CHEMOTHERAPY OF LEPROSY[1,2]

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Because of the inability to grow *Mycobacterium leprae* in artificial medium or tissue culture, or, until about 10 years ago, even in experimental animals, most of the tests of drugs for anti-leprosy activity have consisted of clinical trials. With a few exceptions, the drugs tested for activity against *M. leprae* have been those whose prospects for use in tuberculosis have justified development to the stage that they could be given safely to humans. Thus, application of a drug in leprosy has usually carried with it a body of expressed facts and implicit assumptions based on studies with *M. tuberculosis* and other bacteria. The history of the sulfones is illustrative. The sulfones, first observed in 1937 to be active against bacteria (streptococci) (1–3), were noted in 1940 to be active against mycobacteria [*M. avium* (4) and *M. tuberculosis* (5)], and they were first tried in human leprosy in 1942 (6). However, confusion over the minimal effective dosage led to the conclusion that the most effective sulfone, 4,4′-diaminodiphenylsulfone (DDS, dapsone), was too toxic, and not until 1949 was it learned that DDS was active in sub-toxic dosage, roughly less than 200 mg per day, [reviewed in (7)]. Recent evidence stemming from investigation of the minimal effective dosage against *M. leprae* in mice indicates that even the standard dosage (50 to 100 mg per day) provides 50 to 100 times the minimal inhibitory concentration (8-10).

Basis for Clinical Trials

Unique features of the disease and the microorganism determine the form of the clinical trials. The clinical response is not very decisive over the period of a year or so because the general progress of the disease is slow. The organisms multiply very slowly [in mice the generation time in the logarithmic phase averages 12.5 days (11)], and after the organisms are killed chemotherapeutically they disappear from the tissues of the patient only very slowly [a half-life of about 100 days in lepromatous leprosy

[1] The survey of literature pertaining to this review was concluded in June 1968.

[2] Abbreviations used in this review are: DDS (4,4′-diaminodiphenyl sulfone), DADDS (4,4′-diacetyl-diaminodiphenyl sulfone), G6PD (glucose-6-phosphate dehydrogenase), MADDS (monoacetyldiaminodiphenyl sulfone).

(12, 13)]. Furthermore, the normally indolent clinical course may be interrupted by periods of reaction and clinical worsening that are not caused by bacillary increase. A helpful feature of the disease, however, is that the most widely involved tissue, the skin, is on the surface where it can be sampled by skin-slit smears and biopsies.

In different patients the disease can manifest a wide spectrum of clinicopathological forms, from the milder tuberculoid disease with few bacilli to the more serious lepromatous disease with many bacilli. Clinical trials are best confined to the pure lepromatous type because of the relative uniformity of the patients' tissue response and because bacilli are present in adequate numbers (14). Bactericidal effects are most conveniently observed by determinations of the proportion of bacilli that stain solidly and completely (morphological index, MI, solid ratio). This measurement is based upon the observation that in a patient being treated with DDS, *M. leprae* undergoes degenerative morphological changes (15) that resemble those of dying *M. leprae-murium* and *Escherichia coli* (16), and that the mouse infectivity of *M. leprae* is directly related to the number of solidly staining organisms rather than the total number (11). The morphological index decreases to baseline levels a few months after the institution of DDS therapy (10, 14, 15). Another procedure that reflects bacillary viability is measurement of the number of *M. leprae* in the nasal washings (10). The least ambiguous and most sensitive method of testing bacillary viability is the inoculation of mice (13).

Confusion has arisen in the past because bactericidal activity was illogically confused with disappearance of the organisms from the tissues. Several inconsistencies arose, among which was the inclusion in some clinical trials of large numbers of patients who had already been treated with DDS (17). However, even a 3-month treatment with DDS is enough to kill more than 99 per cent of the viable bacilli (13). In untreated lepromatous patients the proportion of viable *M. leprae* is usually 1 to 10 per cent, so even a bacteriostatic drug could, by preventing the replacement of the numbers of nonviable bacilli, cause the total numbers of bacilli to decrease.

The most rigorous and conclusive test of a drug is to follow the patients until bacteria can no longer be found in the tissues. Since this requires more than 5 years for many lepromatous patients, only a few reports of this kind are present in the literature, and these are confined to DDS (18–21). However, in the course of long-term studies several drugs have been observed to cause initial improvement for a year or longer, followed later by clinical deterioration and increase in numbers of bacilli (18, 22–25).

