CHAPTER VI.

ON CHLORAMPHENICOL
During a systematic search for antibiotics conducted at the Yale University, in which 6,000 soil samples and 20,000 moulds were examined, Burkholder, in 1947, made the pioneer observation that the growth of both gram-positive and gram-negative bacteria was inhibited on an agar streak culture by an actinomycete (Ehrlich, Bartz, Smith, Joslyn and Burkholder, 1947). This finding was destined to have far-reaching results. The particular actinomycete was obtained from the soil of a mulched field near Caracas in Venezuela and was later named Streptomyces venezuelae (Ehrlich, Gottlieb, Burkholder, Anderson and Pridham, 1948).

From the culture filtrate of this organism a crystalline substance was isolated and found to be inhibitory to a wide range of gram-positive and gram-negative bacteria, rickettsiae and the pitt-tacosis virus, (Ehrlich et al, 1947 and Smadel & Jackson, 1947.). This antibiotic, which was given the name of 'Chloramphenicol' with its impressive array of antimicrobial activity, its stability and low toxicity for animals, held great future promise.

At about the same time, another team of workers at the University of Urbana in Illinois, independently isolated a Streptomyces from the compost of a farm. This was subsequently found to be identical to chloramphenicol and the study at Illinois was abandoned, (Carter, Gottlieb & Anderson, 1947 and Gottlieb, Bhattacharya, Anderson & Carter, 1948).

This new antibiotic proved to be very intriguing to the chemist
for when Bartz (1948) succeeded in isolating it in a crystalline form, the product was found to have an unusual composition with chlorine in a non-ionic, organically bound inert form along with a nitro group. Both these features are rare in naturally occurring substances. Besides this unique composition, it was found to be a white crystalline substance with a melting point of 149.7°C and was stable enough to be sublimed in-vacuo. It was also stable in neutral and acid solutions but was rapidly destroyed at pH 10.82. It was further found that in distilled-water solution there was no loss of activity by heating at 37°C for one month or to 100°C for 5 hours. It is a relatively insoluble substance, the solubility in water being about 2.5 mg./ml. but is readily dissolved in methanol, ethanol, butanol-1 and ethyl ether and is insoluble in benzene and petroleum ether. It is optically active and the absorption spectrum in water or 0.1 N-hydrochloric acid shows a peak level at a wave length of 278 mp (Bartz, 1948).

After its isolation, methods for its synthesis were soon devised and adapted to a commercial scale (Rebstock, Crooks & Controulis, 1949 and Controulis, Rebstock & Crooks, 1949). The synthetic product was found to be equally effective as the naturally occurring substance (Smadel, Jackson, Ley & Lewthwaite, 1949).

The structure of the antibiotic was established and confirmed by Rebstock et al (1949) and Controulis et al (1949). It is a D (-) three-ß-nitrophenyl-2-dichloracetamido-1,3-propanediol. Hahn et al (1956) have considered the molecule as follows:
The propanediol moiety

II Dichloracetamide side chain

III The p-nitrophenyl group

By synthesis, two pairs of stereo-isomers corresponding to the two asymmetrical carbon atoms in the propanediol moiety have been obtained (Controulis et al., 1949). But only the D (−)threo-isomer corresponding to the one produced by Streptomyces venezuelae has a significant antibacterial action (Maxwell & Nickol, 1954) thus emphasizing the importance of the steric configuration. The antibiotic can be destroyed by reduction of the nitro group to formaryl amines, by hydrolysis of the amide bond to split away the dichloracetic acid side chain, and by degradation of the propanediol chain. There is evidence that this can be brought about by enzymes derived from bacterial and animal origin (Smith & Worrel, 1949a; Smith, Worrel & Lilligran, 1949; Smith & Worrel, 1950 and Glazko, Dill & Wolf, 1952). A theoretical analysis of the chemical structure and antibiotic activity among members of the chloramphenicol series has been carried out by Hahn et al. (1956). They have concluded that the aromatic character of the ring structure is essential for biological activity and that the steric configuration of the substi-
tuent attached to the two asymmetric carbon atoms of the propandiol moiety controls the antibiotic activity. Further variations beyond narrow limits in the molar volume of the electronegative bead of the acylamide side chain abolish the antibiotic activity.

Owing to the demonstration of anti-microbial activity in-vitro and the success obtained with infections of rickettsiae in chick embryos and of the psittacosis virus in embryonated eggs and mice the possibility of the application of this finding to human-beings was considered and the pharmacology was studied at great length.

