REVIEW ARTICLE

NEW ANTIBIOTICS

CHLORAMPHENICOL, AUREOMYCIN, TERRAMYCIN AND NEOMYCIN

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A WIDESPREAD search for new antibiotics was provoked by the discovery of the therapeutic properties of penicillin at Oxford in 1940, and since that time the capacity of many thousands of micro-organisms to produce antibacterial substances has been surveyed in the laboratories of academic institutions and commercial firms. Following the discovery of streptomycin in 1944 the antimicrobial properties of the actinomycetes have probably been investigated more extensively than those of any other group of micro-organisms. These investigations have proved rewarding, for in recent years they have led to the isolation in the U.S.A. of three new antibiotics—chloromycetin, aureomycin and terramycin—that are powerful chemotherapeutic agents, and of a fourth substance—neomycin —that is undergoing clinical trial.

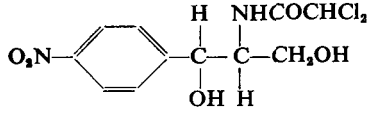
Investigations with other groups of micro-organisms have so far been less fortunate. The further study of antibiotics from fungi has yielded no substance comparable to penicillin in biological interest, and none that has found a place in medicine. The polypeptide antibiotics obtained from bacteria include the bacitracins[1,2], licheniformins[3], and polymyxins[4], which have chemotherapeutic properties and have sometimes proved of value in the clinic[5,6], but the toxicity of these substances to the kidneys has prevented their general use in man. Whatever their source, however, only a very small proportion of the antibiotics that are detected have the properties that are required of a chemotherapeutic substance. It is scarcely possible to say whether substances with these properties are less likely to be produced by fungi and bacteria than by actinomycetes; possibly the practical success that has attended the investigation of the last group of micro-organisms is merely a consequence of the immense number of strains that have been examined.

Chloramphenicol, which has a relatively simple constitution, is remarkable in being a natural compound that contains a nitro and a dichloracetyl group. It can now be readily obtained synthetically. The structures of aureomycin and terramycin are apparently not yet known, but both contain nitrogen and the former, like chloramphenol, contains nonionic chlorine. These three substances have proved effective in the treatment of many important bacterial diseases and have also enabled chemotherapy to be extended to rickettsial and some viral infections.

CHLORAMPHENICOL

The isolation of an antibiotic called chloromycetin[7], from the culture fluid of a species of *Streptomyces* found in the soil near Caracas, Venezuela, was first reported in 1947. The same antibiotic was obtained

independently from a *Streptomyces sp.* present in compost collected in Illinois[8]. Intensive work on this substance was carried out in the laboratories of Parke, Davis and Co. Within two years its structure had been found, it had been synthesised by several methods, and had received successful clinical trials. Chloromycetin is D-(−)*threo*-2-dichloroacetamido-1-*p*-nitrophenyl-1,3-propanediol (I) and is now known by the generic name chloramphenicol.

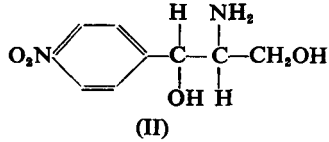


(I)

Production and Isolation. Chloramphenicol[9] was produced by growing the species of *Streptomyces* in a medium containing glycerol, molasses, and meat products such as peptone, under conditions of submerged fermentation at 23° to 27°C. The active culture fluid was filtered, adjusted to *p*H 8·5, and stirred with ethyl acetate, which extracted the antibiotic. The extract was concentrated and mixed with a quarter of a volume of kerosine, and the resulting solution washed successively with dilute acid, sodium bicarbonate, and water. The solution was then dried, concentrated, and cooled, when chloramphenicol separated in crystalline form. It was recrystallised from water or ethylene dichloride[10].

Chemical properties and structure. Chloramphenicol forms colourless needles, m.pt. 150°C., $[\alpha]_D^{25°C.}$ − 25·5° (ethyl acetate), and has a characteristic absorption spectrum with a single maximum at 278 mμ. Its solubility in water is about 2·5 mg./ml., but it is very soluble in ethyl alcohol. It is quite stable in acid or neutral aqueous solution, but is inactivated in alkali. Several methods for estimating the substance have been described[11,12,13].

