Experimental Chemotherapy in Leprosy, Then and Now Charles C. Shepard²

Although 100 years have passed since Hansen discovered the leprosy bacillus, much of what we now consider to be experimental chemotherapy is less than ten years old. Indeed, the field of experimental chemotherapy has recently developed so rapidly that what appears in publications is already old, and much of the communication among scientists working in the field is informal and oral. Unfortunately, when a research front advances rapidly, a gap always seems to develop between those engaged in research itself and those occuped with medical and public health practice. There are, of course, many reasons for this, but one that is not always appreciated is that the scientists contributing the most to the advance tend to have backgrounds and ways of thinking that differ considerably from those engaged in practice. For example, the professional language of a biochemical pharmacologist and the concepts that lie behind it are necessarily very different from those of a practicing leprologist. Yet some of the biochemical pharmacologists' findings are pertinent indeed to the welfare of the patient. The solution to this general problem seems to lie, first, in the recognition that there is a problem of communication and, second, in the acceptance that a realistic and continuing effort must be made from both sides to achieve understanding of new information in terms of the patient. Reviews such as the present one should be considered in this light.

EXPERIMENTAL CHEMOTHERAPY BEFORE 1962

None of the common remedies for leprosy used in Hansen's day survived. Although chaulmoogra oil had been known in India and China for centuries and its use in leprosy recorded in Burmese legends, it was not used widely in leprosy until after World War I. A great optimism about chaulmoogra oil then spread over the world. Later its efficacy became suspect, and the clearly greater effect of sulfones led to the feeling that chaulmoogra oil was completely inactive. Only recently, purified preparations of hydnocarpic acid, one of the components of chaulmoogra oil, have been shown to have a definite effect against M. leprae (¹⁰). The degree of activity against M. leprae is unimpressive, however, when compared to that of the best compounds now available.

The discovery of the antibacterial activity of the sulfonamides and sulfones in the thirties and research on the mechanisms of their action led to the recognition that bacterial metabolism is greatly different from mammalian metabolism. We now have more quantitative concepts about evolution and realize how little DNA homology there is between bacteria and mammals and that even when similar metabolic reactions are carried out in bacteria and in mammals, the protein structures of the responsible enzymes have become very different. Since the discovery of the sulfonamides, many antimicrobial drugs have been found; this has been done by empirically screening compounds of known structure or filtrates of other microorganisms, especially fungi, and by experimental modifications of compounds discovered by the screening process. Leprosy could not partake fully in this antimicrobial chemotherapeutic revolution because the essence of the new approach was the ability to test the activity of large numbers of compounds and culture filtrates against the infectious agent. Nevertheless, some of the most active of the new drugs, especially those active against M: leprae and M. lepraemurium, were tried in leprosy patients. Out of this era in the development of antileprosy drugs came several sulfone compounds, thiambutosine (thiourea, 1906), and clofazimine (B663). Many other drugs were report-

¹ Charles C. Shepard, M.D., Chief, Leprosy and Rickettsial Diseases Unit, Virology Section, Center for Disease Control, Atlanta, Georgia 30333, U.S.A.

² Abbreviations used: BI = Bacterial Index; B663 = clofazimine; DADDS = acedapsone; DDS = dapsone; MADDS = monoacetyl-dapsone; MI = Morphological Index; INH = isoniazid; RMP = rifampin or rifampicin.

ed to be active, but why some were widely accepted and others were not is not always clear from reading the literature. Perhaps much testing went unpublished, or perhaps acceptance of a drug was often based upon the personal experience of leprologists who were widely accepted as authorities.

During this period, the only objective evidence available on the activity of a drug was the response in the patient. The ultimate acceptability of a drug, of course, has to be based on clinical experiments, but the methods of a clinical trial must be as clean and the results as objectively acceptable as those of any other experiment. Unfortunately, decisive clinical trials of antileprosy drugs are very difficult to carry out. It is helpful to confine the trials to lepromatous or near-lepromatous leprosy because spontaneous arrest without therapy is very rare in these cases. Unfortunately, however, lepromatous leprosy does not clear before five years or more of DDS therapy, and there is apparently only one report in which a high percentage of the patients entering the trial received regular treatment and were followed to clinical and bacteriological clearing (13). Thus, most reports concern short-term response and consequently, concern only the beginning of the therapeutic response.

The clinical response itself is not easy to quantify objectively, and in some "blind," controlled trials, the observed improvement in sulfone-treated patients was not decisively better than that in untreated controls (5). The variability is so great that several dozen patients had to be followed for a year or more to be sure that any clinical improvement at all had occurred; comparison of the efficacy of different regimens was very difficult indeed.

The number of *M. leprae* in the skin smears proved easier to quantitate than the clinical response, so chief reliance came to be placed on improvements in the Bacterial Index (BI). In contrast to most other bacteria, however, dead *M. leprae* disappear from the tissues very slowly. Apparently as a result of this slow disappearance, the basic theory underlying clinical trials has been confused. Antibacterial drugs are selected for their ability to prevent the

growth of bacteria, or to kill them, but not to dissolve them. Thus, the rate at which dead bacteria disappear from the tissues depends on the bacterial structure and the tissue reaction, and not necessarily on the drug used to kill them. Studies indicate that most of the *M*. *leprae* are dead even in the untreated patient, and, unfortunately, many drug trials have involved patients who had already received some treatment. Consequently, during the actual trial the first observed decrease in BI describes the disappearance of bacteria that were dead before the trial was started, sometimes as a result of the action of another drug. If the drug being tested is not effective, the living bacteria multiply and replenish the bacterial load in the tissues. When there has been previous treatment, however, it is difficult to predict how long the replenishment will take, so comparisons between groups of patients that have received previous treatment are hazardous.

