ANTIBACTERIAL CHEMOTHERAPY[1]

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SEMISYNTHETIC PENICILLINS

The most important development in bacterial chemotherapy in the last few years is the development of the semisynthetic penicillins (1). Modification of the penicillin molecule has been the aim of organic chemists ever since its structure was established in 1943 (2). Synthesis of the seemingly simple molecule proved more difficult than anticipated, owing to the fact that all the known ring-closing agents, when used to bring about the formation of the β-lactam ring from the penicilloic acids, relatively readily accessible by synthesis, caused the formation of the penicillenic acids which are isomeric with the penicillins, but contain an exazolone ring system (Fig. 1)

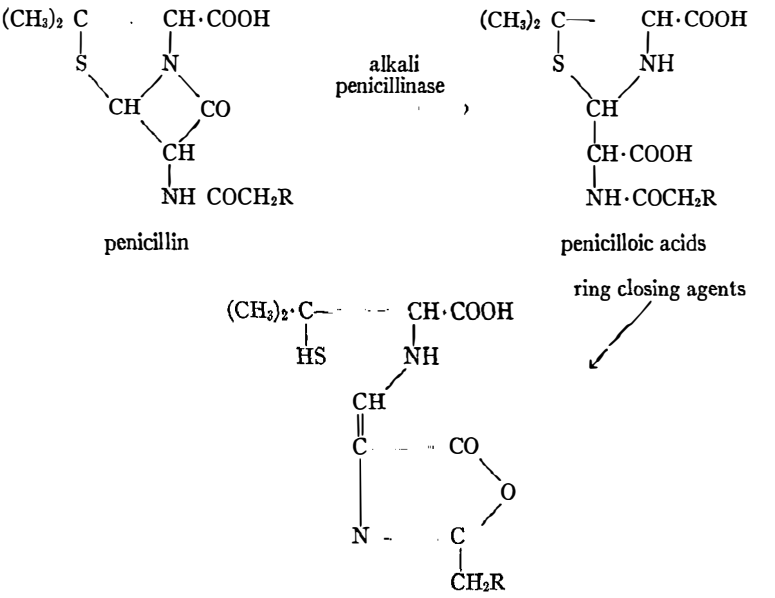

Fig. 1. Action of penicillinase and ring-closing agents.

The problem of the total penicillin synthesis was solved brilliantly in 1957 by Sheehan and his collaborators (3) through the introduction of a new class of ring-closing agents, the carbodiimides, which abstract in aqueous solution

[1] The survey of literature pertaining to this report was concluded on August 1, 1963.

and at neutral pH, the elements of water from carboxyl and amino groups of suitable amino acids with the formation of peptide bonds, being themselves transformed in the process into water-insoluble urea derivatives. The penicillin synthesis made in principle possible the synthesis of penicillins with different side chains; the process was, however, complex and costly.

Another approach to modified penicillins became possible when it was found that the nucleus of the penicillin molecule 6-aminopenicillanic acid (6-APA), became available by direct fermentation (3 to 6) and by enzymatic removal of the side chain from natural penicillins (7 to 9). The latter method gives yields of 6-APA of over 90 per cent and is used widely industrially; it has made 6-APA a readily accessible chemical. The free amino group of this substance can be readily acylated and a large number of new penicillins with different side chains have been synthesised in this way (10). For a comprehensive review of these compounds, with a full bibliography, see Doyle & Nayler (11).

BACTERIOLOGICAL AND CLINICAL ASPECTS OF NEW PENICILLINS

Acid-stable Penicillins

The first semisynthetic penicillins to appear in clinical practice were compounds with an antibacterial spectrum similar to that of benzylpenicillin, but sufficiently stable to acid to be effective after oral administration. The following have all received clinical trial:

Approved name	*Side chain based on penicillanic acid 6-Aminopenicillanic acid*
Phenoxymethylpenicillin	6-(α-phenoxyethylamido)-
Phenethicillin	6-(α-phenoxypropionamido)-
Propicillin	6-(α-phenoxybutyramido)-
Phenbenicillin	6-(α-phenoxyphenylacetamido)-

In addition, some of the penicillins with a changed antibacterial spectrum are acid-stable, e.g., the penicillinase-resistant penicillins, oxacillin and cloxacillin, and ampicillin which has a "broad spectrum." These are not indicated for the treatment of infections due to bacteria sensitive to benzylpenicillin and will be discussed below under the appropriate headings.

Antibacterial activity in vitro.—Although the four penicillins listed above have a similar antibacterial spectrum to that of benzylpenicillin they differ from it and from each other in the degree of activity. Moreover, they vary in the degree to which they are affected by human serum (12, 13, 17). Their relative activity in comparison with that of benzylpenicillin is shown in Table I. It will be seen that against gram-positive cocci phenoxymethylpenicillin has a similar activity to that of benzylpenicillin in routine laboratory medium, but is rather less active in human serum. Phenethicillin has a similar activity against staphylococci, but is rather less active against *Str. pyogenes*

TABLE I

ANTIBACTERIAL ACTIVITY *In Vitro* OF ACID-RESISTANT PENICILLINS COMPARED WITH THAT OF BENZYLPENICILLIN (14)

	Phenoxymethyl penicillin	Phenethicillin	Propicillin	Phenbenicillin
A. In Routine Laboratory Culture Medium				
Staph. aureus	1	1	2	4
Str. pyogenes	1	2	2	2
Str. pneumoniae	1	4	2	1
N. gonorrhoeae	2	8	8	8
B. In 95 per cent Human Serum				
Staph. aureus	2	2	8	16
Str. pyogenes	2	8	8	32

Figures represent fold increase in minimum inhibitory concentration compared with that of benzylpenicillin.

and pneumococci. Propicillin is less active than phenoxymethylpenicillin against all gram-positive cocci and is more adversely affected by serum. Phenbenicillin is very much less active than the other compounds in the presence of serum. Against the gonococcus, only with phenoxymethylpenicillin does the activity approach that of benzylpenicillin (14, 15).

Blood concentration after oral administration.—If the merits of these four penicillins are compared by estimating total blood concentrations after a single oral dose in human subjects the order of merit is the reverse of that of their order of antibacterial activity, i.e., the highest levels are obtained with phenbenicillin and the lowest with phenoxymethylpenicillin. However, if plasma binding is taken into account the picture changes once again, since the degree of binding is very high indeed with phenbenicillin and propicillin. Thus, if the free blood concentrations are compared, phenethicillin gives the highest figures, and phenbenicillin the lowest.

