THE CHEMOTHERAPY OF LEPROSY

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I. INTRODUCTION

Although *Mycobacterium leprae* was discovered by Hansen in 1868, some 12 years before Koch discovered *Mycobacterium tuberculosis* in mammalian tuberculosis, a rational approach to the chemotherapy of leprosy has not been possible because the organism has not yet been grown with certainty, either on artificial media or in tissue cultures, nor has inoculation into animals of leprous material rich in organisms produced a progressive lesion suitable for chemotherapeutic studies. Chronic granulomatous lesions have been produced in monkeys (52, 242, 243) but there is no convincing evidence that the organisms multiply in these lesions.

Some workers have explored the possibility of using *Mycobacterium lepraemurium*, the causal organisms of rat leprosy, for making a preliminary assessment of the antileprotic activity of drugs. This organism, like *M. leprae*, has not been grown in artificial media or tissue cultures but the disease can readily be transmitted to rodents. In spite, however, of *M. lepraemurium* appearing to be more closely related to *M. leprae* than is *M. tuberculosis*, the value of using it in experiments for assessing new drugs is still in doubt, and Domagk (78) has stated that "rat leprosy is no test for finding substances useful in the treatment of leprosy." Experiments with this organism are discussed separately.

So, with the absence of a reliable screening test, the preliminary assessments of new drugs have had to be made in patients with the disease. Almost all of the drugs known to be chemotherapeutically active in bacterial infections have been tried but because of the morphological and antigenic relationships between *M. leprae* and *M. tuberculosis*, the antitubercular ones have been given the most

extensive trials. The sulphones were the first of these drugs to be used and their efficacy imposed a further handicap on the development of newer drugs; their therapeutic activity although slow is definite and to use alternative untried drugs raises the ethical problem of withholding for months an effective treatment whilst an unknown one is being tried.

This ethical difficulty, however, is not the only factor in the problem of assessing the value of new drugs. Leprosy is a disease in which the value of specific treatment is particularly difficult to appraise. In one of the two common forms, the organisms are present in very large numbers causing little tissue reaction—a state which would be expected to respond readily to chemotherapy and therefore be eminently suitable for assessing new drugs—and yet appraisal is very difficult because the disease has a natural tendency for spontaneous remission and exacerbation. The other common form of the disease is even more unsuitable because the organisms are very scanty and much of the clinical state is due to secondary features of the disease.

The drugs have usually been assessed in a few selected patients without controls, or less often in a relatively large group of patients by comparing the changes that occur during treatment with those occurring either in an untreated group or in a group receiving a standard drug. When the assessment has been made on a few selected patients the evaluation has depended entirely upon the judgment of the observer, and although the variable course that leprosy normally runs makes this method of assessment very difficult, it has an advantage of being economical with the drug—an important factor when the trial may have to run for a year or more. By either method however, assessment of the drugs depends on detecting clinical and bacteriological changes in the patients, and as leprosy is in many ways a unique disease, this review includes a brief description of the disease.

II. THE DISEASE

Leprosy (44, 48, 98, 150, 151, 199) has much in common with tuberculosis but there are some major differences. It is always a very chronic disease and the organisms are almost entirely intracellular. Studies of the metabolism of *M. lepraemurium* suggest that multiplication takes place intracellularly, the extracellular fluids being inhibitory, and this possibility would also seem to apply to *M. leprae* (page 33).

The fully established disease can be divided into two main types, tuberculoid and lepromatous. Their fundamental differences are apparently immunological and cases rarely if ever change from one type to the other. In the tuberculoid form the host shows high resistance to the organisms associated with a state of allergy and marked tissue reaction; circulating antibodies are however absent or present in only a low concentration (175, 180, 226). The organisms are very scanty and the lesions consist of focal masses of epithelioid cells with giant cell formation and lymphocytic infiltration; apparently these epithelioid cells destroy the bacilli for the organisms are rarely found within them. Since these

lesions resemble those of tuberculosis histologically, this form of the disease is termed tuberculoid.

In lepromatous leprosy there is no evidence of the host being resistant to the organisms and there is apparently a state of anergy, although circulating antibodies, readily detectable by the Middlebrook and Dubos technique, are present at high titres (175, 180, 226). The lesion is essentially a granuloma consisting of numerous macrophages, the lepra cells of Virchow, and, in contrast to the cells of tuberculoid leprosy, these cells contain numerous bacilli. The cells apparently harbour the organisms for there is no evidence of their destroying the organisms except during spontaneous remissions or during effective chemotherapy. The principle lesion in this form of the disease is known as the *leproma* and there may be as many as 7×10^9 bacilli per g of infected tissue (126). In an infected nerve, the bacilli may be found in any of its structures.

Other forms of the disease are recognised (141). Cochrane (50) suggests that the disease passes from its earliest stage, the silent-phase, through an indeterminate stage into either the lepromatous or the tuberculoid form. In some patients, however, it persists for variable periods in forms intermediate between these two extremes, and these are classed by him as *dimorphous* cases. These intermediate cases are unstable and their allergic state varies.

In tuberculoid leprosy the infection is mainly confined to the skin and peripheral nerve trunks, whereas in the lepromatous form it is the superficial lymph glands, the eyes and the nose that are most conspicuously involved. The spleen, liver and testes may also be infected, especially in the lepromatous form. Degeneration of the nerve trunks, which is often very severe in the tuberculoid form, causes anaesthesia and paralysis leading to trophic changes and ulceration in the extremities. These trophic changes may become aggravated by trauma. In spite of the extensive tissue destruction, leprosy cannot be regarded as a fatal disease and although there may be myriads of bacilli present, as there are in the lepromatous lesions, leprosy is rarely a direct cause of death.

The primary infection usually occurs in childhood and the onset of the disease is insidious; as long as 10 years may elapse before the disease becomes clinically evident (81). Infection seems to depend upon prolonged and intimate contact with the disease in the open lepromatous form, and there is evidence that the structures primarily infected are the finer terminals of the nerves (51, 150).

Diagnosis depends upon the demonstration of bacilli in scrapings from the lesions, anaesthesia of the skin lesions to tactile and thermal stimuli and thickening and tenderness of the peripheral nerves. In the tuberculoid form, however, it is rare to find organisms in the smears, and diagnosis then rests mainly on the demonstration of anaesthesia.

The typical skin lesion of lepromatous leprosy is a *macule*. It is usually hairless and hypopigmented, showing varying degrees of anaesthesia, and although these macules are usually small they increase in number as the disease progresses. Eventually, the skin becomes furrowed and nodulated and it is these changes which cause the typical leonine facies. As the lesions age, the bacilli gradually

diminish in number and finally disappear leaving the skin wrinkled and with gross nerve destruction. Cochrane (44) considers that this elimination of the organisms is not fundamentally curative but is due to incidental biochemical changes in the tissues which render the cells such that they can no longer nourish the organisms.

From time to time apparently acute exacerbations of the lepromatous disease occur and these can be divided into two types. One, the acute lepra reaction, is a true exacerbation and is in fact a rapid extension of the disease. The other, *erythema nodosum leprosum*, often referred to simply as the lepra reaction, appears to be a sudden defensive reaction of the host against the previously harboured bacilli; although it is not necessarily the result of treatment it resembles the Herxheimer reaction. This reaction varies in intensity and although it may be very troublesome to the patient, it is usually beneficial, there being much fragmentation and destruction of the bacilli followed by a regression of the lesions. The reaction is characterized by pyrexia, an increase in size, inflammation and ulceration of the lesions, acute neuritis, iridocyclitis and orchitis.

The course of tuberculoid leprosy varies greatly; in some patients it may be very slow and limited to one large macule (sometimes referred to as the *lepride*), but in others the lesions may be multiple and spread to cover the whole skin surface. These macules differ from those of lepromatous leprosy in being raised and reddened, especially at the edges, and the sensory changes are usually more marked. Involvement of the larger nerves is also more extensive and, although the prognosis is better than in lepromatous leprosy, gross mutilations due to nerve damage may develop before the lesions regress. Lepra reactions, which appear to be manifestations of hypersensitivity, also occur in this form of the disease but the symptoms are less severe although the nerves are usually more affected. The more severe the reaction however, the greater is the chance of a spontaneous cure.

The immunological state of the patient is determined by the lepromin test. In this test an antigen prepared from lepromatous tissue, rich in bacilli, is injected intradermally. Two reactions may occur at the injection site: an early one, the *Fernandez reaction*, developing in one to two days and disappearing in three to four days, and a late one, the *Mitsuda reaction*, appearing in about one week and disappearing in three to four weeks. The early reaction consists of erythema and oedema; the late reaction consists of local infiltration developing into a firm nodule, which may ulcerate, and is the one on which positivity is usually based. Dharmendra (68) considers that the same protein antigen causes both reactions, the early one being due to the antigen from the disintegrated cells and the late to the same antigen slowly liberated from the dead bacilli. He considers both reactions to be evidence of an allergic state, but other workers (95, 237) consider that the late reaction is evidence of immunity. The reaction, however, is not specific for exposure to other mycobacteria; *e.g.*, *M. tuberculosis* can apparently also produce a lepromin-positive state (page 27), and similar reactions can be obtained with extracts of normal skin, indicating that the test is basically a foreign body reaction (154).

The fundamental cause of the lepromin-state of the patient is unknown but

it is rare, except in a dimorphous case, for the state to change qualitatively (99), although the positive state varies quantitatively as the activity of the disease varies either naturally or from the effects of treatment (175). It may be that during the development of the initial lesions all infected persons are lepromin-positive, and if the infection is effectively controlled, the allergic state persists. If it is not controlled the organisms multiply freely, a state of anergy develops and the patient becomes lepromin-negative (49, 124).

III. THE TREATMENT OF LEPROSY BY CHEMOTHERAPY

The efficacy of chemotherapy is based on clinical and bacteriological observations. No treatment aimed at the elimination of the causal organism can correct the physical deformities which are the most pitiful aspect of leprosy, but successful treatment should eliminate the bacilli so that smears taken from a number of sites, usually five, on repeated occasions contain no acid-fast bacilli. With the effective forms of treatment so far used, the changes resemble those occurring during natural remissions and the final state of fully established lesions is identical with that of the natural terminal stage sometimes referred to as the "burnt-out" state. Electrophoretic analysis of the plasma proteins may be useful (11); the persistence of a raised serum γ-globulin is indicative of the persistence of the infection, and an increase in the α- and β-lipid content is suggestive of amyloidosis.

In lepromatous leprosy, the mucosal lesions usually heal more quickly than the cutaneous lesions, the rapid subsidence of inflammation and the healing of the ulceration in the nasal and laryngeal mucous membranes being particularly gratifying to the patient. Early cutaneous lesions may completely disappear but older ones may leave permanent pigmentation, atrophy, "tissue paper" wrinkling and scarring. The organisms, however, disappear only slowly. Histological examination of healing lepromas shows that they become invaded by the surrounding healthy corium while the uninvaded areas remain densely bacteriologically positive (218). Hair may or may not regrow, sensation may or may not return, and trophic ulcers do not always heal naturally (143, 198).

In tuberculoid leprosy, the cutaneous lesions subside rapidly but the nerve lesions take much longer. The tenderness and pain disappear but the nerves may remain permanently thickened due to the fibrosis which develops during treatment, and so the paralysis may increase (176).

A. Chaulmoogra oil

Until recently chaulmoogra oil and the esters of its two active constituents, hydnocarpic acid and chaulmoogric acid, were the specific drugs used for the treatment of leprosy. Sir Leonard Rogers describes how he first used these drugs, having learned from the Indians that they had been used for many years (223). He first gave chaulmoogra oil orally and then, because of the poor response, he gave it parenterally. These drugs show no activity in tuberculosis but there is some evidence that they stimulate the phagocytic action of the reticulo-endothelial system (118, 148).