Chloramphenicol is a neutral compound which has the molecular formula $C_{11}H_{12}Cl_2N_2O_5$. Its ultra-violet absorption spectrum suggested that it was a nitrobenzene derivative[14]. On acid or alkaline hydrolysis it yielded dichloracetic acid and an optically active base with the formula $C_9H_{12}N_2O_4$. The base was oxidised by periodic acid to ammonia, formaldehyde and *p*-nitrobenzaldehyde and thus appeared to have the structure (II). It followed that (I) was the probable structure of chloramphenicol[14].

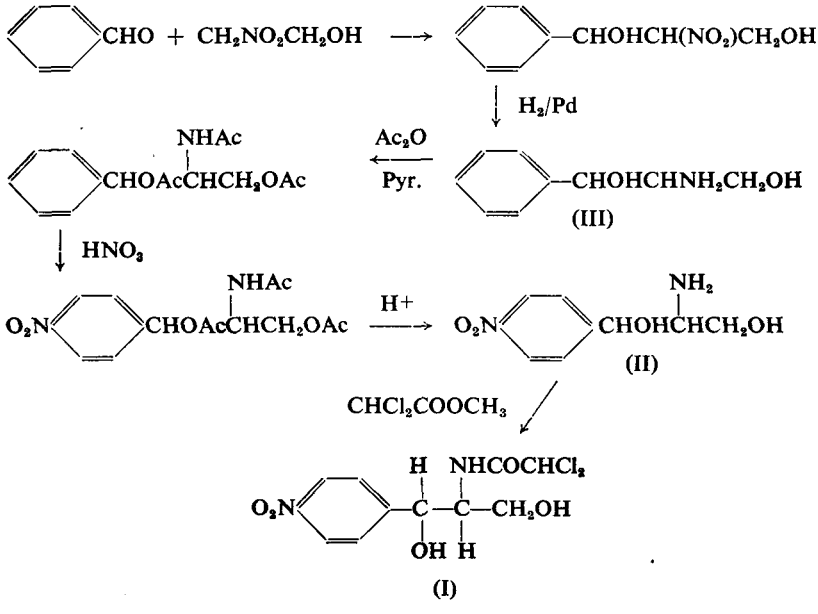


(II)

The structure (I) was confirmed by the following synthesis, starting from benzaldehyde and β-nitroethanol.[15].

The base (III) consisted of the racemates of two stereoisomers and was separated into racemates belonging to the *threo* and the *erythro* configuration. The racemic base (II) from the *threo* series was resolved

by crystallisation of the *d* and *l*-salts of *d*-camphorsulphonic acid, and the *N*-dichloracetamide from the (−) base was found to be identical with



chloramphenicol. The corresponding derivative from the (+) base showed less than 0·5 per cent. of the activity of chloramphenicol against *Shigella paradysenteriæ* (Sonnei).

This work represented the first practical synthesis of an antibiotic of medical importance. Two other syntheses, starting from α-acylamidoacetophenone and *p*-nitroacetophenone respectively, were reported almost immediately afterwards[16,17].

Antimicrobial properties. Chloramphenicol inhibits the growth of a wide range of Gram-positive and Gram-negative pathogens at a dilution of 1 in 10^6 or more[7,9]; sensitive organisms include *Staphylococcus aureus, Streptococcus pyogenes, Bacterium coli, Brucella abortus, Brucella melitensis, Salmonella typhi, Salmonella schottimuelleri* and *Salmonella paradysenteriæ*. It was found to be from 7 to 36 times as active as penicillin and twice to 10 times as active as streptomycin against a variety of Gram-negative organisms, although only about one fiftieth as active as penicillin against a strain of *Staph. aureus*. It was only about one-tenth as active as streptomycin against various strains of *Mycobacterium tuberculosis*[7,9].

Some bacteria contain enzymes able to inactivate chloramphenicol. *Bacterium coli, Bacillus mycoides, Bacillus subtilis* and *Proteus vulgaris* can degrade the drug by reducing the nitro group, hydrolysing the amide linkage, oxidising the secondary hydroxyl group, and cleaving the molecule between the first and second carbon atoms of the propanediol chain[18]. At least 18 different decomposition products can be formed as a result of these reactions.

