Susceptibility of Mycobacterium leprae to Dapsone as a Determinant of Patient Response to Acedapsone

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In the course of a clinical trial of acedapsone therapy in 17 patients with lepromatous leprosy, the rate of response to therapy was measured by inoculation of mice with Mycobacterium leprae recovered from biopsy specimens of skin lesions obtained before treatment and at intervals of 4, 12, and 24 weeks after institution of treatment. The susceptibility of each isolate of M. leprae to dapsone (4,4'-diaminodiphenylsulfone [DDS]) was measured by passaging organisms that had multiplied in mice to new groups of untreated mice and to mice treated with DDS incorporated in the mouse chow in concentrations of 10⁻³, 3 × 10⁻³, and 10⁻⁴ g/100 ml. The rate of response to acedapsone therapy and the susceptibility of patient strains of M. leprae to DDS varied widely among patients. All isolates were inhibited from multiplication by treatment of mice with 10⁻⁴ g of DDS per 100 ml; all but two isolates were susceptible to 3 × 10⁻³ g of DDS per 100 ml; and 17 of 36 isolates, representing nine patient strains, were susceptible to 10⁻⁴ g of DDS per 100 ml. Plasma levels of DDS measured in the mice administered these diets show that the minimal inhibitory concentration of DDS for M. leprae isolated from untreated patients is about 3 ng/ml. No relationship could be demonstrated between DDS susceptibility of pretreatment isolates of M. leprae and the rate at which patients responded to acedapsone therapy. Neither acedapsone treatment of patients nor DDS treatment of mice appeared to select genotypically more resistant M. leprae.

Acedapsone (4,4'-diacetamidodiphenylsulfone [DADDS]) is a relatively insoluble derivative of dapsone (4,4'-diaminodiphenylsulfone [DDS]) which produces a prolonged low plasma level of DDS after intramuscular administration (3). First developed for use in malaria chemoprophylaxis (27), DADDS was found to be effective against Mycobacterium leprae in footpad infection in mice (16) and in patients with lepromatous leprosy (21, 25), to whom the drug was administered intramuscularly at a dosage of 225 mg every 11 weeks. This dosage of DADDS yielded a maximal plasma DDS concentration of 50 to 80 ng/ml 1 to 5 weeks after the dose; plasma DDS levels fell to a minimum of 30 to 60 ng/ml 11 weeks after the dose (10, 13). Even these low levels of DDS would be expected to produce a chemotherapeutic result, because they are substantially higher than the minimal inhibitory concentration of DDS for M. leprae. This has been established at less than 10 ng/ml of plasma in studies with mice (2, 10) and rats (11) infected with M. leprae. However, the DDS levels obtained after DADDS administration are much lower than the maximal levels of 2,000 ng/ml of plasma found in patients receiving oral doses of 100 mg of DDS (11).

A trial of DADDS therapy in 10 patients with previously untreated lepromatous leprosy showed that, in terms of the rate of killing of M. leprae, three patients responded as rapidly and seven patients responded more slowly than did patients receiving 50 mg of DDS daily (21). The staging of a trial of DADDS therapy in Cebu, the Philippines, among patients with previously untreated lepromatous leprosy provided the opportunity for a prospective study of possible determinants of the rate of response of patients during treatment with DADDS. (In a collaborative effort of the U.S. Leprosy Panel and the Leonard Wood Memorial, the efficacy of DADDS [225 mg intramuscularly every 77 days] has been compared with that of DADDS [225 mg] plus oral rifampin [1,500 mg] every 77 days and with that of oral rifampin [600 mg] daily.) In this paper, the results of a study of the relationship between the rate at which pa-

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Materials and Methods

Skin biopsy specimens from 17 patients with lepromatous leprosy participating in a clinical trial of DADDS in Cebu were obtained before treatment was begun and at intervals of 4, 12, and 24 weeks after beginning treatment with DADDS at a dosage of 225 mg intramuscularly every 11 weeks. Portions of the skin biopsy specimens were shipped by air on ice to San Francisco, California.

Rate of response to DADDS. The response of patients with lepromatous leprosy to treatment with DADDS was measured both in Cebu and in San Francisco in terms of the rate at which M. leprae were killed, i.e., rendered noninfectious for mice, during therapy. This procedure has been described previously (6, 19, 21–23, 27). In brief, all pretreatment specimens contained organisms infectious for mice; this was a prerequisite for admitting patients to the trial. In terms of their response to therapy, the patients may be divided into four groups. (i) Group 1 includes patients no. 1, 5, 11, and 37; no multiplication of M. leprae occurred in mice inoculated with organisms recovered from any of the skin biopsy specimens obtained 4, 12, or 24 weeks after beginning DADDS treatment. (ii) Group 2 includes patients no. 12, 13, 18, and 30; organisms multiplied in mice after 4 weeks but not after 12 or 24 weeks. (iii) Group 3 includes patients no. 4, 25, 29, 41, 44, 49, and 50; organisms multiplied in mice after 4 and 12 weeks but not after 24 weeks. (iv) Group 4 includes patients no. 20 and 34; organisms recovered from skin biopsy specimens obtained from these patients after 4, 12, and 24 weeks multiplied in mice.

