REVIEW ARTICLE

THE PRESENT STATUS OF THE CHEMOTHERAPEUTIC DRUGS

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The Sulphonamides

The sulphonamides may eventually be replaced by antibiotics with wider antibacterial activity but they continue to be very popular in general practice because of the ease with which they are administered, and their relative freedom from serious side effects. Hypersensitivity is probably their only common disadvantage especially when applied locally in skin infections.

Although experimentally the sulphonamides are the least active of the chemotherapeutic agents they are sometimes more efficient clinically than the antibiotics. For instance, in a recent comparison[1] between the sulphonamides and penicillin for the treatment of pneumonia, there was no difference amongst males in the incidence of pleural complications but in females for some unexplained reason the sulphonamides were superior to penicillin; and again in controlled trials[2] in the treatment of infantile diarrhœa, sulphadiazine has proved to be superior to chlortetracycline or chloramphenicol. Also because succinylsulphathiazole and phthalylsulphathiazole provide good cover with little risk of serious side effects they have been regarded[3] as more desirable than the wide-spectrum antibiotics for prophylactic therapy against bowel infection in abdominal surgery.

The sulphonamides are also recommended for combined therapy, e.g., with streptomycin in *H. influenzæ* meningitis[4] and in brucellosis[5], or with neomycin as an intestinal antiseptic[6].

Penicillin

Penicillin is often reputed to be the least toxic of all the antibacterial drugs and therefore it may be surprising to read that "To-day penicillin heads the list of medicinal agents in the frequency, diversity and severity of the sensitivities which it induces. In current experience it has replaced foreign serum as the commonest cause of fatal shock. It is responsible for a growing number of deaths due to irreversible vascular allergy, e.g., periarteritis nodosa[7]." Unfortunately this statement is well supported by published examples and the seriousness of the anaphylactic reactions is emphasised by the suddenness with which death can follow an injection. One patient had been given three uneventful courses of penicillin, then a penicillin troche caused a "queer feeling" in the chest and a brief fainting spell. Three months later the patient died within seconds of being given an intramuscular injection of penicillin[7]. Similar cases of hypersensitivity have been published by other workers, and reports of reactions, happily not often fatal, appear as a slow but steady stream to support

these statements. Procaine penicillin is especially liable to cause these allergic reactions[9].

Fortunately, these severe reactions can be prevented[7,8] by enquiring before any penicillin is administered whether the patient or parents of the patient are allergic subjects, and if penicillin has been used before (it is unlikely that there will be a severe reaction on first contact with the drug), whether there were any reactions such as swelling or itching at the site of injection, rashes, wheezing or fainting. Any patient suspected of sensitisation should first be tested intradermally, the test being observed especially for a delayed reaction, but a negative reaction will not however, necessarily exclude a state of hypersensitivity. Desensitisation has been accomplished by the usual procedure of injecting daily increasing doses of the drug.

Sensitisation of workers preparing and administering chemotherapeutic drugs is a genuine hazard that needs guarding against. A 3·5 per cent. incidence of sensitisation in nursing staff, quoted in a Memorandum from the Ministry of Health[10], emphasises the need for precautions. The greatest risk, according to this report, is incurred when working with streptomycin and the risk with penicillin is about half as great. In every case the hypersensitivity took the form of a skin manifestation, though in several cases there was also angioneurotic œdema. The sensitisation may develop after the first contact with the drug or after as long as 5 years continuous contact. Preventive measures consist of avoiding direct contact, but if contact accidentally occurs the antibiotic should be removed by washing with copious running water. Desensitisation is difficult but feasible[11].

Penicillin still remains the most active of the antibiotic drugs on a weight basis but its rate of clearance from the body has necessitated large and frequent doses. The use of depot preparations has undoubtedly helped to overcome this disadvantage, but it is only the oily ones, especially those of procaine penicillin, which are really efficient depots giving consistently detectable blood levels for 24 hours or longer. But even these are probably unsatisfactory for the treatment of deep and walled-off foci of infection especially when the organism is relatively insensitive[12].

A new repository penicillin, *NN'*-dibenzylethylenediamine dipenicillin G, benzathine, has recently been introduced[13]. It is extremely insoluble in water (about 0·02 per cent.) and when injected a dose of 300,000 units gives detectable blood levels over periods of up to 17 days[14]. But these levels are intermittent and low intermittent levels are said to present the optimum conditions for the emergence of penicillin-resistant organisms[15]. Nevertheless these infrequent injections apparently prevent the recurrence of rheumatic fever by eliminating the streptococcal-carrier state, and single injections of 600,000 or 1,200,000 units were effective in the treatment of 36 of 49 adults with lobar pneumonia[16]. This salt is also available as a mixture with procaine penicillin, the object being to ensure higher initial blood levels.

The soluble penicillin salts are poorly absorbed after oral administration and this route has therefore not been recommended except in

the treatment of infants infected with sensitive organisms, such as the pneumococcus[17], or of adults infected with the gonococcus. The addition of an antacid to the penicillin is claimed to protect it from the acid of the stomach and thus to give more consistent blood concentrations. Daily doses of 48,000 I.U./lb. of body weight taken in divided doses without an antacid have, however, been shown[18] to give reliable bacteriostatic blood concentrations in infants, provided the drug was taken on an empty stomach; an antacid did, however, halve the necessary dose.

A recent innovation for oral administration is the insoluble salt benzathine, mentioned above. Claims have been made that it produces more consistent, though not necessarily higher, blood levels than comparable doses of the soluble salts[19], but this has not been substantiated by other workers[20,21], who find it behaves no differently from the soluble salts. That it should behave similarly to the soluble salts is not surprising, for it rapidly hydrolyses at the *p*H of normal gastric juice and significant hydrolysis also occurs in slightly alkaline solutions[22]. It would seem therefore that the only real advantage of this new salt is that it is stable as an aqueous suspension and can be used as an elixir in a palatable form for children.

