INTERNATIONAL JOURNAL OF LEPROSY

Volume 35, Number 4
Printed in U.S.A.

Drug Resistance of *Mycobacterium leprae* Particularly to DDS[1]

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The emergence of drug resistance is an important and common occurrence during the chemotherapy of nearly all bacterial diseases. Therefore drug resistance could be expected with the introduction of chemotherapy for leprosy, and it is not surprising that such resistance to several of the antileprosy drugs has been reported, for example, thiacetazone ([2, 10]), thiambutosine ([4]), ditophal ([3]), and isoniazid ([7]). However, despite the claim of Wolcott and Ross ([27]), it was still a matter of debate whether or not *Mycobacterium leprae* ever develops resistance to the sulfones, although these have been used for more than 20 years, in the treatment of millions of patients. Because very prolonged treatment is required in leprosy, relapse may result from patients' failure to take adequate treatment, rather than from the emergence of drug resistance. Unfortunately, all the evidence for drug resistance in leprosy has been based entirely on clinical grounds, since hitherto there has been no method for testing the drug sensitivity of *M. leprae*. Now, with the application of the mouse foot pad infection for determining the drug sensitivity of *M. leprae* ([19, 21, 23, 24]), this method can be used also for studying the emergence of drug resistance.

This paper reports the first successful application of these methods for the detection of dapsone (DDS)—and thiambutosine-resistant strains of *M. leprae*.

[1] Presented at Symposium on Sulfones, U. S.-Japan Cooperative Medical Science Program, San Francisco, California, 11 May 1967.

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METHODS

The methods used for harvesting and counting the bacilli from foot pads are the same as those previously described ([18, 22]). All the tests have been carried out on mice inoculated in one or both hind foot pads with 10^4 *M. leprae* obtained either directly from skin lesions in patients or from infections already established in the mouse foot pad. The chemotherapeutic activity of a drug has been assessed by comparing the yield of bacilli in the foot pads of untreated mice with that in mice treated with the drug, either fed in the diet or given by injection, from the first day of infection. Individual mice from the untreated group are killed, usually beginning six months after the infection, to establish that the infection has taken and that multiplication has resulted in yields of bacilli in the order of 10^5–10^6 bacilli/foot pad. When this level of infection has been reached, usually within a period of 6-10 months, depending on the viability of the inoculum, the remaining mice in the untreated group and all the mice in the treated groups are killed, for determination of the number of bacilli in their foot pads.

RESULTS

DDS resistance. Once it was established that multiplication in the mouse foot pad of *M. leprae* derived from untreated patients could be inhibited by drugs, this infection provided, for the first time, a specific test for detecting the emergence of drug-resistant strains of bacilli from treated patients. The first study of this type was on DDS resistance as determined in 10 selected patients with lepromatous leprosy who, despite 15 years or more of sulfone thera-

py, showed active disease with a high bacteriologic index (BI) and a high proportion of solidly staining bacilli (morphologic index, MI). Nine of the patients were from Malaysia ([16, 17]) and one was from England ([1]). Biopsy specimens were obtained from each patient, and, from these, bacilli were inoculated into mouse foot pads and tested against DDS. All strains were tested against 0.1 per cent DDS in the diet daily, and three were tested also at 0.025 per cent. The patients were started on a carefully controlled six-month test period on 300 mgm. DDS by injection twice weekly, initiated from the time the biopsy specimen was taken. Although the response of the patients during the test period on DDS was assessed clinically, histologically and bacteriologically, most weight was placed on the fall in the MI as the most sensitive measure of the chemotherapeutic activity of a drug. Studies ([26]) consistently demonstrated that previously untreated patients with lepromatous leprosy show a reproducible and significant diminution in the MI within a period of 4.5–6 months on standard doses of 300 mgm. DDS injected intramuscularly twice weekly.