In the last ten years, methods have been developed that allow the measurement of bacterial viability in patients through the Morphologic Index (MI, solid ratio) and mouse inoculation, and the theoretical distinctions between short-term and long-term trials have become more clear; these newer developments are discussed below.

EXPERIMENTAL CHEMOTHERAPY SINCE 1962

Methods of testing drugs against M. leprae in animals. M. leprae grows in the cool, peripheral tissues of several small rodents, but the growth in foot pads of mice is probably best. The relative consistency of the results in mice and the convenience of the model have been particularly advantageous. Some have objected that mice are less susceptible to leprosy than lepromatous patients, who are said to be completely susceptible, but this objection is based on a confusion of terms, Mice are apparently susceptible to infection with about five living M. leprae; the minimal infectious dose in humans is not known, but it obviously cannot be much less. In mice during the logarithmic phase of increase, M. leprae multiply with a generation time (doubling time) that averages 12.5 days. At this rate,

tenfold increases occur every 41 days, and in a year the increase would be nearly 109-fold (which corresponds to an increase of 9 BI units on a decade scale, such as that of Ridley). Systematic observations have not been made in untreated lepromatous patients, but occasional observations in untreated patients and more careful measurements in dapsone-treated patients with completely dapsone-resistant M. leprae indicate that the BI increase seldom exceeds one unit per year. Thus, by the criterion of rate of growth the lepromatous patient is much more resistant than the mouse in the logarithmic phase. During this phase in mice, the bacilli multiply freely without tissue reaction. Nearly all the work with M. *leprae* in mice involves the logarithmic phase. The opinion regarding susceptibility of the lepromatous patient is, of course, based on the observation that large numbers of bacilli can be present, and that, in contrast to the experience in tuberculoid patients, spontaneous arrest (without chemotherapy) is rare and relapse with inadequate therapy is common. Clearly, it is illogical to generalize from these observations to all the manifestations of infection that are sometimes, incorrectly, lumped under the headings of susceptibility.

In the main part, two methods have been used to test the effects of drugs on M. *leprae* in mice. One is the continuous method, whereby the drug is administered from the day of infection to the end of the experiment. Bacillary counts are performed in untreated controls, and when they show that the plateau level has been reached, counts are made in the treated groups. This method, which is analogous to a test in vitro in which the drug is incorporated in the medium, was the method used in the beginning when we thought that anti-M. *leprae* effects might be difficult to see (³¹). Experience showed, however, that anti-M. leprae activity is guite common (²⁸), especially among drugs active against M. tuberculosis. The continuous method was not discriminating enough, and it left too much to be learned during the subsequent human trials. Consequently, the kinetic method was developed (26, 27) by taking advantage of the consistency of the

bacillary growth curve in mice. By this method the drug is administered for a limited period only, for example, from the 70th to the 130th day after infection. Bacilli are counted at appropriate intervals in control and in treated mice, and the growth curves are compared so that the amount of growth delay can be stated. Bactericidal drugs can be differentiated from those that are merely bacteriostatic in the presence of drug, and the drug is subjected to a more severe test. Theoretically, a drug that exerts a bacteriostatic effect that continues after the drug has disappeared from the tissues can also produce a bactericidal-type result. Nevertheless, with the kinetic technic, the rather large list of drugs with activity against M. leprae by the continuous method is reduced to a reasonably small number from which, as far as we can tell, no drugs of real promise in leprosy have been eliminated. The bactericidal drugs are discussed below.

An important consequence of the availability of laboratory methods for measuring anti-*M. leprae* effect has been to open the door to the methods of modern chemotherapeutic pharmacology. Much can be done in humans, but studies of pharmacokinetics, drug metabolism, and toxicology are handicapped without the quantitative insights that animal experimentation can provide.

A valuable benefit of animal technics for the study of *M. leprae* has been the ability to test strains of *M. leprae* for drugresistance (²¹). For such tests the drug is administered by the continuous method in a dosage known to be effective against all strains from untreated patients. When growth reaches plateau levels in untreated controls, the bacterial harvest in the treated mice is compared. With this technic, dapsone (DDS)-resistance can be proven and the level of resistance measured.

Methods of testing drugs against *M. leprae* in man. The availability of more efficient methods of testing drugs in animals and man has led to a clarification of the theoretical basis of drug-testing in man. Short-term and long-term tests are now differentiated. Short-term tests give information on the initial stages of bacterial

41, 3

killing; long-term tests, on the final stages. Thus, short-term tests seek to determine the effects of drug on the vast majority of M. leprae in the body, whereas long-term tests are directed toward learning whether small numbers of M. leprae survive the treatment either for physiologic or anatomic reasons or because they are genetically drug-resistant. The two types of tests require different administrative arrangements. Because of the more intensive laboratory studies generally needed, short-term trials are generally carried out with patients who have been temporarily institutionalized. Because long-term institutionalization is objectionable, long-term trials, at least after the initial stages, are best carried out with outpatients in an administrative arrangement that assures efficient followup. For the reasons stated above, both types of tests should be carried out only with untreated patients.

The methods formerly used for shortterm clinical trials are discussed above. The principle method used then, measurement of the BI, detects the net loss in total (living plus dead) bacilli that occurs when an active drug prevents replacement of bacilli. Perhaps two dozen patients need to be followed for a year or more to be certain of antibacterial activity. Since 1962, however, several new technics have been introduced. First was the measurement of the Morphological Index (MI) (43). When living, M. leprae stain solidly and completely, but when they die they develop irregularity of staining; hence, the MI falls after effective treatment. The solid ratio is a very similar measurement, but its stricter morphological requirements make the number of solid bacilli a direct measure of the number of viable bacilli (14). The MI falls to the base-line level in about three months after the onset of treatment with dapsone. Perhaps four patients are needed in a study if they are selected for a high initial MI, and several times this number is needed if they are unselected.