Until recently only inorganic salts of benzylpenicillin have been used in clinical practice but the diethylaminoethyl ester of benzylpenicillin has been introduced on the grounds that it possesses an affinity for lung tissue and is excreted in relatively large amounts in the sputum[23]; animal experiments suggest that this affinity is more marked when the lungs are infected with *H. pertussis*[24]. Also, high concentrations of penicillin are present in the cerebrospinal fluid after intramuscular injections of this ester[25]. But in spite of the claims made for it, conclusive clinical proof showing any evidence of superiority over ordinary penicillin is still lacking[12]. This penicillin represents a derivative of a new type for the esterification is on the carboxyl carbon of the penicillin molecule, but in 0·9 per cent. sodium chloride solution at *p*H 7·3 and 37° C. it undergoes 50 per cent. hydrolysis within 23 minutes, and although its toxicity is low when given orally or intramuscularly it is highly toxic when given intravenously[26].

Although penicillin has now been widely used for seven or eight years, resistant organisms have not become a serious problem, except with staphylococci[27], and fortunately, alternative drugs are usually available against the resistant strains. The incidence of penicillin-resistant staphylococci naturally tends to be high in hospitals where the use of penicillin eliminates the sensitive ones and where wound infections arise mainly from carriers and cross-infection. In one survey[28] of 915 strains isolated from in-patients, 65 per cent. were resistant to penicillin, 28 per cent. were resistant to streptomycin, 8 per cent. to chlortetracycline and oxytetracycline, and only 1 per cent. to chloramphenicol. Of the strains resistant to chlortetracycline and oxytetracycline 98 per cent. were resistant to penicillin. The incidence of resistant strains in the staff of this hospital is of ætiological significance; 54 per cent. were found to be nasal carriers of staphylococci and of these 47 per cent. were carrying resistant strains. Similar findings have been reported by other workers[29].

Penicillin-resistant strains are usually sensitive to chloramphenicol and often to the newer antibiotics, erythromycin and carbomycin. Bacitracin is also active against most of these resistant strains but unfortunately this antibiotic is not suitable for parenteral use.

There is experimental evidence that the exposure of an organism to the action of one antibiotic changes its resistance to another[30], and in America the incidence of penicillin-resistant strains of staphylococci is reported[27] to have fallen during the last two years, and the fall was attributed to the widespread use of bacitracin and the tetracycline antibiotics.

Streptomycin

This antibiotic is still the most important drug in the treatment of tuberculosis (*vide infra*). It is unfortunate that organisms so readily develop resistance to it because it is also active against many Gram-positive and Gram-negative organisms, and when used for short periods causes few side reactions. In spite, however, of this weakness it has proved very effective in some infections. *In vitro* against *Shigella sonnei* it is more effective than chloramphenicol or the tetracyclines, and in 16 acute cases of dysentery oral streptomycin produced clinical cures within 24 hours and bacteriological cures with no relapses within 6 days[31]. It is still probably the most active antibiotic available for *Proteus vulgaris* and *Klebsiella pneumoniæ* infections[12].

In order to reduce the rate at which organisms develop resistance to streptomycin, this antibiotic is now frequently used in combination with other drugs[12]; combined with sulphadiazine it is usually effective in *H. influenzæ* meningitis[4] and combined with oxytetracycline it is strongly recommended for the treatment of brucellosis[5].

Chloramphenicol

This antibiotic is still unique in being the only one that is manufactured synthetically more cheaply than it is produced naturally.

Although chloramphenicol is one of the wide-spectrum antibiotics, i.e., is bacteriostatic against many Gram-positive and Gram-negative organisms, rickettsia and the larger viruses, it is most effective when used against Gram-negative bacilli[12]. The tetracyclines and the polymyxins have high *in vitro* activity against *Salmonella typhosa*, but chloramphenicol is the only drug which is of real value for the treatment of typhoid fever[12,32]. Again, in a controlled trial with chlortetracycline, chloramphenicol and sulphadiazine in the treatment of infantile diarrhœa, which recent investigations suggest is due to certain strains of *Bact. coli*, chloramphenicol proved superior to chlortetracycline although it was inferior to sulphadiazine. In the treatment of urinary infections by *Proteus vulgaris*, it is frequently effective because many strains of this organism are sensitive to it[33]. In staphylococcal infections it is usually not as effective as penicillin or the tetracyclines but as many strains are now resistant to these other antibiotics, chloramphenicol is becoming increasingly more valuable against staphylococci.

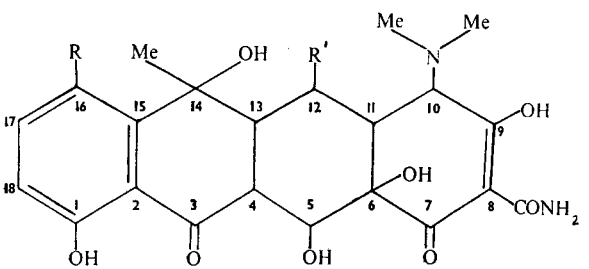
Chloramphenicol is also particularly valuable in the treatment of pulmonary infections not only because of its high activity against most of the bacteria found in the respiratory tract but also because of its activity against the virus of viral pneumonia. In an extensive trial[34] it was also shown to be of value in the treatment of whooping cough, but it had to be used early in the disease and even then the effects were not dramatic. Chlortetracycline was also used in the same trial and it produced similar results.

Although chloramphenicol is the most effective drug available for the treatment of typhoid fever it is far from ideal; the relapse rate is high, and complications such as perforation and cholecystitis are not infrequent[12]. These relapses may be due to inadequate treatment but most probably to the drug being only bacteriostatic.