In addition to its antibacterial power, chloramphenicol showed chemotherapeutic activity in chick embryos infected with *Rickettsia prowazeki* (epidemic typhus)[7] and in mice infected with *Rickettsia orientalis* (scrub typhus)[19]. Subsequent work demonstrated that it was also active against some of the larger viruses, including the agents responsible for psittacosis and lymphogranuloma venereum, but that it was ineffective against most of the smaller viruses[20].

Pharmacological properties. Chloramphenicol is readily absorbed into the blood and body fluids after parenteral or oral administration. In mice, the intravenous LD50 was found to be about 245 mg./kg. When given intravenously in propylene glycol to dogs, a dose of 12·5 mg./kg. had no effect and 100 mg./kg. produced a fall in blood pressure followed by recovery. A dose of 150 mg/kg. caused sudden death due to a fall in blood pressure and respiratory failure. Dogs that received twice daily a dose of 40 mg./kg. intravenously, or 70 mg./kg. orally, for 24 days, showed no significant changes in total white cell counts, blood sugar and non-protein nitrogen, and no evidence of damage to the liver or the kidneys[9]. Even in small amounts, however, it appears that the drug is not entirely innocuous to animal tissues, for concentrations of 10 μg./ml., less than those attained in the blood of patients, were found to retard the growth of epithelial cells and fibroblasts[21].

After administration to dogs by the intravenous, intramuscular, or oral routes, chloramphenicol rapidly appeared in the urine, but less than 10 per cent. of the total dose was excreted, and it could therefore be concluded that some of the drug was inactivated in the body[9]. Later it was found that when chloramphenicol was given by mouth to man it was excreted partly unchanged, partly as D(−)-*threo*-1-*p*-nitrophenyl-2-amino-1:3-propanediol (II), and partly as the 3-glucuronide of chloramphenicol. The glucuronide was the main product. This derivative had no antibacterial activity, but chloramphenicol could be liberated from it by the enzyme β-glucuronidase[22].

AUREOMYCIN

The isolation of a new antibiotic[23] from the culture fluid of *Streptomyces aureofaciens* was announced in 1948. The antibiotic was named aureomycin, on account of the yellow colour of the crystalline substance. Aureomycin is prepared in the Lederle Laboratories Division of the American Cyanamid Company. Little has been revealed about its chemical structure or the methods used for its isolation, but its biological properties have been the subject of many publications. Rapid biological methods for assaying the substance have been described[24,25].

Chemical properties. Aureomycin is an amphoteric compound which contains nitrogen and non-ionic chlorine. The crystalline substance analyses as follows: C, 54·56; H, 5·34; N, 5·77; Cl, 7·16; O, 21·17; Mol.Wt, 508. It melts at 168° to 169°C. and has $[\alpha]_D^{23^\circ C.} - 275{\cdot}0$. It forms a hydrochloride which contains 6·69 per cent. of ionic Cl, decomposes above 210°C., and has $[\alpha]_D^{23^\circ C.} - 240{\cdot}0$.

Aureomycin is only soluble in water to the extent of about 0·5 mg./ml. at 25°C., but is very soluble in alcohol and in aqueous solution above pH 8·5. The hydrochloride is more soluble in water than the free base[26], giving a solution containing 14 mg./ml. with a *p*H of 2·9.

At 37°C. aureomycin is unstable in aqueous solution at *p*H 7 or above, and its instability is markedly increased in the presence of certain bacteriological media[27]. A fluorimetric method for assaying aureomycin has been suggested[28].

Antimicrobial properties. In serial dilution tests read after 24 hours, aureomycin inhibits the growth of a variety of Gram-positive and Gram-negative organisms at dilution ranging from 1 in 10^5 to 1 in 10^6 and more, but when readings are made after 96 hours it appears very much less active. The growth which occurs on continued incubation of the cultures is no doubt due in part to the destruction of aureomycin in the culture fluid[27]. In sufficient concentration the antibiotic may kill the majority of organisms in a culture[29].

Among the bacteria sensitive to aureomycin are *S. typhi, Bact. friedländeri, Bact. coli, Str. haemolyticus* and *Staph. aureus.* In general, the Gram-positive organisms are affected by lower concentrations than the Gram-negative ones. Some strains of *Staph. aureus,* including penicillin-resistant strains, are particularly sensitive. Aureomycin is also active against *E. histolytica*[30,31].