In our work, we have come to rely primarily on mouse inoculations. Skin punch (6 mm) biopsy specimens are removed at appropriate intervals, from the same lesion if possible, and the bacilli are inoculated

into mice. The same number of bacilli is inoculated each time, if possible. Decreases in the proportion of viable bacilli in the patient's tissues are detected first, by increases in the incubation period (time from inoculation of mice until appearance of M. *leprae* in histological sections) and by increases in the generation time (average doubling time between inoculation and harvest of foot pads), and then by complete disappearance of infectivity for mice. The standard inoculum is 5 x 10³ bacilli, but the minimal infective dose of M. leprae from untreated lepromatous patients is about 1% of that, or 5 x 10^1 M. leprae. Hence, the disappearance of infectivity during treatment signifies that less than 1% of the original infectivity remains. In contrast, it is difficult to detect 10% of the original infectivity in measurements of the MI. A further disadvantage of MI measurements is that there is a lag between loss of viability and loss of staining properties, which amounts to several weeks in the case of rifampin (36). Probably for these reasons, the mouse inoculation method can show distinct differences in the activity of antileprosy drugs that seem equally effective by measurements of the MI. Results with four drugs are shown in Figure 1.

Another method is the enumeration of M. leprae in nasal excretions (³⁸). Most untreated lepromatous patients excrete large numbers of M. leprae from the nasal mucosa, and an effective drug stops the excretion by preventing multiplication of M. leprae and by allowing the nasal ulcers to heal. Because the method is somewhat laborious, it has not been widely used.

Long-term trials are necessary to determine whether a drug can cure leprosy, or at least cause bacteria-free arrest. After treatment with DDS has started, the MI reaches the base-line level in about three months. Mouse inoculations become negative in nearly all patients in four months. After these times, these two technics are useful only in detecting failures in therapeutic response. The BI (which should represent the average of at least six sites) decreases much more slowly, at an approximate rate of one BI unit (on the Ridley scale) per year. After four or five years of treatment,



FIG. 1. Comparative drug duration of therapy before loss of bacterial viability detectable by mouse inoculations. Each line refers to a patient. Mice were inoculated with standard numbers of *M. leprae* from skin punch biopsy specimens that were serially removed before and at intervals after the start of treatment. Results are shown only for the last positive and the next negative specimen. The drug dosages were as follows: Rifampin, 600 mg daily; DDS, a dosage ascending to 50 mg daily in a few weeks; B663, 200 mg daily; DADDS, 225 mg every 11 weeks. In general, about 99% of the bacteria must be killed before mouse inoculations become negative. [Reprinted by permission from the EGYPTIAN REVIEW: AIN SHAMS MEDICAL JOURNAL, in press.]

many patients will have BI's of 0 (all six sites negative). The histological examination of skin biopsy specimens also is informative in long-term trials. Although skin smears represent a wider sampling (six sites), they are more susceptible to poor technic (inadequate tissues in the smear, too many red blood cells), and they often fail to sample the deeper dermal tissue. Skin biopsy specimens can include the deeper tissue, and they are especially valuable when skin smears have become negative. Clinical examinations are also necessary; in general, clinical manifestations disappear at the time the skin smears become negative. The careful examination of the patient often leads to the selection of better sites for skin smears or biopsies. Relapses are usually manifested by the appearance of new lesions. Clinical assessments are often made by two leprologists working independently.

THE PRINCIPLE DRUGS TODAY AND THEIR EXPERIMENTAL BASIS

The drugs that were in favor before 1962 were the sulfones, amithiozone, and thiambutosine. Clofazimine (B663) also belongs to this era even though the first clinical reports of its use appeared in 1962, since it, too, was studied in leprosy patients without first being tested against *M. leprae* in animals. Initial enthusiasm about amithiozone was later tempered when the patients' conditions were observed to deteriorate clincially and bacteriologically after two years of treatment (12). The hepatotoxicity of the drug also spoke against its wider use. In mice, however, when tested by the continuous method, the drug was observed to be only partially active $(^{32})$. With thiambutosine, too, initial favorable experience was followed by reports of deterioration after two years (⁴). In mice, thiambutosine was not active against all strains when tested by the continuous method, and by the kinetic method the drug was merely bacteriostatic against the one strain tested (²⁸). The remaining drugs, sulfones and clofazimine, were later found to be bactericidal by the kinetic method (see below).

Results of drug-testing in mice have recently been examined $(^{28})$. Of the more than 100 drugs reviewed or tested since then, those in the following list are the only ones found to be bactericidal by the kinetic method. The list appears to include all drugs of real clinical merit.

- 1. DDS (see below) and several other sulfones that are hydrolyzed to DDS in the gut or metabolized to DDS in the body. The minimal effective dosage of DDS is so small that the activity of sulfones, such as glucosulfone (Promin³), Promizole³, and Promacetin³, that were used in larger dosages, can be accounted for by slight contamination with DDS (³⁴).
- 2. Sulfadimethoxine (Madribon³). Other long-acting sulfonamides may also be bactericidal. Their use in leprosy has been objected to on the ground that the minimal effective dosage is too close to the usual dosage (⁶); drugresistance here would be serious because of probable cross-resistance to DDS.
- 3. B663 (see below) and B1912, another riminophenazine compound. B1912 will probably not become available because of reluctance to finance the required toxicity studies.
- Ethionamide. Wider use of this drug is inhibited by its tendency to produce gastrointestinal distress and hepatitis.
- 5. Rifampin (see below) and streptovaricin, a related antibiotic. The streptovaricin we studied, a mixture

of several natural compounds, was less active than rifampin $(^{39})$.