Therefore it was considered that patients who failed to show a significant fall in the MI during this carefully controlled six-month trial period on DDS were likely to be infected with DDS-resistant strains of *M. leprae*. DDS estimations on blood and urine samples from these patients, taken during the test period, confirmed that absorption of the drug was satisfactory. The figures for the MI at the time of selection and after the six-month test period on DDS and the DDS sensitivity of *M. leprae* from the 10 patients with *prima facie* evidence of DDS resistance, are shown in Table 1. From the mouse foot pad tests five of the strains (cases 1, 2, 5, 7 and 9) proved sensitive to DDS, whereas the other five strains multiplied in the animals treated with DDS, i.e., four strains in mice fed 0.1 per cent in their diet and one strain (case 8) which, while being inhibited at this level, multiplied freely in animals fed 0.025

TABLE 1. *Morphologic index at time of selection and after a 6 month test period on DDS and DDS sensitivity of* M. leprae *from 10 patients with* prima facie *evidence of DDS resistance.*

Case No.[a]	Morphologic index		DDS sensitivity using mouse foot pad infection	
			Per cent DDS in diet	
	At time of selection	After 6 months treatment[b]	0.1	0.025
1	37	12	S	
2	32	4	S	
3	38	32	R	
4	43	49	R	R
5	53	1	S	
6	36	31	R	
7	48	4	S	
8	43	19	S	R
9	34	1	S	S
10	53	63	R	R

[a] Cases 1–9 from Pettit *et al.* ([17]). Case 10 from Adams, A. R. D. and Waters, M. F. R. ([1])
[b] 300 mgm. injectable DDS twice weekly.
S = Sensitive
R = Resistant

per cent DDS in their diet. The results of these tests in the mouse foot pad were in good agreement with the bacteriologic responses, as measured by the fall in the MI, of the 10 patients during the carefully controlled six-month test period on DDS. Thus the five patients with DDS-resistant organisms failed to show a significant fall in their MI's, and with one exception (case 1) there was a significant fall in the MI in the five patients with DDS-sensitive organisms.

The mouse foot pad test has therefore clearly demonstrated the existence of DDS-resistant strains of *M. leprae*. It is of particular interest that five of the 10 patients with *prima facie* evidence of resistance to sulfone therapy and with a history of 15 years or more of treatment with sulfones should have responded favorably to a carefully controlled six-month test period on DDS. With the exception of case 1, these five patients have continued to respond satisfactorily on DDS, thus supporting the evidence provided from the MI and DDS sensitivity test. The exception, case 1, was continued on DDS because, although his MI had fallen to only 12, rather than the more usual 5 or less, during the six-month trial period, it was felt that this small difference was too rigid a criterion to justify immediate change of treatment, and furthermore his bacilli were sensitive to DDS fed at 0.1 per cent in the mouse foot pad test. The subsequent progress of this patient while maintained on DDS and on a long-acting sulfonamide (sulformethoxine) for a period of almost 18 months, has been unsatisfactory, with fluctuations in the MI([15]).

However, valuable information has been obtained during this subsequent period from two further sensitivity tests carried out with bacilli using the mouse foot pad infection. The results of these tests, shown in Table 2, indicate that subsequently the patients' bacilli were resistant to DDS fed

TABLE 2. *DDS and sulformethoxine sensitivities, using the mouse foot pad test with* M. leprae *from case 1 in Table 1 on three occasions during treatment.*

Date of test	Proportion of foot pads showing multiplication of *M. leprae*					Result of sensitivity test
	Untreated mice	Treated mice (% drug in diet)				
		DDS			Sulformethoxine	
		0.1	0.025	0.006	0.04	
March 1963	7/9	0/6	—	—	—	Sensitive to DDS (0.1 %)
May 1964	10/10	2/8	6/6	—	—	Resistant to DDS (0.025 %)
Feb. 1965	12/12	—	6/10	7/12	10/12	Resistant to DDS (0.025 and 0.006 %) and to sulformethoxine (0.04 %)

at 0.025 and 0.006 per cent DDS in the diet and also showed cross resistance to the long-acting sulfonamide, sulformethoxine (fed at 0.04 per cent in the diet), i.e., levels of these drugs that are known to inhibit strains of *M. leprae* from previously untreated patients ([19, 21]). In retrospect it is of interest that at the completion of the six-month trial period on DDS the MI of this patient had fallen somewhat less than that of previously untreated lepromatous patients on standard DDS therapy.