Large quantities of chloramphenicol were used in America before it was realised that the drug occasionally produces aplasia of one or more elements of the hæmopoietic system[35], and recently the Council of Pharmacy and Chemistry has considered it necessary to advise that the use of chloramphenicol be restricted to the treatment of typhoid fever and other serious infectious diseases caused by organisms resistant to other chemotherapeutic agents. Investigation[36] of 31 cases of aplastic anæmia associated with the use of chloramphenicol in this country has shown that in adults the total dose of the drug should not exceed 26 g. and the length of treatment should not be longer than 10 days. In children the maximum total dose should not exceed 100 mg./kg. over a period not longer than 7 days.

The Tetracycline Antibiotics

The very similar antibacterial activity, and "cross-resistance"[37], of "aureomycin" and "terramycin" strongly suggested that the two antibiotics were related in spite of their being produced by different species of *Streptomyces*. The closeness of the relationship was, however, not fully realised till the chemists of the two companies responsible for their respective developments gave details of the structural formulæ of the antibiotics[38]. Among antibiotics this chemical configuration is unique and they differ only in that "aureomycin" has a chlorine atom at carbon 16, and "terramycin" has a hydroxyl atom at carbon 12.



Aureomycin R = Cl; R′ = H

Terramycin R = H; R′ = OH

The terms chlortetracycline and oxytetracycline have now been accepted as the descriptive names for "aureomycin" and "terramycin" respectively.

These antibiotics have wide antibacterial and antiviral activities similar to those of chloramphenicol but in contrast to chloramphenicol they are particularly effective against the Gram-positive cocci. They have proved to be of especial value against penicillin-resistant staphylococci, but recently a high proportion of these penicillin-resistant strains have proved to be resistant to them as well[12,28]. Like chloramphenicol, they are bacteriostatic only and the carrier rate after therapy is much higher than after using a bactericidal drug, e.g., penicillin[39].

Being absorbed like chloramphenicol, from the gut, these antibiotics are usually taken orally, and they are effective in the treatment of a variety of infections, especially of pneumonia, urinary infections[40], actinomycosis[41], brucellosis[43], and non-specific urethritis[44]. Side reactions are rarely serious, and consist of nausea, vomiting and loose stools. Intravenous preparations are available but are seldom used unless the patient cannot take the drug orally; intramuscular injection of the hydrochloride has been recommended for oxytetracycline[42].

The wide antibacterial activity causes almost complete sterility of the gut and therefore interferes with the bacterial synthesis of members of the vitamin B complex, but the deficiency becomes important only after prolonged treatment. More serious however, is the growth of monilia which can sometimes invade the tissues and produce a fatal infection[45]. The risk of moniliasis, especially the pulmonary form, is such that the American Council of Pharmacy and Chemistry in 1951 issued a warning statement to be added to the labelling of this drug drawing attention to the risk. This increase in the incidence of monilia in the sputum, throat and rectum after treatment with oxytetracycline or sulphadiazine was determined in 174 patients with pneumonia[46]. Treatment with the antibiotic increased the incidence of monilia in the sputum from 32 per cent. before treatment to 61 per cent. after treatment; in the throat it was increased from 16 to 42 per cent. and in the rectum from 0 to 59 per cent. Treatment with the sulphonamides caused no immediate increase, but 2 to 4 days later there was an increase, due perhaps, according to the investigator, to cross-infection from patients treated with the antibiotics. The pruritis and rectal soreness which so frequently accompanies the oral administration of the wide-spectrum antibiotics has also been attributed to the moniliasis[47].

The cause of the sudden growth of monilia during treatment with these antibiotics is still unknown but it has been attributed either to the elimination of the sensitive organisms thus permitting a vast increase in the few resistant monilia normally present, or to changes in the mucous membrane, which then permits invasion by organisms normally unable to penetrate the healthy mucosa, or to a direct stimulation of the growth or virulence of monilia. It has been shown[48] that *Candida albicans* and chlortetracycline are non-toxic when injected singly intraperitoneally into mice but together they are fatal, due, it is suggested to chlortetracycline lowering the animal's resistance. In other experiments[49]

chlortetracycline has stimulated the growth of *Candida albicans*, but the fact that chloramphenicol, streptomycin and oxytetracycline, especially the latter, failed to produce the same stimulating effect makes unlikely the suggestion that stimulation is the cause, since moniliasis has followed treatment with all these drugs.

Attention has recently been drawn[50] to another serious complication, antibiotic enterocolitis, following the oral use of the wide-spectrum antibiotics. The direct cause of this infection is not always known but sometimes it is due to the replacement of the normal intestinal flora by strains of organisms not susceptible to the antibiotic and the result can be fatal, especially when the organism is *Staph. aureus*. These complications are, however, not contra-indications for the use of these very powerful antibiotics but stress the need for restraint in using them as prophylactics.

Achromycin or tetracyn is a more recently introduced tetracycline antibiotic which differs chemically from the earlier two members of the series by the absence of both the chlorine and the hydroxyl groups; it has been given the descriptive name, tetracycline. It has been prepared from both chlortetracycline and oxytetracycline and is also produced naturally by a species of *Streptomyces* isolated from Texas soil[51]. Its antibacterial activity is very similar to those of chlortetracycline and oxytetracycline but it is more stable in solution than is chlortetracycline and it does not stimulate the growth of *Candida albicans*. Resistance is claimed to develop more slowly and it is said to be less toxic, and cause less gastro-intestinal disturbance[12,52]. Used in the treatment of 179 patients[53] mainly with urinary and respiratory infections it had effects similar to those that would have been expected from treatment with the other two tetracyclines, except that the side-effects were probably less.