Experiments with embryonated hen's eggs, mice, and guinea pigs showed that aureomycin was active *in vivo* against the rickettsiae of epidemic typhus, scrub typhus, Rocky Mountain spotted fever and Q fever, and the viruses of the psittacosis-lymphogranuloma group, although it appeared to be unable to destroy these agents *in vitro*[32]. Thus, complete protection was afforded to mice infected with lymphogranuloma venereum virus by daily doses of 1 mg. of aureomycin subcutaneously, or 5 mg. orally, for 5 days. Similarly, daily doses of 5 mg., given orally, completely protected mice from as much as ten million lethal doses of the Karp strain of scrub typhus (*R. tsutsugamushi*).

Pharmacological properties. Aureomycin, like chloramphenicol, is readily absorbed from the gastro-intestinal tract, and also passes the blood-brain barrier into the cerebrospinal fluid. After an oral dose it may be excreted in the urine for more than 10 hours. When given intravenously, the LD50 of aureomycin hydrochloride for mice was 134 mg./kg. Dogs tolerated doses of 50 mg./kg. intravenously without symptoms, and 200 mg./kg. per day orally for 12 weeks without evidence of damage to the blood, liver or kidneys. The drug was found to be a mild diuretic, but it did not produce albuminuria[33].

TERRAMYCIN

Terramycin, a product of *Streptomyces rimosus,* was discovered in the Biochemical Research Laboratories of Chas. Pfizer and Co., Inc. Its discovery was the result of an extensive programme of research in which micro-organisms in soil samples from many parts of the world were

investigated for their capacity to produce antibiotics. *S. rimosus* was so named because of the cracked appearance of its growth on the surface of an agar medium[34,35]. Preliminary experiments, in which the actinomycete was grown in liquid media in Erlenmeyer flasks, showed that it produced an antibiotic active against a variety of Gram-positive and Gram-negative bacteria. Activity was measured turbidimetrically[36].

Isolation. *S. rimosus* was grown under submerged aerobic conditions and culture fluids were obtained containing about 200μg./ml. of terramycin. The antibiotic was extracted from the culture fluid with *n*-butanol at *p*H 7·5. After concentrating the butanol *in vacuo*, the antibiotic was extracted into a small amount of 0·1 N hydrochloric acid, from which it was precipitated as a crude brown powder on neutralisation. Further purification was carried out by chromatography of the hydrochloride on a column of florisil. The column was developed first with water, which removed inactive material, and then with acetone, which eluted a light yellow band containing most of the activity. The material from the active band was extracted into butanol at *p*H 7·5 and re-extracted into 0·05 N. hydrochloric acid. On concentrating the acid solution terramycin separated as a crystalline hydrochloride. Neutralisation of an aqueous solution of the hydrochloride yielded crystalline terramycin dihydrate[37].

Crystalline terramycin appeared homogeneous when examined by paper chromatography, using *n*-butanol-acetic acid-water for development, or by counter-current distribution in a two-phase system consisting of *n*-butanol and buffer at *p*H 2·5.

Chemical properties. Terramycin dihydrate, m.pt. 181° to 182°C., appears to have the molecular formula $C_{22}H_{24\text{-}26}N_2O_9,2H_2O$. It shows absorption maxima in the ultra-violet region at 266 mμ and 366 mμ and a number of strong absorption bands in the infra-red. It gives positive ferric chloride, Pauly, and Molisch tests[37]. Terramycin is an amphoteric compound and potentiometric titration reveals one group on the acid side and two groups on the alkaline side of the neutral point. The substance forms a hydrochloride, containing 7·16 per cent. of Cl, and a disodium salt which analyses for $C_{22}H_{22\text{-}24}N_2O_9Na_2,2H_2O$. The solubility of terramycin in water is a minimum at *p*H 5, being only about 0·5 mg./ml. Below *p*H 2 or above *p*H 8, when the substance is in the form of the hydrochloride and the sodium salt respectively, the solubility is greatly increased. Terramycin is quite stable in dilute acid solution. It is less stable in dilute alkali, but appears to be inactivated less rapidly than aureomycin. At 37°C. its half-life is 134 hours at *p*H 2·5, 26 hours at *p*H 7, and 14 hours at *p*H 10.

