955 samples of cephalosporin N and synnematin B were exchanged and it was agreed that the two substances were identical[25]. Synnematin B was found to be a useful chemotherapeutic agent for the treatment of typhoid fever in man[26], but economic difficulties have so far hindered its production on a commercial scale.

*Cephalosporin C*

Examination of partially purified cephalosporin N revealed that it contained a small amount of another hydrophilic antibiotic which was named cephalosporin C. This antibiotic resembles cephalosporin N in some of its chemical and biological properties, but not in others. Cephalosporin C, like cephalosporin N, contains sulphur and a residue of D-α-aminoadipic acid that is linked to the rest of the molecule through its δ-carboxyl group. Unlike cephalosporin N, it is relatively stable in

dilute acid at room temperature and is not inactivated by purified preparations of penicillinase. Its ultra-violet absorption spectrum ($\epsilon$ max at 260 m$\mu$) shows that it contains a chromophore which is not present in the normal penicillins. It yields no penicillamine on hydrolysis with acid, although it does yield valine after the sulphur atom has been removed by treatment with Raney nickel, and this indicates that it differs from the penicillins in the thiazolidine-$\beta$-lactam portion of the molecule[27].

Structural studies on cephalosporin C are not yet complete, but there can be little doubt that the substance is related, biogenetically, to cephalosporin N. Two of its biochemical properties are in harmony with this interpretation of the chemical evidence. Although not destroyed by penicillinase, it is a competitive inhibitor of the enzyme[28] and it induces the formation of the enzyme by *Bacillus cereus*[29].

Cephalosporin C shows about 0·1 per cent only of the activity of benzylpenicillin against many Gram-positive bacteria, but it is virtually non-toxic to animals and its insensitivity to penicillinase endows it with a higher activity than that of benzylpenicillin against penicillinase-producing strains of staphylococci. In adequate doses it gave complete protection to mice infected with a penicillin-resistant strain of *Staph. aureus*[30]. This antibiotic might thus find a place in medicine if the problem of producing it economically on a large scale could be overcome.

*Biogenesis of Penicillin*

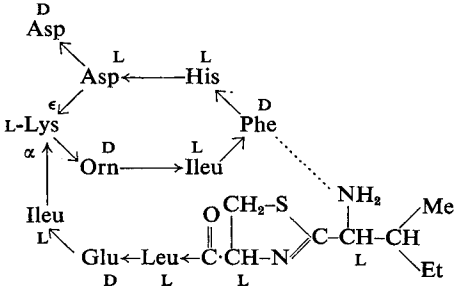
Inspection of the thiazolidine-$\beta$-lactam structure of the penicillins suggests that it is built up from two amino acids. Work with isotopically labelled amino acids has shown that the nucleus of benzylpenicillin is formed, in *Penicillium chrysogenum*, from an intact residue of L-cysteine and from the carbon skeleton, at least, of valine[31,32]. Since much of the valine added to the culture fluid undergoes deamination, or transamination, the origin of the nitrogen of the $\beta$-lactam ring is still uncertain, but it may be that L-cysteinylvaline is an intermediate. The present evidence is consistent with the view that the penicillin nucleus is synthesised first and is then acylated with phenylacetic acid. It has recently been suggested that the formation of the nucleus involves the $\alpha\beta$-dehydrogenation of the valine fragment of an intermediate $\beta$-lactam, and the subsequent addition of a thiol group to the double bond[33].

Cephalosporin N, with its amino acid side-chain, has a more obvious peptide nature than the common penicillins, and it may be regarded, perhaps, as a link between the latter and the larger polypeptide antibiotics. Like the penicillins, these polypeptides do not consist of open chains of amino acid residues but contain one or more ring systems. Most of them are too toxic to the kidneys to be injected into man, but two of them—bacitracin and polymyxin—have had a limited systemic use.

*Bacitracin and Polymyxin*

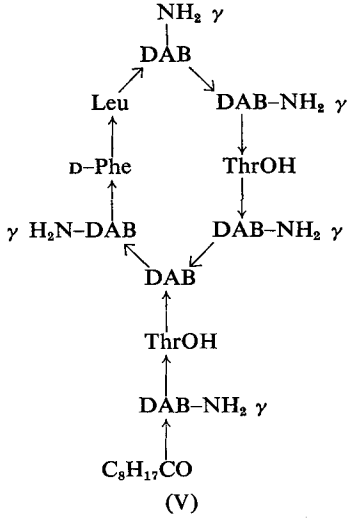
Bacitracin is the name given to a family of sulphur-containing polypeptides formed by *Bacillus licheniformis*[34,35]. The main peptide, bacitracin A, which has two acidic and three basic groups and yields a free

thiol group on mild hydrolysis with acid, has been assigned the provisional structure IV[36,37]. It has at least two ring systems, both of which are of an unusual type. One ring, made up of six amino acid residues, contains lysine linked through the ε-amino group of its aliphatic side-chain, and a branch from this ring arises from the α-amino group of the lysine. The second ring is a thiazoline which appears to have been formed by condensation of both the thiol and the amino group of a cysteine residue with the carboxyl-carbon of an *N*-terminal *iso*leucine residue (IV)[38]. Bacitracin A is the first protein-like substance to be discovered in which a masked thiol group can be attributed to the presence of a thiazoline ring.

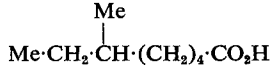



(IV)

(The arrows represent C–N bonds and the dotted line symbolises an interaction whose nature is uncertain. An amide group is probably located at D-Asp.)