Erythromycin and Carbomycin

These new antibiotics are derived from two species of *Streptomyces* and are most active against Gram-positive organisms. They are readily absorbed from the gastro-intestinal tract and having similar activity to penicillin the main interest in them is for use against strains of staphylococci resistant to penicillin and the tetracycline antibiotics.

Erythromycin is a basic substance, and it is active only against multiplying organisms. It is effective in staphylococcal infections, but unfortunately resistance quickly develops[12]. *In vitro*, according to one report[54], after 3 to 5 subcultures staphylococci sensitive to 0·4μg./ml. became resistant to 100μg./ml., and according to another[55], within one month of adopting erythromycin for general use in a hospital, strains of resistant staphylococci were isolated from the nose and throat of the staff and within 5 months the carrier-rate of these resistant strains reached 75 per cent. There is however no cross resistance with any of the other antibiotics in common use, except with carbomycin[56]. It is also effective in streptococcal infections; in the treatment of scarlet fever and in the prevention of the suppurative complications it is apparently as good as penicillin[57].

Carbomycin, a crystalline monobasic substance, is also highly active

against staphylococci and even more so against pneumococci and hæmolytic streptococci. After oral administration the blood concentration is low but the tissue concentration is reported to be high[58]. There are as yet few reports of its clinical use and those available are not promising; pneumococcal pneumonia appears to respond less dramatically than with the more usual drugs[12], and the response in staphylococcal infections is often even more disappointing[12]. It appears, however, to be of some value in the treatment of urinary infections, especially when the causal organism is the enterococcus[59]. Resistance to carbomycin readily develops *in vitro* and there is cross-resistance with erythromycin[60].

Both erythromycin and carbomycin are active against rickettsia and some of the larger viruses and, although there is yet no evidence of rickettsia developing resistance to antibiotics, they may be useful as alternatives to the tetracyclines and chloramphenicol for the treatment of typhus, etc.

The Polypeptide Antibiotics

There are several antibiotics of this class but only bacitracin and the polymyxins are used in man.

Bacitracin is active against many Gram-positive organisms but because of renal toxicity[61] it is usually only used topically, and when applied to the surface of the brain it is apparently far less toxic than penicillin[12,27].

The polymyxins are a group of five antibiotics produced by different species of *B. polymyxa*. Three of them, polymyxins, A, C and D cause transitory proteinuria but polymyxins B and E are almost, if not entirely, free from this effect[62,63]. It is unfortunate that the first polymyxins to be used were the renal toxic polymyxins A and D, for they have undoubtedly helped to create the impression that all the polymyxins are unfit for parenteral use. The adoption of the same name for each of them has also contributed to this impression, for frequently the suffix is omitted when reference is made to any of them. Both polymyxins B and E cause mild paræsthesia when used parenterally, and also some pain at the site of injection but this is less with polymyxin E than with polymyxin B[62,63].

The polymyxins are active against almost all bacteria but they are at least a 100 times more active against the Gram-negative bacilli than against other organisms. As they are not absorbed from the gut, they must be given parenterally for systemic use[62], but they are rapidly bactericidal and injected intrathecally rapidly sterilise the cerebrospinal fluid of patients with meningitis due to sensitive organisms, e.g., *H. influenzæ*[64]. Experimentally the polymyxins are very efficient against *Salm. typhosa*[62], but the produce little or no effect when used in the treatment of typhoid fever[65,66]. In practice they have proved to be the best available drugs for the treatment of *Ps. pyocyanea*[67] infections especially in meningitis[68], burns[69] and eye infections[70], and they are also very effective in selected cases of urinary infections.

The polymyxins and bacitracin have bactericidal activities which are complementary and as the drugs rarely cause hypersensitivity when used

topically[12], they form an excellent combination for the local treatment of infected wounds and dermatological lesions[72], conditions in which most other chemotherapeutic drugs have limited uses because of hypersensitivity. This combination is also recommended[12,27] for reducing the bacterial flora of the intestine since neither of the drugs is absorbed from this site.

Neomycin

Neomycin is particularly active against the tubercle bacillus[73], but it is too toxic for systemic use[74]. It is however, bactericidal for many other bacteria and as it is not absorbed from the gastro-intestinal tract it is used as an intestinal antiseptic, mainly in combination with bacitracin[27], the tetracylines[75], and phthalylsulphathiazole[6]. It is also poorly absorbed from wound surfaces and applied topically for the control of surgical infection it has been particularly useful against *Proteus vulgaris*[27].

Although organisms do not readily develop resistance to neomycin, those that do show cross-resistance with streptomycin[76]. Neomycin as a 0·5 per cent. cream was successfully used alone for the treatment of 93 patients with pyogenic skin infections due to staphylococci resistant to penicillin, chloramphenicol and aureomycin; none developed hypersensitivity[77].

The Antitubercular Drugs

Much progress has been made in the treatment of pulmonary tuberculosis by the use of chemotherapeutic drugs and it seems unlikely that any new drug will be discovered that is more active than some of the present ones. The value of the present ones is probably limited only by the ease with which the tubercle bacillus develops resistance to them and by the inaccessibility of the organisms in the more chronic forms of the disease. The development of resistant organisms has, however, been much reduced by the concomitant use of two or more drugs and advances in surgery are helping to overcome the limiting effect of the disease by enabling the irreparable portions of the lungs to be removed. The surgical advances are, however, only possible because the drugs are able to control the acute spread of the disease which almost invariably follows surgical interference.

The sulphones were the first drugs to show definite though slight *in vitro* and *in vivo* antitubercular activity[78,79], and clinically they were of some value in the treatment of pulmonary tuberculosis[80,81], but in 1946, even before the trials were completed, streptomycin was showing in animal experiments[82,83] how vulnerable is the tubercle bacillus to a really efficient drug. Before long there were very favourable reports of the use of streptomycin in patients with miliary and meningeal tuberculosis[84,85], the two conditions in which previously the prognosis had been hopeless.