In this review most of the clinical trials referred to have been based on long-term trials or careful studies of bacterial morphology. Recent critical reviews and descriptions of current methods for the conduct of clinical trials are available (15, 26).

Basis for Tests in Mice

In normal mice *M. leprae,* when inoculated in the foot pad in a dose of 5×10^3 bacilli, multiplies up to a plateau level of about 2×10^6 in about 6 months (11, 27). Tissue changes are minimal, so microscopic enumeration of bacilli in the foot pad is required to detect the multiplication. The method that has been used longest for the study of anti-leprosy drugs involves administration of drug from the day of infection to the end of the experiment (continuous method) (28). A recent modification (kinetic method) (29) involves the administration of drug for a period of 2 to 3 months beginning early in the logarithmic phase; pure bacteriostatic activity is revealed by a delay in the appearance of bacteria equal to the period that the drug was present in effective concentrations, and bactericidal activity is revealed by a more prolonged delay.

Sulfones

DDS was the first sulfone found to be active against non-acid fast bacteria (1–3) and mycobacteria (4, 5). However, when it was given to patients in the 1 to 2 g daily dosages thought necessary on the basis of mouse experiments with streptococci and pneumococci, it produced severe anemia, methemoglobinemia, and cerebral irritation (30, 31). As a result, a series of less toxic sulfones were introduced. The ones most widely used in leprosy were di-amino substitution products of DDS: Glucosulfone (Promin,[3] the didextrose sodium sulfonate), which was the first sulfone tried, and found effective, in leprosy (6); sulfoxone (Diasone, the disodium formaldehyde sulfoxylate derivative); and solapsone (Sulphetrone, the tetrasodium 3-phenyl-1, 3-disulfopropyl derivative). When administerd orally these sulfones release DDS by hydrolytic cleavate in the gut (32, 33, 47), and their oral activity in mice against tuberculosis (32) and against streptococcal infections (33) is proportional to the blood levels of DDS that they produce.

Glucosulfone and solapsone were usually administered parenterally; they contained 0.2 to 0.3 per cent of DDS as impurity (7, 33). The antistreptococcal activity *in vitro* of these and of two other sulfones was proportional to their content of DDS (33). Injection of 1 g a day would provide more DDS than what is now thought to be the minimal effective dosage (8–10). Another possibility is that they owed their activity to monosubstituted derivatives (34). The case of glucosulfone has been examined in this regard. In the body glucosulfone was found to be converted largely to the mono-N-glucoside; however, this compound (as well as the di-N-gluco-

[3] Use of trade names is for identification only and does not constitute endorsement by the Health Services and Mental Health Administration or by the U.S. Department of Health, Education and Welfare.

side and purified glucosulfone) was distinctly less active *in vitro* against *E. coli* than DDS (35).

Activity of DDS against M. leprae.—DDS is now widely accepted as the drug of choice in leprosy and is used throughout the world, and with considerable safety even under minimal medical supervision. It is usually given orally in a dosage of 50 to 100 mg per day, but is sometimes given by parenteral injection in larger, twice-weekly doses. Long-term follow-ups have shown that with regular intake of the drug nearly all patients eventually convert to negativity, although they may require more than 5 years to do so (18–21). Lowe's data (18, 19) indicate that at least 99 per cent of the patients respond in this manner. If even 1 per cent of lepromatous patients failed to respond at all, they would have accumulated in great numbers in leprosaria and clinics by now. Perhaps 0.1 per cent of patients, presumably after an initial favorable response, eventually develop sulfone-resistant bacilli (see below). A host of reports in the literature describes the usual decrease in numbers of *M. leprae* in skin smears and improvement in clinical condition in the first year or two of DDS therapy.

The activity of DDS in lepromatous patients has also been shown by the reduction in numbers of solidly staining *M. leprae* in skin smears (10, 13, 15, 36, 37), reduction in numbers of *M. leprae* in nasal washings (10, 13), and loss of infectivity for mice (13). In dosages ascending in a few weeks to 50 mg per day, the bactericidal effects of DDS are distinct by 60 days, and less than 0.1 per cent of original infectivity is left by 100 days (13).

The lowest dosage of DDS reported for clinical trials appears to be 50 mg twice weekly; this dosage produced the same decrease in solidly staining *M. leprae* that had been seen with standard dosages (37). Another report states that 50 or 100 mg DDS twice weekly gives good clinical and bacteriological response (38).