(V)



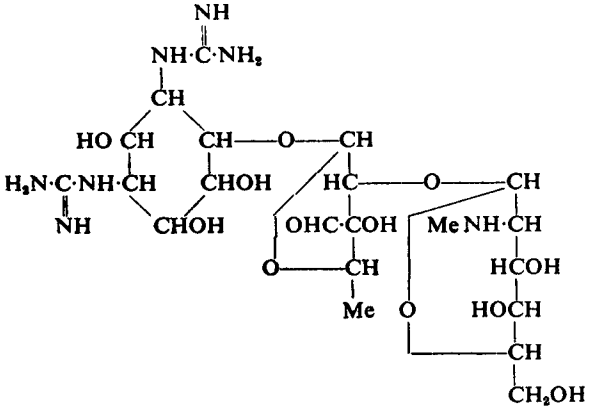

(VI)

Polymyxin $B_1$, a member of a family of polypeptides produced by *Bacillus polymyxa*, probably has the structure V[39]. It has no free carboxyl group, but contains five basic groups owing to the fact that five of its six residues of α,γ-diaminobutyric acid (DAB) have one of their $NH_2$ groups free. The sixth residue of DAB has both its α- and its γ-amino group bound, and forms a bridge between a ring and a side chain comparable

to that formed by lysine in bacitracin A. Another unusual feature of the structure of polymyxin $B_1$ is the *N*-acylation of the terminal DAB residue by the rare $C_9$ fatty acid, 6-methyloctanoic acid (VI). A residue of an *iso*octanoic acid is found in this position in another member of the polymyxin family known as polymyxin $B_2$.

## The Streptomycins

In 1939 Waksman and his colleagues, who had made an extensive study of the actinomycetes, began to search among these organisms for antibiotic-producing strains[1]. After some initial disappointments they discovered streptomycin[40], and this substance quickly became established in medicine. Streptomycin, which is produced by *Streptomyces griseus*, is complementary to penicillin as a chemotherapeutic agent, since it is highly active against many Gram-negative bacteria and against mycobacteria.



Streptidine Streptobiosamine *N*-Methyl-L-glucosamine

(VII)

Streptomycin has the properties of both a strong base and a sugar, its basicity being accounted for by two guanidino groups and an *N*-methylamino group. It also contains a free aldehyde group, the reduction of which to an alcoholic group by catalytic hydrogenation results in the formation of dihydrostreptomycin.

Work on the structure of streptomycin[41] by several groups of organic chemists showed that the molecule could be dissected into three components which all had uncommon features. On treatment with methanolic hydrochloric acid the antibiotic was readily degraded to a strong base, streptidine, and a disaccharide, streptobiosamine, which was a moderately weak base. Streptidine was shown to be a diguanidinocyclitol, and since it was optically inactive it was a *meso*-form of this compound.

Streptobiosamine (see VII) was isolated as methylstreptobiosaminide dimethyl acetal. On further hydrolysis with acid, followed by acetylation of the products of hydrolysis, the acetal yielded a penta-acetyl hexosamine. Removal of the acetyl groups from the latter gave a compound

which was identified as *N*-methyl-L-glucosamine (see VII). The first hint of the nature of the remaining part of streptobiosamine was furnished by the isolation of the $\gamma$-pyrone, maltol, from the products obtained by heating streptomycin with hot alkali for a few minutes. The presence of another six-carbon fragment in the streptomycin molecule was thus clearly established. This fragment, which was named streptose, contained the aldehyde group of streptomycin and its conversion to maltol involved a carbon-carbon rearrangement.

The ease with which streptomycin was cleaved with acid strongly suggested that the streptidine, *N*-methyl-L-glucosamine and streptose fragments were joined by glycosidic linkages. After a great deal of careful work the positions of these linkages were determined and streptomycin was shown to have the structure VII.

A second antibiotic, resembling streptomycin, was found in crude material from *Streptomyces griseus*. This substance was isolated by counter-current distribution and shown to contain a residue of D-mannose in addition to the usual products of hydrolysis of streptomycin itself. It was therefore named mannosidostreptomycin. The D-mannose residue was shown to be glycosidically linked to carbon atom 4 of the *N*-methyl glucosamine residue[42].

It has been suggested that streptomycins are formed which are of the mannosido type but which have polysaccharides, in place of a simple hexose, attached to *N*-methylglucosamine[43]. If this turns out to be so, it may help to throw light on the relationship of the biogenesis of streptomycin to some of the essential metabolic processes of *Streptomyces griseus*.

## The Neomycins

Neomycin[44], which is produced by *Streptomyces fradiae*, is active against a wide range of Gram-positive and Gram-negative bacteria and also against *Mycobacterium tuberculosis*. Neomycin appears to resemble streptomycin more closely than do the other antibiotics considered here, but it can easily be distinguished from streptomycin by its chemical and antibacterial properties. Thus, strains of *Mycobacterium tuberculosis* which are sensitive to both antibiotics remain sensitive to neomycin after they have become streptomycin-resistant[45]. Unfortunately, neomycin is too toxic to be used as a systemic chemotherapeutic agent, but it is used extensively for local application, and, in conjunction with other antibiotics, for pre-operative sterilisation of the gut[46].

Crude neomycin is a complex which has been shown to consist of two isomers called neomycins B and C[47]. These substances are bases, but, unlike streptomycin, they do not contain guanidino groups or give a maltol reaction. Neomycins B and C both have the molecular formula $C_{23}H_{46}N_6O_{12}$. When cleaved by methanolysis they yield the same base, neamine ($C_{12}H_{25}N_4O_5$), and the methyl glycoside of different disaccharides called neobiosaminide B and C respectively. The two neobiosamines are diaminohexosides of D-ribose and differ only in the diaminohexose portion of the molecule.

The partial structure VIII has been proposed for the neomycins[48].

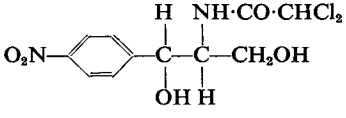
$$C_{12}H_{25}N_4O_5—O—C_5H_7O(OH)_2—O—C_6H_7O(OH)_2(NH_2)_2$$

(VIII)

## Chloramphenicol

Chloramphenicol is not only quite different chemically from the antibiotics already described but also has a wider range of action against micro-organisms. Gram-positive bacteria, Gram-negative bacteria, and rickettsiae (which cause diseases such as epidemic typhus) are susceptible to its action. The term "broad spectrum antibiotic" has been coined to describe antibiotics of this type. Chloramphenicol, which is produced by *Streptomyces venezuelae*, was the first member of the type to be discovered[49,50]. Its isolation, characterisation and synthesis were carried out at the Parke Davis Research Laboratories.