The absorption studies showed that the antibiotic is rapidly absorbed both by the oral and parenteral routes (Smith et al, 1948; Ley, Smadel & Cocker, 1948; Glasko, Wolf, Bill & Bratton, 1949; Gruhzit, Fisken, Reutner & Martino, 1949). In dogs, a single oral dose of 86-150 mg./kg. provides serum levels from 8-35 mcg./ml. at 2 hours, 10.6-20 mcg./ml. at 4 hours and less than 1.0 mcg./ml. at 12 hours. With a higher dosage greater serum levels are obtained (Gruhzit, Fisken, Reutner & Martino, 1949). The peak is reached at about 4 hours (Crooks, 1949). Other animals also appear to show a similar rate of absorption with very slight variations. Thus in rabbits, a subcutaneous injection of 100 mg./kg. produces concentrations of 4.9-5 mcg./ml. in 2 hours and 0.6-4.6 mcg./ml. in 4 hours (Gruhzit and Fisken, 1951). In mice this dose given as two subcutaneous daily injections produces serum levels of 11 mcg./ml. (Gruhzit, Fisken, Reutner & Martino, 1949).

In the guinea-pig when 750 mg./kg. chloramphenicol are adminis-
tered orally the concentration obtained is 2.3 µg./ml. of serum at 2 hours, and at four hours no chloramphenicol can be detected. But with the same dose given subcutaneously, serum concentrations of 7.8 µg./ml. at 30 minutes and 2.2 µg./ml. at 24 hours have been reported (Gruhnitz, Piskon, Reutner & Martino, 1949), indicating that in the guinea-pig, chloramphenicol is poorly absorbed when administered orally or is rapidly degenerated in the body.

The serum levels in normal human subjects after oral administration of chloramphenicol have been studied by Loy et al. (1948). They were able to detect appreciable amounts in the serum within 30 minutes. The serum concentration rose to its peak in about 2 hours and then declined slowly over a period of several hours. Crookes (1949) has reported that after an oral dose of 1.5 gm. maximum concentration in serum is attained in four hours and effective levels are maintained for 6-8 hours. With a larger single dose of 2-5 gm., low levels may persist in the serum for as long as 24 hours. The maximum concentration attained and the time required for the serum concentration to fall to negligible values depends on the size and frequency of the dose and with repeated doses at 4-8 hourly intervals a continuous therapeutically effective concentration can be maintained. Bunnell & Kirby (1951) have estimated serum concentration after intravenous and intramuscular administration, and have concluded that the concentration was slightly higher with the intravenous route as compared to the oral route and persisted for a longer period. They were of the opinion that the intramuscular route produced concentrations which were inadequate for therapy.
After gaining access to the blood, owing to the relatively small size of the molecule, chloramphenicol readily diffuses into tissues and body fluids and passes across different internal barriers, (Glasko et al, 1949; Ross, Bischoff, Presiser and Orr, 1949; Scott & Warner, 1950; Ross, Burke, Sites, Rice and Washington, 1950; Williams & Dart, 1950; Leopold, Nichols & Vogel, 1950; Riley, 1950 and Bender, Pressman & Tashjian, 1953). The concentration in different tissues has also been estimated (Glasko et al, 1949 and Gray, 1955).

The question of whether chloramphenicol is able to penetrate the cell wall has not yet been finally settled. Glasko et al (1949) have reported that unchanged chloramphenicol and one of its hydrolytic products are bound to the formed elements of the blood; whereas the glucuronide derivative, is located almost entirely in the plasma. They are of the opinion that the binding is due to adsorption rather than slow diffusion through the cell-membrane. Magoffin & Spink (1951) could draw no conclusion about the bacteriostatic action of chloramphenicol on Brucella organisms located within the leucocytes. Pramer, in 1955, demonstrated slow but definite penetration of the antibiotic into the cells of an alga called Nitella clavata. An indirect, but more convincing evidence is afforded by the work of Bozeman et al (1956). They showed that by addition of chloramphenicol to tissue culture cells infected with Rickettsia tungsurgusenushi, there is rapid disappearance of the organism from the cells and a fall in the infective titre. Hopps, Jackson, Danzuskae (1959) have shown that L 929 cell cultures heavily infected with R.
tsutsugamushi can be completely freed of the organisms by the use of chloramphenicol. But Smith, Worrel and Swanson (1949) in their study of chloramphenicol on esterase activity, have suggested that there is a barrier at the cell wall, as only 40–50% esterase activity is inhibited when mitochondria were used for the test.

In the blood, the chloramphenicol combines reversibly with the serum albumen and about 45% occurs in this protein-bound form. This amount is relatively little affected by the concentration of the drug in the serum (Smith, Joslyn, Gruhzit, McLean, Penner & Ehrlich, 1948).

