Prolongation of the Lag Phase of *Mycobacterium leprae* by Dapsone[1,2] (36123)

LOUIS LEVY

Leprosy Research Unit, Public Health Service Hospital, San Francisco, California 94118

In a previous report from this laboratory (1), evidence of an effect of dapsone (4, 4′-diaminodiphenylsulfone, DDS) on the lag phase of multiplication of *Mycobacterium leprae* in the mouse footpad was presented. In this earlier experiment, *M. leprae* were permitted to multiply almost to the "plateau level" of 10^6 organisms per footpad, at which time continuous feeding was begun to half of the animals of a meal containing 0.1% DDS. In order more certainly to ascribe the effect of DDS to lengthening of the lag phase of multiplication of *M. leprae*, we have studied the effects of brief periods of DDS treatment in the same experimental system as that used in the earlier work.

Methods. The methods by which the mice were inoculated with *M. leprae*, the organisms harvested, and the viability of the recovered organisms measured are those used in the earlier study (1). The experimental design was somewhat different from that described in the earlier report.

Male BALB/c mice ("experimental mice") were inoculated in the right hind footpad, each with 5×10^3 *M. leprae* originally isolated from a patient with lepromatous leprosy and subsequently carried in mouse passage. Harvests of *M. leprae* were performed from the pooled footpad tissues of 4 experimental mice at weekly intervals beginning 117 days after inoculation. Immediately following the 138-day harvest, these mice were divided randomly among several groups, which were treated as follows: a control group (Group A); DDS 0.1% in the mouse meal for 1 day (Group B); DDS for 3 days (Group C); and DDS for 7 days (Group D). Harvests were made from each of the groups after 1, 3, and 7 days, and at weekly or biweekly intervals thereafter, and organisms recovered from each harvest were passed to a group of 20 BALB/c mice ("passage mice"). Harvests were made periodically from passage mice, growth curves were constructed, and the time required for multiplication in passage mice from the standard inoculum (5×10^3 organisms per foot pad) to 10^6 per footpad ("time to plateau") was calculated.

Results. The recovery of *M. leprae* in each harvest is shown in Fig. 1 as the common

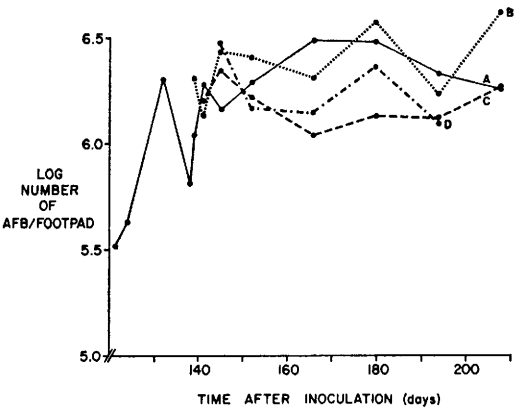

FIG. 1. Results of Experiment m 10-8-69. The common logarithm of the number of *M. leprae* recovered per footpad from experimental mice is shown as a function of the time after inoculation. Experimental mice were inoculated with 5×10^3 organisms per footpad on Day 0; the first harvest was performed 117 days later. The first harvest from Group B mice was performed after one day, that from Group C mice after 3 days, and that from Group D mice after 7 days of DDS treatment.

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[2] A preliminary report of this study was presented at the Sixth Annual Leprosy Research Conference, Atlanta, Georgia, March 1971.

logarithm of the number of acid-fast bacteria (AFB) per footpad for each group of experimental mice. Multiplication of *M. leprae* was somewhat more rapid than in the earlier experiment (1), reaching the plateau level on the 136th day (calculated from the harvests performed on untreated animals between the 117th and the 141st days after inoculation). Thus, the organisms were near or at the end of the logarithmic growth phase when DDS treatment was begun on the 138th day. The number of organisms changed very little during the remainder of the experiment.