Chloramphenicol ($C_{11}H_{12}O_5N_2Cl_2$) is a neutral substance with a characteristic ultra-violet absorption spectrum due to the presence of a *para*-substituted nitrophenyl group[51]. It is hydrolysed by alkali to dichloroacetic acid and an optically active base. The base yields *p*-nitrobenzaldehyde, ammonia and formaldehyde on treatment with periodate, whereas chloramphenicol itself does not react with periodate. Further investigation showed that the side-chain belonged to the *threo* configurational series. It could then be deduced that chloramphenicol was (−)-D-*threo*-2-dichloroacetamido-1-*p*-nitrophenylpropane-1 : 3-diol (IX)[51]. The validity of this structure was confirmed by synthesis[52].



(IX)

The synthetic (−)-D-*threo* isomer of IX was identical with the natural product, but the (+)-L-*threo* isomer had only 0·5 per cent of the antibacterial activity of the D-isomer and the two *erythro* forms were inactive.

The total synthesis of chloramphenicol represented the first efficient synthesis of an antibiotic of medical importance. There followed a period of great activity in the preparation of analogues of chloramphenicol. The antibacterial activity of many of these analogues has not been given, but it appears that changes in the aliphatic side-chain cause a drastic reduction in activity and changes in the aromatic portion of the molecule a smaller reduction. No analogue prepared so far has proved to be superior to the parent compound as a chemotherapeutic agent[53].

## The Tetracyclines

Soon after the clinical value of chloramphenicol had been established two more broad spectrum antibiotics of great importance appeared in medicine. These substances were aureomycin, discovered in 1948 by a research team of the Lederle Laboratories[54], and terramycin, discovered

about a year later by workers at Chas. Pfizer Inc.[55] They were found to be active not only against bacteria and rickettsiae but also against some of the larger viruses, such as those responsible for psittacosis and lymphogranuloma venereum. Aureomycin ($C_{22}H_{23}O_8N_2Cl$) is produced by *Streptomyces aureofaciens* and terramycin ($C_{22}H_{24}O_9N_2$) by *Streptomyces rimosus*. It soon became evident, from a consideration of their chemical and physical properties, that these two antibiotics were closely related[56]. Both are amphoteric substances which contain two acidic groups (forming part of two conjugated enolic systems) and one basic group. The two nitrogen atoms can be accounted for as $-N(CH_3)_2$ and $-CONH_2$ groups respectively and one carbon is present as C-methyl. Other data suggest that both antibiotics contain a core, $C_{18}H_9O_4$, to which similar functional groups are attached, but that aureomycin contains a chlorine atom which appears as an OH group in terramycin.

*Terramycin*

Early attempts to degrade terramycin showed that, unlike streptomycin, it could not be split easily into a number of well defined fragments. It was found later, however, that a relatively simple procedure could be used to throw light on the nature of the $C_{18}$ nucleus. When terramycin was exposed to the prolonged action of zinc and acetic acid, the dimethylamino group and one oxygen atom were removed to give desdimethylaminodesoxyterramycin. Treatment of the latter with acid under anhydrous conditions removed two molecules of water to give a crystalline red substance, called desdimethylaminoterrarubein, which was fully aromatic, and distillation of the terrarubein from zinc dust yielded the parent hydrocarbon, naphthacene[57]. The formation of this hydrocarbon was clearly consistent with the assumption that terramycin itself had a similar tetracyclic structure. The complete structure of terramycin[58] (X, R = H, R′ = OH) was eventually arrived at by skilful deductions based on the properties of a large number of degradation products, most of which had undergone molecular rearrangement during the degradation process. Much use was made of careful analyses of the absorption spectra of the two chromophoric centres in the molecule, and of the spectra of suitable model compounds. Subsequently, the results of degradations of aureomycin[57] were interpreted in the light of the structure suggested for terramycin[59].

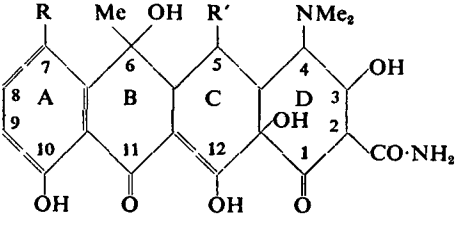
*Aureomycin*

The structural investigations on aureomycin confirmed the suggestion that this antibiotic only differed from terramycin in containing a chlorine atom instead of a hydroxyl group. It was realised at an early stage, however, that the chlorine atom in aureomycin and the corresponding hydroxyl in terramycin were located at different positions on the carbon skeleton[60]. A clue to the placing of the chlorine atom was the formation of 5-chlorosalicylic acid when aureomycin was fused with alkali. Salicylic acid, derived from the aromatic ring A of X, was obtained under similar conditions from terramycin. On the basis of this and other evidence the

chlorine atom of aureomycin was placed at C(7) and the antibiotic was assigned the structure X, R = Cl, R′ = H[57,59].

*Tetracycline*

Catalytic hydrogenation of aureomycin removed the chlorine atom and led to a product (X, R = H, R′ = H) which is now known by the generic name tetracycline[59–62]. Subsequently tetracycline was found to occur naturally in the culture fluid of a *Streptomyces*[63]. This substance may be regarded as the parent from which both aureomycin and terramycin are derived: aureomycin is a chlorotetracycline and terramycin an oxytetracycline. Tetracycline is similar to aureomycin and terramycin in biological activity and is itself used in medicine[64].




(X)

*Demethyltetracyclines*

A mutant of *Streptomyces aureofaciens* has recently been found which produces a tetracycline without a methyl group in the C(6) position (6-demethyltetracycline). The same mutant can also produce 7-chloro-6-demethyltetracycline when grown in the presence of chloride.