The claims made for these drugs have varied greatly. McCoy (188), in review-

ing them, concluded that there was no evidence that chaulmoogra therapy affected the natural course of the disease; he believed that too little consideration was given to the natural tendency for spontaneous improvement. Schujman (234), on the other hand, maintained that the failures were due to improper use and that only regimes which did not include the intradermal injection of 400 ml in a year would bring hydnocarpus oil into disrepute, and with this Cochrane (45) concurred. Schujman (235) claimed that 40% of his cases became clinically and bacteriologically negative, and he maintained that the results during the first eighteen months' treatment were very similar to those obtained with intravenous promin. There is little doubt, however, that very few workers now use chaulmoogra therapy (142) although the oil is recommended as a vehicle for intramuscular sulphone therapy (page 18) and recently it has been used in chemical combination with a sulphone (page 17).

B. The sulphones

Glucosulfone sodium (Promin sodium, Promanide sodium, 4:4′-diaminodiphenyl sulphone-N,N′-di[dextrose sodium sulphonate]) (see Fig. 2) was the first sulphone to be tried for the treatment of leprosy. It was used at the National Leprosarium, Carville, following the demonstration of its antitubercular activity in animals (91). Although the parent sulphone, Dapsone, B.P.C. (DDS, 1358 F. Avlosulfon, 4:4′-diaminodiphenyl sulphone) (see Fig. 1) was first synthesized in 1908 (111), it was not until 1937 that its antibacterial activity was discovered (24, 109). Its examination as a possible chemotherapeutic agent was

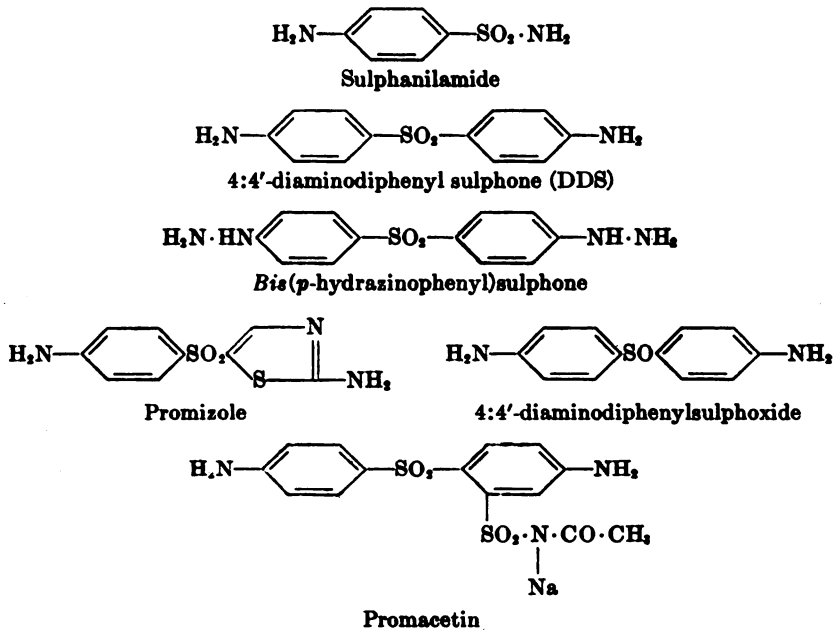


FIG. 1. Sulphanilamide, DDS, and related sulphones

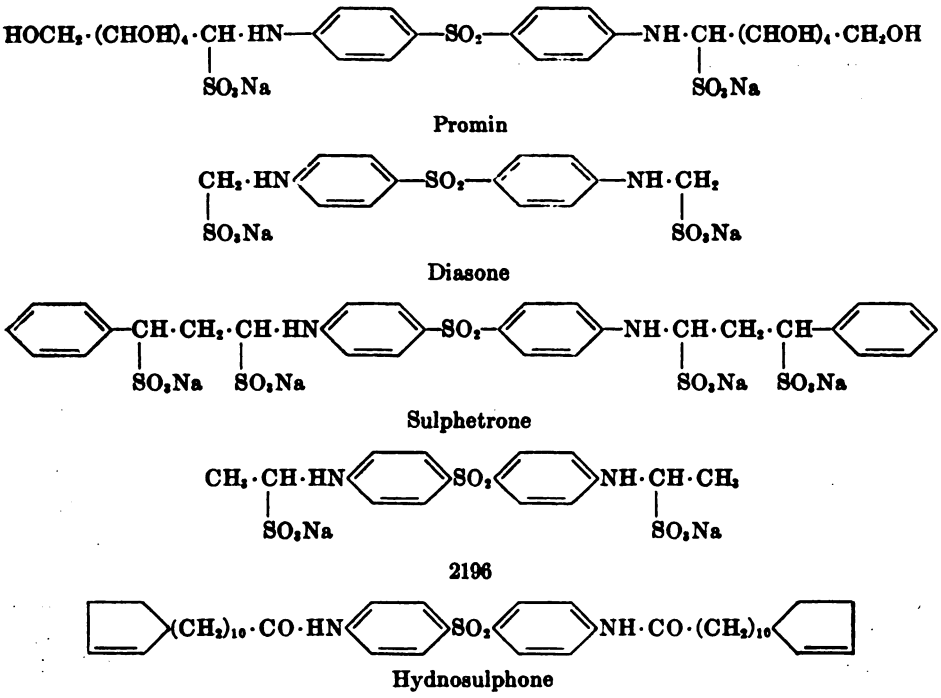


FIG. 2. Disubstituted derivatives of DDS

a natural step in the attempt to increase and widen the antibacterial activity of sulphanilamide (see Fig. 1). Although DDS is about one hundred times more active than sulphanilamide against *Streptococcus pyogenes* in mice, it is much more toxic, and when it was first used in man in 1938 for the treatment of acute streptococcal infections with doses of 1–2 g/day the treatment had to be stopped because of the severe haemolytic anaemia which developed (23, 165). At that time, no attempt was made to assess the chemotherapeutic activity of non-toxic doses, the assumption being made that the drug must be given in doses similar to those of the sulphonamides (110). Attempts were made, however, to produce less toxic derivatives of DDS, and a number of analogues with substituents on the amino groups were prepared. None of these disubstituted derivatives is as active as DDS, either *in vitro* or *in vivo*, although some are apparently less toxic for larger doses are tolerated. The antitubercular activity of DDS was first shown in 1940 against *Mycobacterium avium* in rabbits (219) and at about the same time the disubstituted derivative of DDS, promin, was shown to be active in guinea-pigs infected with human tubercle bacilli (92).

Although, in man, promin had only doubtful antitubercular activity (135, 136), it was apparently the reports of its activity in animals that encouraged Faget *et al.* (91) in 1941 to try it in the treatment of leprosy; they had previously found sulphanilamide to be inactive (88). This trial with promin marked a beginning of the most important advance yet made in the treatment of leprosy,

but the further history of the use of these sulphones is an instructive example of the difficulties that can arise in developing antibacterial drugs when there is no experimental knowledge to guide their use.

Promin is usually administered intravenously, in daily doses of 2–5 g, because, when given orally, it causes severe cyanosis and anaemia. As the intravenous route is usually considered to be inconvenient the demonstration that two other disubstituted forms of DDS could be given orally without serious untoward effects marked an important advance. The drugs were Sulfoxone sodium, U.S.P. (Diasone sodium, 4,4′-diaminodiphenylsulfone disodium formaldehyde sulfoxylate) (see Fig. 2) (197) and Solapsone, B.P. [Sulphetrone, tetra sodium 4:4′-di-(3-phenyl-1:3 disulphopropylamino) diphenyl sulphone] (see Fig. 2) (268), and they were given in daily doses of 2 g and 6 g respectively.

Two other sulphones were also used during this early developmental period. One was Acetosulphone (Promacetin, sodium 2-acetylsulphamyl-4:4′-diaminodiphenyl sulphone) (see Fig. 1) (145) and the other was Thiazolsulfone (Promizole, 2-amino-5-sulphanilylthiazole) (see Fig. 1) (90). The first is a derivative of DDS which differs from the aminosubstituted derivatives in having both amino groups free, the substitution being on a carbon atom of one of the benzene rings; the daily dose of this derivative is 3–4 g. The second is an unsubstituted sulphone which is like DDS in having both amino groups free but differs by having a thiazole ring in place of one of the benzene rings; the daily dose is 5–7 g. Neither of these drugs continued to be used very extensively.

More recently, other aminosubstituted derivatives of DDS have been tried (page 16), and of these the monosubstituted compounds are the most interesting.

DDS, itself, was not used for the treatment of leprosy until 1947. It was introduced into veterinary medicine in 1941 (189), and its activity and the low toxicity, shown in animals with the doses used, encouraged Cochrane *et al.* (53) to use it parenterally in leprosy and Lowe and Smith (179), soon afterwards, to use it orally.

1. Effects of treatment. In adequate doses, DDS and its disubstituted derivatives have similar and probably equal therapeutic effects; Laviron and Lauret (158), however, thought that sulphetrone at a dose of 3 g daily was more rapidly effective than DDS. With effective sulphone therapy, the clinical and bacteriological changes are similar to those which occur in natural remissions (page 4), and, although a clinical improvement may be obvious within three months or less, bacteriological improvement, as judged by the number of organisms present in smears, is usually delayed; fragmentation of the bacilli, however, may be seen within twenty days (48). Lepromatous patients seldom become bacteriologically negative for at least two years (48), but more usually it takes five years (66); Paul (210) found that only 79 of 206 cases, after four or more years' treatment with various sulphones, were symptom-free and bacteriologically negative. The rate at which the organisms disappear varies with the site; in one report (26) on 146 cases treated for 6 to 10 years, although the nasal mucosae of 50% of the cases were free from organisms within one year, in less than 10% of the cases was the skin free from bacilli after up to 10 years' treatment.

This lag in bacteriological improvement, however, may be more apparent than real. The clinical improvement is associated with shrinkage of the lesions and, unless the organisms can be destroyed at a faster rate than the lesions shrink, the organisms must become concentrated. Smears taken from the lesions would therefore show no bacteriological improvement and, in fact, may suggest a deterioration. Killed organisms persist in the tissues for long periods (page 26), therefore it is unlikely that bacteriological improvement, as measured by this method, could ever parallel clinical improvement (264).

The response to sulphone therapy in tuberculoid leprosy is less dramatic than in the lepromatous form. Cochrane (48) believes that the improvement is proportional to the number of bacilli present and the ability of the treatment to cause the lesions to pass into an active phase. Lowe and Davey (176) maintain that a clinical response to the sulphones is apparent within a month and that the skin lesions disappear within six months, although those in the nerves take much longer. Trophic lesions and paralysis may increase during treatment due to the fibrosis which accompanies healing of the lesions.

Soon after the commencement of treatment many patients develop lepra reaction (page 4), and although this reaction may be uncomfortable it is generally regarded as a favourable sign. Muir (200) considers not only that the dose of the sulphones should be increased till a reaction occurs but that the reaction should be induced by the concomitant administration of iodides. During the reaction there seems to be an acceleration in the rate of disappearance of the organisms, especially the fragmented forms which are phagocytosed by monocytes and even neutrophils (201). In cases not reacting to treatment, the fragmentation occurs but the fragments persist.

The prognosis of patients on sulphone therapy is undoubtedly good, but a permanent cure cannot be certain in any particular case and it may be necessary, as a precaution, for the patient to take the drug for life (51).

The failures that occur with sulphone treatment are usually due to relapses after cessation of treatment or to exacerbations during treatment. Erickson (85) noted that in 33 lepromatous cases treated with either promin or diasone and which had been presumed to be arrested after having been bacteriologically negative for 12 months, 45% relapsed within six to sixty months whereas only 4.5% of the patients relapsed who had continued treatment for this same period of time since the arrest. In an analysis (173) from East Nigeria of the results of 252 cases who had completed treatment, the lepromatous patients having been bacteriologically negative for at least twelve months and the tuberculoid patients for six months, 15 of the 139 lepromatous cases and 8 of the 69 tuberculoid ones showed slight evidence of reactivation within a year; all the patients, however, quickly responded to further treatment. Lowe (173) considers, from his wide experience of treatment with the sulphones, that if a relapse occurs it is usually within two years of the cessation of treatment. Cochrane (47), however, points out that as leprosy may take years after contraction of the disease to become clinically evident, relapses may not become evident for five to ten years after the patients are apparently cured.