Metabolites in urine.—In comparing blood levels of different penicillins it has to be borne in mind that during passage through the body many of the penicillins are partly converted to other active compounds. According to the findings of Rolinson & Batchelor (16) with phenoxymethylpenicillin and phenethicillin the extent of metabolite formation is too small to be of importance; and with propicillin, although metabolite formation is greater, the metabolite has a similar activity to that of the parent compound. With phenbenicillin, however, two metabolites were found in the urine which contributed more antibacterial activity than the unchanged compound.

Clinical application.—Bond et al. (17) devised a method of comparison which took into account *in vitro* activity, absorption from the gut and plasma binding. Thus they compared the ratio of total blood levels to the minimum

TABLE II

RATIO OF "FREE" BLOOD LEVEL* TO MINIMUM INHIBITORY CONCENTRATION

	Str. pyogenes	*Staph. aureus*
Phenoxymethylpenicillin	19	5
Phenethicillin	18	9
Propicillin	8	2
Phenbenicillin	4	0.5

* Average levels obtained at 1 and 2 hours after a single oral dose in 20 normal subjects. Based on the findings of Bond et al. 1963.

inhibitory concentration in serum and that of the free blood levels to the minimum inhibitory concentration in broth. Comparative figures based on their findings by the second method are given in Table II. From these results it would appear that phenethicillin is probably the most effective of the four for staphylococcal infections, but that phenoxymethylpenicillin is equal, if not superior, to it for those due to *Str·pyogenes*. It is, however, realised that the conditions *in vivo* are complex and factors other than those which at present can be quantitatively measured (such as *in vitro*, activity, absorption from the gut and plasma binding) may influence the effectiveness of antibacterial agents and, indeed, of all drugs. For this reason, all *in vitro* tests are only indicative and the final assessment of a drug can only be made by the clinical test. In the case of the acid-stable penicillins, however, which show only marginal differences in their *in vitro* behaviour, the design of appropriate significant trials presents very considerable difficulties.

PENICILLINASE-STABLE PENICILLINS

Perhaps the most valuable semisynthetic penicillins from the clinical standpoint are those which are resistant to staphylococcal penicillinase. The following have all been shown to have a high degree of resistance:

Approved Name	*6-Aminopenicillanic acid derivative*
Methicillin	6-2′:6′-dimethoxybenzamido
Oxacillin	6-(3′-phenyl-5′-methyl-isoxazole-4′-carboxamido-
Cloxacillin	6-(3′-o-chlorophenyl-5′-methyl-isoxazole-4′-carboxamido-
Nafcillin	2-ethoxy-1-naphthamido-
Ancillin	2-biphenyl-
Quinacillin	3-carboxyquinoxaline-2-yl

The therapeutic effectiveness of these compounds depends, though other factors may also be involved, essentially on their rate of hydrolysis and affinity to the staphylococcal enzyme. For a full discussion of this problem and bibliography on penicillinase see Pollock (18).

The first penicillinase-resistant penicillin, methicillin, entered the clinical field in 1960 (19) and the isoxazolyl penicillins, oxacillin and cloxacillin made their appearance the following year (20, 21). These three compounds are now of proved value in the treatment of penicillin-resistant staphylococcal infection. The other compounds, listed above are more recent arrivals and since they have so far not been shown to have any serious advantage over the first three, they are only of academic interest and will only be briefly referred to below. None of these compounds can compare with benzylpenicillin in activity against penicillin-sensitive bacteria and only their antistaphylococcal activity will be discussed.

Anti-staphylococcal activity in vitro.—Methicillin inhibits the growth of nearly all strains of *Staph. aureus* in a concentration of 1 to 4 μg/ml regardless of penicillinase activity and of size of inoculum. Oxacillin and cloxacillin have an activity of about 10 times that of methicillin against penicillin-sensitive staphylococci and a small inoculum of a penicillinase-producing strain, but with a large inoculum of an active penicillinase-producing strain the activity of cloxacillin is halved and that of oxacillin reduced to about one-third (14). Moreover, the activity of oxacillin and cloxacillin is further reduced in the presence of serum, whereas serum has practically no effect on the activity of methicillin so that with a large inoculum of a penicillinase-producing staphylococcus in 95 to 100 per cent serum, the activity of methicillin and the isoxazolyl penicillins is similar.

Mode of action.—Like other penicillins, methicillin and cloxacillin are actively bactericidal and show the typical zone phenomenon (14). Their main site of action, as for other penicillins, is on the synthesis of the cell wall (22, 23).

Resistance to penicillinase.—The different results obtained with a large and a small inoculum reflect the fact that oxacillin and cloxacillin are slightly less resistant to staphylococcal penicillinase than is methicillin. All three compounds have a very low affinity for the enzyme, but they are hydrolysed to some extent. With methicillin the maximal rate of hydrolysis is about 30 times lower than with benzylpenicillin, and Novick (24) has calculated that the low affinity together with the low rate of hydrolysis means that the "half life" of methicillin in the presence of penicillinase is 5×10^5 times longer than that of benzylpenicillin when both compounds are present at concentrations just sufficient to inhibit bacterial growth. The isoxazolyl penicillins are hydrolysed at about twice the rate of methicillin.

Of the new compounds, nafcillin is similar to methicillin in its degree of resistance to staphylococcal penicillinase and in the absence of serum is two to four times more active, but in 90 to 100 per cent serum the activity of the two compounds is similar (25, 26). Ancillin appears to resemble the isoxazolyl penicillins in antistaphylococcal activity and in the effect of serum (27).

Pharmacology.—Methicillin is rapidly inactivated by acid. It has to be administered parenterally and in order to ensure adequate blood levels a dose

of 1 g must be given every 4 to 6 hours (28). Oxacillin and cloxacillin on the other hand are sufficiently stable to acid to give adequate blood levels after oral administration in a dose of 250 to 500 mg every six hours (21, 29).

These advantages of the isoxazolyl penicillins are somewhat counterbalanced by studies of plasma binding. Methicillin shows a lower degree of plasma binding than any other clinically useful penicillin except ampicillin, including benzylpenicillin, whereas the isoxazolyl penicillins show a high degree of plasma binding. A study in dogs by Verwey & Williams (30, 31) shows that, as might be expected, this is reflected in the proportion of the blood level which reaches the lymph, and that, although the degree of binding to protein in lymph is less than in the blood, the relative amount of binding with different penicillins is similar. Taking all these facts into account the authors found that the percentage of the total plasma concentration present in a free state in lymph was 90 per cent with methicillin and only 30 per cent with oxacillin.