The demethyltetracyclines are very much more stable under acidic and alkaline conditions than the parent compounds, yet they retain most of the antibacterial activity of the latter[65]. If the demethyltetracyclines can be produced economically it is conceivable that they may replace the tetracyclines that are now in use.

## The Macrolides

By 1950 most bacterial infections could be controlled either by the well established antibiotics, such as penicillin and streptomycin, or by the newer broad spectrum antibiotics. However, as time went on, the need was felt for substances which would be effective against strains of staphylococci that were resistant to penicillin or the tetracyclines. There followed an intensive search for chemotherapeutic agents active against Gram-positive bacteria and this revealed, among others, a large group of interesting antibiotics called the macrolides. These antibiotics, of which erythromycin is perhaps the best known, are found in the culture fluids of *Streptomyces*. Their range of activity is similar to that of penicillin, but they are active against penicillin or tetracycline-resistant strains of micro-organisms[66].

The term macrolide was coined to express the fact that each member of this group of substances has a large lactone ring. The macrocyclic lactone

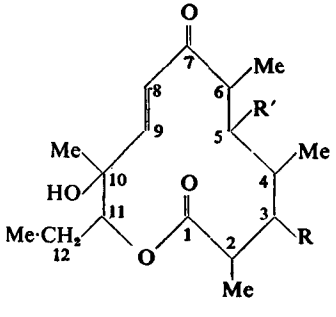
which contains from 12 to 17 carbon atoms, is linked glycosidically to one or more dimethylamino sugars. In some cases other pyranose sugars are linked to the lactone ring or to the dimethylamino sugar.

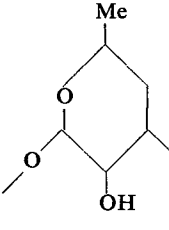
Table I shows the molecular formulae and the types of sugar that the various macrolides contain. Methymycin[67] and pikromycin[68,69] are isomers having very similar structures, and the foromacidines[76] and the spiramycins[77] have been shown to be identical. Several of the known macrolides contain the same dimethylamino sugar, desosamine, and at least two others contain a closely related dimethylamino sugar, mycaminose.

TABLE I

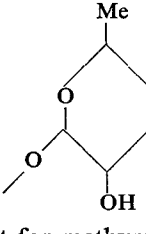
| | *Streptomyces* Sp. | Molecular formula | Dimethylamino sugar | Other sugar | Macrolide ring | |
|---|---|---|---|---|---|---|
| | | | | | Name | No. of atoms |
| Methymycin[67] | M-2104 | $C_{25}H_{43}O_7N$ | Desosamine | None | Methynolide | 12 |
| Pikromycin[68,69] | | $C_{28}H_{43}O_7N$ | ,, | ,, | Kromycin | 12 |
| Narbomycin[70] | *narbonensis* | $C_{28}H_{47}O_7N$ | ,, | | | |
| Griseomycin[71,78] | *griseolus* | $C_{28}H_{48}O_6N$ | ,, | | | |
| Oleandomycin[72] | *antibioticus* | $C_{35}H_{61}O_{12}N$ | ,, | Oleandrose | | |
| Erythromycin[73] | *erythreus* | $C_{37}H_{67}O_{13}N$ | ,, | Cladinose | Erythronolide | 14 |
| Magnamycin[74,75] | *halstedii* | $C_{42}H_{67}O_{16}N$ | Mycaminose | Mycarose | | 17 |
| Foromacidines[76] } Spiramycins[77] } | *ambofaciens* | $C_{48}H_{82}O_{16}N_2$ | Mycaminose and another[81] | Mycarose | | |
| Angolamycin[79] Miamycin[80] | *eurythermus ambofaciens* (?) | $C_{49-51}H_{87-91}O_{18}N$ | | | | |

The foromacidines (or spiramycins) contain a second dimethylamino sugar, 5-dimethylamino-6-methyl-2-hydroxytetrahydropyran[81]. There is more variation in the nature of the non-nitrogeneous sugar component than in the dimethylamino sugar. Cladinose, found in erythromycin[73], and mycarose, in magnamycin[74] and foromacidine[76], are new sugars. But oleandrose, obtained from oleandomycin[72], had previously been found in the cardiac glycoside oleandrin[82].




(XI) (R = 

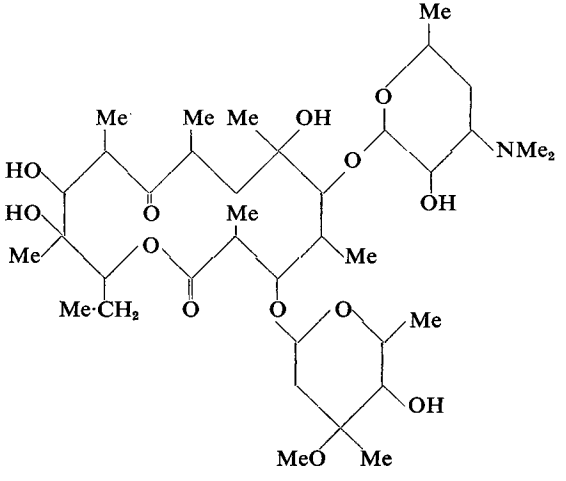
, R′=H)

(XII) (R=H, R′= 

)

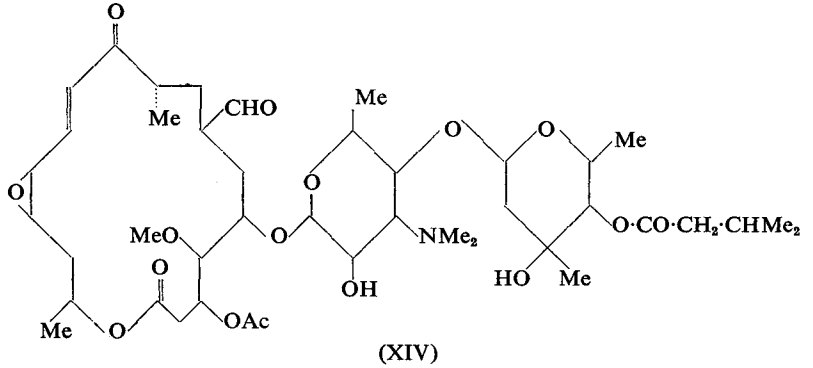
Complete structures have now been worked out for methymycin[67,83,84] (XI), pikromycin[68,69] (XII), erythromycin[85,86] (XIII) and magnamycin[87–89]