- Cephaloridin and cephaloglycin (^{30,-39}). The bactericidal activity of these two cephalosporin-C derivatives is not great, but it is interesting because the mechanism of action is through inhibition of cell-wall formation.
- 2 Amino 5 (1 methyl 5- nitro 2imidazolyl)-1, 3, 4-thiadiozole (¹⁰). Development of this compound has ceased because of reported carcinogenicity.

DDS and DADDS. One of the two most surprising findings to date in experimental chemotherapy is the smallness of the minimal effective dosage of DDS. (The other is the rapidity of the bactericidal effect of rifampin.) Published reports of experiments on the size of the minimal effective dosage of DDS in man were not satisfactory, perhaps because clinical opinion was unanimous that at least 50 mg a day was needed. We originally expected that the minimal effective dosage in mice would be 0.01% in the diet; this produced about the same blood concentration as 50 mg a day in man. In fact, however, the minimal effective dosage was only 1/100th as much as expected. This finding in mice led to the prediction, later confirmed, that DDS would be effective in man in milligram dosages.

The equivalency between DDS dosages in the mouse and in man is given in Table 1. These considerations are applicable to DDS because it is evenly distributed in the body (outside of the liver and kidneys); hence, measurements of DDS in blood or plasma reflect the concentration in the immediate environment of the organism. Because the mouse eliminates the drug from the blood much faster than man (a half-life of about 3 hours in the mouse compared to an average of about 24 hours in man), a higher per kilo dosage is needed in the mouse to maintain the same blood level as in man. The minimal effective dosage in mice, 0.0001% in the diet $(^{34}, ^{37})$, produces about 0.010 μ g DDS/ml. Therefore, the minimal inhibitory concentration is somewhere between 0.010 and 0.001

³ Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health, Education and Welfare.

TABLE 1. Blood levels according to dosage and activity for M. leprae (taken from Ozawa et al (16) except where indicated).

DDS do	osage in	
Mouse (% in diet)	Man (mg/day)	Remarks
	100	Standard dosage in man
0.01		
0.001		
	2.4	Released from DADDS injection
	1.0	Active therapeutically in man
0.001		Minimal effective dosage in mice
0.00001		Usually not effective in mice
	DDS do Mouse (% in diet) 0.01 0.001 0.001 0.0001	DDS dosage in Mouse Man (% in diet) (mg/day) 100 0.01 0.001 2.4 1.0 0.001 0.0001 0.00001

^a Determined in plasma by Ellard *et al* (⁷)

 μ g/ml; the latter blood concentration is produced by 0.00001% DDS in the diet, a dosage that is effective against only some strains of *M. leprae*.

The relevancy of the mouse results was then shown in man in short-term trials in lepromatous leprosy. First, 2.4 mg DDS/day, as supplied by the repository sulfone DADDS, was found to be active, as judged by decreases in numbers of solid bacilli in skin smears and disappearance of M. leprae from nasal washings (38); the response in ten DADDS patients was as great as that in ten patients treated with 100 mg DDS per day. Next, 1 mg per day of oral DDS was found to be active $(^{44})$; the initial fall in MI was as rapid as that expected with full dosages of DDS.

DADDS, a very slightly soluble compound, is suspended in a suitable particle size range in benzyl benzoate and castor oil and injected intramuscularly in a dosage of 225 mg every 75 days. DDS is released slowly at an average rate of 2.4 mg per day. DDS blood levels average about 0.050 $\mu g/ml$. DADDS is not completely absorbed in 75 days, so there is a slight buildup in DDS concentrations in the blood after the second injection, but not later. The rate of fall in MI with DDS and DADDS treatment seems to be about the same. Mouse inoculations, however, which can follow the bactericidal curve down through about 1% of the starting value, show that the bactericidal rate with DADDS is frequently slower (Fig. 1) (³⁵). Thus, skin biopsy specimens from 17 of 20 DDS patients (50 mg/day) have

converted to negativity by 100 days. Only three of ten DADDS patients were negative by the 100th day, and some were still positive after 300 days. The survival of bacilli in DADDS patients has not been associated with the development of drugresistance; many of the isolates after 100 days of treatment were tested against the minimal effective dosage of DDS and found to be normally susceptible.

DADDS has been used chemoprophylactically in the Caroline Islands of the Pacific Trust Territory (⁴¹). It was administered for three years, beginning in 1967, to the entire Pingelapese population, a group with high endemicity. On the basis of past experience, about 12 new cases a year were expected. In fact, however, only six cases were seen in 1968, and none were found in 1969 and 1970. Continued follow-up is needed to see the permanent effect of the drug on the appearance of new cases.

DADDS is being used for the treatment of all leprosy patients in the Karimui, an area of difficult access in New Guinea. More than 400 patients of all types have completed their fourth year of DADDS therapy, and their clinical progress has been favorable (25, unpublished results). Of the lepromatous patients, 29 had sufficient bacteria in their skin smears to allow the MI to be followed. They all responded well initially with decreases in MI to the base-line level. The BI's also fell satisfactorily at rates that averaged one BI unit a year. In the last year or two, two patients have not been responding well bacteriologically, that is, a few solid bacilli have been seen and the BI's are no longer decreasing. Serum samples of these patients contained normal amounts of DDS and MADDS. M. leprae were isolated from the skin biopsy specimens of one patient and shown to be normally susceptible to the minimal effective dosage of DDS (0.0001% in the diet). Whether similar results would have been obtained if the patients had been treated with dapsone is not known; DDS controls were not possible in the Karimui, and no group of DDS-treated patients had been followed as carefully. Lowe reported in his long-term study of DDS therapy that a few patients ceased to improve occasionally, but they all responded eventually and moved toward skin negativity (13). MI's were not determined at that time, of course. The mechanisms of survival can only be speculated on: a physiologic suspension of bacterial metabolism leading to decreased uptake of DDS, or decreased incorporation of DDS into folate, or decreased requirement for folate, or a histologic location inaccessible to DDS. Similar survival of a small fraction of the infectious agent in the presence of inhibitory concentrations of other drugs have, of course, been noted with other bacteria, especially tubercle bacilli. In leprosy, the phenomenon could be responsible for the relapses that occur in lepromatous patients when DDS treatment is stopped prematurely. The experience in the short-term trial of DADDS that was monitored by mouse inoculation is perhaps an earlier reflection of the same phenomenon. The surviving fraction is presumably greater with lower levels of DDS. With DADDS, the surviving fraction after the first six months is apparently too small to be detected by the MI until enough of the nonsolid acid-fast bacteria disappear (perhaps 99% to 99.9% or a drop in the BI of 2.0 or 3.0 units), a decrease that required three years in the case of the first patient mentioned.