In addition to this special study the detection of DDS-resistant strains of *M. leprae* has been further extended by our group to other patients who have shown relapse under prolonged treatment with sulfones. A summary of all these results, including the special study already referred to, together with the DDS sensitivity of strains of *M. leprae* from previously untreated patients, is given in Table 3. From the rapidly accumulating data in this entirely new field of leprosy research the detection of DDS-resistant strains of *M. leprae* from patients previously treated with sulfones can be determined precisely only when substantial data are available for the DDS sensitivity of bacilli from untreated patients. Although many more strains of *M. leprae* from previously untreated patients need to be fully titrated against falling doses of DDS in the diet of mice, using the foot pad infection, a provisional assessment of DDS resistance in 22 strains of bacilli from previously treated patients, compared with the DDS sensitivity of 13 strains of bacilli from previously untreated patients, is set out in Table 3. From the present, though incomplete data, a pattern of response is beginning to take shape which suggests that strains of *M. leprae* that are capable of multiplying in patients under treatment with DDS (clinically resistant strains) are those that multiply in the foot pad in animals fed 0.01 per cent or more DDS in their diet.

More detailed studies have been undertaken on some of the DDS-resistant strains of *M. leprae*. For example, the infectivity and ability of resistant strains to multiply in the foot pads of untreated mice is the same as for sensitive strains (Table 4). However, it can be seen from the same table that the DDS-resistant strains of *M. leprae* multiply less freely, thus resulting in lower total yields of bacilli, in DDS-treated than in untreated mice. So far, the four DDS-resistant strains of *M. leprae* that have been tested against sulfonamides, i.e., two against sulfadimethoxine and two against sulformethoxine, have all shown cross resistance (Table 5). On the other hand, no cross resistance with thiacetazone or thiambutosine has been demonstrated in the five DDS-resistant strains of *M. leprae* so far tested. Investigations have revealed that DDS resistance is a stable characteristic when the resistant strains are maintained in animals treated with the drug, and that, in general, resistance persists also in strains passaged in untreated animals, although more recently two out of five originally resistant strains have become sensitive to DDS after two passages in untreated mice.

Thiambutosine resistance. Other studies have been undertaken to detect resistance to thiambutosine in patients showing clinical relapse during treatment with this drug. Strains of *M. leprae* from eight such patients have been investigated; the results are shown in Table 6, comparing the drug sensitivities against maximum tolerated doses of thiambutosine (0.1 per cent in the diet) and thiacetazone (0.2 per cent in the diet) with the sensitivities of six strains of *M. leprae* from previously untreated patients. These studies, again for the first time, provide direct evidence for the existence of thiambutosine-resistant strains of *M. leprae* and show, moreoever, that such strains are also resistant to thiacetazone, thus indicating cross resistance between the two drugs, similar to that reported against *M. tuberculosis* ([9]). Because far less data are as yet available on the thiambutosine sensitivity of strains of *M. leprae* from previously untreated patients, compared with the data on DDS, the full significance of the results in the group of relapsed-sensitive patients has still to be determined. However, a pattern of response has been obtained in relapsed patients under thiambutosine treatment which is similar to that obtained in the preliminary studies on DDS resistance, in that not all strains of

organisms from such patients are resistant to the maximum tolerated doses of these two drugs in mice.

DISCUSSION

The most important result from these studies has been the demonstration that a proportion of relapses that occur in patients on chemotherapy are due to the emergence of drug-resistant strains of *M. leprae*. For the first time the evidence for drug resistance has been based on sound bacteriologic data instead of reliance, as hitherto, on clinical evidence. However, it is of interest that these precise studies have shown that only a proportion of the relapsed patients had at that time drug-resistant organisms. Because leprosy is such a chronic infection, requiring prolonged treatment, past claims that relapses were due to the emergence of drug resistance were quite rightly criticized as possibly due to failure on the part of the patient to have continued taking any or adequate doses of drug. The results of our studies completely support this possibility, since at least half of our specially selected relapsed patients were infected with DDS-sensitive strains of *M. leprae* and these same patients responded satisfactorily to a supervised course of DDS on injection. It is, of course, possible that a proportion of these particular patients, who, at the time of relapse, were taking DDS by mouth, may have been suffering from a malabsorption syndrome resulting in inadequate tissue concentrations of DDS. However, these considerations are academic, since no relevant data were available on these patients before entry into our studies.