Cases of exacerbation during treatment are quoted by Wolcott and Ross

(270). They could discover no common factor causing the extension of the lesions during treatment, and other drugs (TB1/698, PAS, isoniazid or streptomycin) were unsuccessful in controlling the spread. These workers also noted that in some cases the disease reaches a low state of activity where the lesions are still bacteriologically positive and do not improve even when the treatment is changed to other drugs. Lowe (173) on the other hand said that he had never seen an exacerbation in patients in East Nigeria.

2. Mode of action. The sulphones act on streptococci and similar organisms in the same manner as do the sulphonamides; their action is essentially bacteriostatic and is reversed by *p*-aminobenzoic acid. Their activity against *M. tuberculosis* is also reversed by this metabolite and it is generally assumed that their action against *M. leprae* is similar. Although treatment with the sulphones has to be of long duration, Muir (200) considers, from the speed in which lesions of the skin and nasal mucosa heal and eye lesions become controlled, that the sulphones must induce an immediate and profound change in the bacilli; fragmentation undoubtedly occurs within a few weeks of commencing treatment, and he suggests that the bacilli are killed in large numbers although they remain in the tissues for months or even years. Cochrane (46), on the other hand, suggests that fragmentation is a change induced by an unfavourable environment and that the granules remain viable and capable of reversion to the more normal bacillary form for many years.

de Souza and de Souza Lima (252), from evidence derived from careful microscopic observations, conclude that, although the sulphones probably have a direct action on the bacilli, their principal action is on the Virchow cell. They maintain that the changes which occur in these cells during sulphone therapy precede those seen in the bacilli, and that these changes are similar, if not identical with, those which occur during a spontaneous remission, *i.e.*, swelling of the cell with pyknosis of the nucleus, vacuolation of the cytoplasm, and granulation of the bacilli. Millar (190) supports this view and maintains that the prime effect of the sulphones is to liberate the bacilli from the cells, thus exposing them to the inhibitory substances in the extracellular fluids (page 34), and he quotes Cochrane as stating that during sulphone therapy the bacilli are found more frequently extracellularly. Chatterjee (36), also, considers that the sulphones act primarily on the Virchow cells.

Mitsuda (191), however, claims that during therapy with the sulphones or other antileprotic drugs the bacilli are destroyed intracellularly and then take the granular form. The lepra cells swell, becoming filled with phosphatide-lipid granules from the lysed organisms, and he considers that some of these products of lysis are liberated and cause the lepra reaction.

Malfatti and Jonquieres (182) have studied with the electron microscope the changes that occur in the bacilli during sulphone therapy. The peripheral envelopes, which surround isolated bacilli and the globi, disappear (the authors believe that this envelope depends upon normal bacillary metabolism) and granular forms appear. TB1/698 and isoniazid cause similar changes.

3. Metabolism of DDS and the disubstituted derivatives. Most of the earlier

workers assumed that the disubstituted derivatives of DDS are broken down in the body to the parent substance, this assumption being based on the knowledge that stable substitutions of the amino group of the sulphonamides abolished their antibacterial activity. Brownlee *et al.* (16), however, maintained, mainly from toxicity and antibacterial studies *in vitro*, that sulphetrone was not degraded and that it possessed activity *per se*, but later studies by other workers, especially Francis and Spinks (110), do not support this contention (see below).

Titus and Bernstein (258) confirmed the general assumption by demonstrating with solvent partition methods and chromatography that up to 75% (w/w) of the diazotizable drug present in the blood of mice after the oral administration of equi-molar doses of diasone, promin or sulphetrone existed as DDS. They also showed that compared with DDS these drugs are very poorly absorbed from the alimentary tract. These findings were later confirmed independently by Boyer *et al.* (13) by paper chromatographic analysis of rabbit urine and by Francis and Spinks (110) using solvent partition methods with rabbit, rat and mouse urine. These latter workers also showed that the relative chemotherapeutic potencies of DDS and its derivatives in mice infected with streptococci were: DDS—100, 2196 [a previously undescribed acetaldehyde bisulphite disubstituted derivative of DDS (see Fig. 2)]—43, diasone—18, promin—16 and sulphetrone—1. As this order of activity corresponds with the rate at which these derivatives are hydrolysed to DDS in 0.1 N hydrochloric acid, the authors consider that it reflects the degree of hydrolysis which occurs *in vivo*. Using solvent partition methods, Lowe (170) confirmed that diasone, promin and sulphetrone changed to DDS when given orally to man. That the hydrolysis of sulphetrone occurs in the alimentary tract, presumably in the stomach, is shown by the fact that little or no breakdown occurs when the drug is given parenterally (21,110,170). With promin some hydrolysis may occur when it is given intravenously to man for, according to Floch *et al.* (106), 50% of the diazotizable drug excreted in the urine is present as free DDS, but this breakdown was not confirmed by Lowe (170) or, in animals, by Francis and Spinks (110). These latter workers showed that appreciable breakdown occurs with 2196 when given intravenously but this derivative is known to be less stable.

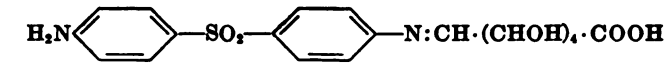
Various groups of workers (21, 110, 170, 244, 258) also noted that, when DDS was administered orally, a portion of the diazotizable drug present in the blood and urine was in a form which did not partition into organic solvents. The proportion varied, but in man, according to Lowe (170), about 20% (w/w) of the drug in the blood and about 80% of that in the urine was in this water-soluble form. Titus and Bernstein (258) concluded from their studies that the water-soluble derivative was labile in dilute acid and that although the solubilizing substituent was not determined it is presumably attached to the amino-nitrogens.

More recently Bushby and Woiwod (21) demonstrated, by paper electrophoresis and chromatography, the presence of two metabolites of DDS in the urine of rabbits after the oral administration of the drug. One of these was present in a much greater proportion than the other and represented the major diazotiz-

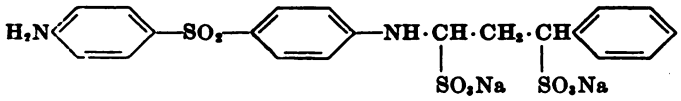
able constituent. It was isolated and shown to be a glucuronic acid mono-conjugate of DDS (see Fig. 3), possessing one free amino group and the glucuronic acid linked through its aldehyde group (22); it was synthesized and shown to be acid-labile. The minor derivative was present in too low a concentration to be identified. When the urine was excreted in an alkaline state no free DDS was present, thus suggesting that DDS is not excreted by the kidneys in the free form but that hydrolysis may occur in the bladder when the urine is acid. The glucuronic acid mono-conjugate was also present in the rabbit's plasma; two hours after an oral dose of 0.1 g/kg when the concentration of diazotizable drug in the blood was equivalent to 1 mg of DDS per 100 ml, at least 70% (w/w) of the drug was in the conjugated form.

These workers also noted that the excretion products of DDS in human urine matched exactly those of the rabbit, both chromatographically and electrophoretically. The major component was, however, less acid-labile than that of the rabbit, and Bushby and Woiwod suggested that it too was a glucuronic acid mono-conjugate but that its conjugating bond was stronger. In patients on continuous DDS therapy with alkali given to render the urine neutral, less than 5% (w/w) of the excreted diazotizable drug in the urine was present as free DDS.

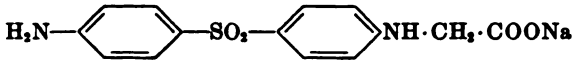
Bushby and Woiwod (21) also confirmed that the excretion products of sulphetrone after oral administration are identical with those of DDS, and that after parenteral administration the sulphetrone is excreted unchanged. They showed, however, that commercial sulphetrone contains as much as 15% of semi-sulphetrone [*i.e.*, DDS with the sulphetrone substitution on only one of the amino groups (see Fig. 3)], and that, when pure disubstituted sulphetrone



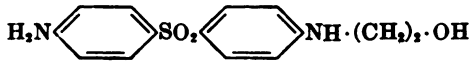
Major metabolite of DDS in rabbit urine



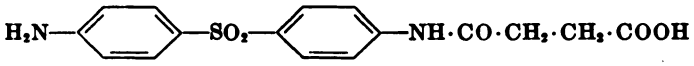
Semi-sulphetrone



Sulphone Cilag



Hydroxyethyl sulphone



Succisulphone

FIG. 3. Monosubstituted derivatives of DDS

was injected into rabbits, both it and semisulphetrone were excreted in the urine, thus showing that some conversion occurred *in vivo*. A similar conversion occurs *in vitro*, for they found that 5 % solutions (w/w) of pure disubstituted sulphetrone changed until an equilibrium mixture of sulphetrone, semi-sulphetrone and DDS was reached. Commercial preparations of both promin and diasone may also be mixtures of disubstituted and monosubstituted derivatives of DDS, for minor components with a similar chromatographic R_F to semi-sulphetrone have been detected in both (12); a similar change from the disubstituted to the monosubstituted form *in vivo* has been suggested for promin (254).

Although there is now little doubt that the antileprotic activity of oral sulphetrone is due to the liberation of DDS in the stomach, the activity of parenteral sulphetrone must be due to an intrinsic activity, for little or no degradation occurs. Unless, however, the mode of action of this sulphone against *M. leprae* differs from that against streptococci, the parenteral activity of the commercial product must be due to the minor, monosubstituted component. Although the parenteral antileprotic activity of promin can be similarly accounted for, that of the bis-acetaldehyde bisulphite derivative of DDS (260) is probably due to the liberation of the parent sulphone; Francis and Spinks (110) showed that this derivative is extensively hydrolysed when administered parenterally to rabbits.

Most workers have assumed that the activity of DDS *in vivo* is due to the presence of unaltered drug in tissues. The observation that DDS circulates in the body in a free form and in very low concentrations for probably only short periods makes this assumption unlikely, especially as the major metabolite is a mono-conjugate with antibacterial activity *per se*. The significance of this possibility in relation to the monosubstituted derivative of DDS is discussed on page 16.

4. Absorption and excretion. Studies of the drug concentrations in blood and urine after the administration of DDS and its disubstituted derivatives have been made by many workers using the diazotizing method of Bratton and Marshall, but the fact that the drug is present as metabolites has usually been ignored.

Studies by Smith (248), in man, showed that as much as 85 % of the daily oral dose of DDS is excreted in a diazotizable form in the urine, whereas only 53 % of that of diasone and 11 % of that of sulphetrone are excreted by this route. Estimations of the quantities of the drugs present in the faeces confirm that the remainder of the dose of each is excreted by this route. These findings agreed with those of Titus and Bernstein (258) who showed in mice that although DDS was well absorbed, its disubstituted derivatives, especially sulphetrone, were poorly absorbed. Ross (225) also confirmed these findings, but she found that when promin was administered intravenously none was excreted in the faeces, thus demonstrating that little of the substance found in the faeces after the oral administration of these drugs, is due to excretion *via* the bile or the ileum as has been suggested to occur with sulphetrone (15). Although no chromatographic studies appear to have been made to confirm that the drug which is

excreted in the faeces is in its original form, there can be little doubt, especially in view of the almost complete absorption of DDS, that in the case of the disubstituted derivatives it represents that portion of the original drug which escaped hydrolysis.

Estimations of the concentration of diazotizable material present in the blood after the oral administration of DDS show that the peak level is reached within one to three hours and that the concentration then falls slowly (16, 110, 248, 258). With daily doses of 0.2 g the concentration in the blood taken just before the next dose is about 1.2 mg/100 ml, and with single doses of this size, traces of diazotizable material are present in the blood 8 to 12 days later; with repeated doses, traces may be found as long as 35 days after the last dose (75). The concentrations of the drug in the urine are usually about ten times higher than those in the blood. When injected as a suspension in arachis oil, 25% of a 0.4 g dose of DDS is excreted in the urine within 36 hours, and the concentration in the blood of patients receiving weekly doses of 0.5 g ranges from 0.1–0.9 mg/100 ml (193).