Measurement of susceptibility to DDS. The technique for measuring the susceptibility of strains of M. leprae to DDS was a combination of Shepard's "continuous" and "kinetic" methods (18). When M. leprae inoculated in our laboratory were found to have multiplied in mice, a passage was made of 5 × 10⁶ organisms into each hind footpad of 60 locally bred male BALB/c mice, divided into four groups of 15. One of the groups was left untreated; the other three groups received chow containing DDS in concentrations of 10⁻³, 3 × 10⁻³, and 10⁻⁴ g/100 ml for about 150 days, beginning on the day of inoculation. Both the diets and water were allowed ad libitum. Harvests of M. leprae were made from pools of four footpads of the untreated mice about 100 and again about 120 days after inoculation. Just before drug administration was stopped, organisms were harvested from pools of eight footpads of the treated mice; additional harvests were made from the treated mice at intervals thereafter. The methods by which mouse tissues were processed and the mice were inoculated and harvested have been described previously (15, 24).

Monitoring DDS dosage to mice. The DDS-containing chow was prepared by the addition of appropriate quantities of a 1% solution of DDS (K & K Laboratories, Inc., Hollywood, Calif.) in 95% ethanol to 5-kg batches of commercially prepared mouse meal (Wayne Lab-Blox, Allied Mills, Inc., Chicago, Ill.) in a liquid-solid twin-shell blender (Patterson-Kelly Co., East Stroudsburg, Pa.). Drug-containing mouse chow was stored at 4 C. At first, the dosages of DDS to the mice were monitored by three means. Firstly, each batch of DDS-containing diet was sampled at the time of preparation; these samples were frozen and stored at −70 C for later analysis. Secondly, at the time of the 150-day harvest of M. leprae from DDS-treated mice, samples of the diets were removed from the cages and stored frozen. And thirdly, the four mice killed for each 150-day harvest were bled by heart puncture, and the heparinized plasma was separated, pooled, and stored frozen for later analysis. For the greater part of this study, DDS dosage to the mice was monitored by means of the plasma levels of DDS.

Levels of DDS in plasma were determined by a chromatographic-fluorometric technique (9) which was recently modified (8) to obtain a sensitivity of 0.1 ng of DDS per ml of plasma. Levels of DDS in diets were determined colorimetrically after double extraction by using a modification (14) of the method of Bratton and Marshall (1). DDS was extracted from 10-g portions of the powdered diets by shaking with 100 ml of 1 N HCl. After removal of the insoluble material by centrifugation, 75 ml of the acid extract was neutralized with 8 ml of ice-cold 10 N NaOH, and the mixture was extracted with 25 ml of ethylene dichloride. After phase separation by centrifugation, the upper aqueous layer was removed by aspiration. The organic phase was then washed twice with 5 ml of 0.1 N NaOH, removing the aqueous phase each time after shaking and centrifugation to separate the phases. Finally, the DDS was extracted from 20 ml of the ethylene dichloride solution into 1.5 ml of 2 N HCl, and 1.0 ml of the acid extract was diazotized and coupled. The practical limit of sensitivity was 1,000 ng of DDS/10 g of diet, which corresponds to a diet containing 10⁻³ g of DDS per 100 ml. The extractability of DDS from diets was the same as from water. All assays were performed in duplicate; the variability in the procedure was ±2.1%.

Results

Dosage of DDS. As a preliminary study, DDS concentrations were measured during the first 3 months of this study in the batches of mouse chow as prepared, in the chow recovered from the mouse cages at the time mice were sacrificed for the 150-day harvests, and in the plasma of the mice sacrificed for harvests. The results of these analyses, summarized in the second, third, and fourth columns of Table 1, show that the concentrations of DDS in the mouse chow recovered from the cages averaged 60 to 100% of expected, whereas the average concentration measured in the batches of prepared diets was that expected. The relationship of plasma levels of DDS in mice to the concen-
trations of DDS measured in the batches of prepared diets and found in the mouse chow recovered from the cages was examined by a linear regression technique (4). The results are shown in the footnotes to Table 1, in which the regression equations are presented in the form: \( y = \bar{y} + (b \pm t_{0.08}SD)(x - \bar{x}) \). The regression of \( \log_{10} \) plasma DDS level (\( y \)) on \( \log_{10} \) DDS concentration in the chow recovered from the mouse cages (\( x \)) was parallel to that of \( \log_{10} \) plasma DDS level on \( \log_{10} \) DDS concentration in the batches of prepared diets, but significantly different from it except at \( x = -4.0 \) (dietary concentration, \( 10^{-4} \) g/100 ml).