Two drawbacks to streptomycin soon became apparent. Firstly, the initial clinical improvement was often not maintained, especially in the

more chronic forms of the disease, due to the organisms becoming resistant to streptomycin[86]. Secondly, streptomycin damaged the nucleus of the eighth nerve causing vertigo and ataxia from vestibular disturbances, and hearing impairment, often to the extent of complete deafness, was caused by damage to the auditory mechanism[87]. This toxicity has now been minimised by reducing the dose of streptomycin[12], and giving it less frequently and for shorter periods. In 1948 dihydrostreptomycin was introduced[88], on the grounds that it was less toxic than the parent antibiotic, but experience has shown that it causes greater hearing impairment, and this is more serious than vestibular damage because the latter can be recognised early when the damage is reversible whereas the auditory nerve damage is insidious and not reversible[12,74,89]. However, from the results of a questionnaire[90] sent to 19 sanatoria in this country it appears that the risk of complete deafness from dihydrostreptomycin is small provided the daily dose does not exceed 1 g. Since the two streptomycins have additive actions on the tubercle bacillus and yet tend to produce different side effects, streptomycin and dihydrostreptomycin are being given in combination in an attempt to lessen the side effects[89].

The importance of streptomycin-resistant organisms in the sputum of any particular patient is difficult to assess[91]. In one series of cases[91], although resistant organisms were isolated from the 42nd day of treatment onwards, and the number of bacilli present in the sputum having fallen initially rose again, the early clinical improvement was maintained in most of the patients, in spite of the persistence of resistant strains. Moderate drug resistance seems to be of little clinical significance[92].

In 1946, *p*-aminosalicylic acid was shown to have high *in vitro* antitubercular activity[93] and to be active in guinea-pigs[04], and early clinical reports suggested that it was also active in the treatment of tuberculosis in man[95]. Although 1 to 10 μg./ml. inhibit growth of the tubercle bacillus *in vitro* it is less active in animal experiments than streptomycin[96] and more recent clinical reports showed that although it may have some effect it is inferior to streptomycin. Tubercle bacilli develop resistance to *p*-aminosalicylic acid but to a lesser degree than to streptomycin[74,86,92,97]. It has low toxicity, usually causing only anorexia, mild nausea, and diarrhœa but it not infrequently causes hypersensitivity[97]. The sodium salt causes less gastro-intestinal upset than the free acid but, due to the rapid rate of excretion, the dose of *p*-aminosalicylic acid has to be large, 12 to 15 g. per day, and the quantity of sodium may be too high for patients requiring a low sodium intake[89]. The calcium salt is reported to cause even less gastro-intestinal upset than the sodium salt[98].

By now the use of *p*-aminosalicylic acid would probably have been limited to chronic cases requiring prolonged treatment but for the fact that given with streptomycin it delays the development of streptomycin-resistant strains. This effect has been demonstrated experimentally, both *in vitro* and in animals[99,100], and clinically in man[86,92,97]. In a trial in this country[86], patients treated with combined *p*-aminosalicylic acid (20 g. daily) and streptomycin (1 g. daily) showed improvement somewhat greater

than those who received streptomycin alone, and while from the 49 patients treated with streptomycin alone, 33 streptomycin-resistant strains were isolated, only 5 such strains were recovered from the 48 patients on combined therapy.

In 1946 a new class of compounds, the thiosemicarbazones, was shown[101] to be effective *in vitro* and experimentally *in vivo* against the tubercle bacillus, and in Germany, *p*-aminobenzaldehyde thiosemicarbazone (T.B. 1/698) has been widely used in the treatment of tuberculosis[102,103]. In this country and in America, however, it has been little used because of toxic side effects, which have included malaise, dizziness, photophobia, liver necrosis and bone marrow hypoplasia[102], This drug has no effect on miliary or meningeal tuberculosis[102], it is probably less active than *p*-aminosalicylic acid and resistant strains can be isolated from the patients as early as 4 weeks after commencement of treatment[104]. Other thiosemicarbazones have been tried, e.g., *p-iso*butoxybenzaldehyde thiosemicarbazone but the clinical effects have not differed from that obtained with *p*-aminobenzaldehyde thiosemicarbazone[105].

In 1952 the high antitubercular activity of isoniazid, *iso*nicotinyl hydrazide, was announced independently by 3 groups of workers, 2 in America[106,107], and the third in Germany[108]. Isoniazid is highly specific against mycobacteria, but it has been reported to be effective also in the treatment of actinomycosis[109]. Although the *in vitro* activity is of the same order as that of streptomycin, in animal experiments it is even more effective[106]. The first reports of its clinical use were most encouraging[110], but more prolonged use showed that the effects were only temporary[111,112], the relapses coinciding with the isolation of isoniazid-resistant strains; in one trial[112] in which 264 patients were treated, 30 still had tubercle bacilli in their sputum after 6 months treatment and of these 28 had isoniazid-resistant strains.

The importance of isoniazid resistance is even more difficult to assess than streptomycin resistance because the degree of resistance varies so much and many of the more highly resistant strains have been shown to be of very low virulence to mice and guinea-pigs[113,114,115,116], and there is the rather disturbing experimental evidence which suggests that some of the less highly resistant strains are actually made more virulent by the presence of isoniazid[115,116]. Clinical experience, however, suggests that the patients with organisms resistant to 0·2 μg./ml. but not to 1 μg./ml will continue to respond favourably to treatment with isoniazid[117].