Nafcillin.—is rather more acid-stable than methicillin, but nevertheless is readily inactivated at pH 2.0. In a study in humans (32) adequate blood levels were not obtained after an oral dose even when given together with probenecid, but in a therapeutic trial in mice (33) oral administration was satisfactory. After intramuscular injection the peak levels are about 60 to 70 per cent of those obtained with a similar dose of methicillin, but detectable levels of nafcillin were still present at six hours, whereas this was not the case with methicillin (25). Nafcillin shows a degree of plasma binding similar to that of oxacillin and cloxacillin.

Ancillin is resistant to acid and adequate blood concentrations are obtained when an oral dose of 0.5 to 1.0 g is given. The compound is very rapidly excreted so that the dose needs repeating every four hours. Like nafcillin and the isoxazolyl penicillins it shows a high degree of plasma binding.

Toxicity.—The penicillinase-resistant penicillins share the low toxicity of other penicillins, but show cross-hypersensitivity and are not, therefore, suitable for administration to patients allergic to other forms of penicillin.

Clinical application.—Fairly extensive clinical trials have now been undertaken with methicillin (34 to 38), and the isoxazolyl penicillins (21, 39, 40, 41). Both have been shown to be effective in the treatment of severe penicillin-resistant staphylococcal infection, including cases of septicaemia. Preliminary trials with nafcillin (42) and ancillin (27) suggest that these penicillins are also effective *in vivo*.

One of the penicillinase-resistant penicillins is undoubtedly the drug of choice for the treatment of severe infection due to penicillinase-producing staphylococci. At present the choice must lie between methicillin and oxacillin or cloxacillin since these are of proven value. The advantages of the two latter are greater activity, at least in the absence of serum, and the possibility of oral administration. Methicillin on the other hand shows greater stability towards staphylococcal penicillinase and a much lower degree of plasma binding.

Penicillinase-stable Penicillins and Hospital Staphylococci

Since their introduction into clinical practice, now almost three years ago, hospital outbreaks of staphylococcal infections untreatable by penicillinase-stable penicillin have not been recorded. It must be remembered, however, that there exist methicillin-resistant staphylococci which have been isolated from patients in a number of hospitals in different parts of the world (43 to 50). Moreover, there is some evidence to suggest that the incidence of such strains has increased (51) since the introduction of methicillin. These strains appear to be naturally resistant since in most cases they have been isolated from patients who have not been treated with any of the penicillinase-resistant penicillins.

The degrees of resistances are not great and all the strains so far recorded appear to have similar rather peculiar features. Thus in the presence of quite low concentrations of methicillin (5 to 10 μg) they cease to resemble typical staphylococci; on solid medium they tend also to grow massed together at the site of heavy inoculum, and individual colonies, if present, are small and semi-transparent. The strains show a similar increase in resistance to all the other penicillinase-resistant penicillins; the resistance is of the drug-tolerant type and these strains do not show an increased capacity to inactivate methicillin and isoxazolyl penicillins (45, 52).

However, the methicillin-resistant strains appear to be fully virulent in the absence of methicillin (48, 49, 52), and for this reason it is of the utmost importance to limit the spread of those organisms, through cross infection, by the application of stringent hygienic measures. While the methicillin-resistant staphylococci have at present not become a serious clinical problem, it seems reasonable to suggest that the new penicillinase-resistant penicillins should be reserved for the treatment of patients with severe penicillin-resistant infections, and that such patients should be in single-bedded rooms, or in wards free from cross infection.

"Broad Spectrum" Penicillins

Adicillin.—The first penicillin noted to have increased activity against gram-negative bacilli is the compound now known as adicillin. It was originally described under the names synnematin B(53) and cephalosporin N (54), but was subsequently shown to be a penicillin with a side chain derived from D-α-amino-adipic acid (55). Although a hundred times less active than benzylpenicillin against penicillin-sensitive cocci it was noted to have slightly greater activity than benzylpenicillin against some gram-negative bacilli, particularly *Salmonella* species. A preliminary clinical report from Mexico (66) suggested that it was superior to chloramphenicol in the treatment of typhoid fever but further clinical trials are indicated before the value of the drug can be assessed.

Ampicillin.—Ampicillin is a semisynthetic, acid-stable penicillin discovered in the recent search for penicillins with new properties (57). It has a

phenylglyoyl side chain and its chemical name is 6-(D(−)-α-aminophenylacetamido) penicillanic acid.

The *in vitro* activity of ampicillin has been investigated by several authors. Rutenberg et al. (58), and Barber & Waterworth (14) report that ampicillin is considerably less active against gram-positive bacteria than benzylpenicillin; Ross et al. (59) on the other hand, found that the activity of ampicillin against strains of penicillin-sensitive staphylococci and haemolytic streptococci closely paralleled that of benzylpenicillin. Against most strains of *Neisseria*, ampicillin was found to be less active than benzylpenicillin, but Odegaard (60) found that though the highly benzylpenicillin-sensitive strains of *N. gonorrhoea* were less sensitive to ampicillin, the strains showing some penicillin resistance were more sensitive to ampicillin.

Against the gram-negative bacteria, ampicillin was found to be four to eight times as active as benzylpenicillin against many species of gram-negative bacilli and of about equal activity against *Stra. faecalis* (59, 61). It is particularly active against *Haemophilus influenzae*, (some strains of which are sensitive to 0.25 μg/ml or less), *Salmonella typhi*, and *Shigella* species.

The other coliform bacilli vary in their sensitivity towards ampicillin. This is partly due to the fact that some of them produce penicillinase which hydrolyses ampicillin almost as readily as benzylpenicillin. In these cases their sensitivity towards ampicillin is roughly proportional to the penicillinase producing capacity of the strains (62, 63, 64).

Strains of *Escherichia coli* usually produce small amounts of penicillinase, and, moreover, their penicillinase-producing capacity is not increased by the substrate (63, 64, 65) as is the case in other penicillinase-producing species, such as *B. subtilis* and the staphylococcus, in which penicillinase production is readily inducible (18). These strains are sensitive to 2–8 μg/ml of ampicillin. However, the capacity to produce penicillinase is not the only reason for the resistance of gram-negative organisms to ampicillin. Some species produce little or no penicillinase, yet are completely resistant to ampicillin, for instance *Pseudomonas pyocyanea*, and some strains of *Klebsiella*, though some strains of the latter are reported to be sensitive (61, 66).