A number of studies directed toward ascertaining the explanation for the uniformly decreased levels of DDS in the samples of mouse chow recovered from the cages yielded inconsistent results. We found no evidence that the DDS concentrations declined with storage of the diets at 4 C. Because the powder feeders in the mouse cages were emptied, cleaned, and refilled twice weekly, none of the diets remained in the cages for longer than 4 days. DDS concentrations did not appear to decrease during this time at room temperature. There was no important variation of DDS concentration from batch to batch. The dosage of DDS to the mice was therefore monitored by measurement of plasma DDS levels.

Analysis of the DDS concentration of 110 plasma samples obtained from mice sacrificed for the 150-day harvests yielded values that were not different from the plasma levels found in the preliminary study (column 4, Table 1). The linear regression of \( \log_{10} \) plasma DDS concentration on \( \log_{10} \) DDS concentration measured in the batches of mouse chow as prepared compares well with that calculated from the data summarized by Ellard et al. (2), as shown by the plots in Fig. 1.

The variation of the plasma concentrations of DDS from month to month during the current study is shown in Fig. 2. Although the plasma DDS concentrations may be noted to vary within the range -50% and +100% of the mean, no trend with time is discernible. There is virtually no overlap of plasma DDS concentrations between the groups of mice receiving different dosages of DDS.

Susceptibility of M. leprae to DDS. The susceptibility to DDS of 17 pretreatment isolates of M. leprae was measured in mice treated with \( 10^{-5} \), \( 3 \times 10^{-5} \), or \( 10^{-4} \) g of DDS per 100 ml. The method by which the effects of DDS treatment on bacterial multiplication were used to evaluate susceptibility to DDS is exemplified by the results of studies in patients no. 5 (a patient from group 1, organisms noninfective for mice after only 4 weeks of treatment with DADD) and no. 29 (a group 3 patient, organisms multiplied in mice after 4 and 12 weeks of DADD treatment but not after 24 weeks). These results are presented in graphic form in Fig. 3. The organisms isolated from the pretreatment biopsy specimen of patient no. 5 had multiplied to the level of \( 10^{8} \) acid-fast bacilli 119 days after the inoculation of untreated mice with \( 10^{7} \) organisms/footpad (Fig. 3). A harvest of M. leprae from the footpad tissues of mice administered \( 10^{-5} \) g of DDS per 100 ml yielded \( 10^{8} \) organisms/footpad on day 154, the day treatment was stopped. On that same day, only \( 10^{11} \) organisms were recovered in a harvest from mice treated with \( 3 \times 10^{-5} \) g of DDS per 100 ml, and less than \( 10^{9} \) organisms/footpad were recovered from mice fed \( 10^{-5} \) g of DDS per 100 ml. These results indicate that patient no.
5's organisms were susceptible to $10^{-4}$ and $3 \times 10^{-5}$ g of DDS but not to $10^{-5}$ g of DDS per 100 ml.

The *M. leprae* isolated from the pretreatment specimen of patient no. 29 proved more susceptible to DDS. These had multiplied to the level of $10^6$/footpad in untreated mice by day 150, the day drug administration was stopped, whereas no multiplication was found in the mice fed any of the DDS-containing diets.

The susceptibility of an organism to the action of an antimicrobial drug is traditionally measured in terms of the ability of the drug to inhibit multiplication of the organism. The data presented in Fig. 3 suggest a second criterion of susceptibility. When multiplication of *M. leprae* has been inhibited during administration of a drug, multiplication of the organisms may resume immediately after the cessation of drug treatment, or only after some delay longer than can be accounted for by the presence of effective concentrations of the drug in the mouse, or not at all. Once treatment has been withdrawn, multiplication of *M. leprae* will resume immediately if the effect of the drug is primarily bacteriostatic, whereas no multiplication may occur if the drug has exerted a bactericidal effect. A delay between cessation of treatment and resumption of multiplication may result from killing of a portion of the inoculum, with only the surviving fraction giving rise to later multiplication. Alternatively, delayed multiplication may represent prolonged bacteriostasis, with later multiplication arising from the entire bacterial population (5). Shepard has shown (17) that the duration of the delay is proportional to the dosage of DDS, suggesting that the delay between cessation of DDS treatment of mice and resumption of multiplication of *M. leprae* may represent a useful criterion of susceptibility of the organism to the drug.