The results of the present studies have thus demonstrated the existence of both thiambutosine- and DDS-resistant srains of *M. leprae*. While in the case of thiambutosine most clinicians have reported a proportion of their cases relapsing between the second and the third year, there has been little or no evidence of relapse occurring in patients on regular treatment with DDS. Present estimates from our own detailed studies of DDS resistance in Malaysia ([11, 17]) confirm the rarity of such resistance, since less than 20 cases of DDS resistance are likely to be found from a population of not less than 5,000 lepromatous patients treated with DDS.

On the basis of our own findings that strains of *M. leprae* from previously untreated patients in both Malaysia and India are sensitive to thiambutosine fed at a concentration of 0.1 per cent in the diet of mice, we have concluded that relapsed-treated patients from these same regions of the world whose organisms have multiplied freely at these concentrations are resistant to thiambutosine. Shepard and Chang ([24,23]), however, failed to show inhibition of multiplication of *M. leprae* in mice fed 0.1 per cent thiambutosine in their diet. Undoubtedly their strains of *M. leprae* came from different regions of the world, and, although they may have come from patients previously treated with either thiambutosine or thiacetazone, it is possible that strains of *M. leprae* vary in their susceptibility to these two drugs in different parts of the world, since such differences have been shown with wild strains of *M. tuberculosis* coming, for example, from India and Hong Kong, on comparison with those coming from England and Africa ([12]).

In addition to the direct evidence provided from these studies for the existence of drug-resistant strains of *M. leprae*, much more fundamental information can be expected from such studies in relation to the whole field of chemotherapy in leprosy. The discussion of these more basic principles will be confined to DDS, since this is the standard treatment for leprosy, and at present more precise data are available for this drug than for other antileprosy agents. For practical purposes in chemotherapy, a drug-resistant organism is one that multiplies in the presence of a drug at concentrations above the maximum that can be achieved in the patient. The chemotherapy of other bacterial infections has evolved, in the last 20 to 30 years, from data available on the minimal inhibitory concentration (MIC) of the drug *in vitro*, the pattern of emergence of resistant mutants from *in vitro* studies, and the concentration of a particular drug found in the serum and tissues of man.

TABLE 3. *DDS sensitivity of strains of* M. leprae *from untreated and DDS-treated patients, using the mouse foot pad test.*

Clinical status	Strain No.	Country of origin	DDS sensitivity: per cent DDS in diet							
			0.1	0.025	0.01	0.01 (once weekly)	0.006	0.001	0.0001	0.00001
Untreated	1	Malaysia	S							
	2	"	S							
	3	"	S	S						
	4	Burma	S							
	5	Malaysia	S	S						
	6	"		S	S	S	S			
	7	India		S						
	8	"		S						
	9	"		S						
	10	"		S						
	11	Malaysia			S					
	12	"		S	S			S	S	R
	13	"		S	S			S	S	R
Treated (A) Relapsed-sensitive	1	W. Indies	S							
	2	Malaysia	S							
	3	"	S							
	4	"	S							
	5	"	S	S						
	6	W. Africa	S	S						
	7	"		S						
	8	India		S						
	9	Malaysia		S	S					
	10	"		S	S					
	11	"		S	S			R	R	R

TABLE 3. *Continued.*

(B) Relapsed-resistant	1	Malaysia	R	R									
	2	"	R										
	3	"	R		R								
	4	"	S		R								
	5	"	S		R								
	6	India	R		R	R							
	7	Malaysia			S	R							
	8	India				R							
	9	W. Africa				R		R					
	10	India				S	R						
	11	Malaysia				S	R		R	R		R	R
	12	"				S	R		R	R		R	R