When the disubstituted sulphones are given orally the concentrations of diazotizable material in the blood are similar to those found with DDS, but when calculated on a dosage basis they are relatively lower due to the poorer absorption of these derivatives. Sulphetrone when injected intramuscularly is excreted fairly rapidly; Relwicz (217) found that 4 hours after the injection of 2 g of sulphetrone the blood concentrations, expressed as sulphetrone, ranged from 4–8 mg/100 ml and at 24 hours from 2–4 mg/100 ml, but at 48 to 72 hours only traces were present. The urinary concentrations at these same times ranged respectively from 50–200 mg, 20–45 mg, 1–30 mg and from a trace to 8 mg/100 ml. Similar figures have been reported by Lowe (170).

Promin, like sulphetrone, is rapidly excreted when it is injected intravenously. Floch *et al.* (106) found that in man 36% (w/w) of a 5 g intravenous dose was excreted within 2 hours, and of this 42% (w/w) was present as promin and 58% (w/w) as DDS. With similar daily doses, Ross (224) found that the concentration of diazotizable material present in the blood just before giving the next dose was 1–1.6 mg/100 ml, but detectable quantities were present 9 days after the drug had been discontinued.

The sulphones show a tendency to be retained in the skin and muscle and especially in the liver and kidney; traces of diazotizable material may be found in these tissues up to three weeks after the last dose (225). Chatterjee and Poddar (37), using DDS labelled with radioactive S^{35}, found that the concentration of the drug in diseased skin was ten times higher than in normal skin. Dresbach (82) showed that the sulphones are excreted in the milk of nursing mothers and that diazotizable material is present in the urine of the babies.

Although these estimations are instructive, their precise value will remain in doubt until the actual concentration of the drug or its metabolite necessary to affect the viability of *M. leprae* is known. Without a suitable *in vitro* test or susceptible experimental animals such knowledge is difficult to obtain, but Lowe (170) endeavoured to determine the minimum dose of DDS that is clinically ef-

fective. He found that as little as 30 mg of DDS a day, which gave blood levels ranging from 0.15 to 0.25 mg/100 ml, gave a clinical response although the response was probably not maximal.

5. Toxicity. As stated earlier (page 7), Buttle and Long had to discontinue the administration of DDS at a dose level of 1–2 g a day because of severe cyanosis and anaemia, and it was this toxicity which led to the development of the disubstituted derivatives. Although DDS weight for weight is more toxic than the sulphonamides, the early conception that it is too toxic for clinical use arose from the preconceived idea that it must be given in similar doses to the sulphonamides and that comparable blood levels are necessary. More recently it has been shown that doses less than one-hundredth of the usual sulphonamide dose give a clinical response in leprosy (170). The disubstituted derivatives are less acutely toxic than the parent substance when given orally, but this is due to their poor absorption and depends on their rate of hydrolysis in the stomach. The toxic effects of DDS, promin, diasone and sulphetrone are identical but those from the parent substance may be more severe because of its total absorption (248).

In animals, when DDS is administered orally the LD50 dose is 0.6 g/kg for mice (12) and 0.3 g/kg for guinea-pigs (104). In mice severe tremors developed with doses of 0.12 g/kg but not with doses of 0.025 g/kg. With a weekly dose of 0.1 g/kg, there is concentration of the drug in the liver and kidneys, but no toxic effects are observed unless the concentration in the liver rises above 25 mg/100 g (104).

In man, the most constant toxic effect of oral sulphone is anaemia of the regenerative type (94, 214, 249). Brownlee (15) considered that with doses of sulphetrone of 3–6 g daily, the nature of the anaemia is complex; there is a haemolytic process, an iron deficiency and a deficiency of a nutritional factor probably due to an alteration of the intestinal flora. With doses of 100 mg of DDS a day, however, Dharmendra and Chatterjee (76) found that anaemia occurred in only about 25 % of the patients, and the fall in haemoglobin, which took six to twelve weeks to develop, was about 1.6 g/100 ml; this fall could not be prevented by iron or yeast therapy. With a higher dose of DDS, methaemoglobinaemia is not uncommon; Brownlee (15) noted in many patients on sulphetrone treatment, a leaden blue coloration of the skin which he considered was not due to the methaemoglobinaemia, and these patients excreted a violet-blue dye in the urine.

Psychosis is not uncommon with high oral doses of DDS (113, 166), but this is rarely seen when minimal effective doses are used. The incidence is highest in the more educated and sensitive patients. There may also be an acute febrile reaction with nausea, migraine and erythematous skin lesions, surrounded by papules and nodules resembling tuberculoid eruptions (253). An allergic type of dermatitis may also develop which can be controlled by desensitization with small doses of the sulphone used (176). Hepatitis has also occurred (4).

Erythema nodosum leprosum is common. It is not, however, regarded as a toxic effect but rather as evidence of the activity of the drug. The reaction is discussed on page 4. It may be severe and as an acute inflammatory reaction may cause

severe damage to infected eyes and nerves. The intensity of the attacks should be kept to a minimum, and this may be done by introducing the treatment gradually (176). In tuberculoid leprosy, it is customary to suspend the specific treatment as soon as the reaction is established because, although the reaction is itself favourable, when severe its consequences in the form of residual deformities may be serious (51).

On intramuscular administration, the sulphones are relatively free from toxicity. The low solubility of DDS at the pH of body tissue prevents excessive blood concentrations, and sulphetrone because of its high solubility is rapidly excreted in the urine.

In spite of these side-effects the sulphones, used properly, have a high safety record. At Carville more than 1,100 patients have been treated with various sulphones for prolonged periods without a single fatality or serious illness attributable to the drug (35).

6. The monosubstituted sulphones. Only three of these derivatives of DDS have been used clinically. They are the sodium salt of 4-amino-4′-carboxymethylaminodiphenyl sulphone (Sulphone-Cilag) (see Fig. 3), 4-amino-4′-β-hydroxyethylaminodiphenyl sulphone (Hydroxyethyl sulphone) (see Fig. 3) and 4-amino-4′-succinylaminodiphenyl sulphone (Succisulphone) (see Fig. 3). The substitution links of these drugs are acid-stable and there is no evidence of appreciable breakdown to DDS when given orally. According to Boyer (12), however, chromatographic analysis shows that when Succisulphone is given orally to rabbits it is excreted in a changed form, and Bushby (18) has confirmed this and shown that Sulphone-Cilag is also excreted in an altered form; these metabolites have not been identified but they are different from those of DDS. After intravenous injection in man, at least 90% of the Succisulphone (w/w) is excreted unchanged in the urine (105).

These monosubstituted derivatives are of especial interest in view of the probability that the *in vivo* activity of DDS is due to a monosubstituted metabolite (page 13). Smith *et al.* (250) examined some 20 monosubstituted forms of DDS and found that their *in vivo* antitubercular activity varied, and although most were less active than DDS at least four were more active; the maximum tolerated oral dose of these drugs was three times as large as that of DDS. There is, therefore, evidence that it is possible to synthesize more active and less toxic monosubstituted derivatives of DDS than the one which is formed *in vivo*.

None of these derivatives, however, has been used extensively for the treatment of leprosy. Floch and Destombes (101) treated 56 patients with 0.2–1.0 g of Succisulphone by mouth or 1 g intravenously each day. No toxic side-effects were noted and they claimed that there was good bacteriological and clinical improvement, but it was apparently not better than it would have been with DDS; they thought, however, that they detected an additive effect when the drug was given with DDS. Ramanujam (213) gave 0.5 g of Sulphone-Cilag intravenously or subcutaneously each day or 1 g subcutaneously twice weekly to 14 patients but he was not impressed with the results because the bacteriological improvement was not proportional to the clinical improvement. Severe neuritis

occurred in one case, severe lepra reactions in two cases and a moderate anaemia in another. Cochrane (47) tentatively concluded from the results of treatment of a few patients that Sulphone-Cilag was as active as DDS but hydroxyethyl sulphone was less active. Browne (14) showed in 67 patients that daily oral doses of 0.2 g of Sulphone-Cilag produces bacteriological and clinical improvement, but the duration of treatment was too short for a proper assessment of the drug. Floch and Lecuiller (103) successfully used Succisulphone with DDS for the treatment of patients whose dose of DDS had had to be reduced because of lepra reactions.

7. Other sulphones and related compounds. 4:4′-Diaminodiphenyl sulphoxide, a close analogue of DDS (see Fig. 1), is about one-fifth as acutely toxic as DDS and has similar antitubercular activity *in vitro* and in guinea-pigs. Buu-Hoï *et al.* (25) treated 34 leprosy patients with daily doses of 0.1 g and the clinical and bacteriological improvements were similar to those that would have been expected from treatment with DDS; similar results were also reported by Davey *et al.* (61).

Di-atox-argentique, a silver diazotized derivative of DDS, was claimed by Tzanck *et al.* (261) to be completely non-toxic to guinea-pigs when administered orally in doses of 1 g/kg, and to have antileprotic activity in man. They treated 15 leprosy patients with 0.2 g a day and gave details of the response with 3 patients. They claimed rapid improvement, and, although a few lesions were still bacteriologically positive after 2 years' treatment, they too eventually became negative. According to Rist *et al.* (220), this drug consists of two compounds—diphenyl sulphone 4:4′-*bis*azo-*p-iso*-propyl*meta*cresol and a silver derivative of this. Neither compound has antistreptococcal activity *in vitro* and therefore any *in vivo* activity presumably depends on hydrolysis to DDS. In mice, 5 mg has the same protective action against streptococci as 0.5 mg of DDS. These investigators were unable to detect any antitubercular activity in guinea-pigs and therefore they doubt whether it can be as effective against human leprosy as the less stable disubstituted derivatives or as DDS itself.

Bis-(*p*-hydrazinophenyl) sulphone (see Fig. 1) is chemically very similar to DDS, but with hydrazino radicals replacing the amino groups, and it is alleged to have antileprotic activity (273). This claim is based upon the treatment of only 7 cases with daily doses of 30–50 mg, but good effects were observed. The bacilli diminished and disappeared from the nasal discharge and skin lesions within three or four months.

An interesting derivative of DDS is di-hydnocarpyl-4:4′-diaminodiphenyl sulphone (Hydnosulphone) (see Fig. 2) which is a chemical combination of DDS and hydnocarpic acid. According to Dharmendra and Chatterjee (73), it is insoluble in water but inhibits the growth of streptococci and of certain acid-fast bacilli at a concentration of 50 μg/ml. It is non-toxic to laboratory animals at a daily oral dose of 50 mg/kg. A daily oral dose to man of 0.15–0.2 g gave blood concentrations of 0.5–0.8 mg/100 ml and 85–90% (w/w) of the total intake was excreted in the urine. Excretion is slow and traces of the drug may be found in the blood a month after the cessation of treatment. These authors claim that there

is no breakdown to free DDS. Bushby (19), in attempting to confirm these observations, has failed to demonstrate activity at 1 mg/ml against *Streptococcus pyogenes* or *M. tuberculosis var hominis* in media in which DDS inhibits these organisms at concentrations of 1 and 20 μg/ml respectively, or in mice infected with *St. pyogenes* and treated with doses of 10 mg; in this infection 3 doses of 0.1 mg of DDS gave full protection. No protection has been shown against *M. lepraemurium* in mice infected intravenously and treated with daily doses of 0.1 g per mouse (Table 1). Also, although Dharmendra and Chatterjee used the Bratton and Marshall method for estimating the drug, Bushby found no diazotizable material before or after acid hydrolysis of blood and urine of dogs receiving a daily dose of 0.1 g Hydnosulphone per kg for 10 days; he too found the drug to be insoluble in water and also resistant to hydrolysis at 120°C. There are, therefore, some discrepancies between the findings of these workers. From the findings of Bushby this drug would not be expected to have antileprotic activity unless it possesses a mode of action different from that of DDS; a different mode of action is also implied by Dharmendra and Chatterjee, for, although they maintain that it is not degraded to DDS in the body, they find that it is active in leprosy. They treated 26 patients, 22 of whom had previously failed to respond to DDS, and had very encouraging results. Hydnosulphone is therefore an interesting drug and it is to be hoped that it will be given more extensive trials.