In Fig. 3, multiplication of *M. leprae* may be noted to have resumed without delay in the case of two growth curves, that of patient no. 5's organisms in mice treated with $3 \times 10^{-5}$ g of DDS per 100 ml and that of patient no. 29's organisms in mice treated with $10^{-5}$ g of DDS per 100 ml, whereas multiplication resumed only after some delay in both groups of mice treated with $10^{-4}$ g of DDS per 100 ml and in mice infected with organisms from patient no. 29 and treated with $3 \times 10^{-5}$ g of DDS per 100 ml. The time of resumption of multiplication is estimated by extrapolating the growth curve to the level of $5 \times 10^5$ acid-fast bacilli/footpad, the size of the inoculum; the duration of the delay...
Fig. 2. Results of analysis of DDS concentration in 110 pools of plasma, each pool representing four mice, as a function of the calendar month during which each sample was obtained. The closed circles represent mice administered 10^-5 g of DDS per 100 ml, the triangles mice administered 3 \times 10^{-5} g of DDS per 100 ml, and the squares mice administered 10^{-4} g of DDS per 100 ml. The solid lines connect the means for each diet at each time interval.

Fig. 3. Studies of the susceptibility to DDS of pretreatment isolates of M. leprae from patients no. 5 (left) and 29 (right). The number of acid-fast bacilli per footpad is shown as a function of the time from inoculation of passage mice. Symbols: ●, control mice; ○, mice administered 10^{-5} g of DDS per 100 ml; ×, mice administered 3 \times 10^{-5} g of DDS per 100 ml; +, mice administered 10^{-4} g of DDS per 100 ml. The shaded bars along the abscissae represent the period of DDS administration to the mice. The solid circles on each ordinate represent the inoculum (5 \times 10^6 acid-fast bacilli/footpad). No organisms were found in the harvests represented by the points with the downward extending arrows; the points were calculated as if one organism had been found in the counting procedure.
is then the number of days between cessation of DDS administration and resumption of multiplication of M. leprae. 

Multiplication of patient no. 5's organisms may be noted to have begun without apparent delay when treatment with $3 \times 10^{-4}$ g of DDS per 100 ml was stopped. On the other hand, multiplication after cessation of treatment with $10^{-4}$ g of DDS per 100 ml began only after a delay of 91 days. Harvests of M. leprae isolated from the pretreatment biopsy specimen of patient no. 29 showed that multiplication began without delay once treatment with $10^{-5}$ g of DDS per 100 ml was stopped, whereas there was a delay of 37 days after treatment with $3 \times 10^{-5}$ g of DDS per 100 ml was stopped before multiplication of M. leprae began; and multiplication was delayed for 52 days after stopping treatment with $10^{-4}$ g of DDS per 100 ml. These results suggest that the susceptibility of an isolate of M. leprae to DDS might be characterized both by the level to which the organisms had multiplied during treatment with a given dosage of DDS and by the duration of delay between cessation of treatment and resumption of multiplication.

The results of the studies of DDS susceptibility and the relationship between the rate at which patients responded to DADDS and the susceptibility of patient strains of M. leprae to DDS are summarized in Table 2, in which the susceptibility to DDS of the strains of M. leprae is compared for the four groups of patients. Patient groups are ranked according to the rate of response; group 1 patients are those responding most rapidly and group 4 patients are those responding most slowly to DADDS treatment. The results of the studies in patients no. 5 and 29 are included in this table.

The data of Table 2 suggest a broad range of susceptibility to DDS among isolates of M. leprae from patients with previously untreated lepromatous leprosy. Eight of 17 patient strains of M. leprae (column 3) were inhibited from multiplication to the level of $10^0$ organisms/footpad by treatment of mice with $10^{-5}$ g of DDS per 100 ml; these strains may be considered susceptible to this dosage. On the other hand, M. leprae of two patient strains multiplied at least 20-fold (i.e., to $10^9$ organisms/footpad) but less than 100-fold (i.e., $<5 \times 10^5$ organisms/footpad) during treatment of mice with $10^{-5}$ g of DDS per 100 ml (column 4); these strains may be considered partially susceptible.