DDS is partially converted to the mono-N-acetyl derivative (MADDS) in the body by the enzymatic mechanism described for acetylation of isoniazid (INH) and certain sulfonamides, e.g., sulfamethazine (^{8, 18}). The percentage of DDS present in plasma as MADDS varies in different persons from less than 10% to 40%. The distribution is bimodal and is about the same in Caucasian, Philippine, Indian, and African subjects. Rapid acetylators of INH and sulfamethazine acetylate DDS more extensively. If MADDS is administered, it is deacetylated by another enzyme system, and after a few hours the percentage of MADDS is the same as that attained after administration of DDS. After intramuscular injection of DADDS, a low percentage of DADDS is present in the plasma, together with DDS and MADDS in a characteristic ratio (¹⁹). DADDS is not, however, demonstrable after administration of DDS or MADDS.

The ratio of MADDS to DDS varies considerably among mammalian species (¹⁷). Mice and dogs have very little MADDS, squirrel and rhesus monkeys have large amounts, and rats, intermediate amounts. Lines of rabbits that have been bred for rapid or for slow acetylator status of INH have high or low percentages of MADDS, respectively.

The therapeutic implications of the acetylation capability are not clear. In the case of INH and tuberculosis, acetylated INH is inactive. Rapid acetylators have shorter half-lives of nonacetylated INH, and therefore respond less well bacteriologically when treated with ordinary dosages of INH. The half-life of DDS, however, is not related to the acetylator status. Nevertheless, there is a suggestive excess of rapid actylators among patients where dapsone therapy has failed because of the emergence of DDS-resistant M. leprae (19). Because mice deacetylate MADDS rapidly, the therapeutic activity of this compound for M. leprae cannot be studied in mice. Against a cultivable mycobacterium, M. sp. 601, MADDS is less active than DDS, especially in the presence of bovine albumin (¹⁵).

The rate of elimination of DDS varies markedly in different persons (²⁰). The half-life of DDS in plasma varies from about 13 hours to more than 40 hours, and averages about 24 hours. A similar variation has been found in Caucasian, Philippine, Indian, and African populations. The variation appears to be great enough to affect the therapeutic response to DDS, especially when the drug is given at wide intervals (see below). For example, following a dose of 25 mg, the plasma level would be expected to fall below 0.005 μ g/ml in seven half-lives, i.e., in 3.7 days with a half-life of 13 hours, compared to 11.7 days with a half-life of 40 hours. Preliminary results indicate that there is an excess of persons with short DDS half-lives among patients who have developed DDS-resistant infections.

Another metabolite has recently been demonstrated in an *in vitro* system involving rat liver microsomes (³). The monohydroxylamine (DDS-NOH) was formed; this compound and perhaps the nitroso derivative, are believed to be responsible for the production of methemoglobin during DDS therapy. Methemoglobinemia is a serious complication with high dosages of DDS, but in dosages of less than 100 mg per day, it is infrequent.

B663. This drug produces bactericidaltype results against *M. leprae* tested by the kinetic method, but it is deposited in the tissues and cleared only very slowly, so, theoretically, one cannot be sure that the effect in mice is true bactericide. Results of mouse inoculation in short-term experiments in human patients, however, show that B663 is truly bactericidal (11). Earlier studies of MI in B663-treated patients had shown that the drug had a bactericidal effect (2^2) , to the extent detectable with that technic. The rate of bactericide in man during B663 therapy with 200 mg per day has been measured by mouse inoculations of M. leprae and found to be very similar to that during DDS therapy with 50 mg per day, once it starts (11). The onset of bactericide with B663 was, however, delayed about 50 days; hence, conversion to mousenegativity did not occur until 100 to 150 days (Fig. 1).

The minimal effective dosage of B663 in mice varies from 0.001% to 0.0001% in the diet. The pharmacokinetics of this drug are complicated because the drug is very insoluble and because it deposits unevenly in the tissues; hence, the minimal inhibitory concentration cannot be estimated at all accurately from the mouse data available. In mice, the drug is active against *M. leprae* when administered as infrequently as once every four weeks (40). Because intermittent administration would simplify therapeutic coverage and because results of the human trials mentioned (11) had indicated that the drug might need to accumulate in the tissues before it became bactericidal, a human short-term trial of various regimens was carried out with monitoring by mouse inoculations (2). A total of 1200 mg every four weeks was given as 600 mg on two subsequent days or as divided dosages three times a week, once a week, and once very two weeks. Frequent administration was found to be more rapidly effective. Since collateral experiments indicated complete absorption of B663 from the gut, the results indicate that the drug deposited in human tissues is not as available for anti-M. leprae activity as is recently absorbed B663.

The mechanism of action of B663 is not known, but it is active against DDS-resistant M. *leprae*. A low degree of one-way cross-resistance between rifampin and B663 has, however, been demonstrated with the cultivable mycobacterium M. sp. 601.

Rifampin. The first report on rifampin and *M. leprae* described activity in mice, as tested by the continuous method of drug administration, and in man, as measured by decrease in MI (24). The MI decreased to base-line level in about four weeks with 600 mg per day of rifampin as compared to about 20 weeks with DDS.