A very similar DDS derivative to Hydnosulphone was used by Gaté (114). It is the condensation product of DDS and hydrogenated chaulmoogric acid and it therefore differs from Hydnosulphone only by having a saturated fatty acid instead of an unsaturated one in the molecule. Gaté treated 17 patients with doses increasing up to 0.5 g intramuscularly two or three times weekly and claimed excellent responses, with the bacilli diminishing in number.

8. Current sulphone therapy. DDS is now the most generally used sulphone, and as the usual oral dose is 0.3 g twice weekly a year's treatment costs less than one pound sterling (176). Treatment should start with a low dose which should be slowly increased so that the maximum is reached in about three months (51).

Although most workers prefer to give DDS orally some consider that under certain circumstances, as for instance when the Medical Officer has to travel long distances, it is better to administer it intramuscularly (266). Laviron *et al.* (159) recommend that a dose of 1.25 g in 5 ml of equal parts of chaulmoogra oil and its ethyl ester be given intramuscularly each fortnight. Floch and Gélard (102) find that adequate blood levels, lasting for 30 days, can be obtained by injecting 1.8 g of DDS, particle size 200 μ, in 0.2% agar saline, making only monthly injections necessary. Parenteral sulphetrone, 1.5 g twice weekly, is recommended by some leprologists (51, 239) and a few still prefer to give promin, starting with an intravenous daily dose of 1 g 6 days a week, rising to 5 g daily after 4 to 6 weeks, and giving a rest period of one week in three. Others prefer to give oral diasone, starting with 0.3 g daily and increasing slowly to 1 g daily (35).

Treatment with the sulphones should be continued for at least 18 months after all activity has ceased in non-lepromatous leprosy, and perhaps for life, but

certainly for 2 years in lepromatous leprosy (51); Scohier (239) considers that the dose should always be kept as close as possible to the toxic threshold.

C. Thiosemicarbazones

Certain thiosemicarbazones have been widely used in Germany for the treatment of tuberculosis. As they have high *in vitro* activity against *M. tuberculosis* it was logical that they should be tried for the treatment of leprosy, and, in 1950, several workers gave preliminary reports of encouraging results with Thiacetazone (TB1/698, Conteben, Tibione, Amithiozone, *p*-acetamidobenzaldehyde thiosemicarbazone) (149, 227, 232, 233, 263). In 1952, Lowe (168), in describing his results from the treatment of 126 patients with TB1/698 for five to seventeen months, claimed that apart from 1 case of agranulocytosis no other serious toxic signs were seen and that the clinical and bacteriological responses were satisfactory. The dose was 0.2 g twice daily for 6 days a week, and drug fever and dermatitis were rarer and much milder than with DDS. In 1953, Lowe (171) reported on further experiments with a total of 182 cases. He claimed that TB1/698 was a most useful therapeutic agent in the treatment of leprosy, the results closely paralleling those of DDS and sometimes possibly being better; he suggested that in spite of some toxic effects, consisting of hepatitis, nephritis, agranulocytosis, allergic dermatitis and recurrent drug fever, TB1/698 might prove to be the best treatment for leprosy. These promising early results were confirmed by a number of other workers (2, 10, 17, 62, 70, 152, 195, 228, 236), but, in 1954, Lowe (172) concluded that the late results of treatment were disappointing; during the second year of treatment improvement was slower than during the first year, and during the third year there was sometimes a rapid deterioration. He suggested that the relapses were due to the organism becoming resistant to the drug and recommended that TB1/698 be used only for patients who cannot tolerate the sulphones. Davey (59) confirmed that deterioration occurs after prolonged treatment and noted that, if the deterioration is due to resistant organisms, the organisms are not cross-resistant with DDS, for the patients responded to treatment with this drug. Davison (64) considers from his experiments that the action of TB1/698 is too weak for the treatment of lepromatous leprosy, but that the drug is of value in tuberculoid leprosy.

Of the other thiosemicarbazones tried in leprosy, the β-pyridine aldehyde analogue of TB1/698 is active (107), but if the acetyl group is replaced with a hydroxyl group, or the aldehyde with a ketone, or if there are two thiosemicarbazone radicals per molecule the activity is lessened (160).

The thiosemicarbazones may be regarded as monosubstituted thioureas, and a number of disubstituted thioureas have also been shown to possess antitubercular activity (187). Two of these have been used in the treatment of leprosy and reported to be active. Buu-Hoï *et al.* (25) treated 13 patients orally for 6 months with 0.1 g daily of 4:4′-diethoxydiphenylthiourea, and obtained promising results; Davey and Currie (60) treated 41 patients with daily doses of 1.5–3.0 g of 4-butoxy-4′-dimethylaminodiphenylthiourea (SU 1906) for periods of up to sixteen months and considered that the clinical and bacteriological improve-

ments observed during the first nine months were at least equal to those that would have been produced by DDS. After this period, however, there was a similar lessening in the rate of improvement in some cases, as occurs during treatment with the thiosemicarbazones. Konopka *et al.* (153) observed that strains of tubercle bacilli resistant to TB1/698 were also resistant to SU 1906, and they therefore considered that these drugs probably act upon similar if not identical biological systems within the tubercle bacillus. The two groups of drugs would therefore be expected to behave similarly in the treatment of leprosy.

D. Isoniazid

Isoniazid, B.P.C., U.S.P. (Nydrazid, Rimifon, *iso*nicotinic acid hydrazide) has proved to be very disappointing for the treatment of leprosy. Its very high activity both *in vitro* and *in vivo* against *M. tuberculosis* encouraged the hope of similar activity against *M. leprae*. In 1952, however, Lowe (169) published a preliminary report on the treatment of 27 patients with doses increasing up to 0.3 g daily for 5½ months, in which he concluded that isoniazid is possibly of slight beneficial effect but its activity is much less than that of the sulphones. Since then, this conclusion has been supported by a number of workers (39, 96, 125, 157, 185), although others have reported results which seem to be more promising (140, 156, 164, 240, 241).

This poor response to isoniazid can be explained either by the relative insensitivity of *M. leprae* to isoniazid or by the fact that the organism, although initially sensitive, rapidly develops resistance. Without suitable *in vitro* or *in vivo* tests, it is not possible to decide whether either of these possibilities is the true explanation. Mycobacteria undoubtedly vary in sensitivity to isoniazid; 50–200 μg of isoniazid per ml is necessary to inhibit the growth of *Mycobacterium paratuberculosis* (Johne's bacillus) as compared with 0.3 μg/ml for the H37Rv strain of *M. tuberculosis* growing on the same medium (247), but Barnett and Bushby (6) have shown, in mice, that *M. lepraemurium*, the organism probably most closely related to *M. leprae*, is as sensitive as *M. tuberculosis* to this drug. As these latter workers and others (page 30) have also shown that *M. lepraemurium* readily develops resistance to isoniazid, it seems probable that the failure of isoniazid in human leprosy is due to this rapid development of resistance. This supposition is supported by the clinical experiences of some leprologists (71, 108, 192), who noted early, sometimes even spectacular, improvement for a few months, followed by a deterioration.

Changes in resistance to isoniazid by the tubercle bacilli are often associated with changes in the pathogenicity of the organism and similar changes might be expected to occur with *M. leprae* if it became resistant. The changes in the tubercle bacillus are complex, and seem to be related to a decrease in "general virulence", *i.e.*, ability to invade, rather than to a change in "local virulence", *i.e.*, ability to produce local disease (116). Such changes occurring with *M. leprae*, already an organism of very low virulence, in established infections, would probably not be readily detected. It is interesting to note that Barnett and Bushby (6) found that mice infected with isoniazid-resistant variants of *M.*

lepraemurium survived longer than those infected with the parent sensitive strain.

If the rapid development of resistance is the limiting factor in the use of isoniazid in the treatment of leprosy, then giving it in combination with another drug would be expected to decrease this limitation, as it has in the treatment of tuberculosis; this is discussed on page 24.

Cyanacetic acid hydrazide is chemically related to isoniazid and, according to Valdecasas *et al.* (262), it has high antitubercular activity and is clinically active against isoniazid-resistant strains of tubercle bacilli. Barnett *et al.* (8), however, found that it was less active *in vitro* than isoniazid, that resistant variants developed quickly and that in general there was cross resistance between strains resistant to either of the two drugs. Capurro and Wilkinson (27) treated 7 lepromatous patients with 0.3 g of the drug for up to eight months and noted an outstanding bacteriological improvement; promin, however, was given as an alternate treatment, so the significance of the observation is doubtful.

E. Streptomycin

Streptomycin, including dihydrostreptomycin, is another antitubercular drug which has not proved spectacular in the treatment of leprosy. In 1946, Faget and Erickson (87) started the treatment of 10 patients with lepromatous leprosy with 2 g streptomycin every 24 hours for 4 months and then halved the dose and continued treatment till their time of writing. Encouraging results were obtained which, however, were no greater than the authors would have expected from treatment with the sulphones. Erickson (86) later, reporting the results of treatment with the dose reduced to 0.5–1.0 g daily or of giving 0.5–1.0 g of dihydrostreptomycin daily, concluded that these antibiotics have a beneficial effect on cutaneous and mucous membrane lesions. The effects were similar and sometimes occurred earlier than with the sulphones, and this he attributes to the elimination of secondary invaders. There was, however, no decrease in the number of *M. leprae* in smears from the lesions, but as the lesions were becoming smaller the total number of bacteria had obviously decreased. He maintained that vertigo, tinnitus and deafness precluded continuous treatment for periods of longer than 6 months. Similar conclusions to these were obtained by other workers (208, 215).

Sinha (245) conducted a most extensive trial by treating 245 cases using both forms of the antibiotic. He found toxicity no hindrance but the cost of the treatment very heavy; the responses to treatment were similar to those noted by Erickson. The Committee on Treatment of the VI International Congress of Leprosy (142) found that, while there had been no reports of striking results with these antibiotics and the early response to treatment was slower than with the sulphones, by the end of a year's treatment there was little difference between the results from the two drugs; dihydrostreptomycin, 1 g thrice weekly, was considered to be preferable to streptomycin because it less frequently caused damage to the eighth nerve.

F. Other drugs

Before the advent of the sulphones, a number of drugs, besides chaulmoogra oil and its derivatives, had been used apparently on an empirical basis. These included small doses of potassium iodide, which was claimed to have a specific action against the leprosy bacillus, fluorescein [10 grains (0.65 g) daily] and methylene blue or trypan blue [4 grains (0.25 g) daily] (196). More recently, all the common antibiotics have been tried although usually for only relatively short periods.

Penicillin was used in its early days but only in small doses, 50,000 units being given daily for several weeks without beneficial effect (89, 194). Oxyprocaine penicillin (the diethylaminoethanol-*p*-aminosalicylic salt of penicillin) has a higher antitubercular activity *in vitro* than has procaine penicillin (203), and Percy (212) administered a daily dose of 300,000 units of this salt of the antibiotic to 5 lepromatous and 7 tuberculoid cases of leprosy for 3 months without finding any change in the number of bacilli in the lesions, although there was repigmentation of the macules and subsidence of the areas of infiltration. Chloramphenicol (Chloromycetin) was unsuccessfully tried for a very short period by Pardo-Castello *et al.* (209); they treated only 3 patients with doses of 50 mg/kg at 4, 6 or 8-hourly intervals for 6 days, repeating the course after 6 days' rest, but the alleged risk of damaging the haemopoietic system probably deterred them and other leprologists from giving more prolonged periods of treatment with this antibiotic.

Chlortetracycline (Aureomycin) was given by Johansen and Erickson (144) to 13 patients in daily doses of 1–1.5 g for 1 to 1½ years during which time there was healing of the specific lesions and some bacteriological improvement. These beneficial effects were, however, not seen in 6 patients given total doses of oxytetracycline (Terramycin) ranging from 136–625 g. Neither of these drugs was given long enough for a final assessment, but it is unlikely that the tetracyclines have a marked beneficial effect in leprosy.