Finally, the organisms of seven strains (also column 4) underwent at least 100-fold multiplication from the standard inoculum of $5 \times 10^5$ organisms/footpad during administration of the DDS in the smallest dosage. The organisms of only two patient strains demonstrated greater than 20-fold but less than 100-fold multiplication during treatment with $3 \times 10^{-5}$ g of DDS per 100 ml, indicating partial susceptibility to this dosage of DDS. As already noted, the organisms of all patient strains were susceptible to DDS administered in a dosage of $10^{-4}$ g/100 ml.

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**Table 2. Rate of response to DADDS therapy and susceptibility of pretreatment isolates to DDS**

| Patient group | No. of patients | No. of isolates multiplying with following characteristics | M. leprae per footpad after 150 days of treatment with DDS | Delay between withdrawal of DDS and resumption of multiplication (days) |
|---------------|-----------------|----------------------------------------------------------|----------------------------------------------------------|
|               |                 |                                                          | $10^{-4}$ g/100 ml$^b$ | $3 \times 10^{-4}$ g/100 ml | $10^{-3}$ g/100 ml | $10^{-2}$ g/100 ml | $3 \times 10^{-2}$ g/100 ml | $10^{-1}$ g/100 ml |
|               |                 |                                                          | <10$^3$         | $\geq 10^3$ | $<10^4$         | $\geq 10^4$ | $<10^5$         | $\geq 10^5$ | $<30$ | $\geq 30$ | $<30$ | $\geq 30$ | $<30$ |
| 1             | 4               | $2^c$                                                   | 2              | 2              | 2              | 2              | 1              | 1              | 2              | 2              | 2              | 2              |
| 2             | 4               | 3                                                       | 1              | 1              | 1              | 1              | 1              | 1              | 1              | 1              | 1              | 1              |
| 3             | 7               | $4^c$                                                   | 4              | 4              | 4              | 4              | 4              | 4              | 4              | 4              | 4              | 4              |
| 4             | 2               | 1                                                       | 1              | 1              | 2              | 2              | 2              | 2              | 2              | 2              | 2              | 2              |

$^a$ Patients grouped according to rate of response as defined in Materials and Methods.

$^b$ Amount of DDS.

$^c$ One of these two strains actually multiplied to $7.34 \times 10^8$ M. leprae/footpad.

$^d$ These strains actually multiplied at least to $5 \times 10^9$ organisms/footpad.
The number of *M. leprae* per footpad in the harvests performed from the pooled tissues of eight footpads just before stopping the 150-day course of treatment with 10^-5 g of DDS per 100 ml appears to be a useful criterion of susceptibility, because it discriminates between more and less susceptible strains of *M. leprae*. However, criteria based on the multiplication of *M. leprae* during treatment with the two larger doses of DDS would not be useful, and a more discriminating criterion is needed. Columns 9 and 10 of Table 2 show that the resumption of multiplication of *M. leprae* from no specimen was delayed as long as 30 days after cessation of treatment with 10^-3 g of DDS per 100 ml. On the other hand, as shown in the last four columns of this table, the time between cessation of treatment with 3 x 10^-5 g of DDS per 100 ml and resumption of multiplication was found to vary among the patient strains and represents, therefore, a more discriminating criterion. The results of treatment of mice with 10^-4 g of DDS per 100 ml are too uniform to be useful in distinguishing more susceptible from more resistant strains of *M. leprae*.

The measurements of susceptibility to DDS produced results that appear internally consistent. There was good correspondence between the two criteria of susceptibility to DDS, the number of organisms per footpad at the end of the period of administration of 10^-3 g of DDS per 100 ml and the duration of the delay before multiplication of *M. leprae* began after cessation of administration of 3 x 10^-5 g of DDS per 100 ml (Table 2). Only one of eight isolates multiplying to a level lower than 10^6 acid-fast bacilli/footpad during treatment with 10^-2 g of DDS per 100 ml (an isolate from a group 2 patient) multiplied with a delay shorter than 30 days after withdrawal of 3 x 10^-5 g of DDS per 100 ml, whereas seven of nine isolates multiplying to a level of at least 10^6 acid-fast bacilli/footpad during treatment with 10^-3 g of DDS per 100 ml began to multiply within 30 days after cessation of treatment with 3 x 10^-5 g of DDS per 100 ml. The probability that this distribution could have been encountered by chance is less than 0.015 (4).

The four most rapidly responding patients (those of group 1) appear to harbor some of the least susceptible strains, whereas one of the two most slowly responding patients (from group 4) harbored a strain of *M. leprae* that proved quite susceptible to DDS by both criteria (Table 2). There appears, therefore, to be no relationship between the rate of response of patients to treatment with DADDS and the susceptibility of the patient strains of *M. leprae* to DDS in mice. Alternative explanations of the variation of the rate of response among patients will be considered below.