(XIV). The first two substances contain twelve membered lactone rings while the other two contain a fourteen and a seventeen membered ring respectively. Methymycin has a desosamine residue glycosidically linked to the lactone ring (called methynolide) at C(3). Neomethymycin[90], a



(XIII)

minor component of crude methymycin, has a hydroxyl group at C(12) instead of that at C(10) in methymycin. Pikromycin appears to differ from methymycin in that the desosamine is linked to C(5) of the methynolide. Erythromycin contains desosamine and also cladinose, each sugar being linked separately to the erythronolide core. Erythromycin B[91], found in



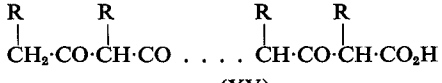
(XIV)

the mother liquor from the crystallisation of erythromycin, differs from the latter in having no hydroxyl substituent at C(12). Magnamycin, on the other hand, contains the dimethylamino sugar, mycaminose, linked directly to the macrolide ring and another sugar, mycarose, which is linked glycosidically to the mycaminose. The mycarose is acylated with an *iso*valeryl residue. Magnamycin B[92], which is found as a minor component in the mother liquors from the crystallisation of magnamycin,

differs from the major component in having a double bond between C(14) and C(15) in place of an epoxide ring.

The non-nitrogenous sugar which is found (in addition to the dimethylaminosugar) in erythromycin and magnamycin can be removed by mild methanolysis, leaving compounds which have been named erythrolosamine and carimbose respectively. Much more drastic conditions are required to remove the desosamine or mycaminose fragment from erythrolosamine or carimbose, since the dimethylamino group, which is positively charged in acid media, has a protective effect on the glycosidic linkage. Indeed, the amino sugar can be removed hydrolytically from erythromycin and magnamycin only under conditions which result in the break up of the acid-labile nucleus. Reduction of the C(9) carbonyl group of erythromycin led to the isolation of dihydroerythronolide, but no well defined product consisting only of the intact macrolide nucleus of magnamycin has been obtained. For these reasons the determination of the structures of the large lactone rings has been a difficult task. Nevertheless, the structure of methynolide was elucidated by Djerassi and his colleagues at Wayne State University[67,83,84] and later the structure of erythronolide was discovered in the Lilly Research Laboratories[85,86] and that of the lactone from magnamycin was determined by the combined efforts of chemists at the Pfizer Research Laboratories and Harvard University[87–89]. Somewhat later, the structure of kromycin (the macrolide from pikromycin) was determined by Brockmann and Oster[68] and by Anliker and Gubler[69].

Although these macrocylic lactones are complex, it is likely that they are built up in nature by the condensation of units of acetic acid (and possibly propionic acid) to give polyketonic acids of the type XV, in which R is H or Me.

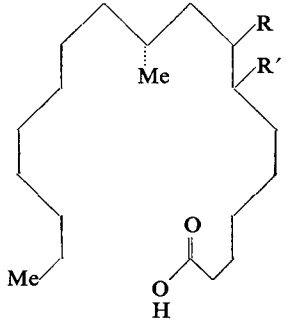


(XV)

This hypothesis is based on the way in which oxygen atoms (or functions such as double bonds which can be formed by the elimination of oxygen) and methyl groups are distributed in the rings. In methynolide we find oxygen on each odd numbered carbon atom except C(5) and in erythronolide on each odd numbered carbon except C(7). Moreover, in methynolide we find methyl groups on four of the even numbered carbon atoms and in erythronolide on all of them. The fact that it is possible to dissect the structure of erythronolide into seven consecutive three-carbon units, each responsible for a branched methyl group, has led to the suggestion that it may be possible to apply to some compounds a "propionate rule",[85] analogous to the "isoprene rules" which have facilitated the prediction of structures among the terpenes.

A lactone built from acetate or propionate units should have a carbon chain with an even number of atoms. The lactone ring of magnamycin, however, contains an odd number of carbon atoms. To overcome this difficulty Woodward has suggested that the aldehyde branch at C(7) is

formed by a pinacol-pinacolone rearrangement[88,89]. Thus, the hypothetical precursor of magnamycin, which has been called protomagnamycin[88,89], would have a $C_{18}$ carbon skeleton (XVI, R = R′ = OH). This skeleton is very similar to that of the fatty acid, tuberculostearic acid (XVI, R = R′ = H), which has been obtained from the tubercle bacillus, the analogy even extending to the absolute configuration of the carbon atoms from C(10).




(XVI)

The common fatty acids are now known to be built up by the stepwise addition of acetic acid residues (in the form of acetyl coenzyme A) to give β-keto acids[93]. In some instances, at least, it seems that oxygen is eliminated from the β-carbon atom of the chain before each new acetate unit is added. However, the structures of the macrolides, and of a number of other natural products, indicate that the chain can also be lengthened while oxygen on odd numbered carbons is retained. The enzymic mechanisms which govern the varying degrees of oxygenation of these fatty acid-like substances are a subject for future biochemical investigation.

## Novobiocin and Vancomycin

Recently, two new antibiotics which are highly active against the staphylococcus and against Gram-positive bacteria in general have been introduced into medicine. They have been named novobiocin and vancomycin. Although they probably have few chemical properties in common, they are placed together here as very little information about the chemistry of vancomycin has yet been published.