Acidomycin [2-(5-carboxypentyl)-4-thiazolidone], an antibiotic which has activity *in vitro* against the tubercle bacillus, is claimed by Yukichi Satani *et al.* (230) to clear leprous skin lesions of the bacilli when injected directly into the lesion, but it has no therapeutic effect in tuberculosis or leprosy when injected either subcutaneously or intravenously.

Para-aminosalicylic acid (PAS), the antitubercular drug which, when administered in combination with streptomycin and isoniazid, has proved to be invaluable for delaying the development of resistance to these drugs, has no striking effect in leprosy (69, 142, 166). In the controlled clinical trials (page 25), however, it was shown to have a slight but definite effect. The low antileprotic activity of PAS is not surprising for, in spite of high activity *in vitro*, the drug has little effect in tuberculosis when used alone.

A phenazine dye, B 283 (2-anilino-3-amino-5-phenylphenazine) (9), which has high activity *in vitro* and *in vivo* against experimental tuberculosis, was given in an average daily dose of 0.5 g continuously for a year to 10 cases of lepromatous leprosy (1); 3 became bacteriologically negative, 3 others improved, 3 showed

no change either clinically or bacteriologically and in 1 the disease became worse. In 3 patients, hepatitis developed when the dose was 0.75 g a day but disappeared when it was reduced to 0.5 g. Another report (2) claimed that B 283 seems to have activity similar to that of DDS, but too few cases were treated for an accurate assessment.

Cepharanthin, an alkaloid which is derived from *Stephania cepharantha*, is claimed by Hasegawa and Shinozuka (134) to have antitubercular activity but Sirsi and De (246) were unable to show any activity in mice infected with tubercle bacilli and Ebina *et al.* (83) noted no beneficial effects in 34 tuberculous patients. According to Sato (231), it is also ineffective in the treatment of leprosy, and Dharmendra and Chatterjee (72) observed definite improvement in only 3 of 15 patients after 41 to 52 weeks of treatment. Ranade and Gokhale (216), however, observed clinical but no bacteriological improvement in 5 lepromatous cases after 6 months' treatment with 0.1 mg by mouth daily and 0.1 mg intramuscularly twice a week.

Lupulon (*beta* lupulic acid), an antibiotic from hops, produced no beneficial effects in 8 patients given a total dose of 136–625 g over a period of 10 months (144).

G. Combined therapy

Although the additive or synergistic activities shown *in vitro* between several pairs of antibacterial chemotherapeutic drugs are rarely of clinical value, a number of leprologists have given two drugs simultaneously in the hope of producing an enhanced effect. Others have used combined therapy because they suspected that when administered singly, one or both of the drugs fail through *M. leprae* becoming resistant; they have thus followed the procedure that has been so successful against *M. tuberculosis*.

Sir Leonard Rogers (222) considered the antileprotic activity of the chaulmoogra drugs and the sulphones to be complementary; according to him, the former act rapidly but primarily on the bacilli present in the nodules and other lesions whereas the sulphones act more slowly and especially on the organisms in the softened tissues. He therefore recommended combined treatment with these two drugs, and Tolentino (259) published figures which showed that the combination gave better clinical and bacteriological improvement than did either drug when given singly.

Further examples of two drugs being given with the hope of obtaining an additive effect were TB1/698 with DDS or isoniazid, and streptomycin with DDS. Davison (65) noted that the thiosemicarbazone produced a favourable clinical response without a corresponding bacteriological improvement, so he first added DDS to the TB1/698 treatment and then isoniazid, but as DDS alone gave equally good or better results the combined therapy was discontinued. Lowe (172) considered that the deterioration which occurred after prolonged treatment with TB1/698 was due to the organisms becoming resistant, and so he also tried using DDS in combination with this drug. The toxic effects of the drugs, however, were increased and the results were no better than with DDS

alone. Ramos e Silva and Peryassú (215) treated tuberculoid leprosy with streptomycin and added DDS to the treatment because they thought that the bacilli liberated from the cells through the action of streptomycin might become disseminated and form fresh lesions. The lesions disappeared from all but one of their 27 patients, but the authors attributed this mainly to the high activity of the streptomycin. Sinha (245), who considered that streptomycin produced a very rapid response, claimed that a similar response could be obtained with smaller doses if DDS was given at the same time. Lundin (181) tried the effects of administering 0.25 g of cycloserine (Seromycin) with DDS every 12 hours to 5 patients but he observed no enhanced effect.

The success of using combined therapy to delay the emergence of isoniazid-resistant strains of *M. tuberculosis* encouraged the hope that in leprosy markedly superior results would be obtained from isoniazid if it were given in combination with another drug. Although the investigations most likely to give an answer are controlled trials, such as those discussed on page 25, there are a number of uncontrolled observations which suggest that this hope is not being fulfilled. Chaussinand *et al.* (39) gave 0.15–0.3 g of isoniazid and 0.1 g of DDS daily to patients who could not tolerate larger doses of DDS and, from the results, concluded that the importance of isoniazid in leprosy lies in its use in combination with the sulphones, but that this is due to its favourable effect on the general condition and appetite of the patient rather than to a specific effect on *M. leprae*. Garrett (112) treated a group of patients, who had responded poorly to DDS, with isoniazid at a dose of 6 mg/kg daily and DDS at 0.3–0.4 g twice weekly. He decided that, although more rapid bacteriological and clinical improvement can be expected from the combined therapy than from DDS alone, the difference is not marked, and therefore the extra expense of the combination is not warranted for routine treatment. At the end of 2 years of treatment, Jopling and Ridley (147) similarly found no advantage in adding isoniazid to sulphone therapy. Therefore, if the development of drug-resistant variants is the factor that makes isoniazid so much less dramatic in the treatment of leprosy than in tuberculosis, DDS does not appear to affect their emergence. In contrast to these observations, Dharmendra and Chatterjee (74) found from the results of 24 cases that treatment with isoniazid and DDS in combination is definitely superior to giving either drug singly, and they therefore consider that DDS may delay the emergence of isoniazid-resistant variants.

Davidson (63) noted that when patients who were undergoing treatment with isoniazid were given sulphetrone at the same time, their condition deteriorated but it improved again when the treatment with the sulphone was discontinued. The improvement noted with isoniazid alone, however, was only temporary and it produced its maximum effect between six and nine months. Results which the author thought to be more promising were obtained by giving the combination of 0.2 g of TB1/698 with 0.3 g of isoniazid daily; this combination was given to 47 patients who had failed to improve with other drugs, and 11 of these became bacteriologically negative, 22 showed improvement, 10 remained static and only 2 deteriorated.

H. Controlled clinical trials

Few, if any, of the claims for antileprotic activity so far referred to in this review can be assessed objectively because in very few instances have the effects of treatment with a drug been compared with the results in similar cases of leprosy, treated under the same conditions, but receiving a placebo in place of the drug.

Some carefully controlled and extensive trials of the antitubercular drugs which are used for the treatment of leprosy have been organized by the Leonard Wood Memorial (American Leprosy Foundation). These trials are being conducted at leprosaria in the Philippines, South Africa and Japan, and only patients with lepromatous leprosy, who are bacteriologically positive and lepromin-negative, are accepted for the trials. The patients are divided into groups with similar age and sex distribution. Before and during treatment, each patient is examined clinically by a consultant who is unaware of the type of treatment, and all the bacteriological examinations of smears taken from at least five sites, and of biopsy material are made at one centre.

The first of these trials was designed 1) to compare the effects of treatment with diasone (1 g daily) with those of DDS (0.2 g daily), 2) to determine whether DDS is more effective than dihydrostreptomycin (1 g thrice weekly), and 3) to compare the effects of this dose of dihydrostreptomycin when given alone with the effects produced by giving it with diasone (1 g daily) or PAS (15 g daily). A control group was also included, and although at two of the centres these patients received a placebo by injection, at the third centre this group received PAS. At two of the centres the observation period was 48 weeks but at the third it was 32 weeks; in all, 852 patients completed these periods of treatment.

The results have been extensively analysed (79) and the conclusions reached were that both of the sulphones and dihydrostreptomycin proved to be of definite and approximately equal value. The combination of diasone and dihydrostreptomycin was no better than either of the drugs separately, and PAS did not augment the effect of the streptomycin. The proportion of patients showing clinical improvement, however, was not high; at the centre where treatment was for 32 weeks it was one-fifth of the group, and at the two centres where treatment was for 48 weeks it was one-quarter and three-tenths respectively. At the centre where the patients of the control groups received a placebo, only a few of these controls showed improvement, but at the centre where PAS was given, one-sixth of those treated with this drug showed some improvement, thus indicating that, in spite of reports to the contrary, PAS has some effect in leprosy.

At the centre where treatment was for only 32 weeks, bacteriological improvement occurred in all the treated groups, but this improvement was not very different from that observed in the control group which received the placebo; the groups receiving the drugs singly at this centre showed most bacteriological improvement. Of the centres where the treatment was for 48 weeks, at one all the treated groups showed equal bacteriological improvement, but at the other centre the group which received streptomycin showed most bacteriological improvement. At only one of these centres, however, was there agreement be-

tween the bacteriological changes and the clinical improvement; bacteriological improvement was as frequently seen in patients showing no clinical improvement as in those showing improvement.

The organizers of this trial considered that it had proved to be too complicated, and so the second and third trials were simplified. In the second trial 499 patients were divided into groups and treated with either diasone, isoniazid plus diasone, dihydrostreptomycin, isoniazid or dihydrostreptomycin plus isoniazid. Only a preliminary report of this trial is available (80) but equal results were claimed for all the drug regimes. In the third trial, the value of BCG vaccination in combination with drug therapy is being assessed.

The failure of these trials to show any difference between the effects of treatment with the sulphones, dihydrostreptomycin or isoniazid is perhaps surprising, for the impression gained from other reports is that the sulphones are the most effective of these antitubercular drugs for the treatment of leprosy. It must be remembered, however, that the periods of treatment in these trials were relatively short. The failure of combined therapy to show any increased beneficial effect is probably not unexpected, for in tuberculosis there is no evidence that combined therapy has any advantage other than that of delaying the development of resistant variants; the duration of these trials was probably too short for a deterioration in the clinical state to occur from this cause. The most surprising fact emerging from these trials is the low proportion of patients on any regime who showed a definite improvement within the periods of observation. This raises the pertinent question as to the type of improvement that can be expected from chemotherapy.

Lepromatous leprosy has these two unique characteristics: there are large numbers of organisms in the lesions and there is a small tissue response to their presence except during a lepra reaction (page 4). There is evidence that dead leprosy bacilli injected intradermally remain at the site for many months (176, 272); heat-killed *M. lepraemurium* inoculated into mice can readily be demonstrated in the tissues some four months later (84). Accordingly, even if a drug immediately killed every one of the myriads of organisms present in a lepromatous patient, no marked improvement could be expected in the bacteriological state or probably in the clinical condition of the patient for many months. It therefore seems possible that, apart from a drug which could eliminate relapses no drug could act significantly more rapidly or more effectively than the present ones. If it is true that the granular forms of the bacilli which occur during treatment are the result of an unfavourable environment (46), then it is possible that bacteriostatic drugs, *e.g.*, the sulphones, may appear bacteriologically to act more rapidly than bactericidal drugs, *e.g.*, isoniazid; in the unfavourable conditions produced by the bacteriostatic drug the organisms would change into the granular forms whereas with a bactericidal drug, the organisms would apparently be unaffected as they would be killed before they could undergo this change. The controlled trials described above, however, give no indication that the sulphones act more rapidly than isoniazid. Uncontrolled observations by other workers are conflicting on this point.