**Change of DDS susceptibility during DADDS treatment.** This study provided an opportunity to examine the effect of treatment on the susceptibility to DDS of the *M. leprae* that survived treatment. Nineteen isolates of *M. leprae* were obtained 4, 12, or 24 weeks after beginning DDS treatment of the 13 patients in groups 2, 3, and 4. These specimens are identified in Table 3. The tests of DDS susceptibility were carried out only on *M. leprae* isolated in San Francisco, whereas measurement of the rate of response to DADDS was based on isolations of *M. leprae* both in Cebu and in San Francisco; therefore, the number of post-treatment isolates tested is sometimes smaller than the number of patients.

The susceptibility to DDS of the 19 isolates of *M. leprae* recovered during DADDS treatment of the 13 group 2, 3, and 4 patients was measured. In Table 4, the susceptibility of each post-treatment isolate is compared with that of the corresponding pretreatment isolate at each dietary level of DDS. The pretreatment isolates have been pooled without regard for the patients' response group. The post-treatment isolates have also been pooled without regard for the patient group and without attempting to distinguish among multiple isolates from the same patient. The *M. leprae* isolated before treatment from eight of the 13 patients multiplied less than 20-fold (to <10^5/footpad) during treatment of mice with 10^-2 g of DDS per 100 ml (Table 4). Twelve isolates were made from these patients during DADDS treatment, of which eight multiplied to <10^6 *M. leprae*/footpad and four to at least 10^6 organisms/footpad during treatment of mice with 10^-3 g of DDS per 100 ml. The pretreatment isolates from five patients multiplied at least 20-fold during treatment of

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**Table 3. Change of susceptibility to DDS of *M. leprae* isolated during treatment with DADDS for 4, 12, or 24 weeks: identity of specimens**

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. of patients</th>
<th>No. of post-treatment isolates tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 weeks</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

*Patients grouped according to rate of response as defined in Materials and Methods.*
mice with $10^{-5}$ g of DDS per 100 ml; of the seven post-treatment isolates from these patients, only one multiplied less than 20-fold during treatment of mice with the same concentration of DDS. The probability that this distribution of post-treatment isolates could have occurred by chance is $<0.05$ (4). Thus, post-treatment isolates of *M. leprae* were likely to exhibit the same degree of susceptibility to DDS as did the corresponding pretreatment isolates.

The susceptibility of *M. leprae* isolated before and during DADDS treatment to $3 \times 10^{-5}$ g of DDS per 100 ml is compared in terms of the duration of the delay between withdrawing DDS treatment of the mice and the resumption of multiplication of *M. leprae* (Table 4). Here, too, the patients whose pretreatment isolates were more susceptible to DDS (delay $>30$ days) were likely to have more susceptible organisms isolated during treatment. The likelihood that this distribution of post-treatment isolates of *M. leprae* could have occurred by chance is smaller than 0.01. As was the case with the pretreatment isolates of *M. leprae*, the *M. leprae* isolated during DADDS treatment were almost uniformly susceptible to $10^{-4}$ g of DDS per 100 ml in the mouse diet (Table 4).

In summary, there was no striking or consistent change of DDS susceptibility of *M. leprae* during treatment of patients with DADDS. Certainly, no progressive change of DDS susceptibility was seen in the specimens from patients yielding more than one isolation of *M. leprae* during treatment with DADDS.

This study also provided an opportunity to retest the susceptibility to DDS of *M. leprae* that had been inhibited from multiplication during treatment of mice with DDS. Four strains of *M. leprae* from a patient from each response group, two of them pretreatment isolates and two isolates recovered after 4 weeks of DADDS treatment, were reisolated from DADDS-treated mice after DDS treatment of the mice had been terminated and the organisms had resumed multiplication.

In Table 5, the susceptibility of the reisolated *M. leprae* is compared with that measured in the original test at each dietary level of DDS. Organisms that had multiplied after cessation of treatment of mice with $10^{-4}$ g of DDS per 100 ml (the strain of patient no. 5), for example, were as susceptible to DDS as the original isolate. The results of this study (Table 5) demonstrate no consistent change in susceptibility to

### Table 4. Change of susceptibility to DDS of *M. leprae* isolated during treatment with DADDS for 4, 12, or 24 weeks: comparison of pre- and post-treatment isolates

<table>
<thead>
<tr>
<th>No. of <em>M. leprae</em>/footpad after 150 days of treatment with $10^{-4}$ g of DDS per 100 ml</th>
<th>No. of days elapsed between cessation of DDS treatment and resumption of multiplication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$3 \times 10^{-4}$ g/100 ml</td>
</tr>
<tr>
<td></td>
<td>$\geq 30$ g/100 ml</td>
</tr>
<tr>
<td></td>
<td>$\geq 30$</td>
</tr>
<tr>
<td>Pre</td>
<td>Post</td>
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<td>---</td>
<td>---</td>
</tr>
<tr>
<td>8*</td>
<td>8</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

* Number of isolates multiplying with characteristic shown.