Antimicrobial properties. Terramycin inhibits the growth of a variety of Gram-positive and Gram-negative pathogens for 20 hours at dilutions of the order of 1 in 10^6. Its antibacterial range was found to be similar to that of aureomycin, but in general it showed a somewhat higher activity and was less susceptible to inactivation by serum. As with aureomycin, cultures inhibited by terramycin tended to grow out on prolonged incub-

ation, and this was ascribed to the deterioration of the antibiotic in the medium[38]. Terramycin inhibited the growth of the H 37 Rv strain of *Myco. tuberculosis,* and of streptomycin-resistant tubercle bacilli at a dilution of about 1 in 100,000[39]. Like aureomycin, terramycin is active against *Entamœba histolytica,* inhibiting the growth of this amoeba *in vitro* at a dilution of 1 in 40,000[40]. Like chloramphenicol and aureomycin, terramycin is active against a number of rickettsiae and against certain viruses in chick embryos[41,42]. In doses of 300 μg. per egg it suppressed the multiplication of *R. prowazeki* (epidemic typhus) and *D. rickettsi* (Rocky Mountain spotted fever), and the amounts required to protect half the embryos for 12 days were smaller than the corresponding amounts of aureomycin and chloramphenicol. About 2 mg. of terramycin per day, given orally, protected mice from infection with as much as 10^5 LD50 of *R. tsutsugamushi* (scrub typhus).

Terramycin suppressed the multiplication of influenza virus in chick embryos if the toxic level of the drug was approached, but it exerted no effect on a Type A influenza virus infection in mice. It was also ineffective against herpes simplex virus and rabies virus[43,44].

Pharmacological properties. Terramycin is absorbed from the gastrointestinal tract, and becomes widely distributed throughout the animal body. It is able to cross the blood-brain barrier in greater amount than either aureomycin or chloramphenicol. After oral administration of terramycin considerably more of the drug is recovered both in the urine and in the fæces than is the case with aureomycin or chloramphenicol[45]. When given intravenously to mice, the LD50 of terramycin was 178 mg./kg. The corresponding subcutaneous and oral doses were about 800 mg./kg. and 6·7 g./kg respectively. Dogs given daily oral doses of 465 mg. of terramycin hydrochloride per kg. for over 40 days showed no changes in the blood, or in liver or renal function, and the tissues and organs of these animals appeared normal on histological examination. The only reaction observed was vomiting and loose stools, which disappeared or decreased in frequency on continued administration of the drug.

Neomycin

Neomycin is produced by *Streptomyces fradiae,* an actinomycete that was isolated from the soil during the course of an investigation designed particularly to find new antibiotics active against streptomycin-resistant strains of *Myco. tuberculosis*[46]. The crude product consists of a mixture of antibiotics and is called the neomycin complex. Neomycin is quite different from chloramphenicol, aureomycin, or terramycin; it is probably best compared with streptomycin, although the two substances are easily distinguished by their chemical and antibacterial properties.

Production. Neomycin was produced by growing the streptomyces in deep aerated culture in a medium containing soya peptone or bacto peptone, glucose, and meat extract. The antibiotic complex was concentrated by methods similar to those used for the isolation of streptomycin.

The active material was adsorbed onto charcoal and eluted with 50 per cent. methanol containing 0·05 N hydrochloric acid. The resulting neomycin hydrochloride was then precipitated as the picrate from aqueous solution, and the regenerated hydrochloride was further purified by chromatography on charcoal. Counter-current distribution showed that the product contained three antibiotics and that the components of this "neomycin complex" were different from streptomycin[47]. Further purification of the neomycin complex by counter-current distribution and chromatography, in the laboratories of Merck and Co., Inc., led to the isolation of one of its constituents, called neomycin A, in a pure condition. Treatment of concentrates with *p*-(*p*-hydroxyphenylazo)-benzene sulphonate, methyl orange, and orange II, yielded the corresponding crystalline sulphonic acid salts. The regenerated hydrochloride was obtained as an amorphous powder[48]. Investigations of a neomycin complex from *S. fradiae* in the laboratories of Chas. Pfizer and Co., Inc., resulted in the isolation of a crystalline sulphonic acid salt of an antibiotic that was different from neomycin A. This was called neomycin B[49].