IV. CORTISONE AND ACTH

Although there is no evidence that cortisone and ACTH have any direct action on *M. leprae*, they have proved to be a valuable adjuvant to antileprotic drugs in the treatment of leprosy. Roche *et al.* (221) reported that 40–80 mg of ACTH a day controlled the lepra reaction, and Lowe (167) found that 0.1 g of ACTH or cortisone every 12 hours for 2 or 3 days, followed by smaller doses for 2 days, controlled the acute manifestations of leprosy or of sulphone sensitivity (fever, dermatitis and hepatitis), although he considered that repeated use may aggravate the underlying disease. Del Pozo *et al.* (67), however, do not agree that prolonged treatment is harmful; they treated 9 lepromatous patients, who previously could not tolerate treatment with the sulphones, with 24–100 mg of cortisone daily for one year, and claim very favourable effects on the resolution of the disease and the toleration of DDS; there were no undesirable side-effects. A number of workers (142) have confirmed the beneficial effects of these drugs, and their place in the treatment of leprosy has been reviewed by Jopling and Cochrane (146).

Pregnenolone and colchicoside have also been used in the treatment of leprosy; it is claimed that they have similar but less spectacular effects than cortisone (100, 146, 211). Samuel (229), however, found colchicoside to be usually ineffective in controlling lepra reactions.

V. BCG IN LEPROSY

Although vaccination with BCG is not strictly within the orbit of chemotherapy, its use in the control of leprosy may have such an important bearing on the future evolution of leprosy that no review of the disease can ignore the investigations that are being made to evaluate its use. The early history of these investigations was reviewed by Wade (267).

Leprosy patients are divided into two main groups by the lepromin test (page 4). Those who have the malignant lepromatous form of the disease are lepromin-negative, whereas those with the more benign tuberculoid form are lepromin-positive; patients who are affected with the dimorphous forms (page 5) may give variable reactions. Persons who react to tuberculin are also usually lepromin-positive (3, 54, 93, 115, 178).

From these facts two questions arise. Firstly, will BCG vaccination of lepromatous patients render them lepromin-positive, and will the form of their disease then change? Secondly, can BCG vaccination of persons exposed to leprosy infections render them lepromin-positive, and if they subsequently develop the disease will it be the more benign form? These questions assume that there is an immunological relationship between tuberculosis and leprosy but ignore the suggestion that the lepromin-test is merely a foreign-body reaction (page 4). Many extensive investigations designed to answer these questions have been made, especially by workers in Brazil, but the final answers are still unknown.

Lowe and McNulty (177), in attempting to find the answer to the effects of BCG vaccination on patients with lepromatous leprosy, vaccinated 104 cases

undergoing treatment with DDS or TB1/698 and, although 84.6% became tuberculin-positive, only 11.5% became definitely lepromin-positive. The patients were observed for one year and the authors' tentative conclusions were that the lepromin conversion was only temporary and did not affect the prognosis; later observations, however, (given as an addendum to their paper) showed that conversions could occur a year or more after vaccination. In a similar study on active and arrested cases, Schujman (238) was able to induce a lepromin conversion rate as high as 50%; the cases, however, gave only the late Mitsuda reaction, never the early Fernandez reaction, and he concluded that the positive reaction in these lepromatous patients was of no protective value.

The controlled attempts to determine the effects of BCG vaccination as a protection against leprosy have mainly been confined to observations with children born of leprosy patients. Of the many children vaccinated, the majority have become lepromin-positive. The proportion, however, in which the conversion can be attributed solely to the vaccination is probably low. Guinto *et al.* (122), who analysed the conversion rate in groups of children who had previously been vaccinated with BCG, tested with lepromin or un-vaccinated and un-tested, concluded that only 33.4% of the children became lepromin-positive through the vaccination; a further 7.2% became positive through the antigenic effect of the previous testing with lepromin and 11.5% because of natural causes. Exposure to the tubercle bacillus was not the only natural cause because only 2.3% of the children were tuberculin-positive.

Information on the effects that the lepromin conversion by BCG has on the type of leprosy that may subsequently develop is still scanty. de Souza Campos (251), in an observation made on 4987 contacts, noted that of the 1658 vaccinated contacts, 0.6% developed leprosy and all were of the tuberculoid type, whereas during the same observation period 5.6% of the 3329 non-vaccinated contacts developed leprosy and 26.3% of the cases were of the lepromatous form.

VI. RAT LEPROSY

Rat leprosy is a natural disease of rats which can be readily transmitted experimentally to rats and mice. It closely resembles the human disease in that the causal organism, *M. lepraemurium*, has not been cultured with certainty and the infection is essentially intracellular (97). Chaussinand (38), however, considers that it is a mistake to apply the term of *leprae* to organisms of this disease because according to his studies, *M. lepraemurium* is antigenically more closely related to *M. tuberculosis* and Johne's bacillus than to *M. leprae*. He also considers the disease in rats to be more closely related to tuberculosis and to Johne's disease than to leprosy. Tanimura and Nishimura (256) also emphasize that, although both *M. leprae* and *M. lepraemurium* produce nodular lesions of epithelioid-like cells and giant cells loaded with bacilli in skin and many other organs of the body and although the site of predilection of both organisms is the tissues of mesodermal origin, *M. lepraemurium* has no affinity for nerves and does not show the selective tendency of *M. leprae* to produce lesions in the nasal mucosa, testes and adrenal glands. The absence of nerve involvement was also noted by Sellards and Pinkerton (242).

Although there are these important differences between the organisms, the evidence available indicates that of all the mycobacteria, *M. lepraemurium* has the closest relationship to *M. leprae* and would therefore be expected to be the most valuable for selecting chemotherapeutic drugs for trial in leprosy. However, although experiments were reported in 1929 by Markianos (183, 184) and since then by many other workers, there is still doubt as to whether the results are a more valuable guide than those obtained in experimental tuberculosis.

Rats, mice and hamsters have been used in these experiments and the disease has been transmitted by injecting homogenized tissue containing the bacilli by various routes. When injected subcutaneously, a leproma is produced which is purely local with little tendency to metastasize, and the effects of drugs on its development have been used by some workers to evaluate their activity (28, 40, 55). This method, however, has the disadvantages that mice are not very susceptible when injected by this route and in rats the observation period required is long, ranging from five to eighteen months (29).

After intraperitoneal injection, multiple lepromata develop in the peritoneum, mesentery, omentum and the pelvic fatty pads within two to three months; the liver and spleen also become involved. Grunberg and Schnitzer (120) infected mice by this route and assessed the activity of antileprotic drugs by comparing the number of organisms present in the lepromata of treated and untreated animals, 29 to 56 days after infection. Chang (29) also used this route of infection in mice, but he assessed the efficacy of the drugs by comparing the weights of the omentum and pelvic fatty pads and the number of organisms in smears of these organs and of the portal, paravertebral and tracheobronchial lymph glands, spleen, liver and lungs of treated and untreated animals 3 months after infection.

When the homogenized lepromatous material is injected intravenously a widely disseminating infection is produced, and chemotherapeutic activity may be measured by comparing either the number of organisms in various organs or the survival times of treated and untreated animals. A most extensive study of this method, in which activity was measured by the effects on the multiplication of the organisms, was made by Hobby *et al.* (138). They concluded that a rapid appraisal of activity could be made by counting the number of organisms present in spleen homogenates at various intervals after infection. The effects of continuous, intermittent or delayed treatment were readily assessed, and the periods of observation ranged from 14 to 78 days. Barnett and Bushby (6) found assessment by the comparison of the average survival time more convenient, especially for demonstrating the development of drug-resistant variants of *M. lepraemurium*.

Levaditi and Chaigneau-Erhard (161) used the intracerebral route of inoculation and assessed the effects of treatment by the extent of the disease in the meninges. The method involved a histological assessment and, although the duration of the experiment was only 77 days, it does not appear to possess any advantage over methods which do not necessitate the preparation of sections. Goulding *et al.* (117) showed that, after the injection of organisms intracorneally in mice, a lesion slowly developed which after about six months involved the whole cornea.

Although no drug has yet been found to eradicate the disease completely, several have been shown to have suppressive action. Grunberg and Schnitzer (120) have reviewed the results of some of the experiments made prior to the advent of the sulphones. In these experiments chaulmoogra oil and some related drugs were shown to have a favourable effect on the disease.

Since then almost all the antibacterial drugs have been assessed for activity in this infection by one or more groups of investigators. Table 1 summarizes the results of experiments for which data were available to the writer.[1]

The activity shown by most of the drugs has varied with different investigators, but the only drugs to show activity, apart from chaulmoogra oil and its related drugs, are those which are active against the tubercle bacillus, and the most active of these are isoniazid and streptomycin. In experiments of relatively short duration, both of these drugs, and especially isoniazid, have almost completely suppressed the infection. Longer experiments, however, show that this suppression is only temporary and in the case of isoniazid, the relapse is undoubtedly due to the organism becoming resistant to the drug; Bushby and Barnett (20) showed that the organisms were resistant by passaging the strain from the relapsed mice into fresh mice which were then not protected, even temporarily, by the drug. This resistance develops very rapidly; in experiments in which treatment with isoniazid was delayed for 66 days until the infection was well established, Barnett and Bushby (6) found that isoniazid-resistant variants were present in the animals after 30 days of treatment. The resistance has since been confirmed by Chaussinand *et al.* (42).

The relapses which occur with streptomycin are apparently not due to the emergence of streptomycin-resistant variants, because when the organisms are inoculated into fresh mice, streptomycin still gives the same primary suppressive effect (6, 206); it was not till the fourth passage through streptomycin-treated mice that the suppressive action of the drug was lost (6).

Compared with isoniazid and streptomycin the activity shown by the other antitubercular drugs has usually been of a relatively low order even at the maximum tolerated doses. The relatively low activity of the sulphones, found by most investigators, has been most striking; a notable exception was reported by Levaditi and Chaigneau-Erhard (161), who found that when the infection was introduced intracerebrally, DDS was superior to streptomycin.

There are several reports of experiments in which two or more drugs were given together. Although Ichihara *et al.* (139) found isoniazid and streptomycin to be more active together than either drug singly, Hobby and Donikian (137) found that when these two drugs were given together there was no significant increase in the suppressive effect on the multiplication of organisms in mice. Yasumoto and Hiramoto (271) found that the combinations of TB1/698 with streptomycin and PAS with streptomycin were superior to the drugs given singly, but TB1/698 and PAS given together were antagonistic. Chang (33) found that, when sub-maximal therapeutic doses of DDS, isoniazid or streptomycin were given in combination, the suppressive effects were greater than when the individ-

[1] Much other work has been done in Japan and is published in Japanese.