### Table 5. Change of susceptibility to DDS of *M. leprae* passaged through DDS-treated mice: comparison of pre- and postpassage isolates

<table>
<thead>
<tr>
<th>Patient (group)</th>
<th>Duration of DADDS treatment before original test (weeks)</th>
<th>No. of <em>M. leprae</em>/footpad ($\times 10^{9}$) after 150 days of treatment with $10^{-4}$ g of DDS per 100 ml</th>
<th>No. of days elapsed between cessation of DDS and resumption of multiplication</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$3 \times 10^{-4}$ g/100 ml</td>
<td>$10^{-4}$ g/100 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Original</td>
<td>Retest</td>
</tr>
<tr>
<td>5 (1)</td>
<td>0</td>
<td>7.34</td>
<td>2.48</td>
</tr>
<tr>
<td>13 (2)</td>
<td>4</td>
<td>1.42</td>
<td>3.18</td>
</tr>
<tr>
<td>4 (3)</td>
<td>0</td>
<td>6.17*</td>
<td>2.83</td>
</tr>
<tr>
<td>20 (4)</td>
<td>4</td>
<td>0.63*</td>
<td>2.06</td>
</tr>
</tbody>
</table>

* The organisms isolated from mice treated with this concentration of DDS were selected for retest of DDS susceptibility.
* ND, Not done.
DDS of *M. leprae* that have multiplied after withdrawal of DDS treatment. Certainly, there is no evidence that DDS treatment of mice selected genotypically more resistant individuals in the inoculum of *M. leprae*.

The previously unpublished data of some other work carried out in this laboratory are relevant to this presentation. Mice were inoculated with *M. leprae* of a strain that had been used for all of the drug studies carried out in this laboratory and found to be susceptible to $3 \times 10^{-5}$ g of DDS per 100 ml but not to $10^{-5}$ g of DDS per 100 ml. Treatment with $10^{-4}$ g of DDS per 100 ml was begun 75 days after inoculation and continued for the duration of the experiment. A harvest performed on day 509 showed that some multiplication of *M. leprae* had occurred. A harvest and passage were performed 77 days later. Multiplication of the organisms was inhibited by treatment of the passage mice with $10^{-4}$ g of DDS per 100 ml.

In a second experiment, mice were inoculated with the same strain of *M. leprae*, and $10^{-4}$ g of DDS per 100 ml was administered for 90 days beginning 60 days after inoculation. By day 322, the organisms were found to have multiplied and were harvested and passaged twice through untreated mice. Multiplication of the organisms in the third serial passage was inhibited by treatment of the mice with $10^{-4}$ g of DDS per 100 ml. Thus, treatment of mice with DDS did not appear to select genotypically more resistant strains of *M. leprae*.

**DISCUSSION**

In an earlier study (20), it was shown that *M. leprae* isolated from previously untreated patients with lepromatous leprosy were inhibited from multiplying in mice by administration to the mice of a diet containing $10^{-4}$ g of DDS per 100 ml. Some isolates were found to be inhibited by DDS administered in a concentration of $10^{-3}$ g/100 ml. The current study was carried out to learn if differences of DDS susceptibility among patient strains of *M. leprae*, all susceptible to $10^{-4}$ g of DDS per 100 ml, might explain differences of the rate at which *M. leprae* are killed during DADDS therapy of patients with previously untreated lepromatous leprosy.

The technique by which the susceptibility of strains of *M. leprae* to DDS was measured in the current study was a combination of Shepard's "continuous" and "kinetic" methods (18). In the continuous method, mice are treated from the day of inoculation, whereas in the kinetic method treatment is begun only after the beginning of logarithmic multiplication. The continuous method is, therefore, a more sensitive test. Because it requires the cessation of therapy after multiplication of the organisms in untreated mice has reached its maximum, the kinetic method permits a distinction between merely bacteriostatic and bactericidal activity. The use of the combined technique in this study provided two criteria by which the susceptibility to DDS of each strain could be evaluated. Beginning treatment on the day of inoculation amplified the effects of the smallest dosage of DDS. Because virtually all of the isolates were inhibited from multiplication by treatment of mice with the two larger dosages, use of the continuous method alone would have provided very little additional information. Withdrawal of treatment after about 150 days, on the other hand, permitted the distinction of degrees of susceptibility to the larger dosages of DDS.