The subsequent fate of chloramphenicol has been studied by the assay of excretion products. Several methods have been devised of which the most important are the colorimetric and the microbiological methods (Glasko, Wolf & Dill, 1949; Berman & Stevens, 1950; Joslyn & Galbraith, 1950 and Lovine & Fischbach, 1951). The former is based on the estimation of the reduction of the aromatic nitro group to amino group and determination of the resulting amines by a modification of the Bratton Marshall diazo reaction. This method, however, includes the nitro compounds formed by the degradation of the chloramphenicol. The microbiological methods on the other hand assay only the active form of the drug. They are usually performed either by the turbidimetric or by the diffusion plate assay method.

Applying these methods of assay to urine, Glasko et al (1949) have estimated that in man, after oral administration, about 75–90 per cent of the drug is excreted within 24 hours in forms which retain the aryl nitro group intact. But microbiological assay has
shown that only about 10% is present in the active form. Later in 1950, Glazko, Dill & Rabstock studied the metabolic products of chloramphenicol in the urine and have found that in the rat, dog and man, the principal nitro compounds are made up of unchanged chloramphenicol, a hydrolysis product, and a glucuronic acid conjugate. And of all these only the unaltered drug exhibits significant antibiotic activity. In the guinea pig the metabolic fate of chloramphenicol appears to differ from that observed in other animals in that large amounts of aryl compounds are found in the tissues.

The urinary excretion rate of nitro compounds as related to the serum level of the drug was also studied (Glazko et al., 1949) and found to be directly proportional. Although a large percentage of the drug is converted to an inactive form, the urinary concentrations of the active product are high. Ley, Smadel & Crocker (1948) have shown that with a single oral dose of 1 gm., a urinary level of 200 mcg./ml. was obtained at three hours and this fell to 50 mcg./ml. at 8 hours.

The microbiological and colorimetric values diverge considerably after the first two hours (Glazko et al., 1949). Inactive nitro compounds are excreted for more than 24 hours after the administration of the drug, though the microbiological method reveals only very low concentrations of the active drug.

In man, the principal mechanism for renal excretion of the unaltered product is by glomerular filtration, whereas the inactive nitro compounds are excreted mainly by tubular excretion (Glazko et al., 1949).
A considerably smaller fraction of the administered dose of chloramphenicol is excreted in the bile and faeces as compared to urine. Glazko et al (1949) have reported that in a man with a biliary fistula after a dose of 1 Gm, only 2.7% of the drug could be recovered from the bile by chemical assay and the active form was even less, being about 0.14%. As compared to this, the concentration of nitro compounds in the stool is negligible and the authors suggest that this is due to reabsorption of the products from the gall-bladder. Gray (1953) has also found that in a patient with cholecystitis in whom gall-bladder drainage was established, after a dosage of 100 mg./kg./day, the gall-bladder received 5-6 mg. of active chloramphenicol per day. The stool, however, accounted for only 0.8-1 mg. of chloramphenicol. He has also expressed the view that this discrepancy is due to reabsorption.

Opinion regarding the relative concentrations of chloramphenicol in bile and serum and about the presence of the active form of the drug in the gall-bladder are conflicting. Woodward, Samedel & Ley (1950) have found that three hours after oral administration, the antibiotic level in bile estimated by the bio-assay method, was approximately 50% of that in the blood. Other workers have obtained much higher concentrations in bile (Danopoulous, Angelopoulous and Ziordron, 1954 and Pulaski & Fusillo, 1955). It has been suggested that the drug may not all be in the active form (Berrel, 1951 and Danopoulous et al, 1954) but Gronroos (1952) stated that active chloramphenicol in bile corresponds to that in serum.

Studies of the excretion products in dogs, guinea-pigs and rats,
indicate that the metabolic handling of chloramphenicol is different in man and some of the lower animals. In the dog the amount accounted for by urinary excretion is slightly less than in man and the high concentration of nitro compounds in bile suggest that it may also be a route for excretion. In rats and guinea-pigs, on the other hand, the bile and faeces form the chief route for excretion of the drug in the form of aromatic derivatives produced by reduction of the nitro group. (Glazko, Dill & Wolf, 1952). The subsequent metabolic changes have been studied by these authors in the rat. They state that a small portion of the glucuronide which is the chief excretory product, is reabsorbed and subsequently excreted in the urine, but a larger portion passes into the caecum where it is reactivated by bacterial hydrolysis and ultimately reduced to inactive amines by bacteria.

The chief site for the conjugation of the chloramphenicol appears to be the liver. Incubation of the antibiotic with rat liver brings about inactivation of the drug to the extent of 1/3 of the initial concentration (Glazke et al, 1949). Similar inactivation has also been observed by them with the liver and kidneys of rats & guinea-pigs.