There is no cross-resistance between strains resistant to isoniazid, *p*-aminosalicylic acid or streptomycin, and there is now much evidence to show that combined treatment with isoniazid and *p*-aminosalicylic acid or streptomycin delays the development of resistance, thus enabling the initial clinical improvement to continue. In one trial[118] doses of 100 mg. of isoniazid daily with 1 g. of streptomycin daily or 5 g. of *p*-aminosalicylic acid 4 times a day reduced the incidence of resistant strains to a degree comparable to that produced by the combination of streptomycin and *p*-aminosalicylic acid on streptomycin-resistant strains. The clinical improvements in both of these groups were satisfactory, and did not

significantly differ from each other. In a supplementary comparison in this trial the streptomycin was reduced from daily to twice weekly doses but the incidence of isoniazid-resistant strains was higher than in the group receiving the daily doses.

It is now generally agreed that isoniazid should never be used alone[74], and from the activity shown in animal experiments it was expected that isoniazid would be at least as effective as streptomycin if the development of resistant strains were prevented. In the acute human infections where the lesions are more vascular and the drug can readily reach the organisms, e.g., in the miliary and meningeal forms of the disease[119,120], this expectation appears to have been fulfilled for it appears to be even more effective than streptomycin. Although both isoniazid and streptomycin are bactericidal[121,122], histological examination of tissue from patients with miliary and meningeal tuberculosis, dying during treatment with these drugs also suggests that isoniazid is the more efficient drug because with it the lesions show resolution whereas they become fibrosed during treatment with streptomycin[123]; when combined treatment with these drugs is used the isoniazid effect predominates. The higher efficiency of isoniazid may be due to the fact that tuberculosis is essentially an intracellular infection and isoniazid penetrates cells more readily than does streptomycin[124,126]. Studies with radio-active isoniazid show that it also diffuses into caseous lesions[125].

In spite of the experimental evidence of isoniazid possessing advantages over streptomycin and that *in vitro* the bactericidal activity of the drugs together is greater than when acting alone, especially when tested at maximal therapeutic concentrations, there is no conclusive clinical evidence that the combination of streptomycin-isoniazid is more effective than the combinations, isoniazid-*p*-aminosalicylic acid and streptomycin-*p*-aminosalicylic acid in pulmonary tuberculosis[127,128]. The triple combination, streptomycin-isoniazid *p*-aminosalicylic acid, is being tried and if it proves to suppress, completely or almost completely, the development of resistant strains, then this will obviously be the combination of choice. According to one report[128], however, the combination shows no significant advantage and if this is confirmed then it would seem unwise to use streptomycin and isoniazid together. Undoubtedly these two drugs are the only really effective antitubercular drugs available and it would therefore seem better to use only the combinations of streptomycin-*p*-aminosalicylic acid or isoniazid-*p*-aminosalicylic acid so that should resistance develop, which it invariably does in a proportion of patients, there will still be available the alternative powerful drug[74].

Of the double combinations, isoniazid-*p*-aminosalicylic acid seems to be the more desirable for routine treatment as it avoids the use of the hypodermic syringe and although isoniazid is not completely free from side reactions, these are not as serious as the damage caused by streptomycin to the eighth nerve. The side reactions attributed to isoniazid have included central nervous system stimulation (hyperactive deep reflexes, twitching, insomnia, changes of temperament) and muscle weakness but no serious effects on the liver, kidney or bone marrow have been reported[89].

Many derivatives of isoniazid have been examined *in vitro*[106,129], and some have been used clinically but none has so far proved superior to the parent substance[74,110]. An analogue of nicotinic acid, pyrazinamide, also has *in vivo* antitubercular activity[130] and has been used clinically[131] but drug resistance quickly develops. Recently it has been used with isoniazid, and according to one report[132] the combination rapidly eliminated tubercle bacilli from the spleen of mice infected experimentally and in 90 per cent. of 61 patients with pulmonary tuberculosis, completing a course of 3 months duration, the sputum became negative and in 70 per cent. there were substantial radiographic improvements. The authors were of the opinion that the antitubercular activity of the combination is superior to that of any other combination in current use, but as the incidence of hepatitis was high, 6 patients developing liver damage from which one died, it is unlikely that the combination will be widely used. Similar results but with less evidence of liver damage have been reported by another group of workers[133].

Most of the controlled studies with the antitubercular drugs, singly or in a combination have been on patients with the pulmonary form of the disease but there is no doubt that they are of equal value in the treatment of extra-pulmonary lesions, e.g., meningeal or renal tuberculosis[74,134]. The treatment of tuberculous meningitis is one of the outstanding successes of chemotherapy for previously this condition was almost invariably fatal. Treatment with intrathecal streptomycin alone increased the survival rate by about 50 per cent.[74] and on a regime of intramuscular streptomycin and oral *p*-aminosalicylic acid[135] or oral and intrathecal isoniazid it is much higher[136,137]; in one report, only 6 deaths occurred in 100 cases, and in another only 1 out of 30. The administration of intrathecal drugs is however a very disturbing procedure for the patient, but as adequate concentrations of isoniazid have been shown to be present in the cerebrospinal fluid in tuberculous meningitis after oral administration[120,138], and as treatment with intramuscular streptomycin and oral isoniazid with or without *p*-aminosalicylic acid has been successful[139] the intrathecal route is probably unnecessary with isoniazid. Intrathecal streptomycin is also probably unnecessary for, although streptomycin does no pass the normal blood-brain barrier, it apparently gets through when there is a meningitis for in one series of patients 8 of 19 were alive 3 to 5 months after beginning treatment with streptomycin and all except one had received the drug intramuscularly only[140].