Among *Proteus* species, most strains of *Pr. mirabilis* do not produce penicillinase and are sensitive to 2–8 μg/ml of ampicillin, but other *Proteus* species such as *Pr. morganii* and *Pr. rettgeri* are resistant (67, 68). In ampicillin-resistant penicillinase-producing strains of *Proteus* the penicillinase production is inducible. (63).

A careful comparison between the activity of ampicillin, tetracycline and chloramphenicol against over 600 fresh clinical isolates, mainly from cases of urinary tract infection, was made by Sutherland & Rolinson (68). These consisted of strains of *E. coli* (40 per cent), *Pr. mirabilis* (24 per cent), other species of *Proteus*, i.e., *Pr. vulgaris*, and *Pr. morganii* (2 per cent), *Aerobacter* (12 per cent), *Streptococcus faecalis* (10 per cent), *Pseudomonas pyocyanea* (7 per cent), and miscellaneous organisms, mainly paracolon and *Acromobacter* (6 per cent). At a concentration of 5 μg/ml of the antibiotics 78 per cent were

inhibited by ampicillin, 52 per cent by tetracycline, and 64 per cent by chloramphenicol; however, at the lower concentration of 2.5 μg/ml only 19 per cent were inhibited by ampicillin, 45 per cent by tetracycline and 8 per cent by chloramphenicol. Of the tetracycline and chloramphenicol-resistant strains of *E. coli* about 70 per cent—80 per cent were sensitive to 5 μg/ml or less of ampicillin.

Of the *Pr. mirabilis* strains tested, 83 per cent were highly sensitive to ampicillin, being inhibited by 5 μg/ml and less; these strains were much less sensitive to tetracycline and chloramphenicol. *Pr. morganii* and *Pr. mirabilis* were resistant against all three antibiotics; of the strains of *Streptococcus faecalis* tested 95 per cent were inhibited by 2.5 μg/ml of ampicillin or less; they were much less sensitive against tetracycline and chloramphenicol. *Pseudomonas* and *Aerobacter* strains were found to be rather resistant to all three antibiotics.

The same authors carried out comparisons of the sensitivity of 20 strains of each of the main groups tested against ampicillin, tetracycline, and chloramphenicol, and also polymyxin sulphate and methane sulphonate, cycloserine, kanamycin, nitrofurantoin, and streptomycin. Ampicillin was more effective than any of the other antibiotics against most strains of *Pr. mirabilis* and *Str. faecalis;* and against *E. coli*, kanamycin was usually the most effective antibiotic, followed by polymixin and streptomycin. Against *Pseudomonas* polymyxin sulphate was by far the most effective, inhibiting all strains tested at 5 μg/ml. Against *Aerobacter aerogenes* kanamycin and streptomycin exhibited the greatest activity.

Like other penicillins, ampicillin is bactericidal.

Pharmacology.—The pharmacological properties of ampicillin have been studied extensively (69). Satisfactory blood levels can be maintained by oral administration and very little is bound to plasma proteins. About 30 per cent of an oral dose is excreted in the urine in 6 to 8 hours, so that very high urinary levels are obtained (70). Ampicillin is also excreted in the bile, which may contain as much as 300 times the blood concentration (71).

Clinical application.—Ampicillin has been shown to be a valuable antibiotic for the treatment of chronic bronchitis and acute exacerbations. It appears to be as effective for this purpose as tetracycline and less liable to cause gastrointestinal disturbances (72, 73). It is also effective in the treatment of many urinary infections due to *E. coli*, *Pr. mirabilis*, and *Str. faecalis* (66, 61).

In preliminary trials in the treatment of typhoid fever ampicillin in spite of its greater bactericidal action, appears to be less effective than chloramphenicol.

For other clinical reports see references (74, 75, 76).

CEPHALOSPORINS

The various antibiotics produced by *Cephalosporium* species have been reviewed by Abraham (77). Up to date, only members of the cephalosporin C group show any prospects of clinical use. Cephalosporin C is a substance

related to the penicillins containing a fused dihydrothiazine β-lactam ring instead of the fused thiazolidine β-lactam ring, and α-amino adipic acid as side chain (Fig. 2)

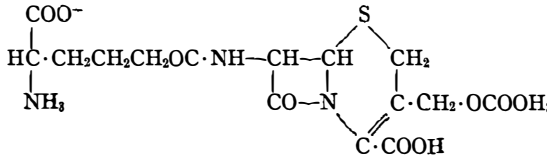

Fig. 2. Cephalosporin C

The side chain can be split off by chemical means (78) leading to the formation of 7-amino-cephalosporanic acid (7-ACA) but so far no enzymic process achieving their hydrolysis has been found, despite intensive screening (79).

It is of interest that the penicillin structure can be converted to the cephalosporin structure by chemical means (78). Also, 7-amino-cephalosporanic acid can be acylated in the same way as 6-amino-penicillanic acid, giving rise to different derivatives with antibacterial properties (80).

Antibacterial activity.—Three compounds in this group have now been studied bacteriologically and clinically, namely, cephalosporin C, 7-phenylacetylamidocephalosporanic acid, and cephalothin (7-thiophene-2-acetamidocephalosporanic acid).

All the antibiotics of this series are highly resistant to *B. cereus* and the staphylococcus. They are thus equally active against penicillin-sensitive and penicillinase-producing staphylococci. The affinity of these compounds to the enzyme varies; in the case of cephalosporin C it is low; in the case of the phenylacetyl and the α phenoxy propionyl derivatives of 7-ACA, it is very high (18). Cephalosporin C and other derivatives of 7-ACA are potent inducers of penicillinase (18, 77).

Strains of many species of bacteria, including *B. cereus*, the staphylococcus, *E. coli*, *Klebsiella* and *Proteus*, produce an enzyme which opens the β-lactam ring of the 7-ACA derivatives. The enzyme is obviously closely related to penicillinase. Whether it is identical or not with penicillinase, has not yet been decided in all cases; the organisms attacking the cephalosporin β-lactam ring do not always inactivate the penicillins (81 to 84). Cephalosporinase can be induced in *Proteus* species by cephalothin as well as by benzylpenicillin and methicillin (84). It has recently been shown, however, that highly purified staphylococcal penicillinase displays some cephalosporinase activity and the analysis of all the available evidence suggests that the action on the two substances is due to the same enzyme (85). The cephalosporins are actively bactericidal and, like the penicillins, inhibit cell wall synthesis (77).