Clinical trial of a repository sulfone, 4,4′diacetyldiaminodiphenyl-sulfone (DADDS), was recently reported (10). DADDS, though insoluble in its vehicle or in the body tissues, releases DDS (or the monoacetylated derivative) through the action of tissue enzymes. It was given intramuscularly in a dose of 225 mg every 77 days. The patients' urinary excretion data indicated steady release of DDS at the average rate of 2.4 mg per day. The patients treated with DADDS responded as rapidly as those in a control group treated with standard (100 mg per day) dosages of DDS. In mice, DADDS and a series of other discrete repository sulfones, when administered every 2 months, suppressed growth of *M. leprae* (9).

In mice, activity against *M. leprae* has been shown repeatedly at dosages of 0.01 per cent in the diet or higher by the continuous method (28, 39–46). The minimal effective dosage for isolates from previously untreated patients is 0.0001 per cent or lower (8, 9, 44–46). The effect noted by this method is complete suppression of multiplication. The same oral dosage of DDS caused 50 per cent suppression of parasitemia due to *Plasmo-*

dium berghei in mice (47); when given by injection, about three times as much was required to produce the same effect (48). In Group A streptococcus infections in mice, 0.00625 per cent DDS in the diet was able to delay death distinctly (33).

By the kinetic method, DDS is bactericidal even at the minimal effective dosage, 0.0001 per cent in the diet. With low concentrations the onset of bacteriostasis is delayed several weeks, but after growth is stopped bactericidal action seems to proceed at the same rate as at higher dosages. The fraction of surviving bacteria falls to the range 6 to 0.01 per cent in 2 to 3 months of administration. After only 1 month of administration there is pure bacteriostasis without bactericide (49).

The dosages of DDS in mice are correlated with dosages in humans through the following considerations. In both species DDS is absorbed completely from the gut and well distributed throughout the body. In man the standard dosage of 100 mg per day produces an average of 2 μg "free" DDS per g of blood or nonhepatic tissue. In mice a diet containing 0.01 per cent DDS produces about the same blood and tissue concentration. Lower intakes proportionately lower blood levels in the range measurable with the Bratton-Marshall procedure (8, 44). Extrapolation to 0.0001 per cent, the minimal effective dosage in the mouse, indicates 0.02 μg per ml to be the minimal inhibitory concentration; 0.00001 per cent, which is usually not effective in mice, would produce a level of 0.002 μg per ml. The patients receiving DADDS were estimated to have 0.06 μg DDS per ml. The estimated minimal inhibitory concentration, 0.02 μg per ml, is very much lower than that reported for other mycobacteria growing *in vitro* (50).

Studies of other sulfones in mice are limited. One report states that solapsone (43) is completely suppressive of *M. leprae* when administered subcutaneously at a level of 2.5 mg per kg. This would put the minimal effective dosage of solapsone nearly as low as that of DDS on a molar basis, an unexpected result since solapsone was considerably less effective than DDS against *M. tuberculosis* (32) and Group A streptococci (33), and the activity it did exert was accounted for by the DDS it released.

The di-formyl derivative of DDS has been tested against *M. leprae* by the kinetic method by oral administration at a single level, and has been found at least as bactericidal as DDS (49).

Toxicity of DDS.—Early studies in leprosy patients in Nigeria (7) led to the conclusion that the maximum well-tolerated daily dosage was 200 mg; higher dosages regularly caused anemia with methemoglobinemia and Heinz bodies. Psychosis sometimes occurred. More recently, the toxicity of DDS has come under active investigation again. With intakes of 100 to 200 mg DDS a day (51), and 25 to 100 mg a day (52) the survival of ^{51}Cr-labelled red cells is shortened, even though anemia may not appear with lower dosages. Studies with ^{51}Fe-labelled red cells in a patient receiving sulfoxone (and who was consequently absorbing DDS from the gut) showed that the hemolysis occurred in aged cells (those more than 50 days old) (53). It

was suggested that the frequent anemia that occurs in the first few weeks of sulfone therapy represents loss of aged cells, which contain lower amounts of glucose-6-phosphate dehydrogenase (G6PD) and glutathione reductase, and that the "recovery" when patients continue on sulfones represents compensation of the marrow to a shorter red cell age span. DDS-induced anemia is more severe in persons with G6PD deficiency, a genetic condition found in 10 per cent of American negroes. Daily dosages of 25 to 50 mg, however, even when given with weekly doses of 300 mg chloroquine and 45 mg primaquine, caused only a transient anemia in G6PD-deficient negroes (54). The early studies of the maximal tolerated dose of DDS in leprosy (7) were carried out in populations in which G6PD deficiency is common (55). Studies of the toxicity of DDS and its use as a falciparum malaria-suppressive in areas with frequent resistance to other drugs have been reviewed (56).