### *Novobiocin*

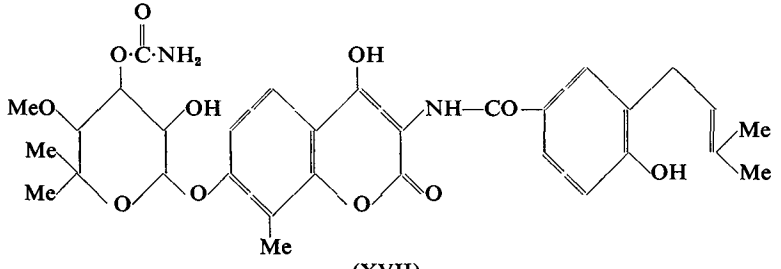
Novobiocin was discovered independently at the research laboratories of both the Upjohn Co., and Merck Sharp and Dohme. The Upjohn workers obtained the antibiotic from the culture fluid of *Streptomyces niveus* and originally called it streptonivicin[94]. The Merck group obtained the same substance from *Streptomyces spheroides* and named it cathomycin[95]. It is now apparently called novobiocin[96]. Novobiocin has been shown to have the structure[97–100] XVII. It is readily cleaved into three fragments by treatment with hot ethanolic hydrochloric acid. These fragments are a substituted benzoic acid, a substituted coumarin, and a

new sugar, noviose, respectively. The enolic group on C(4) of the coumarin moiety imparts an acidic character to the molecule.

At pH 10 novobiocin is converted in about 30 per cent yield to an isomeric product called *iso*novobiocin. Analysis of the equilibrium mixture shows that the carbamyl group in the noviose fragment has migrated from C(3) to C(2)[101]. This change is accompanied by the loss of about one third of the original activity.

*Vancomycin*

Vancomycin is produced by *Streptomyces orientalis* and has been obtained in a highly purified form at the Lilly Research Laboratories. It is an amphoteric substance with an isoelectric point of 5·0, and has a molecular weight of 3200–3500. Its hydrochloride contains 7 per cent of nitrogen and from 16 to 17 per cent of carbohydrate[102].




(XVII)

## Polyene Antibiotics

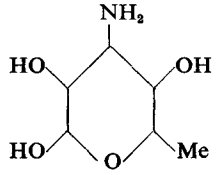
Although a number of human diseases, some trivial, some disabling, and others fatal, are caused by fungi, the progress of chemotherapy has been less rapid in this field than in others. The introduction of the broad-spectrum antibiotics into medicine raised the problem of fungal infections in a new form, because these antibiotics may eventually eliminate much of the normal bacterial flora of the gut, leaving the way open for pathogenic fungi to become established. The need to cope with this situation undoubtedly stimulated the search for antifungal antibiotics.

Since 1950 we have seen the discovery of a group of antifungal substances which are produced by streptomyces and have certain chemical features in common. The substances in this group contain a chromophoric centre characteristic of a conjugated polyene and are therefore referred to as polyene antibiotics[103]. Tetraenes, a pentaene, hexaenes and heptaenes with antifungal properties have been isolated. Nystatin[104] (fungicidin), produced by *Streptomyces noursei*, and amphotericin B[105], produced by another *Streptomyces* sp., are probably the best known members of the group.

Little information about the structure of nystatin and amphotericin B, apart from that provided by their ultra-violet absorption spectra, is yet available. Both substances are amphoteric and are practically insoluble in water. Nystatin, which contains a conjugated tetraene, has the molecular formula $C_{46}H_{77}NO_{19}$, and amphotericin, which contains a

heptaene, has the formula $C_{46}H_{73}NO_{20}$[76]. On prolonged acetolysis both antibiotics yield the same tetra-acetate of an aminodesoxyhexose, mycosamine[107] (XX).

In spite of their low solubility in water, both nystatin and amphotericin B are active against systemic infections when administered by mouth, and their toxicity, when they are given in this way, is low[108]. They appear to have been used successfully, by the oral route, to eliminate pathogenic fungi from the stools of patients in whom fungal infections of the gut had followed treatment with broad spectrum antibiotics[109].




(XX)

## Chemical Structure and Antibacterial Activity

It will now be clear that the antibiotics form an extremely heterogeneous collection of substances and that no single type of chemical structure can be regarded as the seat of their antibacterial activity. Chloramphenicol and the tetracyclines provide an illustration of this point. These substances show a similar pattern of activity against a variety of bacteria and are classed in every clinician's mind as "broad spectrum antibiotics"; but it would be hard to find two types of compound having fewer structural features in common.

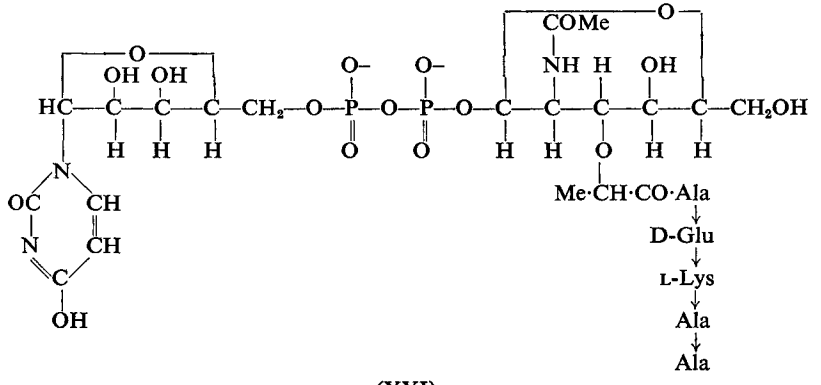
Nevertheless, the antibiotics appear to have a common property in the possession of structural features which have not so far been found in animal products. The penicillins contain the unique $\beta$-lactam-thiazolidine ring system and amino acids belonging to the D-configurational series. The polypeptide antibiotics also contain new ring systems and D-amino acids. Chloramphenicol is almost unique in possessing an aromatic nitro group and a dichloroacetyl group, and the tetracyclines have a naphthacene ring system which is not known to be formed by animal cells. Streptomycin and the macrolides contain new sugars. Large lactone rings, which are present in the macrolides, have also been found in the peptide antibiotics enniatin[110], etamycin[111], amidomycin[112] and valinomycin[113], but apparently not elsewhere in nature.