Pharmacology.—The cephalosporins are moderately resistant to acid, but

are not well absorbed from the intestinal tract, so that they have to be administered parenterally. They are deacetylated to a considerable extent in the liver, and the resulting hydroxy acids have a much lower antibacterial activity. Early clinical studies suggest that this occurs much more readily in small laboratory animals than in humans. The cephalosporins show some degree of plasma binding. Like the penicillins they are rapidly excreted by the kidneys (86).

Toxicity.—The toxicity of the cephalosporins for laboratory animals is even lower than that of the penicillins. A fact of great practical importance is that they show no cross hypersensitivity with the penicillins and many patients with penicillin allergy have now been safely treated with cephalothin (87).

Clinical application.—Possible uses for one of the cephalosporins are the treatment of (*a*) penicillin-sensitive infection in patients hypersensitive to penicillin; (*b*) urinary infections with some strains of coliform bacilli; and (*c*) penicillin-resistant staphylococcal infection. Rather high doses (4 g daily) are necessary for generalized infection, but good results have been reported in the treatment of septicaemia due to *Staph. aureus*, haemolytic streptococci and various coliform bacilli (87, 88). Cephalothin in a dose of 0.5 g every six hours has also proved effective in the treatment of many coliform infections of the urinary tract (89).

FUCIDIN

Chemical structure.—Fucidin belongs to the steroid group of antibiotics. The first of these to be discovered was helvolic acid (90) produced by *Aspergillus fumigatus* var. *helvola* Yuill. Subsequently similar substances were isolated from culture filtrates of cephalosporin cultures; chemical and biological properties of one of these, cephalosporin P., have been studied in greater detail (77). Fucidin is the sodium salt of fucidic acid, a metabolic product of *Fusidium coccineum* (91).

The structures proposed for helvolic acid, cephalosporin P_1 and fucidic acid are shown (Fig. 3):

Fucidin is at present the only steroid antibiotic which has found clinical use.

Antibacterial activity.—Fucidin is active against many species of gram-positive bacteria, and particularly so against staphylococci, most strains of which are inhibited by a concentration of 0.06 μg/ml. Streptococci are relatively resistant. Among other susceptible bacteria are *Myco. tuberculosis* and *N. gonorrhoeae* and *N. meningitidis*, but fucidin is not the drug of choice for infections with these organisms (92).

A large inoculum of most strains of *Staph. aureus* contains a few cells which are resistant to fucidin and for this reason fucidin-resistant strains of *Staph. aureus* develop rapidly *in vitro* and these strains show cross resistance with cephalosporin P (95).

In vivo *protection*.—Therapeutic tests on mice infected with staphylococci gave disappointing results. Only a small percentage of treated animals survived though their life time was extended in comparison with the untreated animals (personal communication). In this respect fucidin behaved like helvolic acid (90, 93) and cephalosporin P (94). In view of the fact that inhibitory blood levels were maintained during the period of treatment, the reason for the failure of these compounds to afford protection in animals is not understood and presents an interesting problem for study.

Apparent synergy with penicillin.—It has been claimed that fucidin and benzylpenicillin act synergically against penicillinase-producing staphylo-

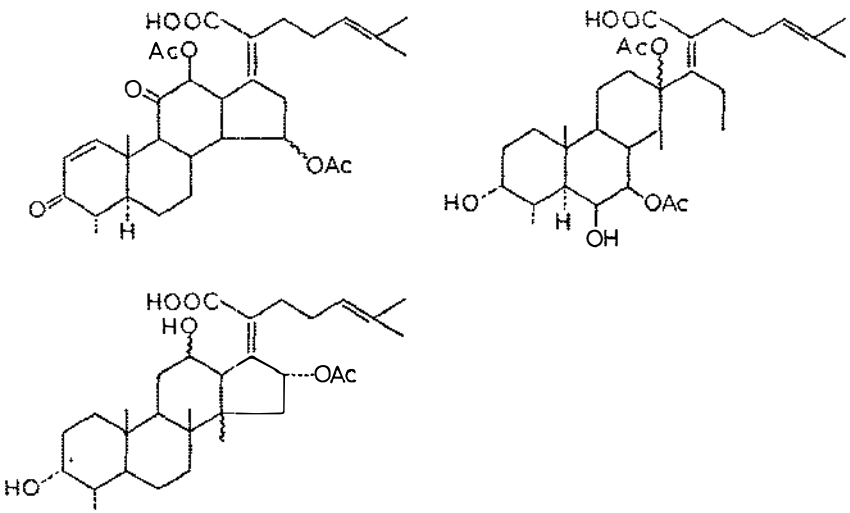

Fig. 3 Structures for Steroid Antibiotics

cocci (92) and that such a combination is bactericidal even for a large inoculum. This is not a true synergy and is dependent on the fact that with some penicillin-resistant strains of *Staph. aureus* fucidin delays bacterial multiplication and therefore penicillinase production for long enough (a few hours) to permit penicillin to kill the cells resistant to penicillin. This only occurs with strains which are relatively weak penicillinase producers (95).

Bacteriostatic synergy occurs with erythromycin and novobiocin and combination with either delays the emergence of fucidin-resistant strains.

Pharmacology and toxicity.—Fucidin is readily absorbed after oral administration. Adequate blood levels can be maintained for eight hours after a single dose of 500 mg, but there is a high degree of plasma binding.

Fucidin is excreted and concentrated in the bile and about 2 per cent of the dose is excreted in active form in the faeces. A large proportion of the dose is inactivated in the body. Little or none is found in the urine (92).

After oral administration fucidin is of very low toxicity and is well tolerated. Parenteral administration causes necrosis at the site of injection.

Clinical application.—Fucidin is now of proven value in the treatment of severe staphylococcal infection, including cases of septicaemia (96 to 100). It is particularly effective in eliminating staphylococci from lesions including infected burns (101). If the organisms are not rapidly eliminated there is a danger that the infecting strain will become resistant, and such a change has been recorded in burns (101) and in several pulmonary infections (98, 99). To avoid this danger it may be wise to give fucidin in combination with erythromycin, novobiocin, or penicillin.