The tetracycline antibiotics have some *in vitro* and experimental *in vivo* antitubercular activity[141], and have been tried in pulmonary tuberculosis. Although treatment with chlortetracycline or oxytetracycline is ineffective[142], the latter apparently delays the development of streptomycin resistance, for of 66 patients given 5 g. of oxytetracycline daily in combination with 2 g. of streptomycin every third day for 120 days, at the end of treatment 25 still had tubercle bacilli in their sputa but all the strains were still streptomycin- and oxytetracycline-sensitive[143]. This effect has been confirmed by other workers[144] and it has also been shown[145] that 2 g. doses of oxytetracycline are insufficient.

Neomycin is more active *in vitro* than is streptomycin[73] against the tubercle bacillus but as it causes auditory and renal damage[12], it is not used systematically in tuberculosis[74].

Viomycin is active *in vitro* against some Gram-negative organisms, but it is more active against the tubercle bacillus, 2·5 to 12·5 μg./ml. inhibiting most strains[146]. In animals, however, it is less effective than streptomycin, having activity approximately equal to that of *p*-aminosalicylic acid[147]. It was introduced in 1951[148], but it has been little used in man because toxic side reactions are common. The most important of these have been renal damage, vestibular disfunction, partial deafness, and pain at the site of injection[149], and they have occurred even when the patients were given only 2 g. every third day. In future it will probably be used in conjunction with other drugs only in cases where the organisms haue become resistant to the less toxic drugs. Trials of such combinations are being run, using *p*-aminosalicylic acid and streptomycin, but the preliminary results are not promising for the incidence of toxic reactions and of the emergence of viomycin-resistance strains is high[150].

Antileprotic Drugs

Leprosy is caused by *Myco. leprœ*, and although this organism has much in common with *Myco. tuberculosis*, it has never been cultivated with certainty, or produced a progressive disease when inoculated into animals, thus making the usual antibacterial screening tests impossible for detecting the antileprotic activity of drugs. The only method available has been to try drugs in persons suffering from leprosy but even this means of assessment is extremely difficult for the disease runs a very chronic course during which it shows a natural tendency to progress and retrogress.

When the first antitubercular compounds, the sulphones, were shown to have some effect against tuberculosis it was natural to try them in the treatment of leprosy. The first sulphones used were the di-substituted forms of 4:4′-diaminodiphenylsulphone (dapsone), e.g., promin, diasone and sulphetrone, and these were reported to be very active in the lepromatous form of the disease.

The di-substituted sulphones were chosen because of the high toxicity of the parent substance, but small doses of dapsone (400 mg. twice weekly) have more recently been shown to be well tolerated and as effective as the larger doses of the less acutely toxic but more expensive derivatives There is some evidence that the derivatives owe their activity when taken orally to being broken down to dapsone[151], but the di-substituted sulphone, sulphetrone, is also active when given parenterally[152] when little or no breakdown occurs. Sulphetrone, however, contains an appreciable quantity of mono-substituted dapsone (semi-sulphetrone)[153] and it is possible that sulphetrone owes its activity to this, for at least one other mono-substituted form of dapsone is active; i.e., the monoacetyl ester of dapsone, sulphone cilag[154].

The activity of these sulphones with only one free amino group is interesting in view of the fact that dapsone is excreted almost entirely in a conjugated form, probably as a mono-substituted derivative[153]. The

antileprotic activity of dapsone may be due to this derivative and not to unchanged dapsone because the parent substance circulates in the body for only a very short time after oral administration.

All the active antitubercular drugs have been tried in leprosy. Streptomycin apparently has some activity[155], but it is not so spectacular in this disease as in tuberculosis, and it is usually regarded as less active than the sulphones. Thiosemicarbazone has definite activity[156], and isoniazid is still undergoing trial but the results so far are not very promising[157].

Whether the development of resistant organisms is the reason for the poor results from streptomycin and isoniazid in this disease is problematical, but the conditions of treatment, being so prolonged are undoubtedly conducive to the development of resistance. Resistance to isoniazid can develop very readily with *Myco. lepræmurium*[158], the organism morphologically indistinguishable from *Myco. lepræ*, and which produces a disease in rodents very similar to the human disease. The answer should come from trials now being made with combinations of the antitubercular drugs, but it may be that leprosy is a disease in which chemotherapy, especially with a bactericidal drug, cannot produce spectacular results. Patients with the lepromatous form of the disease have myriads of organisms present in the infected areas and experience with the lepromin test suggests that even if they were all killed they would continue to behave as living organisms for many weeks. In the lepromin reaction boiled bacilli are injected intradermally and these organisms may continue to produce a local reaction for many weeks. With the bacteriostatic drugs, the sulphones, morphological changes occur in the organism and eventually they appear to disintegrate into acid-fast dust. These changes may be an active process by the organisms to an unfavourable environment, and in the opinion of one authority[154] the granules are capable of reverting back to the active bacillary form if sulphone therapy is discontinued. This authority suggests that 10 to 15 years may be needed to be certain that a patient is cured of leprosy by sulphone therapy. If the morphological changes induced by a bacteriostatic drug result in a clinically inactive form then a better immediate response may be produced by bacteriostatic than by bactericidal drugs.

Combined Therapy

Combining two or more drugs in the treatment of infection is becoming common. Combined therapy can justifiably be used for (1) delaying the development of resistant strains, (2) increasing the activity by the additive or synergistic effect of two drugs against an organism not sufficiently sensitive to the drugs when acting singly, (3) the treatment of mixed infections by drugs with narrow antibacterial spectra, (4) the treatment of infections where the causal organisms are unknown.

Precisely just how the presence of a second therapeutic drug prevents an organism developing a resistance to the first is unknown but it is generally presumed, that an organism becomes insensitive by using a metabolic pathway other than that interfered with by the drug. When

two drugs are presented with different modes of action the organism has difficulty in developing simultaneously two alternative routes, and when three drugs are present the difficulty must be immense. The use of combined therapy has found its widest application with the anti-tubercular drugs.