Chemical properties. Neomycin A hydrochloride melts at 250° to 260°C. and has $[\alpha]_D^{25°C.}$ + 83°. It gives a positive ninhydrin test for amino groups, but unlike streptomycin it gives negative glucosamine and maltol tests, and a negative Sakaguchi test for guanido groups[48]. It shows only end absorption in the ultra-violet region. Neomycin B forms a crystalline *p*-(*p*-hydroxyphenylazo)-benzene sulphonate which has $[\alpha]_D^{25°C.}$ + 30°C. and analyses as follows: C, 46·2; H, 5·3; N, 10·1; S, 8·4 per cent. The analysis of the sulphate obtained from this salt was: C, 29·4; H, 6·9; N, 9·21; $SO_4^{=}$, 28·4 per cent.[49] Crude neomycin was stable at room temperature and in aqueous solution between *p*H 1·5 and *p*H 12.

Antibacterial properties. Crude neomycin is highly active against many Gram-positive and Gram-negative bacteria and against mycobacteria. Like a number of other basic substances[50], it is most active at an alkaline reaction. It antibacterial range is quite different from that of streptomycin, and organisms that are originally susceptible to both antibiotics remain sensitive to neomycin when they have acquired resistance to streptomycin[51]. An interesting characteristic of neomycin is its high activity *in vitro* against some strains of *Proteus* and *Pseudomonas*, since it appears to be the only known antibiotic which attacks both these organisms[52].

Pharmacological properties. Little has so far been published about the pharmacological properties of neomycin. The crude neomycin complex is reported to have a relatively low toxicity to mice, but different batches appear to vary in their effects[51] and there are indications that the drug can damage the kidneys[53,54]. Nevertheless, the material is said to have a high chemotherapeutic index and to be more effective than streptomycin in suppressing infections in mice with *Staph. aureus*, *S. schottmuelleri*, and *S. typhi*[55]. In man, therapeutic levels of the drug have

been obtained in the blood and urine following intramuscular administration[56].

Mode of Action and Acquired Resistance

Chloramphenicol, aureomycin, and terramycin have been said to be mainly bacteriostatic in their action, but there is no doubt that in sufficient concentration they are able to kill susceptible bacteria. Unlike penicillin, however, they do not readily sterilise a culture, for a proportion of the organisms often survive and eventually multiply[38,57,58]. Whether such substances are described as bacteriostatic or bactericidal is purely a matter of definition. Neomycin, in contrast to the other three antibiotics, is stated to have powerful bactericidal properties[46,51].

Although there have been many investigations of the mode of action of antibiotics *in vitro*, relatively little is yet known of the precise manner in which the substances with chemotherapeutic properties affect the bacterial cell. Possibly they interfere with the functioning of specific bacterial enzymes, and since penicillin, streptomycin, and recently chloromycetin[59] have been shown to be more effective against growing than against resting organisms, special importance may attach to enzymes concerned with the synthesis of new cell material.

One approach to this difficult subject is to look for an enzyme system in the cell which is affected by the antibiotic, although even if such a system is found, it is often not easy to decide whether its inhibition is the primary cause of the antibacterial activity. As a consequence of work on these lines with the newer antibiotics, some interesting observations have been made on the inhibition of certain enzyme reactions by chloramphenicol and aureomycin.

Chloramphenicol does not affect the respiration or protein breakdown of resting or growing bacteria, but it disturbs the metabolism of fats by inhibiting the action of esterases[60]. Its action on the esterases of animal tissue cells is much weaker than on those of sensitive bacteria and it has been suggested that the animal cell wall presents a barrier to the drug. Aureomycin, like gramicidin and 2:4-dinitrophenol, appears to be able to inhibit a process by which the energy of cellular oxidation is made available for synthesis, for it inhibits phosphorylation in mitochondria without affecting respiration[61].

The remarkable way in which bacteria acquire resistance to many antibacterial substances when grown in their presence is of theoretical interest and considerable practical importance. If organisms became highly resistant to an antibiotic *in vivo* its clinical value will be seriously diminished. The development of such resistant strains has been one of the factors that have limited the chemotherapeutic power of streptomycin.

From a practical point of view, at least, it seems profitable to distinguish two kinds of acquired resistance. In the first kind, which is frequently encountered with streptomycin, the organisms develop a high and apparently permanent resistance in one stage. In the second kind, which is found with penicillin, resistance develops gradually in a stepwise fashion, and the resistant organisms sometimes regain their sensitivity when grown in the absence of the drug[62].