ANTIBIOTICS FOR GRAM-NEGATIVE BACILLI

Polymyxins.—It is more than 25 years since the discovery of the polymyxin group of antibiotics (102, 103), but the recent increase in infection due to gram-negative bacilli has reawakened interest in them. Five polymyxins have been described, named A, B, C, D, and E, all of which are basic polypeptides and all of which contain the fatty acid D-6-methyloctan-1-oic acid and the amino-acids L-diaminobutyric acid and L-threonine. They differ in other amino acid components. Some confusion occurred at first over the relationship to this group of colistin, an antibiotic isolated in Japan in 1950 (104), but it has now been shown to be identical with polymyxin E (105). The formula of polymyxin B is shown in Figure 4.

Two polymyxins are now commercially available under the names polymyxin (polymyxin B) and colistin (polymyxin E). Pure preparations are not yet available and activity and dosage is usually referred to in units. The unit of polymyxin is such that 1 mg of pure polymyxin B base is equivalent to 10,000 units, but unfortunately the Japanese introduced a new unit for colistin of one-third this value. Commercial preparations of polymyxin contain approximately 6000 British units of polymyxin B per mg and those of colistin approximately 12,500 Japanese units of polymyxin E per mg. The polymyxins are supplied as the sulphate or their so-called methane sulphonate. The latter bases in the body are converted back to the polymixins and exert their antibacterial activity as such.

Antibacterial activity.—The antibacterial activity of polymyxin and colistin are almost identical (106, 107). Nearly all species of gram-negative bacilli are highly sensitive, with the notable exception of *Proteus* species, all of which are highly resistant. All species of gram-positive bacteria and the pathogenic gram-negative cocci are resistant, so that the antibiotic spectrum of the polymyxins is the reverse of that of penicillin. The polymyxins are actively bactericidal.

In an investigation of a large number of coliform bacilli isolated in a hospital in the United States (107) it was found that polymyxin and colistin were at least as effective as, and usually much more effective than, streptomycin, kanamycin, chloramphenicol, and tetracycline against 94 per cent of *E. coli*,

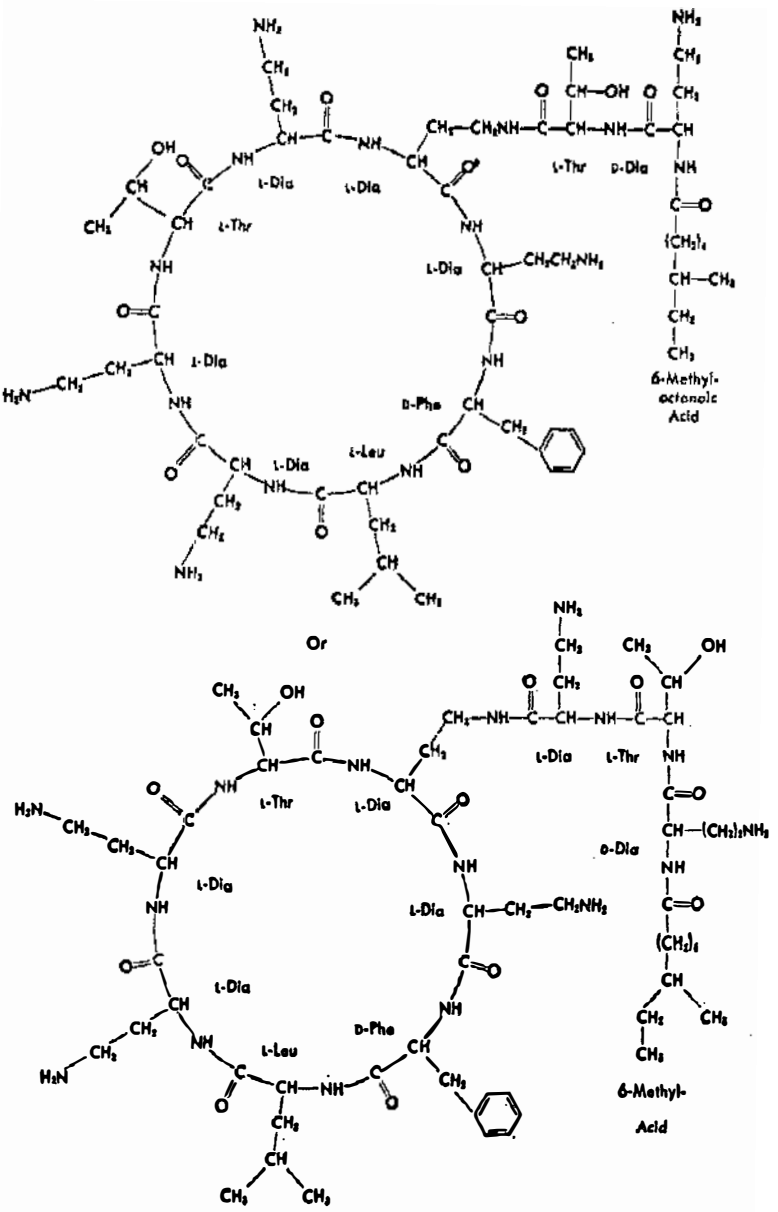

FIG. 4. Alternative structures in polymyxin B.

86 per cent of *Klebsiella*, 93 per cent of *Pseudomonas pyocyanea*, and 50 per cent of paracolon. These findings applied to bactericidal as well as bacteristatic tests.

Pharmacology.—The high activity of the polymyxins is somewhat offset by the fact that not only are they not absorbed from the intestinal tract but even after parenteral administration blood levels tend to be rather low. Moreover, about 50 per cent of their activity is lost in the presence of serum.

They are excreted by the kidneys, but there is usually some delay, so that little or none is present in the urine during the first 12 hours. Thereafter quite, high urinary levels are obtained.

Toxicity.—All the polymyxins are nephrotoxic, but B and E are much less so than A, D, and C. With polymyxins B and E the effect is minimal (108) and the danger of serious renal damage is slight with the usual therapeutic doses even in patients with some degree of renal insufficiency (107).

In the form of the sulphate the polymyxins give rise to severe pain and tissue injury at the site of injection, but this is largely overcome by the use of the methane sulphonates.

Clinical application.—Polymyxin or colistin is the drug of choice in the treatment of serious infections due to *Ps. pyocyanea* and sometimes in infections with coliform bacilli. The poor blood levels are a disadvantage in the treatment of generalised infection, but satisfactory urinary levels can be obtained, meningeal infections can be treated by intrathecal injection, and superficial infections can be treated by local application.