Later work has shown that rifampin is very much more rapidly bactericidal than DDS or B663. In mice even single doses by gavage are effective, with increasing effect in the range 10-40 mg/kg (36). To produce as much bactericide, DDS has to be administered for two to three months. In different experiments the minimal single dose having detectable bactericidal effect has varied from 10 to 30 mg/kg (29). Single doses in the range 25-40 mg/kg may eliminate the infection (containing approximately 10³ living *M. leprae* at the time of administration).

The minimal effective dosage of the drug administered over a long period has been found at different levels from 0.01% to 0.0003% in the diet (^{9, 36, 39}). Values do

not agree in different laboratories for unknown reasons, so the minimal inhibitory concentration of the drug cannot be estimated with any confidence. In the mouse and in man, per kilo dosages are equivalent, however, since the drug is completely absorbed from the gut, evenly distributed in the body (outside the liver and kidney), reaches the same peak levels and disappears from the blood with approximately the same half-life (about three hours). The much shorter half-life and much more rapid bactericidal effect of rifampin operate to make consideration of the minimal inhibitory concentration much less important than it is with DDS.

The rapid bactericidal effect is also seen in man (Fig. 1). In lepromatous patients treated with 600 mg per day, conversion to negativity by mouse inoculation occurred by seven days (36), the time the first specimen was taken, and preliminary results indicate that this loss of viability may occur as early as three to four days (30). This rate of loss of viability would correspond to a half-life of eight hours or less, a surprisingly rapid kill, about 30 times as short as the estimated ten day half-life for the kill of *M. leprae* with DDS (33).

IMPLICATIONS OF THE EXPERI-MENTAL CHEMOTHERAPEUTIC RESULTS FOR THE TREATMENT AND CONTROL OF LEPROSY

DDS and MADDS. The surprisingly low minimal inhibitory concentration of DDS for M. leprae has led to some use of low dosages of DDS in routine therapy. The reasons for low dosages are not clear, however, since toxic reactions to drug at a dosage of 50 mg daily to adults are very rare. The most common toxic manifestation to DDS is anemia. It is unacceptably severe with 200 mg daily, moderate in some patients with 100 mg daily, but nearly negligible with 50 mg daily. There is little evidence that ENL is less frequent at low dosages, although there is some suggestion that its severity is less. Some have suggested that borderline reactions (reversal reactions) are less severe at very low dosages, but here one must suspect that bacterial killing is not being achieved if the

dosage actually avoids these reactions. The disadvantage of low dosages, on the other hand, is quite obvious: namely that therapeutic coverage may not be maintained unless administration is frequent and very regular.

Infrequent administration is also risky. The bactericidal effect of DDS is so slow that it probably provides little "hammer effect." Thus, any regimen should probably provide a constant therapeutic coverage by assuring that at least 0.010 μ g/ml is present in the tissue at all times, that is, that the tissue level never falls below the minimal inhibitory concentration. Since the regimen needs to provide for all patients, it needs to be suitable even for those with a short DDS half-life. To estimate the frequency with which DDS should be given, one can consider the peak level after a single administration and the rate at which the blood level falls. Thus, following a single dose of 100 mg, the peak blood level would be somewhat more than 1 μ g/ml, and, in a patient with a DDS half-life of 14 hours, the blood level would fall to less than 0.010 μ g/ml in 7.5 half-lives, or 4.4 days. Increasing the single dose to 300 mg would extend the desired coverage only to 5.3 days. Thus, once-weekly doses could not provide therapeutic coverage, whereas twice-weekly doses could. With thriceweekly doses, three days' coverage would be needed; theoretically, this could be barely provided by 25 mg, but not by significantly lower doses. Doses of 50 or 100 mg would, of course, be safer. From these considerations one can appreciate the risks involved with irregular intake. In practice, intake of medication has been found to be very irregular; in different studies the proportion of patients failing to take medications as directed has ranged, with few exceptions, from 20% to 82% (42).

The advantages of DADDS, in this regard, are obvious. The injected material releases DDS constantly, to produce an average blood level of 0.050 μ g DDS/ml, and levels below 0.020 are infrequent even immediately preceding the next injection. The therapeutic coverage can be provided with visits five times a year, and in some areas of difficult access, such as Karimui of Papua, New Guinea, it is the only feasible drug. Wide-scale use of the drug will probably necessitate restructuring of the practices of some antileprosy services, since regularity of the injections would be important.

B663. This drug has come into wide use in patients with treatment failure due to the emergence of DDS-resistant *M. leprae.* In large dosages, 200 to 300 mg a day, it is also helpful in controlling ENL. Its disadvantage is that it causes skin pigmentation that is objectionable in lighter skinned patients, especially if they are outpatients. Even with low dosages, such as 100 mg thrice weekly, the pigmentation can be marked. Under special circumstances, the administration of 1200 mg once every four weeks may be convenient, but as stated above, the results of a trial indicated that more frequent dosing is more effective (²).

Rifampin. This drug is much more rapidly bactericidal than any other antileprosy drug known. The side effects are not serious with daily dosage, but with intermittent schedules several types of side effects have been observed that apparently are associated with antibody formation (1, 23). Of these reactions the more common ones, such as the "cutaneous" syndrome, are not serious enough to make interruption of treatment necessary. Some are more serious, and if thrombocytopenia develops, rifampin must be stopped. Consequently, at present, unless accurate thrombocyte counting is practiced, treatment with rifampin should probably be limited to daily schedules.

The high cost has also been an obstacle to the widespread use of rifampin in leprosy. Cost reductions seem probable in the future, however, because of improvements in manufacture. In considering the high cost of the drug one would like to be able to take into account the more rapid bactericidal effect. Unfortunately, clinical trials designed to test the feasibility of shortened treatment are very difficult to carry out for a variety of reasons.