There is no doubt that bacteria can acquire resistance to chloramphenicol, aureomycin, terramycin, and neomycin. With chloramphenicol and a strain of *Bact. friedländeri*, not only were resistant organisms formed but organisms emerged that were dependent on the drug for their growth[63]. A strain of *Bact. aerogenes* was also reported to have increased its resistance to chloramphenicol 15-fold *in vivo*[64].

A number of bacteria, including *S. typhi*, *Bact. coli*, *Pr. vulgaris*, *Strep. hæmolyticus*, and *Bact. friedländeri*, were shown to become more resistant to aureomycin *in vitro*, but with the exception of *Pr. vulgaris* the increase in resistance was less than 70 fold after 14 transfers. *Staph. aureus* showed no change in sensitivity. Under comparable conditions the resistance of bacteria to streptomycin often increases many thousand times[27,65].

No change was noticed in the sensitivity of *Myco. tuberculosis* to terramycin after 8 transfers in the presence of the drug[57], and attempts to increase the resistance of *Myco. ranæ* and *Bact. coli* to terramycin to any great extent were unsuccessful[62].

Resistance to neomycin was said to develop more slowly than to streptomycin and to be relatively impermanent in character[46,51,62], although some investigators denied that this was the case with *Myco. tuberculosis*[66,67].

It seems clear that bacteria acquire resistance to the new antibiotics much less easily than to streptomycin, and it is possible that the emergence of resistant strains will not prove a serious disadvantage to the use of these substances in the clinic[64]. It is also established that bacteria that have become highly resistant to streptomycin retain their sensitivity to chloramphenicol, aureomycin, terramycin, and neomycin. On the other hand the phenomenon of cross resistance is encountered with chloramphenicol, aureomycin, and terramycin, and emphasises the similarity in biological properties of these compounds. Strains of *Bact. coli* or *Bact. aerogenes* made resistant to either chloramphenicol, aureomycin, or terramycin showed an increased resistance to all three antibiotics. When strains of *Strep. fæcalis* and *M. pyogenes* were made resistant to aureomycin there was a substantial increase in resistance to terramycin, and *vice versa*, but with these organisms the sensitivity to chloramphenicol remained unchanged[68].

Whether acquired resistance to antibacterial substances is a consequence of mutations that occur independently of the drug, or of an interaction between the drug and the organisms, or whether both mechanisms play a part in the phenomenon, is still a matter of controversy[69]. Work with the new antibiotics has so far contributed little to the solution of this problem.

The New Antibiotics in Medicine

It is now established that chloramphenicol, aureomycin, and terramycin are valuable systemic chemotherapeutic agents, and the numerous publications on their clinical use have been the subject of several reviews[64,65,70,71,72,73].

These antibiotics are usually given by mouth in capsules containing 250 mg. of material. 2 or 3 capsules may be given 3 or 4 times a day. The drugs exert a strong suppressive action on the intestinal flora[74] since they are absorbed slowly and incompletely, and they may persist in the blood for 12 hours after a single dose. No serious toxic reactions have followed their use, but there are sometimes unpleasant side-effects, such as flatulence, diarrhœa, nausea, muscular weakness, and changes in the tongue[75].

The three antibiotics have a wide range of antibacterial activity, but in general chloramphenicol is more effective against Gram-negative organisms, and aureomycin and terramycin against Gram-positive ones. In some bacterial diseases, such as undulant fever due to *Br. abortus* and *Br. melitensis*, and certain urinary tract infections, chloramphenicol, aureomycin, and terramycin appear to be equally effective[76]. In brucellosis, fever is terminated within a few days and the cure may be permanent after treament for 2 weeks. All three drugs can cure gonorrhœa[77,78]. On the other hand, chloramphenicol is far more effective than aureomycin or terramycin in the treatment of typhoid fever. The value of chloramphenicol in this disease was discovered accidentally while the drug was being used in the treatment of scrub typhus[79,80]; although relapses may occur a permanent cure can often be effected if treatment is continued for more than 8 days[81]. The interesting observation has recently been made that the acute manifestations of the disease terminate more promptly if chloramphenicol is given together with cortisone[82].