On dosages approximating 100 mg DDS a day, dermatitis was observed in about 2 per cent of patients in Africa (20); it is apparently less frequent elsewhere. Hepatitis occurs less often. Patients with dermatitis can usually be desensitized with gradually increasing daily injections of sulfetrone (20), and can then resume oral or parenteral DDS.

Resistance of M. leprae *to DDS.*—Early reports suggesting such resistance were confusing because clinical relapse was said to respond to further treatment and the so-called "bacteriological relapse" sometimes cleared in the absence of treatment. However, a more recent study (40, 57) of poorly responding patients in a leprosarium in Malaya left no doubt that sulfone resistance exists. Organisms were isolated in mice and shown to be resistant to DDS, and the response in the patient (decrease in morphological index) to DDS given by injection was shown to correlate with the level of resistance of the isolates in mice. The proportion of resistant cases that were found was estimated to represent about one for each thousand lepromatous patients entering treatment.

Patients with resistant organisms have also been observed in England (58) and the United States (59). Of the ten patients in the latter study, seven had begun treatment with glucosulfone which presumably would have provided only low and frequently interrupted levels of DDS; in several patients treatment had been erratic. The patients so far shown to have resistant organisms had been treated for 11 to 20 years. The resistant isolates have not had reduced capability to multiply in mice. They have come from infections acquired throughout the world (46, 59). Resistance of *M. leprae* to DDS and other drugs has been recently reviewed (46).

Metabolism and excretion of DDS and DADDS.—Orally administered DDS is absorbed almost completely from the gut (60) and distributed evenly throughout the body with only moderate excess in liver and kidney (39, 61). Studies by one group (62–64) using ^{35}S-labelled DDS in a single dose led to the conclusion that DDS concentrated in diseased skin, but the time required to achieve equilibration between blood and tissue was not

taken into account. Other studies using chemical (7, 60) and radioisotopic technics (65) have not found the excess. With daily dosage the blood concentrations reach maximal levels in a few days; 100 mg daily in an adult produces an average of 2 μg per ml blood (7, 10) of "free" (ethyl acetate- or benzene-extractible DDS), and lower dosages proportionately less (7). About 50 per cent of the total sulfone in serum is bound to proteins (34). Nearly all measurements have been made by modifications of the Bratton-Marshall procedure. Extraction at neutral or alkaline reaction into organic solvents, followed by re-extraction into the usually 1 *N* HCl in which the color is developed, allows determination of DDS in the presence of the water-soluble di-substituted derivatives, such as sulfoxone, glucosulfone, and solapsone, and probably also certain of the monosubstituted derivatives, such as the glucuronide.

Urinary excretion accounts for 70 to 80 per cent of the intake (7, 10, 60, 66, 67). On average, 22 per cent of the sulfone in urine is extractible into methyl isobutyl ketone at neutrality, but 38 per cent became extractible after hydrolysis in 1 *N* NCl at room temperature, and 40 per cent after hydrolysis at 96° (67). How well the partition into the first two fractions represents renal excretion is not clear, since nonenzymatic glucuronide formation in the bladder (67), as well as hydrolysis of the glucuronide in acid urine (34, 68, 69), have been suggested.

In the urine of rabbits (34, 68–70) and humans (70) given DDS, an acid-labile mono-N-glucuronide has been demonstrated by chromatography, and by electrophoresis in neutral solvents. In human urine an acid-stable metabolite was observed with identical chromatographic and electrophoretic mobilities in neutral solvents (68), but its glucuronic acid content was not demonstrated. The mono-N-sulfamate has been described in human urine (70). In the urine of humans receiving either DDS or DADDS by mouth a compound was observed with the electrophoretic mobility in 5 *N* acetic acid of the mono-acetylated derivative (MADDS) (71). Different individuals varied markedly in the relative proportion present as DDS and MADDS. In another study the proportion of sulfone present as acid-stable conjugate in the urine of patients receiving DDS by mouth or DADDS by injection also varied distinctly by individual (10). In another study (72) the urine of patients treated with DDS was shown by chromatography in neutral solvents to give three minor Ehrlich-positive spots and a major one corresponding to DDS, in addition to those seen with normal urine. The activity against *M. leprae* of the metabolites is not known, but the activity of the mono-N-glucuronide and mono-N-sulfamate against *E. coli* and *M. tuberculosis* was distinctly less than that of DDS (35). The need for definitive information in this area is great since the current moves toward lower or less frequent doses of DDS or slowly-releasing repository sulfones may uncover new problems; individual or racial differences in sulfone metabolism may have been obscured with the standard dosages of 50 to 100 mg DDS a day.