Combinations of rifampin and DDS (or DADDS) have many theoretical advantages. We do not know yet how low the resistance ratio is for rifampin and *M. lep*- rae, that is, whether rifampin-resistant forms of *M. leprae* are frequent enough to prevent the cure of leprosy by treatment with rifampin alone. Trials to determine this do not seem to be ethically justified; instead one should probably proceed directly to tests of combinations. An initial rapid kill of M. leprae is clearly desirable, so initial courses of a few weeks or few months with daily rifampin (600 mg) in combination with DDS or DADDS are being tried. Experiments in mice have shown that dapsone does not interfere with rifampin's bactericide, even if the M. leprae are in dapsone-induced bacteriostasis. DDS or DADDS would then be continued alone after the initial course of rifampin, until bacterial, histological, and clinical cure had been achieved.

Conclusion. The course of development of new antileprosy drugs and drug regimens is now similar to that in other infectious diseases. Microbiological research in animals, in connection with studies of pharmacokinetics and drug metabolism, can now elucidate many of the factors that can be expected to govern the results in man. More efficient methods of monitoring clinical trials, especially those of the short-term type, are now available. Consequently, when the clinical trials are carried out, they can be designed more efficiently. More can be learned from fewer patients, and fewer patients need to be exposed to the risks of the experimental treatment. Only after the results of well-designed short-term and long-term clinical trials are available is one in a position to make rational recommendations for drug regimens to be used in actual practice.

REFERENCES

- AQUINAS, SR., M., ALLAN, W. G., HORS-FALL, P. A. L., JENKINS, P. K., HUNG-YAN, W., GIRLING, D., TALL, R. and FOX, W. Adverse reactions to daily and intermittent rifampicin regimes for pulmonary tuberculosis in Hong Kong. Brit. Med. J. 1 (1972) 765-771.
- Collaborative effort of the U. S. Leprosy Panel and the Leonard Wood Memorial. Spaced clofazimine therapy of lepromatous leprosy. Presented at Seventh Annual Leprosy Research Conference, Menlo Park, Calif., Feb. 29-March 1, 1972.

41, 3

- CUCINELL, S. A., ISRAILLI, Z. H. and DAY-TON, P. G. Microsomal N-oxidation of dapsone as a cause of methemoglobin formation in human red cells. Am. J. Trop. Med. Hyg. 21 (1972) 322-331.
- DAVEY, T. F. Some recent chemotherapeutic work in leprosy; with a discussion of some of the problems involved in clinical trials. Trans. Roy. Sec. Trop. Med. Hyg. 54 (1960) 199-206.
- DOULL, J. A. Clinical evaluation studies in lepromatous leprosy. First series: Diasone '(Diamidin), 4-4'-diaminodiphenyl sulfone, dihydrostreptomycin. Internat. J. Leprosy 22 (1954) 377-402.
- ELLARD, G. A., GAMMON, P.T. and REES, R. J. W. The minimal inhibitory concentrations of sulphadimethozine and sulphadoxine against *Mycobacterium leprae*. Leprosy Rev. 41 (1970) 223-228.
- ELLARD, G. A., GAMMON, P. T., REES, R. J. W. and WATERS, M. F. R. Studies on the determination of the minimal inhibitory concentration of 4,4'-diamino-diphenyl-sulfone (dapsone, DDS) against Mycobacterium leprae. Leprosy Rev. 42 (1971) 101-117.
- GELBER, R., PETERS, J. H., GORDON, G. R., GLAZKO, A. J. and LEVY, L. The polymorphic acetylation of dapsone in man. Clin. Pharmacol. Ther. 12 (1971) 225-238.
- HOLMES, I. B. and HILSON, G. R. F. The effect of rifampicin and dapsone on experimental *Mycobacterium leprae* infections: minimum inhibitory concentrations and bactericidal action. J. Med. Microbiol. 5 (1972) 251-261.
- 10. LEVY, L. Unpublished results cited in (28).
- LEVY, L., SHEPARD, C. C. and FASAL, P. Clofazimine therapy of lepromatous leprosy caused by dapsone-resistant *Mycobacterium leprae*. Am. J. Trop. Med. Hyg. **21** (1972) 315-321.
- Lowe, J. The chemotherapy of leprosy. Late results of treatment with sulfone and with thiosemicarbazone. Lancet 2 (1954) 1065-1068.
- Lowe, J. The late results of sulphone treatment of leprosy in East Nigeria. Leprosy Rev. 25 (1954) 113-124.
- MCRAE, D. H. and SHEPARD, C. C. Relationship between the staining quality of *Mycobacterium leprae* and infectivity for mice. Infec. Immunity 3 (1971) 116-120.