These peculiarities of the antibiotics are clearly reflections of structural and enzymic patterns peculiar to the micro-organisms themselves. The presence of such patterns makes chemotherapy possible, for a chemotherapeutic agent must interfere with some process which is vital in a pathogenic organism but is unimportant, or inaccessible, in animal cells, and it must also be resistant to destruction by the enzymes of animal tissues.

In general, we still know little about the specific biochemical reactions with which antibiotics interfere, but we are beginning to obtain some idea,

at least, of the kind of disorganisation that can be brought about by penicillin. Recent work has indicated that penicillin prevents the synthesis of bacterial cell walls[114], and that these walls are quite different, in chemical structure, from the outer membranes of animal cells.

The cytoplasm of a Gram-positive bacterium lies within a fragile membrane which is surrounded by a rigid wall. The contents of the cytoplasm appear to exert a high osmotic pressure, but the cell is prevented from bursting by the strength of its wall[115]. The cell walls of some organisms can be dissolved by the action of the enzyme lysozyme[116]. In ordinary media, removal of the wall is followed by the rupture of the cytoplasmic membrane and escape of the cytoplasm, but in media containing a high concentration of sucrose the membrane and its contents may survive as a spherical body called a protoplast[117].




(XXI)

Methods are now available for separating the bacterial walls from the remainder of the cell[118], and analysis of the walls of staphylococci has shown that they contain residues of D- and L-alanine, D-glutamic acid, lysine, glycine, glucosamine, and the *N*-acetyl derivative of a new amino sugar which is thought to be 3-*O*-carboxyethyl hexosamine[119] and has been given the trivial name "muramic acid". This new sugar is widely distributed in the walls of certain micro-organisms, but has not so far been found in other forms of life.

It has long been known that penicillin causes no damage to resting bacteria, but that sensitive organisms are killed, and often lysed, when they begin to grow in the presence of the drug[120]. More recently it has been found that certain growing bacteria are not lysed by penicillin in a medium of high osmotic pressure, but are converted to protoplasts, and that the latter undergo lysis when the medium is diluted with water[121]. These facts have led to the suggestion that bacteria growing in the presence of penicillin may die because they fail to maintain their rigid cell walls[114].

Evidence in support of this hypothesis has come from investigations of a different kind. Several years ago, Park[122] found that three uridine nucleotides accumulated in staphylococci which had grown for a short time in the presence of penicillin, while very little of these substances

were present in the normal cells. Later, Park and Strominger[114] showed that the principle nucleotide consisted of uridine diphosphate linked glycosidically to *N*-acetylmuramic acid and a peptide composed of D-glutamic acid, D- and L-alanine and L-lysine, and they proposed the provisional structure XXI for this compound. Further work established that the amino sugar and amino acids were present in the same molecular ratio in the nucleotide as they were in the staphylococcal cell walls[123]. Park and Strominger have therefore suggested that the uridine diphosphate *N*-acetylaminosugar-peptide is a precursor of the cell wall and that it accumulates in penicillin-treated staphylococci because the drug prevents the incorporation of the *N*-acetylaminosugar peptide fragment of the nucleotide into new cell wall material[114]. This leads to a picture of penicillin treated cells growing normally except that they soon fail to have enough cell wall to go round. In consequence, the wall can no longer protect the cytoplasmic membrane and cellular organisation is obliterated by lysis.

The polypeptide bacitracin also appears to interfere with the synthesis of bacterial cell walls. This antibiotic, like penicillin, is bacteriolytic to growing staphylococci[124] and can induce the formation of protoplasts in hypertonic media[125]. We have recently shown that the uridine nucleotides which accumulate in staphylococci treated with penicillin also accumulate in staphylococci treated with bacitracin, and that they do so in similar amounts when the two antibiotics are used in dilutions proportional to their antibacterial activities[126]. This finding does not allow us to conclude that the first effects of bacitracin and penicillin on the staphylococcus are identical, but it does suggest that the modes of action of the two antibiotics are very closely related. It is relevant to recall, therefore, that both bacitracin and penicillin contain highly-reactive, sulphur-containing ring systems, for the reactivity of these rings may well be responsible for the primary lesions which the substances produce in bacterial cells.

## Therapeutic Achievements and Problems

The introduction of the antibiotics into medicine has revolutionised the treatment of infections caused by bacteria, spirochaetes, rickettsiae, and some of the larger viruses. Diseases which, twenty years ago, were always dangerous and often fatal can now be treated with every hope of success; the disability which resulted from many less serious diseases has been greatly diminished; and surgical procedures which would once have been attended by grave risk of bacterial infection can now be undertaken with confidence. It would be wrong to assume, however, that the antibiotics are ideal chemotherapeutic agents, even in circumstances in which they are normally used with success.

Although the chemotherapeutic antibiotics are much more toxic to pathogenic micro-organisms than to man, most of them are not entirely harmless to the body tissues. Penicillin is exceptional in being almost devoid of toxicity, in the normal sense of the word, to the patient. Nevertheless, this antibiotic can occasionally cause trouble by inducing sensitivity, for serious discomfort, or even death, may follow the injection of

the drug into sensitised people. Sensitivity to penicillin is not often encountered in this country, but its incidence appears to be as high as 5 per cent among hospital patients in some countries where there is no control over the sale of antibiotics to the public. However, allergic reactions to penicillin can often be controlled by antihistamine drugs and people who are sensitive to benzylpenicillin are frequently able to tolerate the biosynthetic penicillin O[127] (allylmercaptomethylpenicillin). Sensitivity is also encountered among persons treated with novobiocin, but it does not seem to be a problem with the other antibiotics in current use.