Pretreatment isolates of *M. leprae* from the 17 patients in this study were all inhibited from multiplication by the administration to mice of $10^{-4}$ g of DDS per 100 ml. The susceptibility of the 17 patient strains of *M. leprae* to smaller concentrations of DDS was found to vary over a 10-fold range: eight strains were susceptible to $10^{-5}$ g of DDS per 100 ml; two strains were partially susceptible to $10^{-5}$ g of DDS per 100 ml and fully susceptible to $3 \times 10^{-5}$ g of DDS per 100 ml; five strains were resistant to $10^{-5}$ g of DDS per 100 ml and fully susceptible to $3 \times 10^{-5}$ g of DDS per 100 ml; and two strains were resistant to $10^{-5}$ g of DDS per 100 ml and partially susceptible to $3 \times 10^{-5}$ g of DDS per 100 ml.

A by-product of this study is the measurement of the minimal inhibitory concentration of DDS for 17 strains of *M. leprae* from previously untreated patients. The mean plasma concentration of DDS obtained by administering $3 \times 10^{-5}$ g of DDS per 100 ml in the mouse chow may be noted in Table 1 to be slightly smaller than 3 ng of DDS per ml. Because 15 of the 17 patient strains of *M. leprae* were susceptible to this plasma concentration of DDS, the results of this study confirm earlier estimates of the minimal inhibitory concentration of DDS for *M. leprae* (1 to 10 ng/ml in the mouse [2,10], and 1.5 to 4 ng/ml in the rat [11]).

The rate at which *M. leprae* were rendered noninfective for mice during treatment of these 17 patients with DADDS varied as much in this study as in an earlier trial of DADDS treatment of patients with previously untreated lepromatous leprosy (21). No correlation could be found, however, between the rate at which *M. leprae* were killed during DADDS treatment of patients and the susceptibility of the organisms to DDS in mice. Because variation of the
rate at which *M. leprae* were killed could not be explained by variation of the susceptibility of the organisms to DDS, some alternative explanation for the individual variation of the rate of response to DADDS must be sought. Peters et al. have studied the metabolism of DDS and DADDS in many of these same patients and have found no evidence of a relationship between the several parameters of drug metabolism studied and the rate of response to DADDS (12). An explanation is implicit in the description by Mitchison of studies in tuberculosis (7). These studies suggested that *M. tuberculosis* in stationary phase were more resistant to mild heat treatment (33 C for 60 min) than were organisms tested during logarithmic multiplication, and that tuberculosis patients whose sputum contained organisms more susceptible to heat treatment responded more rapidly to isoniazid than did patients whose sputum contained heat-resistant organisms. The technique by which the rate of response to DADDS was measured required repeated biopsies from the same lesion. All of the lesions in a patient with lepromatous leprosy do not appear to evolve at the same rate, some having been established for some time and others having only recently appeared at the time that the patient presents for treatment. It may well be that the rate of response truly measures the rate at which *M. leprae* are killed in a given lesion rather than in the patient as a whole, and that the response will be more rapid when new lesions (organisms in logarithmic phase) are sampled and less rapid when old lesions (organisms in stationary phase) are biopsied. Because the organisms obtained from one lesion are very likely genotypically identical with those of any other lesion of the same patient, one should not expect a good correlation between the susceptibility of the strain of *M. leprae* to DDS and the rate at which the organisms in a given lesion are killed during effective antimicrobial therapy.

That the *M. leprae* of a particular patient are genotypically identical is suggested by the results of the studies of DDS susceptibility after some period of DADDS therapy, or after passage through DDS-treated mice. An obvious shortcoming of the study of changes of DDS susceptibility during DADDS therapy is that the most complete information will become available from those patients who respond most slowly, whereas no organisms can be isolated for measurement of DDS susceptibility once DADDS therapy of the most rapidly responding patients has begun. Nevertheless, a comparison of the DDS susceptibility of isolates obtained later during DADDS therapy with that of the pretreatment isolates suggests that DADDS therapy does not select genotypically more resistant individuals in the bacterial population. The studies of the organisms recovered from DDS-treated mice yielded similar results. These results say nothing about DDS-resistant *M. leprae* (i.e., organisms that multiply in mice treated with $10^{-4}$ g of DDS per 100 ml), which are undoubtedly present in the untreated patient with lepromatous leprosy but in such small numbers that they are unlikely to be encountered among the small numbers of *M. leprae* recovered from skin biopsy specimens and inoculated into mice.

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**LITERATURE CITED**