A more sensitive method for measuring DDS in plasma and urine was

recently described (73). This spectrophotofluorimetric procedure involves a double extraction and allows measurement of DDS down at least to hundredths of micrograms per ml. The Bratton-Marshall technique has a limit of about 0.2 μg per ml. By the new procedure the biological half-life of DDS in human volunteers averaged 20.8 hr. (range 19 to 26) as estimated from plasma concentrations, and 20 hr. as estimated from urinary output. With such a half-life 100 mg DDS could be given at 5-day intervals without the plasma level falling much below 0.02 μg per ml. Following single injections of 300 mg DADDS into 60 to 70 kg volunteers, the plasma levels remained above 0.02 μg per ml for 64 to 106 days.

With the kinetic method, results in mice indicate that DDS does not have a "hammer effect" on *M. leprae;* bactericidal activity did not appear until DDS had been present for more than 1 month (49). Since this point affects therapeutic regimens so much it deserves confirmation; in the meantime it seems wise to select regimens that keep DDS present without interruption.

Molecular structure and activity of sulfones.—This subject has recently been well reviewed (74). Most of the information on activity pertains to *M. tuberculosis.* In general, for high activity the sulfone must contain *p*-aminophenyl groups or groups capable of conversion to such *in vivo.* Considerable activity may remain with nuclear substitution in one (but not both) rings, or with replacement of one (but not both) rings with an isosteric heterocyclic residue. Substitution of one amino group also appears possible if the substitution remains basic. Doubt exists whether mono-alkylated derivatives are active per se or only through conversion to DDS. A similar situation obtains with the aldehyde condensation products, such as the drugs glucosulfone, sulfoxone, and solapsone, since it seems possible that they owe their activity to an intermediate hydrolysis product with only one amino group free. Studies of this point are complicated by the fact that conversion of the monosubstituted compounds to DDS may occur in the body (discussed above). The need for one *p*-aminophenyl group is presumably related to the mechanism of action of sulfones, which in *M. leprae* is apparently a competition with para-aminobenzoicacid since the activity against *M. tuberculosis* can be reversed by the latter agent (75–77).

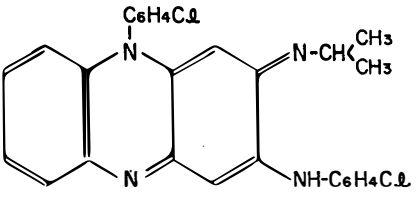
The activity of sulfoxides may apparently be ascribed to their conversion *in vivo* to sulfones; about a quarter of diazotizable compounds present in the urine of patients taking 4,4′-diaminodiphenylsulfoxide arise from DDS (67). The sulfides are not active.

How much these generalizations apply to the anti-bacterial activity of sulfones against *M. leprae* remains to be worked out. Some of the information based on clinical activity in leprosy is invalidated by the possibility that significant amounts of DDS have been present in the drugs tested (see above). The high degree of sensitivity of *M. leprae* to DDS might be related to higher concentrations of DDS at the enzyme site, due to the microorganism's permeability to DDS or even an ability to concentrate it. Such

permeability factors might be governed by structural requirements on one end of the molecule which differ from resemblance to para-aminobenzoic acid and which might therefore be different for *M. leprae*. That sulfones are more effective than sulfanilamides against mycobacteria has not been explained. There are only two reports so far of studies in mice of the activity against *M. leprae* of sulfones other than DDS (43, 49).

Other Drugs

Clofazimine (*B663*).—This drug is now the chief secondary drug for use in patients with organisms resistant to sulfones (78). Its structure is given in Formula I. The Cl atoms are in the para position. Against *M. tu-*



Formula I. Structure of B663

berculosis in guinea pigs B663 was the most active of a series of 67 phenazine derivatives (rimino-compounds) (79). Early reports of the activity of the drug in leprosy were confusing because some of the patients were treated with DDS before receiving B663, and because some of the patients, even some who were also receiving DDS, unaccountably had increases in the proportion of solidly staining *M. leprae* after 12 months of therapy (80). A later study found that the proportion of solidly staining bacilli fell at the same rate as that observed with DDS (81). Some investigators have reported that with 300 mg B663 per day the incidence of reactions of the erythema nodosum leprosum type is less than that seen with sulfones (82, 83). Others have reported that 100 mg B663 per day had no significant effect on reactions (84). Patients receiving the drug develop red and black pigmentation (85), which is objectionable in lighter skinned patients. Although the pigmentation develops more slowly on lower intakes, the minimal effective dosage is not yet known. Pharmacokinetic considerations are complicated by the fact that the drug is unevenly distributed in the tissues and even deposits in crystals (39, 79, 86, 87). The concentration in spleen, liver, lung, and fatty tissue is especially high. The drug is freely soluble in benzene but only sparingly soluble in water; for efficient absorption from the gut it must be administered in very fine crystals.