Aureomycin is highly effective in staphylococcal infections and is of particular value in the treatment of diseases caused by penicillin-resistant staphylococci[83,84]. It is also effective in pneumonia caused by a variety of different bacteria, including pneumococci, streptococci, staphylococci, *H. influenzæ* and *Bact. friedländeri*. Unlike chloramphenicol, aureomycin and terramycin have a pronounced action in syphilis[78] although further investigations will be needed before their value in the various forms of this disease can be assessed.

In addition to their value in many bacterial infections it seems probable that aureomycin and terramycin will prove useful in the treatment of intestinal amœbiasis. Thus daily doses of 1 or 2 g. of terramycin by mouth for 10 days resulted in the disappearance of *E. histolytica* from the stools of all but one of 22 patients[40].

The most remarkable property of the new antibiotics is their chemotherapeutic action in rickettsial and certain viral diseases, since these infections were not previously amenable to chemotherapy. Following the discovery of the antirickettsial activity of chloramphenicol in chick embryos, the drug was tried in man against scrub typhus, a mite-born jungle form of typhus that caused some 25,000 casualties among British and American troops in South East Asia during the last war. The results of clinical trials in Malaya and elsewhere in 1948 left no doubt of its value, for patients were rendered afebrile in 48 hours and convalesced rapidly[85] after receiving a few grams of the drug. Subsequent investiga-

tions have shown that similar curative effects are obtained in epidemic typhus, murine typhus and Rocky Mountain spotted fever. The effects of aureomycin and terramycin in rickettsial diseases appear to be as dramatic as those of chloramphenicol[86,87,88]. It is of interest that aureomycin has proved effective in Q fever, the only rickettsial infection known to exist in the British Isles.

Three virus diseases, primary atypical pneumonia, psittacosis, and lymphogranuloma venereum, can be treated successfully with aureomycin[86,89], and the virus pneumonia has also been shown to respond to terramycin[90]. A number of other virus diseases, however, including poliomyelitis, influenza, and the common cold, are unaffected by any of the new antibiotics.

It would be premature, at the present time, to try to assess the clinical value of neomycin. Reports that the crude substance can damage the kidneys suggest that caution will be necessary in its use, but final judgment must be reserved until more is known about the pharmacological properties of the pure components of the neomycin complex. Recently, the neomycin complex has been used successfully in 10 patients with pyelonephritis or cystitis[56]. The cases were chosen because the infecting organisms, which included *Bact. aerogenes, B. pyocyaneus, Bact. coli,* and *Proteus,* were sensitive to neomycin but insensitive to penicillin, streptomycin, chloramphenicol, or aureomycin. Neomycin was said to be dramatically effective in eradicating organisms sensitive to it from the blood and urinary tract, and the only evidence of toxicity, which was confined to one patient, consisted of impaired hearing and an increased urea nitrogen level in the blood.

The interest that has been aroused in neomycin, however, has centred mainly around the possibility that it may be useful in the treatment of tuberculosis. Of the serious bacterial diseases, this is now the most difficult to deal with by chemotherapy, and although streptomycin has proved of value it has unfortunate limitations. Neomycin is at present undergoing clinical trial. It remains to be seen whether it will prove superior to streptomycin, or useful in the treatment of cases in which the tubercle bacilli are streptomycin-resistant.

With the antibiotics now available most bacterial and rickettsial infections, and some virus diseases, can be treated with a good prospect of success. It should not be forgotten that before these substances reach the clinic they have generally been the subject of extensive chemical and biological investigation. Many of the properties required by a systematic chemotherapeutic agent are well understood and can be determined in the laboratory[91]. Nevertheless, the value of a new antibiotic cannot yet be predicted with certainty in the basis of its antimicrobial activity *in vitro* and its pharmacological behaviour. Chloramphenicol and aureomycin have turned out to be more effective, against some bacterial infections, than would have been anticipated from their activity *in vitro.* For example, aureomycin is said to be 5 to 10 times more effective against *Staph. aureus in vivo* than *in vitro*[92]. On the other hand, chloramphenicol is much more effective than aureomycin against typhoid fever, although

the two drugs have a similar activity against *S. typhi in vitro*, and aureomycin is ineffective against tuberculosis in mice even though it compares favourably with streptomycin in its activity against *Myco. tuberculosis in vitro*[93]. It is evident that much has yet to be learned about the factors which govern the activity of these remarkable compounds in the body.

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