Streptomycin has a relatively low toxicity to man and can be used with some margin of safety, but if given in too large a dose over a prolonged period it may cause permanent damage to the eighth nerve with a resulting loss of the sense of balance[128]. Chloramphenicol has been given to many millions of people without serious ill effects, but in a few cases its administration has been followed by disorders of the blood, including fatal aplastic anaemia[129,130], and it has been suggested that the nitro group in the molecule has been responsible for these changes. The tetracyclines and the macrolides do not appear to show any substantial direct toxicity, but some of them are unpleasant to take, being liable to cause vomiting and diarrhoea. Moreover, prolonged administration of the broad-spectrum antibiotics is attended by the risk that pathogenic fungi and yeasts will replace the depleted bacterial flora of the gut[109].

In contrast to these substances, the polypeptide antibiotics bacitracin and polymyxin only just qualify for inclusion among the systemic chemotherapeutic agents, for they are nephrotoxic in doses no larger than those required to combat infections by sensitive bacteria[131]. These antibiotics are seldom used systemically unless other forms of therapy are unavailing and damage to the kidney can be justified by the severity of the disease.

Unfortunate though they are, the toxic effects of the antibiotics now used in medicine seem trivial when viewed against the saving of life and relief of suffering for which these substances have been responsible. A more serious matter has been the emergence of resistant strains among certain species of bacteria where sensitivity to an antibiotic was at first the rule.

When a micro-organism is subcultured in the laboratory in the presence of an antibiotic, its resistance to the antibiotic tends to increase. With some substances, such as streptomycin, this acquired resistance may rapidly attain a high value; with others, such as penicillin, it often rises gradually. In some cases, the change is brought about by the natural selection, in the presence of the drug, of a resistant mutant, and in others it may represent an adaptation which the drug itself induces[132].

It might have been imagined that bacteria would acquire resistance in patients under treatment with antibiotics, and that strains resistant to a new antibiotic would begin to replace sensitive strains soon after the introduction of the substance into medicine. Fortunately, the development of resistance in this way has not, with most organisms, been an important clinical problem; but with two species of bacteria, *Myco.*

*tuberculosis* and *Staph aureus*, it has been sufficiently serious to endanger the success of chemotherapy.

In the treatment of pulmonary tuberculosis with streptomycin the emergence of streptomycin-resistant strains of *Myco. tuberculosis* has been favoured by the need for prolonged therapy and the ability of bacteria to undergo large and sudden increases in resistance to the drug. During 1949–1950 the problem of resistance, together with the limitation imposed on the use of streptomycin by its toxicity, made it seem possible that this substance would fail to retain its position as an effective chemotherapeutic agent against chronic tuberculosis. However, streptomycin is still firmly established today. This is partly due to the finding that simultaneous administration of streptomycin and *p*-aminosalicylic acid (PAS)[133] or isoniazid[134] delays the emergence of resistant strains and enables smaller quantities of streptomycin to be used.

The problem of the resistant staphylococcus is more serious[30]. By 1949 a high proportion of the strains of staphylococci encountered in some hospitals were penicillin-resistant[135]. This resistance was of a special type, being due to penicillinase-producing strains of staphylococci; the penicillinase-producers survived in the presence of the drug, were carried on the skin and in the nasal passages of nurses and doctors, and passed from patient to patient. However, it was soon found that the staphylococcus became resistant to other antibiotics when the latter were used extensively in medicine. The discovery of the broad spectrum antibiotics greatly improved the position for a time, but today many strains of staphylococci isolated in hospitals are resistant to both penicillin and the broad spectrum antibiotics[136]. Moreover, a strain that has become resistant to one of the tetracyclines is commonly resistant to others. The macrolides, vancomycin, and novobiocin are now available to deal with such strains, but staphylococci readily acquire resistance to the erythromycin (macrolide) group of antibiotics and cross-resistance is found, in some degree, to all the members of the group[66]. A marked increase in the resistance of staphylococci to erythromycin was encountered in some American hospitals two years after the introduction of the drug[137] and it has therefore been recommended, in this country, that erythromycin should be held in reserve for the treatment of staphylococcal infections which do not respond to other antibiotics[66].

Fortunately, other aspects of the staphylococcal problem are more hopeful. Preliminary reports on vancomycin[138,139] and novobiocin[140] are encouraging. These substances are active against staphylococci which are not sensitive to the well-established antibiotics; vancomycin, in particular, is bactericidal in high dilution and staphylococci do not readily acquire resistance to it *in vitro*[141]. Quite a different kind of substance, cephalosporin C, has been shown to protect mice infected with penicillin-resistant staphylococci[30]; although a penicillin-like compound, it is not destroyed by penicillinase[28], and the elucidation of its structure might lead to the production of more powerful substances of a similar type. Furthermore, the proportion of penicillin-resistant strains of staphylococci encountered in a hospital falls considerably when the administration of

penicillin is temporarily restricted[136,137]. Something may therefore be done by the careful use of the different antibiotics available to keep resistant strains in check.

A good deal of effort is now being expended on a search for antibiotics which will extend the range of infections that can be treated, and even for microbial products which will selectively inhibit the growth of human tumour cells[142]. Whether these efforts will be rewarding it is scarcely possible to predict. It is clear, however, that sustained success in some fields where chemotherapy is already well established has been dependent on the slow but steady introduction of new antibiotics into medicine. If this position is to be maintained new drugs will have to be forthcoming in the future. Of the many hundreds of thousands of strains of micro-organisms that have already been examined only a very small proportion have yielded antibiotics which have proved to be clinically useful. The chance of finding further antibiotics able to cope with infections caused by an organism such as the staphylococcus must now decrease as each new substance is discovered. But in the long run, this law of diminishing returns may be offset, to some extent, by an increase in our knowledge of the factors which govern the biological activity of the antibiotics that have already been isolated and characterised. Although little progress has so far been made by the organic chemist in his efforts to improve the antimicrobial substances produced by micro-organisms, we may have seen, in the production of certain new synthetic penicillins[20], the beginning of one successful chapter in this field. Certainly, further studies of the mode of action of antibiotics already in use and of the mechanisms by which micro-organisms become resistant to them will contribute to our understanding of some of the fundamental aspects of microbiology. In doing so they may eventually help to make the rational design of new chemotherapeutic substances possible.

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