B663 is active in mice by the continuous method in a dosage of 0.01 per cent in the diet (39, 40, 43).

Other drugs.—Long-acting sulfonamides have been the subject of many

recent reports. Two of these describe continued clinical and bacteriological progress after several years; with sulfadimethoxine (daily doses) for 4 years (88) and with acetylsulfamethoxypyridazine (weekly doses) for 18 to 36 months (89). In mice, by the continuous method, sulfamethoxypyridazine (42), sulfadimethoxine, and sulphormethoxine (41, 43) were active when tested at relatively high dosages.

Thiosemicarbazones and thioureas have been employed widely in leprosy. The chief drugs used were amithiozone (4-acetylaminobenzaldehyde thiosemicarbazone, TB1), which was found to give an initial improvement followed by clinical and bacteriological deterioration at 3 years (18), and thiambutosine (*p*-dimethylamino-*p'*-butoxydiphenyl thiourea, DPT), which gave similar results (22). Against *M. leprae* in mice, by the continuous method, amithiozone was observed to be partially suppressive (39) or completely suppressive (41, 43); thiambutosine was inactive against two strains from untreated patients (39, 90) but active against eight others (42–44, 46). By the kinetic method thiambutosine was bacteriostatic but not bactericidal against one strain (29). From eight patients who had relapsed while under thiambutosine treatment, three strains of *M. leprae* were isolated that were resistant to thiambutosine. The strains were also resistant to amithiozone (46); cross-resistance between the two drugs in strains of *M. tuberculosis* had been previously described. A number of other diphenyl thioureas have received clinical trials, [e.g. (91)], and four have been tested in mice (43, 44).

Clinical reports on ethionamide in leprosy are somewhat conflicting (92). In mice it was found to be bactericidal for *M. leprae* (49).

Other drugs that have been found clinically to give short initial periods of improvement, followed by clinical and bacteriological worsening are ditophal (diethyl dithiolphthalate, etisul, an ethyl mercaptan) (23), vadrine (2-pyridyl-(4)-1,3,4-oxydiazolone-(5)-*p*-aminosalicylate) (25), and isoniazid (24). In mice, by the method of continuous administration ditophal was found ineffective (39), vadrine effective (43), and isoniazid effective (28, 43). However, by the kinetic method isoniazid was inactive (29).

Aminosalicylic acid (PAS) has given ambiguous clinical results in leprosy (93), but has been active in mice when administered continuously (28, 43). By the kinetic method aminosalicylic acid was inactive (29).

Several antibiotics with activity against tubercle bacilli have given mixed results in clinical trials in leprosy but have been active in mice by continuous administration: streptomycin (39, 43), capreomycin (94), streptovaricin (43), viomycin (43), and rifamycin (43). Cephaloridin was also found active by this method (43). Of these, streptomycin (29) and capreomycin (49) have been tested by the kinetic method; they were found bacteriostatic but not bactericidal.

Several drugs with activity against tubercle bacilli have been inactive when administered continuously against *M. leprae* in mice: ethambutol (39), pyrazinamide (39), the related morphazinamide (43), and tetracy-

cline (43). Cycloserine (39) and gentamicin (43) were only partially effective.

Two drugs with anti-malarial activity were inactive against *M. leprae* in mice: cycloguanil pamoate (9) and 2, 4,-diamino-6(3,4-dichlorobenzylamino)-quinazoline (49). The anti-folate activity of these drugs had suggested possible activity againse *M. leprae*.

Some drugs that have been reported to give favorable clinical results but have not been tested in mice are 2-mercaptobenzimidazole and 2-mercaptobenzothiazole (95). Methimazole had also been reported to give a favorable clinical response, but a later examination of the changes in bacterial morphology in tissue sections indicated that there had been no loss in bacterial viability (96). The study showed how difficult it is to assess a drug by clinical response only, since a favorable anti-bacterial response is sometimes accompanied by clinical exacerbation.

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