The results of the mouse passages for the determination of viability of *M. leprae* appear in Table I and in Fig. 2. The time-to-

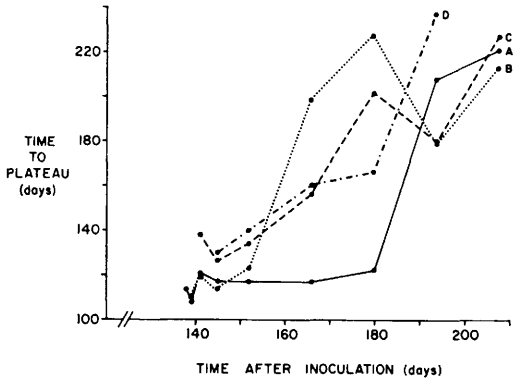

FIG. 2. Results of passages from Experiment m 10-8-69. The time required for the multiplication of *M. leprae* to the plateau level of 10^6 per footpad in passage mice is plotted for each passage as a function of the time from inoculation to harvest and passage from experimental mice. DDS administration to all except Group A mice began on day 138; administration was continued for one day only to Group B, for 3 days to Group C, and for 7 days to Group D mice.

plateau for Group A passages did not change until more than 180 days after inoculation, at which time there was an abrupt prolongation of the time-to-plateau.

Passages from Groups B–D yielded the same measurement for the time-to-plateau as did the Group A passages until the 152-day harvests; at this time, the Group D passage yielded a longer time-to-plateau than did the control passage. For the harvests made at 166 and 180 days, the passages from all three treated groups yielded longer times-to-plateau than did the Group A passages. At 194 days, the time-to-plateau for the Group D passages was longer, and that for the Groups B and C passages shorter than the control value. At 208 days, the times-to-plateau for Groups A–C were not significantly different; no Group D mice remained at 208 days, so that a passage could not be carried out.

Analysis of 71 growth curves of this strain of *M. leprae* is locally bred BALB/c mice, each growth curve based on two harvests yielding at least 10^5 AFB per footpad, has provided an estimate of 8 days for the standard deviation of the time-to-plateau calculated from a single harvest, assuming the doubling time to be 13 days (unpublished observations, 2). Thus, a difference between passages of 16 days for the time-to-plateau is significant at the 95% level of confidence.

In summary, the time-to-plateau for organisms passed from the untreated experimental mice remained unchanged from 138 to 180 days after inoculation. During the next 4 weeks, the time-to-plateau increased markedly, corresponding to a loss of more than 99% of the viable *M. leprae*. The time-to-plateau for organisms passed from treated experimental mice increased earlier than for those passed from untreated mice, but was the same at 208 days after inoculation. The prolongation of the time-to-plateau, relative to that for control organisms, was greatest for the organisms recovered from mice treated for only 1 day, and less marked for the organisms passed from mice treated for the longer periods of time.

Discussion. In the earlier experiment, mice were inoculated with 5000 *M. leprae* per footpad. The organisms were permitted to multiply almost to the plateau level, at which time feeding of a meal containing 0.1% dapsone was begun to half of the animals. Harvests were performed from footpad tissues of animals from both treated and control groups at frequent intervals, and groups of passage mice were inoculated with organisms from each harvest in order to measure their viability. The proportion of viable *M. leprae* decreased in successive harvests from untreated

TABLE I. Harvests From Passage Mice.