Synergy with sulphonamides.—It has recently been shown that there is synergy between polymyxin and sulfonamides against all species of *Proteus* (109, 110). Thus, although polymyxin alone has little or no activity against these organisms and the sulphonamides alone are only weakly bacteriostatic, the two together are bactericidal. This synergy has also been demonstrated in mice experimentally infected with *Pr. vulgaris* (109).

KANAMYCIN

Kanamycin was isolated in Japan in 1957 from a soil bacillus, *Streptomyces kanamyceticus* (111). Crude extracts contain two products referred to as kanamycin and kanamycin B. Both are water-soluble bases and belong to the oligosaccharide group of antibiotics of which streptomycin is a representative. Kanamycin has the empirical formula $C_{19}H_{36}N_4O_{11}$ and consists of three components, (6-amino-6-deoxy-D-glucose, 3-amino-deoxy-D-glucose, and 2-deoxystreptamine) linked glycosidically (112). Its formula is shown in Figure 5. It is commercially available as the sulphate.

Antibacterial activity.—Kanamycin has an antibacterial spectrum similar to that of streptomycin, except that individual strains are less liable to develop resistance. Most strains of all species of *Proteus* and many other coliform bacilli isolated in hospitals today are more sensitive to kanamycin than to any other antibiotic, except the closely related but more toxic antibiotic,

neomycin (107, 113, 114). Most strains of *Ps. pyocyanes* are resistant. Kanamycin is also highly active against many strains of *Staph. aureus* and *Myco. tuberculosis* but it is rarely the drug of choice for infections due to these species. Streptococci are relatively resistant and the spore-bearing anaerobes, highly resistant.

Kanamycin, like streptomycin, is among the most rapidly bactericidal antibiotics.

Staphylococci and coliform bacilli yield kanamycin-resistant strains fairly rapidly, but not as rapidly as is the case with streptomycin. Kanamycin-resistant strains are nearly always resistant to streptomycin, but streptomycin-resistant strains are frequently sensitive to kanamycin (115, 116).

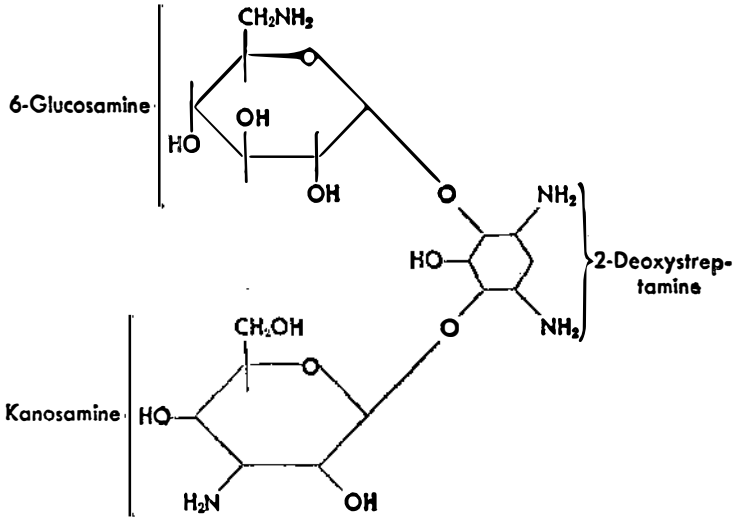

FIG. 5. Kanamycin

Pharmacology and toxicity.—Like streptomycin, kanamycin is not absorbed from the intestinal tract, and for systematic infection has to be administered parenterally. Distribution after parenteral administration is similar to that of streptomycin (117).

Kanamycin is considerably more likely than streptomycin to cause damage to the eighth cranial nerve and much more likely to affect the hearing fibres (118, 119). A short course of 5 to 10 days in doses not exceeding 1 g daily is fairly safe in patients with normal kidneys, but kanamycin should never be given to patients with renal impairment, unless the dose is controlled by testing blood levels.

Clinical application.—Kanamycin is at present usually the drug of choice for severe infection due to *Proteus* species and sometimes those due to other

coliform bacilli. Its rapidly bactericidal action and the high levels obtained in the urine make it particularly effective in the treatment of urinary infections, but because of its toxicity and because coliform bacilli develop resistance to kanamycin fairly readily, treatment should not be prolonged.

TETRACYCLINES

New compounds.—Several new tetracycline derivatives have been prepared in the last few years, but none of these represents a major advance.

Demethylchlortetracycline was introduced a few years ago and according to most investigators is more completely absorbed from the intestinal tract than the three original compounds (120, 121), although there has been one conflicting report (122). Similar or even better absorption has been claimed for methacycline (6-methylene oxytetracycline) [see references 123, 124].

Another line of investigation has led to the discovery of tetracyclines with an increased solubility in water. Three such have been reported, namely, pyrrolidinomethyl tetracycline (125, 126), tetracycline-1-methylenelysine (Tetralysal) (127), and y-β-hydroxyolyl diethylene diamido methyltetracycline (mepicycline) (128).

Deposition in bone, teeth, and tumours.—Recent studies have drawn attention to the fact that tetracyclines are deposited in teeth, bones, and tumors. The first report came from Milch et al. (129) who showed by fluorescent microscopy that tetracycline persisted for at least 10 weeks in the bones of laboratory animals after a single small (0.3 mg/kg) dose. The same group (130) reported a similar deposition in tumours.

Shortly afterwards it was noted that tetracycline administration to young children was frequently followed by yellow discolouration of the teeth (131, 132, 133) and that yellow discolouration of bones might also occur (134). This deposition of tetracyclines in bones and teeth was explained by Hinton (134) on the basis of *in vitro* studies which showed that the tetracyclines chelate with calcium phosphate and form a complex.

Evidence is now accumulating to show that more is involved than simple discolouration. There is little doubt from the studies already mentioned that deposition of tetracyclines in developing teeth interferes with their development and there is now evidence to suggest that, in premature infants at least, tetracycline administration may interrupt the growth of bone (135). Moreover, it has been shown that if tetracyclines are administered during pregnancy, both in humans and laboratory animals, they pass through the placenta, and the infants subsequently show tetracycline discolouration of the teeth (136).