Much attention has been given to the possibility of drugs interfering with each other, and, experimentally, combination of drugs undoubtedly show enhancing or antagonistic effects. When two drugs show enhancement and the increased effect is equal to the sum of the respective activities of the two drugs, the effect is usually regarded as additive but when it is greater it is called synergism. A rather different and narrower conception of synergism has been suggested for chemotherapeutic drugs. It is based on the assumption that the more rapidly a drug kills the organism the higher is its activity, and on the fact that frequently when a bacteriostatic drug is used with a bactericidal drug the former may delay or even prevent the killing of an organism by the bactericidal drug. Synergism, according to this proposed conception occurs when the early bactericidal action is increased and not when there is merely an increase in the bacteriostatic effect[159].

The knowledge that antagonism can occur between two drugs prevents the general use of combined therapy. According to some authorities[159,160], it is impossible to lay down rules that a certain combination of drugs will always show synergism or antagonism against a particular species or organism, because strain variations occur. Nevertheless they suggest a scheme that should be of clinical value for selecting combinations of drugs. In this scheme the drugs are divided into two groups:

(1) those that are essentially bactericidal—penicillin, streptomycin, bacitracin and neomycin;

(2) those that are essentially bacteriostatic—the tetracyclines, and chloramphenicol.

Members of group 1 are frequently synergistic with each other, occasionally indifferent but never antagonistic. Members of group 2 are only additive with each other, and members of group 2 are usually antagonistic to those of group 1.

The results of these various interactions are based on bacteriological studies involving viable counts on organisms exposed to different concentrations of the drugs. Such procedures are unsuitable for routine use for selecting the proper combination of drugs but a recently described technique[161] enables synergism according to the increased bactericidal conception to be deduced with the minimum of labour.

By this technique the drugs of group 1 and group 2 are antagonistic during early incubation but the effects may change on further incubation. Thus penicillin and chlortetracycline may remain antagonistic after 18 to 24 hours incubation or they may become indifferent, and streptomycin and chloramphenicol may become synergistic in 18 to 24 hours. Also the effects observed at 24 hours with any pair of antibiotics is constant for any species suggesting that it is possible to recommend suitable standard combinations of drugs.

Almost all the studies on drug interference have been made *in vitro* and some doubt may be felt as to the extent to which interference acts *in vivo* where the concentrations of the drugs vary and the host's natural defences must play an important part in the effectiveness of treatment. Experiments in mice with induced leucopenia have shown that even with bactericidal drugs the granulocytes assist in killing the organisms[162]. Nevertheless experimental *in vivo* studies in mice have shown interference by the tetracyclines with the action of penicillin[163,164,165], by the tetracyclines and chloramphenicol with the action of streptomycin. Caution, however, should be exercised in assuming that these conditions apply in natural infections, for although the type of interference, whether antagonism, synergism or indifference cannot be changed *in vitro* by altering the concentrations of the drugs[166], there is evidence that it can be changed *in vivo*; in experiments in mice infected with pneumococci an additive or antagonistic effect was obtained with chlortetracycline and penicillin simply by altering the doses of the drugs[167].

Conflicting differences between interference in drugs when tested under experimental conditions and when used in clinical practice occurs with the antitubercular drugs. *In vitro*[168] and in mice[169], streptomycin in sub-inhibitory concentrations antagonises the effect of isoniazid, yet this combination of drugs is very effective in man, where the concentrations of the drugs must be constantly changing.

To obtain definite clinical evidence of interference between drugs is not easy except by statistical methods but what undoubtedly appears to be antagonism between penicillin and chlortetracycline was observed in a series of cases of patients with pneumococcal meningitis. Intramuscular penicillin alone and intramuscular penicillin plus oral chlortetracycline were given in alternate cases, and of 14 patients treated with penicillin only 3 died but 11 died out of 14 treated with both drugs[170]. Also, in a series of patients with meningococcal meningitis penicillin alone was superior to a combination of it and chlortetracycline[171]. A study of the combination of penicillin and chloramphenicol in streptococcal pharyngitis revealed, however, no evidence of interference[172]. It has been queried[173] whether synergistic effects are required for any infection other than *Streptococcus fæcalis* endocarditis. The infection is apparently unique for the bactericidal effect of penicillin even in optimum concentrations is not complete against this particular streptococcus unless streptomycin is also present and in order to ensure a permanent cure every organism on the heart valve must be killed. It therefore seems unlikely that similar conditions exist in any other infection.

Unfortunately combinations of drugs do not show synergism to organisms that are resistant to one of the drugs, and even when the resistance is only partial the synergism may only be demonstrated *in vitro* with a concentration of the drug unobtainable *in vivo*[166].

The use of combinations of drugs in order to cover a wider range of bacterial species is probably rarely necessary with the wide-spectrum antibiotics available, but there may still be conditions in which these antibiotics are less suitable than a combination of other drugs. Penicillin

and streptomycin are very effective for the treatment of peritonitis[12,27]. The polymyxins and bacitracin have relatively narrow ranges of antibacterial activity but their activities are complementary. They are not absorbed from the gastro-intestinal tract and are therefore of value for specifically reducing the bacterial flora of the bowel, and as they rarely cause hypersensitivity they are very useful for topical application.

As one authority[173] points out two or more drugs are often given to an acutely ill patient suffering from an infection when a bacteriological or even clinical diagnosis has not been made, but the genuine necessity for this must be rare. The main objection to this form of treatment is that if commenced before the necessary pathological specimens are taken the diagnosis may be obscured. Sympathy is, however, felt for the practitioner who is fairly certain of his clinical diagnosis but is in doubt about the sensitivity of the causal organisms to the chemotherapeutic drugs and uses two or more of these. Sensitivity tests would disappear if a drug active against all organisms became available, and combined therapy is frequently no more than an attempt to anticipate this drug.

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