TABLE 1

Summary of chemotherapeutic experiments with M. lepraemurium

Treatment			Duration of Experiment	Animal	Method of Assessment	Result	Reference
Drug	Dose	Route					
	g/kg		*days*				
Chaulmoogra oil or derivative	0.3	S.C.	90	rat	leproma	slightly active	257
	0.75	P.O.	180	rat	leproma	inactive	41
Dapsone	2.5	P.O.	28	mouse	organisms	inactive	120
	0.1	S.C.	77	mouse	organisms	active	161
	0.3	P.O.	340	rat	survival	active	186
	0.125	P.O.	180	mouse	organisms	slightly active	5
	0.25	P.O.	90	mouse	organisms	active	30
	0.2	P.O.	35	mouse	organisms	inactive	56
	1.0	I.P.	30	mouse	organisms	slightly active	138
	0.2	P.O.	90	mouse	organisms	active	32
	0.04	P.O.	180	rat	leproma	inactive	41
Promin	0.5	S.C.	56	mouse	organisms	active	120
	4.0	S.C.	137	rat	leproma	active	55
	0.25	P.O.	540	rat	survival	active	28
	2.0	S.C.	115–268	rat	leproma	inactive	257
	1.0	P.O.	42	mouse	organisms	inactive	138
Diasone	0.5	P.O.	28	mouse	organisms	inactive	120
	0.5	P.O.	300	rat	survival	inactive	28
	0.75	P.O.	90	mouse	organisms	slightly active	30
	0.075	P.O.	180	rat	leproma	inactive	41
Sulphetrone	5.0	P.O.	180	mouse	organisms	slightly active	5
	2.0	P.O.	35	mouse	organisms	inactive	56
	0.37	P.O.	180	rat	leproma	inactive	41
Sulphanilamide	2.5	P.O.	365	mouse and rat	leproma	active	155
Succisulphone	0.1	S.C.	180	rat	leproma	inactive	41
4:4′-diaminodiphenyl sulphoxide	39.0	P.O.	240	rat	leproma	slightly active	123
4:4′-diaminodiphenyl sulphide	40.0	P.O.	360	rat	leproma	inactive	123
4:4′-diaminodiphenylamine	15.0	P.O.	420	rat	leproma	slightly active	123
4:4′-diaminodibenzophenone	35.0	P.O.	200	rat	leproma	inactive	123
Hydnosulphone	5.0	P.O.	200	mouse	survival	inactive	19
Thiosemicarbazone TB1/698	0.2	P.O.	180	rat	leproma	inactive	41
	0.5	P.O.	28	mouse	organisms	active	120
	*	"	*	rat	leproma	slightly active	271
	0.5	P.O.	180	mouse	organisms	active	28
	0.4	P.O.	35	mouse	organisms	inactive	56
	*	*	*	rat	leproma	inactive	255
	0.01	S.C.	312	rat	leproma	inactive	257
	2.0	P.O.	90	mouse	organisms	inactive	32

TABLE 1—*Continued*

Treatment			Duration of Experiment	Animal	Method of Assessment	Result	Reference
Drug	Dose	Route					
	g/kg		*days*				
β-pyridine-aldehyde thiosemicarbazone	0.05	I.M.	171	rat	leproma	active	162
PAS	2.5	P.O.	56	mouse	organisms	active	120
	1.0	S.C.	77	mouse	organisms	slightly active	161
	*	*	*	rat	leproma	inactive	271
	5.0	P.O.	35	mouse	organisms	inactive	56
	*	*	*	rat	leproma	inactive	255
	1.2	P.O.	90	mouse	organisms	inactive	32
	4.0	P.O.	180	rat	leproma	inactive	41
Nicotinamide	0.5	P.O. and S.C.	150	rat	organisms	active	43
	25.0	P.O.	90	mouse	organisms	active	31
Isoniazid	0.1	P.O.	180	mouse	organisms	active	5
	0.025	S.C.	127	rat	leproma	active	163
	0.02	P.O.	371	rat	survival	active	57, 58
	0.07	P.O.	90	mouse	organisms	active	30
	0.02	P.O.	35	mouse	organisms	active	56
	*	*	*	mouse	leproma	active	139
	0.01	P.O.	200	rat	leproma	active	41
	0.125	P.O.	29	mouse	organisms	active	121
	0.2	P.O.	42	mouse	organisms	active	138
Pyrasinamide	25.0	P.O.	90	mouse	organisms	active	31
Cepharanthin	*	*	*	rat	leproma	inactive	255
	0.001	P.O.	346	rat	leproma	inactive	257
B.283	1.0	P.O.	90	mouse	organisms	inactive	32
Diphenylthioureas	4.0	P.O.	90	mouse	organisms	inactive	32
Diethyldithiol-*iso*-phthalate	5.0	P.O.	100	mouse	cornea	active	202
Streptomycin	0.05	S.C.	56	mouse	organisms	inactive	120
	0.05	S.C.	77	mouse	organisms	active	161
	0.05	S.C.	60–210	rat	leproma	inactive	40
	0.1	S.C.	180	mouse	organisms	active	5
	*	*	*	rat	leproma	slightly active	271
	0.15	S.C.	90	mouse	organisms	active	30
	*	*	*	rat	leproma	active	204
	*	*	*	rat	leproma	active	255
	0.1	S.C.	42	mouse	organisms	active	138
	0.15	S.C.	90	mouse	organisms	active	32
Penicillin	0.002	I.M.	184	rat	leproma	inactive	77
	0.005	S.C.	150	rat	survival	inactive	28
	0.0012	S.C.	90	mouse	organisms	inactive	32
Tetracyclines	*	*	*	rat	leproma	inactive	204
	0.25	P.O.	30	mouse	organisms	inactive	138
	0.6	P.O.	90	mouse	organisms	inactive	32
Chloramphenicol	0.6	P.O.	90	mouse	organisms	inactive	32
Erythromycin	1.0	P.O.	90	mouse	organisms	inactive	32
Acidomycin	*	*	*	rat	leproma	inactive	204
Viomycin	0.2	S.C.	29	mouse	organisms	active	138
Carbomycin	1.0	P.O.	29	mouse	organisms	inactive	138
Cycloserine	*	P.O.	90	mouse	organisms	active	34

* Data not available.

S.C. = subcutaneous; P.O. = by mouth; I.P. = intraperitoneal; I.M. = intramuscular.

ual drugs in these sub-maximal doses were given alone; the triple combination gave the greatest increased effect, the combination of isoniazid and DDS was next, followed by streptomycin and isoniazid and then streptomycin and DDS. Nishimura and Masuda (205, 206) considered from the results which they obtained in rats that combined treatment with isoniazid and streptomycin was not markedly superior to that of isoniazid alone, and when PAS, TB1/698 or promin was given with isoniazid, the results were a little inferior to those from giving isoniazid alone. These workers were also unable to demonstrate any increased activity by giving PAS with streptomycin. Takayama and Yasumoto (255) from their experiments in rats considered the effects of streptomycin and promin together to be inferior to that of either drug separately, and also that the activity of streptomycin or promin was antagonized by PAS, TB1/698 or cepharanthin. Naguib and Robson (202), however, using the corneal method of assessment, found the suppressive effect of isoniazid and the ethyl mercaptan derivative, diethyldithiol*iso*phthalate, most striking, the combination being superior to either drug alone.

In none of these experiments with combined therapy were the effects on the emergence of isoniazid-resistant variants directly examined. Barnett and Bushby (7) found that with the following daily doses neither streptomycin, 2 mg, DDS, 2.5 mg, TB1/698, 10 mg, pyrazinamide, 5 mg, nor PAS, 50 mg, prevented the development of isoniazid resistance, although streptomycin and DDS had a definite delaying effect and TB1/698 had a slight effect. These delaying effects were shown by an increase in the average survival time of the animals on the combined therapy, the times, expressed as ratios to that of the animals receiving isoniazid alone, being 1.75:1, 1.5:1, 1.27:1, 1.18:1 and 1.17:1 respectively. These observations were made in mice infected intravenously, and the failure to prevent the development of isoniazid resistance was shown by passaging the organisms into untreated mice which were then treated with isoniazid alone. Using the rate of growth of subcutaneous lepromas and the distribution of the bacilli in the lymph glands and viscera of rats as a measure of activity, Nishimura and Masuda (207) also found that streptomycin delays the development of isoniazid resistance.

VII. THE PROPAGATION OF *M. LEPRAE*

There can be little doubt that the successful cultivation of *M. leprae* on artificial media or in tissue cultures would represent a very important advance in the study of the chemotherapy of leprosy. As the failure to propagate this organism cannot be attributed to a lack of effort in the directions which have been so successful for other mycobacteria, Hanks and Gray (119, 127–132) have attempted more fundamental studies by making extensive investigations into the respiration and infectivity of *M. lepraemurium* in the hope that the information gained would apply to *M. leprae*.

Measurements in the Warburg apparatus and the production of formazans from tetrazolium compounds under anaerobic conditions show that *M. lepraemurium* has an endogenous respiration, indicating that although it may compete

with the host's cells it is able to respire and metabolize independently of the cell. Although this endogenous respiration varies, and when low can be restored by the addition of albumin or yeast extract to the suspending fluid, none of the many substrates which affect the respiration of other mycobacteria increases the respiration. Hanks and Gray therefore suggest that until the problem of this inhibited state of being unable to utilize these substrates is solved there is little point in continuing to endeavour to cultivate *M. lepraemurium* or *M. leprae* by the classical methods. Their studies also show that the endogenous respiration and the infectivity of *M. lepraemurium* are rapidly reduced and finally abolished by exposure to plasma, the damage being due to the lipo- and muco-proteins. A similar effect is also produced by anaerobiosis, and the more active the organism the greater is the damaging effect of these conditions.

Studies made with related mycobacteria show that these properties are not unique for this species and therefore they may be a reflection of the essentially intracellular existence of these bacteria. The species of mycobacteria appear to form a series in which the reduced oxidative response to substrates, and the increased sensitivity to anaerobiosis and extracellular inhibiting substances become more apparent as the sequence passes from the saprophytes, through the tubercle bacillus and Johne's bacillus to the leprosy bacillus. It is interesting to note that sensitivity to plasma is not restricted to *M. lepraemurium*; small inocula of freshly isolated strains of *M. tuberculosis* will not multiply in the presence of plasma other than that of their natural host (133).

At concentrations which inhibit the growth of tubercle bacilli, neither streptomycin nor isoniazid affects the endogenous respiration of *M. lepraemurium*, but this finding is not unexpected, as the tubercle bacillus is affected by these drugs only when it is utilizing energy from an exogenous source. The action of the plasma inhibitors and anaerobiosis must therefore be very different from that of these drugs, acting, it would appear, at a more fundamental level. Hanks and Gray speculate on the possibilities of their playing a part in the destruction of the bacilli *in vivo*. They think it unlikely that anaerobiosis plays a decisive role, although the possibility of decreasing the oxygen tension in leprotic lesions should not be overlooked in further studies of the chemotherapy of leprosy. They feel more confident about the natural extracellular inhibitors playing a significant part in the control of the disease, especially during the reactive phases. During these phases, not only is there an increase in extracellular fluids and probably in cell permeability, but the bacilli are more active and thus more sensitive to these inhibitors. They also suggest that increased exposure to these agents occurs during sulphone therapy, thereby agreeing with the leprologists who maintain that the action of the sulphones is primarily one of exposing bacilli to natural inhibiting substances (page 10).

Appreciation of the presence of inhibitory substances in normal plasma explains the failure of leprosy bacilli to grow in standard tissue cultures. It indicates that successful tissue culture might be accomplished by maintaining the cell in solutions free from these agents and by insuring that the cells grow in close contiguity rather than in a single plane.

VIII. CONCLUSIONS

Chemotherapy has profoundly affected the prognosis in leprosy. The outlook for the patient is no longer one of despair and of waiting for the disease to run its long course, with his only hope that his disease would eventually reach the "burnt-out" state. Most leprologists now expect to cure all early cases of the disease, and even when it is firmly established the ultimate outlook is almost invariably good.

The most unsatisfactory aspect of the present treatment is the long duration of treatment necessary to produce first a clinical cure and finally the eradication of the organisms; according to one "probability of arrest" chart, a patient with lepromatous leprosy has a 40% chance of arrest after 8 years of continuous sulphone treatment (269). This rather depressing aspect of the treatment has compelled Ross Innes (see 174) to proclaim that it is time that leprologists express their dissatisfaction with the present drugs. He maintains that the present drugs act too slowly, that bacterial negativity should be attained within six to twelve months; a more dynamic or bactericidal drug or a means of increasing the patient's resistance is needed. Lepromin cannot alter the patient's resistance, nor is there any evidence, in his opinion, that BCG is any more effective. He advocates that the only policy that should be pursued at the present time is to investigate all drugs which have action in human tuberculosis or on the tubercle bacillus.

These provocative views brought prompt responses from Lowe (174) and Wade (265). Although both these leprologists concur with the views expressed, they maintain that the present treatment is far from unsatisfactory, and Wade argues that the effectiveness of the present drugs is probably limited more by the nature of the disease than by their feeble action against the organisms. The harbouring of myriads of bacilli by the cells of the host imposes tremendous difficulties on chemotherapy, and it is conceivable that future progress in the treatment of leprosy may not come from further chemotherapy but from immunology altering the host-parasite relationship.

As for the suggestion that only drugs which are active in tuberculosis should be tried in leprosy, Wade reminds us that not only are the organisms of tuberculosis and leprosy different but that the most widely used antileprotic drugs, the sulphones, were tried in tuberculosis and discarded.

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