It was evident since its discovery, that chloramphenicol had a 'broad spectrum' of antibiotic activity and was hailed as a great advance in the realm of antibiotic therapy. Laboratory studies using growth inhibition techniques in the test-tube and the response to artificial infection in animals were undertaken by numerous investi-
The largest single study undertaken in this field was by McLean, Schwab, Hillages & Schlingman in 1949. No attempt will be made to enumerate the susceptible organisms or to quote their degrees of susceptibility. Suffice it to say that of the Salmonella species numerous strains were tested and the inhibitory concentration lay between 0.75 - 5.0 mcg./ml. for several strains. Experimental studies on laboratory animals were carried out concurrently by innumerable investigators. All of these served to emphasize the broad sweep of the antibacterial range of this antibiotic and also its undoubted effectiveness against the rickettsia and the psittacosis-lymphogranuloma group of viruses, which had hitherto remained uninfluenced.

Therapeutic trials of chloramphenicol were undertaken very soon after its discovery in ever increasing numbers. Although, most of the bacterial infections, caused by organisms proved susceptible to the drug in-vitro, yielded to the treatment, a complete correlation between the laboratory findings and clinical application was not always forthcoming. The typhoid bacillus itself was readily inhibited by chloramphenicol in test-tube experiments (McLean et al., 1949) and the fact was borne out by the clinical response of patients of enteric fever (Woodward, Sandel, Ley, Green & Mankikar, 1948; Cook & Marrion, 1949; Edge, 1950 and El Rasli, 1953). But the results in typhoid infected mice have not been consistent with these findings (Welch, Needy & Wolfson, 1950).

On repeated subculture in the presence of chloramphenicol, the organisms acquired resistance to it in a stepwise manner similar to
penicillin (Eagle, 1950; Demerec & Demerec, 1950; Caffey, Schwab and Ehrlich, 1950 and Monnier & Schoenbach, 1951). The rapidity with which resistance is acquired varies from strain to strain. It has been reported by Coffey et al. (1950) that after 20 transfers, the resistance to Klebsiella pneumoniae increased ten-fold, Pseudomonas aeruginosa and Salmonella typhi fifty-fold and B. coli 125-fold. On subsequent transfer to chloramphenicol free medium, the resistance decreased gradually but did not return to the original level. These authors have suggested that the resistance may be explained either by the development of alternative metabolic pathways, or by increase in the formation of enzymes capable of degrading the antibiotic. In vivo also, the sensitivity pattern of the organisms may alter (Thomson, 1952) but, by and large, the process is slow to develop (Banks, 1952).

Judging from hospital practice, particularly in relation to the sensitivity pattern of staphylococci, the slow development of resistance is due to the restricted use of chloramphenicol. The development of resistance during therapy in clinical cases though rare has been reported (Hoads, Harris, Haslam & Cline, 1950 and Voureka, 1951). It was suggested by Heads et al. (1950) that the resistance was due to a process of selection and successive mutation of drug fast variants that exist in an otherwise susceptible species of bacteria.

Besides the alterations in sensitivity, other characteristics of the organisms, like colonial and morphological characters, antigenic compositions and virulence also undergo change under the influence of the antibiotic (Levaditi, Vaisman & Eveno, 1949 and Voureka, 1951).
Unlike penicillin, chloramphenicol is mainly bacteriostatic in its effect on the organisms (Smadel, 1950 and Woodward, Smadel & Ley, 1950) although, a few authors have described a slow bactericidal effect (Watson, 1955) which appears at drug concentrations well above the bacteriostatic level (Petersdorf, Benett & Rose, 1957).

The problem of the mechanism of this action has been approached from several angles but is not yet clear (Smith & Worrell, 1949). It has been suggested by Smith (1953) that of all the systems which have been tested only the aminoacid utilisation and the metabolism of fats and esters of bacteria show some significant change under the influence of the drug. Eagle & Sax (1955) have conducted a comprehensive review of the subject. Many workers have detected an inhibition of bacterial protein synthesis without a parallel effect on nucleic acid and polysaccharide synthesis.

Owing to its being a nitrophenyl compound, the toxicity data of chloramphenicol was extensively investigated in laboratory animals, and Gruhnzit, Fisken, Reutner & Martino reported in 1949, that it was relatively non-toxic to animals and no cumulative effects were seen with an oral dose of 100–200 mg./kg./day to dogs for four months. With higher doses, the occurrence of anaemia, hypocellularity of the bone marrow and fatty changes in the liver have been reported (Nelson & Radomski, 1954 and Radomski & Nelson, 1953). In man, the toxic effects of the drug can be divided broadly into gastrointestinal reactions, allergic and hypersensitivity manifestations, neurological sequelae and blood dyscrasias. In gastro-intestinal reactions besides the commonplace symptoms like nausea and vomiting, another rarer
outcome of prolonged therapy is the alteration of the intestinal flora, when yeasts like Candida albicans may overgrow the normal flora—(Finland & Weinstein, 1953). The more serious complication of blood dyscrasias is now well-documented and dangers of indiscriminate use of the drug are well recognized.