- MORRISON, N. R. Structure-activity of sulfone compounds. Presented at the workshop on pharmacology of antileprotic drugs of the U. S.-Japan Cooperative Medical Science Program, Bethesda, Maryland, November 12, 1969. Internat. J. Leprosy 38 (1970) 331-335.
- OZAWA, T. SHEPARD, C. C. and KARAT, A. B. A. Application of spectrophotflurometric procedures to some problems in *Mycobacterium leprae* infections in mice and man treated with dapsone (DDS), diacetyl-DDS (DADDS), and di-formyl-DDS (DFD). Am. J. Trop. Med. Hyg. 20 (1971) 274-281.
- PETERS, J. H., GORDON, G. R., BICCS, J. T. and LEVY, L. The disposition of dapsone and monoacetyldapsone in laboratory animals. Internat. J. Leprosy 39 (1971) 101-102.
- PETERS, J. H. and LEVY, L. Dapsone acetylation: Another example of polymorphic actylation. Ann. N. Y. Acad. Sci. 179 (1971) 660-666.
- PETERS, J. H., LEVY, L., TOLENTINO, J. G., JACOBSON, R. R., ENNA, C. D. and KIRCHHEIMER, W. F. Dapsone acetylation in man: Therapeutic implications. Internat. J. Leprosy. 40 (1972) 93.
- 20. PETERS, J. H. Personal communication.
- PETTIT, J. H. S. and REES, R. J. W. Sulphone resistance in leprosy. An experimental and clinical study. Lancet 2 (1964) 673-674.
- PETTIT, J. H. S. and REES, R. J. W. Studies on sulfone resistance in leprosy. 2. Treatment with a riminophenazine derivative (B.663). Internat. J. Leprosy 34 (1966) 391-397.
- 23. POOLE, G., STRADLING, P. and WORL-LEDGE, S. Potentially serious side effects of high-dose twice-weekly rifampicin. Brit. Med. J. 3 (1971) 343-347.
- REES, R. J. W., PEARSON, J. M. H. and WATERS, M. F. R. Experimental and clinical studies on rifampicin in treatment of leprosy. Brit. Med. J. 1 (1970) 89-92.
- RUSSELL, D. A., SHEPARD, C. C., MCRAE, D. H., SCOTT, G. C. and VINCIN, D. R. Treatment with 4,4'-diacetyldiaminodiphenylsulfone (DADDS) of leprosy patients in the Karimui, New Guinea. Am. J. Trop. Med. Hyg. 20 (1971) 495-501.
- SHEPARD, C. C. A kinetic method for the study of the activity of drugs against *Mycobacterium leprae* in mice. Internat. J. Leprosy 35 (1967) 429-435.

318

- 27. SHEPARD, C. C. Further experience with the kinetic method for the study of drugs against Mycobacterium leprae in mice. Activities of DDS, DFD, ethionamide, capreomycin, and PAM 1392. Internat. J. Leprosy 37 (1969) 389-397.
- 28. SHEPARD, C. C. A survey of the drugs with activity against M. leprae in mice. Internat. J. Leprosy 39 (1971) 340-348.
- 29. SHEPARD, C. C. Rapid bactericidal effect of rifampin on M. leprae. Presented at Seventh Annual Leprosy Research Conference, Menlo Park, Calif., Feb. 29-March 1, 1972.
- SHEPARD, C. C. Unpublished results.
 SHEPARD, C. C. and CHANG, Y. T. Effect of several anti-leprosy drugs on multiplication of human leprosy bacilli in foot pads of mice. Proc. Soc. Exp. Biol. Med. 109 (1962) 636-638.
- 32. SHEPARD, C. C. and CHANG, Y. T. Ac-tivity of antituberculosis drugs against Mycobacterium leprae. Studies with experimental infection of mouse footpads. Internat. J. Leprosy 32 (1964) 260-271.
- 33. SHEPARD, C. C., LEVY, L. and FASAL, P. The death of Mycobacterium leprae during treatment with 4,4'-diaminodiphenylsulfone (DDS). Am. J. Trop. Med. Hyg. 17 (1968) 769-775.
- 34. SHEPAFD, C. C., LEVY, L. and FASAL, P. The sensitivity to dapsone (DDS) of isolates of Mycobacterium leprae from patients with and without previous treatment. Am. J. Trop. Med. Hyg. 18 (1969) 258-263.
- 35. SHEPARD, C. C., LEVY, L. and FASAL, P. The death of Mycobacterium leprae during treatment of lepromatous leprosy with acedapsone (DADDS). Am. J. Trop. Med. Hyg. 21 (1972) 440-445.
- 36. SHEPARD, C. C., LEVY, L. and FASAL, P. Rapid bactericidal effect of rifampin on

Mycobacterium leprae. Am. J. Trop. Med. Hyg. 21 (1972) 446-449.

- SHEPARD, C. C., MCRAE, D. H. and 37. HABAS, J. A. Sensitivity of Mycobacterium leprae to low levels of 4,4'-diaminodiphenyl sulfone. Proc. Soc. Exp. Biol. Med. 122 (1966) 893-896.
- 38. SHEPARD, C. C., TOLENTINO, J. G. and MCRAE, D. H. The therapeutic effect of 4,4'-diacetyldiaminodiphenylsulfone (DADDS) in leprosy. Am. J. Trop. Med. Hyg. 17 (1968) 192-201.
- 39 SHEPARD, C. C., WALKER, L. L., VAN LANDINGHAM, R. M. and REDUS, M. A. Kinetic testing of drugs against Mycobacterium leprae in mice. Activity of cephaloridine, rifampin, streptovaricin, vadrine and viomycin. Am. J. Trop. Med. Hyg. 20 (1971) 616-620.
- SHEPARD, C. C., WALKER, L. L., VAN LANDINGHAM, R. M. and REDUS, M. A. 40 Discontinuous administration of clofazimine (B663) in Mycobacterium leprae infections. Proc. Soc. Exp. Biol. Med. 137 (1971) 725-727.
- 41. SLOAN, N. R., WATH, R. M., JANO, B., FASAL, P. and SHEPARD, C. C. Repository acedapsone in leprosy chemoprophylaxic and treatment. Lancet 2 (1971) 525-526.
- 42. STEWART, R. B. and CLUFF, L. E. A review of medication errors and compliance in ambulant patients. Clin. Pharmacol. Ther. 13 (1972) 463-468. WATERS, M. F. R. and REES, R. J. W.
- 43. Changes in the morphology of Mycobacterium leprae in patients under treatment. Internat. J. Leprosy 30 (1962) 266-277.
- 44. WATERS, M. F. R., REES, R. J. W. and ELLARD, G. A. Experimental and clinical studies on the minimum inhibitory concentration (MIC) of dapsone (DDS) in leprosy. Internat. J. Leprosy 36 (1968) 651.

41, 3