Group:	A			B			C			D		
Time after inoculation (days)	Time after passage (days)	No. AFB recovered ($\times 10^5$)	Time-to-plateau (days)	Time after passage (days)	No. AFB recovered ($\times 10^5$)	Time-to-plateau (days)	Time after passage (days)	No. AFB recovered ($\times 10^5$)	Time-to-plateau (days)	Time after passage (days)	No. AFB recovered ($\times 10^5$)	Time-to-plateau (days)
138	134	18.7	114									
139	133	38.6	108	133	33.7	110						
141	131	17.0	121	131	19.0	119	131	7.06	138			
							139	10.5	137			
145	133	23.0	117	133	26.9	114	133	14.7	126	133	11.8	130
152	133	23.7	117	133	17.3	123	133	9.71	134	133	6.96	140
166	126	16.7	117	126	0.44	—	126	0.72	—	126	1.30	164
				162	1.96	193	162	13.5	156	162	13.6	156
				189	5.06	202						
180	141	28.0	122	141	0.30	—	143	0.73	—	143	3.76	161
				168	1.21	208	168	1.53	203	168	8.66	171
				196	2.78	220	196	8.50	199			
				240	5.15	252						
194	147	0.15	—	147	1.90	178	147	1.38	184	147	0.15	—
	168	0	—	168	5.95	178	168	7.29	174	168	0.15	—
	203	7.96	207							203	1.76	236
208	147	0	—	147	0.53	—	147	0	—			
	218	9.14	220	218	14.0	212	218	6.32	226			

mice, as demonstrated by progressive lengthening of the time-to-plateau in passage mice. The time-to-plateau for organisms harvested from treated mice increased for successive harvests at the same rate as for organisms from untreated mice, but was constantly longer; this constant prolongation of the time-to-plateau was interpreted as lengthening of the lag phase (1).

That 0.1 %DDS administered continuously in the mouse diet produced prolongation of the lag phase of multiplication of *M. leprae* rather than death of the organisms was inferred from the demonstration (1) that the time-to-plateau for organisms passed from treated mice increased at the same rate as that for untreated mice, but was constantly longer by a period of time corresponding to the death of 90% of the viable *M. leprae* present in the inocula passed from untreated mice. The alternative explanation—that the remaining 10% of the viable organisms were resistant to this very large dose of DDS, and survived treatment to be killed by host defense mechanisms as in the untreated mice—was rejected on the grounds that this strain of *M. leprae* had been repeatedly demonstrated to be completely inhibited from multiplication by a concentration of DDS 1/1000 that used in the experiment. It was suggested that DDS so damaged the organisms that they were unable to resume multiplying as promptly after passage as was the case for the organisms from untreated mice.

This present experiment was designed to test this inference. If damage to the *M. leprae* produced a prolongation of the time-to-plateau (*i.e.*, a prolonged lag phase), a brief exposure to the drug might be expected to cause prolongation of the time-to-plateau relative to organisms from untreated mice soon after cessation of DDS treatment, whereas repair of the damage might enable a more prompt resumption of multiplication, again relative to the control organisms, when passages were made at longer intervals after withdrawal of the drug. This appears to have occurred. *M. leprae* passed soon after cessation of DDS treatment from mice treated for one to seven days yielded a longer time-to-plateau than did those organisms passed from untreated mice. On the other hand, organisms passed late after cessation of DDS treatment yielded the same time-to-plateau as did organisms passed from untreated mice.

Two anomalies are suggested by the results of this experiment. First, prolongation of the time-to-plateau for control passages did not occur at a constant rate over the entire period of the study, as was the case in the earlier experiment (1). Second, if the severity of the drug-produced damage to the organisms were either proportional to the duration of drug exposure or constant after some minimal effective period of exposure, one might have expected greater prolongation of the time-to-plateau after longer periods of DDS treatment. Yet the results of this experiment suggest that the most marked prolongation of the lag phase occurred with organisms passed from mice treated for only one day. Further work is required to explain these differences.

Conclusions. The results of this study support the inference drawn from an earlier experiment that DDS acts on the organisms to prolong their lag phase of multiplication. The time required for multiplication of *M. leprae* to the plateau level of 10^6 organisms per footpad was shown to be prolonged for early passages from untreated mice; later, the time-to-plateau had decreased for the passages of organisms from treated animals relative to that for the passages from untreated animals. If the differences between the time-to-plateau for control organisms and those for organisms harvested from treated mice may be assumed to represent the prolongation of the lag phase of *M. leprae* by DDS, the lag phase appears to have been prolonged only for the first 6 to 7 weeks after stopping drug administration, subsequently returning to that characteristic of the organisms recovered from untreated animals.

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