To what extent the use of the tetracyclines should be curtailed by these findings remains to be decided. Certainly, as is the case with all antibiotics, they should never be used without a valid reason. In pregnant patients and young children they should probably be avoided, except for the treatment of severe infection, insensitive to less toxic antibiotics.

CHEMOTHERAPY OF TUBERCULOSIS

Recent advances in the chemotherapy of tuberculosis have been mainly concerned with the prevention and treatment of drug-resistant infection. Studies have been made to determine the best treatment regime for the efficient elimination of the bacilli, so that drug-resistant strains do not emerge, and it is becoming clear that the emergence of drug-resistant strains is nearly always the result of inefficient initial treatment (137). In addition many new drugs have been introduced, but most of these are highly toxic and only thiacetazone (see below) can compete with the standard drugs, i.e., streptomycin, isoniazid and *p*-aminosalicylic acid. For a recent comprehensive review on antituberculosis drugs see Robson & Sullivan (138).

Treatment Regimes

Double chemotherapy.—It is now universally accepted that drug combinations must be used for the treatment of tuberculosis. As long ago as 1950 (139) it was shown that the combination of *para*-aminosalicylic acid (PAS) with streptomycin in the treatment of pulmonary tuberculosis delayed the emergence of drug-resistant tubercle bacilli, and gave better clinical and radiological results. The latter findings were confirmed after a five-year follow-up (140). Subsequent reports have shown that similarly good results can be obtained by treatment with combinations of isoniazid and streptomycin or isoniazid and PAS (141, 142).

In a further study by the Medical Research Council, (143), it was shown that in patients with chronic extensive pulmonary tuberculosis with cavitation, initial treatment for six weeks with all three drugs, followed by treatment with two, gave better results than treatment throughout with only two. The most effective pair for continued treatment remains an open question but in the report just quoted streptomycin plus isoniazid is recommended.

Length of treatment.—Recent studies have left little room for doubt that for severe infection treatment should be extremely prolonged. The Medical Research Council Report (143) mentioned above recommends continuing treatment for 18 months to 2 years for extensive pulmonary lesions, but once the patient is out of hospital the two oral drugs, PAS and isoniazid, can be used provided that the physician is satisfied that the patient will take them (144).

Intermittent therapy.—A series of studies carried out in the Tuberculosis Chemotherapy Centre, Madras, have sought to find the optimum dose regimes. A study in patients on isoniazid plus PAS showed that isoniazid in a dose of 7.8 to 9.6 mg/kg bodyweight was more effective when given in a single daily dose, than when divided into two doses given twelve each hours (145). There was evidence to suggest that this was because a high peak concentration in the serum was more effective than a continuous inhibitory level (146).

Working on this hypothesis, studies were initiated to see the effect of intermittent treatment for tuberculosis. This work is still in progress, but a

preliminary study suggests that a regime of streptomycin 1 g plus isoniazid 650 mg for a patient weighing 454 kg, given together twice weekly, was as effective as treatment with isoniazid 200 mg plus PAS 10 g daily in cachets in two divided doses (147).

New Drugs for the Treatment of Tuberculosis

Thiacetazone.—Thiosemicarbazones have been used in Germany for the treatment of tuberculosis since 1946 (148). The most active is 4-acetylaminobenzaldehyde thiosemicarbazone (Thiacetazone). With large doses, (600 mg) anorexia, malaise, headache, and nausea are very common side effects and blood dyscrasias, nephritis, cerebral oedema, liver damage, and hypersensitivity reactions have all been recorded (149). However, in small doses (150 mg daily) serious effects are rare and a trial in East Africa suggests that a combination of isoniazid 300 mg and thiacetazone 150 mg each, in a single dose once daily may be as effective as isoniazid plus PAS, and much cheaper (150, 151).

Ethionamide (alpha-ethyl-thioisonicotinamide).—This compound was introduced by Rist et al. (152). Although clinically ethionamide is effective it is very badly tolerated owing to severe gastrointestinal symptoms, and neurotoxic effects have also been reported (153). The optimum dose has not yet been determined but is between 0.5 and 1.0 gm daily.

Pyrazinamide.—The antituberculous properties of pyrazinamide were discovered during a systematic study of nicotine acid derivatives (154). In experimental infections in mice, isoniazid and pyrazinamide appear to be more effective in eliminating tubercle bacilli than combinations of the standard antituberculous drugs (155). Early clinical trials of isoniazid and pyrazinamide have also been promising, but equally good results were not seen in all cases (156).

Pyrazinamide, however, is another highly toxic drug and liable to cause liver damage (157). A recent report suggests that in a dose of 30 mg/kg, pyrazinamide is effective and reasonably safe (158).

Cycloserine (D-4-amino-3-isoxalidone).—This is a broad spectrum antibiotic which was isolated from a species of streptomyces in 1955 (159). It appears to be more effective in human tuberculosis than in experimental infection in animals (160) but it is a frequent cause of neurotoxic symptoms which may include convulsions (161). The initial dose should not exceed 0.25 gm twice daily, but some patients can tolerate larger doses.

Thiocarbanilides.—One compound of this group, 4,′-diisoamyl-oxythiocarbanilide (isoxyl), has given promising results in a clinical trial in a dose of 500 mg daily in combination with streptomycin (162).

Ethambutol.—This is the dextrorotatory isomer of 2,2′-(ethylenediamino)-di-1-butanol. It has been shown to be effective in experimental tuberculosis in mice and guinea pigs (163, 164), but its toxicity and efficacy in human infection remain to be determined.

Kanamycin.—As indicated earlier, Kanamycin has considerable activity

against tubercle bacilli and it has been used in the treatment of tuberculosis. Its prolonged use is, however, very liable to cause deafness and it should only be considered as a last resort.

Treatment of Drug-Resistant Tuberculosis and General Conclusions

It will be seen from the above notes that all of the more recently discovered antituberculous drugs, with the possible exception of ethionamide, are highly toxic, but they are of value in the treatment of patients with infections resistant to two or more of the three standard drugs. Appropriate regimes of treatment are beyond the scope of this paper, but are discussed by Crofton (137). This excellent review of the treatment of tuberculosis makes it clear that adequate chemotherapy of new infections "scrupulously prescribed and scrupulously taken," controls infection in the vast majority of cases and renders second-line treatment unnecessary. Moreover, the excellent results obtained with such chemotherapy in the absence of bed rest or surgery have demonstrated that the treatment of tuberculosis consists almost entirely of the appropriate administration of antibacterial drugs (143).

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