S = Sensitive
R = Resistant

For treatment of leprosy with DDS, until recently, the only data available have been the concentrations of DDS in the blood of patients receiving therapeutically active doses of the drug, which were selected quite empirically, since no *in vitro* or *in vivo* culture methods were available for determining the MIC of DDS against *M. leprae* from previously untreated patients by treating the infected animals with different doses of the drug and in the same animals determining the resulting tissue and serum levels of DDS (Table 7). Rapidly accumulating data based on these methods by Shepard, in Atlanta, and ourselves in London, indicate that in the mouse the minimal effective dose of DDS is 0.0001 per cent in the diet, giving an MIC *in vivo* of 0.01 to 0.03 μgm./ml. This indicates that *M. leprae* is considerably more sensitive to DDS than any other species of bacteria, including mycobacteria and also *Plasmodium berghei* [25].

It is against the evidence provided from these recent studies that the emergence of resistance to DDS has now to be considered. In man on standard doses of 100 mgm. DDS/day, serum levels of between 1 and 5 mgm./ml. are obtained. Therefore, on the basis of the studies in mice, it seems possible that concentrations of DDS between 100 and 500/fold more than the MIC are achieved. Such a favorable antibacterial ratio is unique in the field of chemotherapy; for example, many perfectly satisfactory chemotherapeutic agents in man have a ratio of only 4, and it is interesting to speculate whether or not such an advantageous ratio would have resulted if *M. leprae* could have been assessed by the standard screening tests *in vitro*. At the time that we applied the foot pad infection to study the emergence of DDS resistance, we chose, empirically, to test the DDS sensitivity of potentially resistant strains of *M. leprae* against the highest tolerated dose of DDS in mice (0.1% in the diet). Then it was not known that strains of *M. leprae* from previously untreated patients were sensitive to doses as low as 0.0001 per cent DDS in the diet. However, fortunately, even at that early stage, strains of *M. leprae* from relapsed patients were resist-

TABLE 4. *Detailed analyses of multiplication of DDS-resistant strains of* M. leprae *in the mouse foot pad.*

Strain No.	Treatment (% DDS in diet)	Proportion of foot pads showing multiplication	Mean yield of bacilli/ foot pad ($\times 10^5$)
1	0	12/12	7.4
	0.006	7/12	2.5
	0.025	6/10	2.2
2	0	10/10	11.3
	0.01	11/12	8.8
	0.025	5/8	2.4
3	0	12/12	10.6
	0.006	7/12	6.7
	0.01	9/12	5.8
	0.025	6/10	1.9
4	0	12/12	7.5
	0.01	8/12	5.5
	0.025	2/12	0.2

TABLE 5. *DDS-resistant strains of* M. leprae *showing cross resistance to sulfadimethoxine or sulformethoxine.*

Strain No.	Proportion of foot pads showing mutiplication				
	Per cent of drug in diet				
	DDS			Sulfadimethoxine (0.1)	Sulformethoxine (0.04)
	0.025	0.01	0.006		
1	6/10		7/12	9/12	
2	11/12			9/12	
3	5/8	11/12			11/12
4		7/12			7/12

ant to mice fed 0.1 per cent DDS in the diet, and since it is known that such animals have serum concentrations of between 10 and 15 μgm./ml., these resistant strains, from more recent knowledge, have a resistance-ratio in excess of 1,500.

However, on the assumption that the emergence of DDS resistance is comparable to the emergence of resistance to other drugs, this may be expected to stabilize at higher or lower levels. Therefore, since standard treatment with DDS in man results in serum concentrations of between 1 and 5 μgm./ml., it would seem likely that all strains of *M. leprae* from relapsed patients that are capable of multiplying at levels above 1 μgm./ml. would be resistant to therapy in man. Serum levels of approximately 1 μgm./ml. are obtained in mice fed 0.01 per cent DDS in their diet, which may be compared with levels of 0.15

TABLE 6. *Thiambutosine (Ciba 1906) sensitivity of strains of* M. leprae *from untreated and from relapsed thiambutosine-treated patients and cross-resistance with thiacetazone.*

Clinical status	Strains	Proportion of foot pads showing multiplication		
		Untreated	Thiambutosine 0.1 % in diet	Thiacetazone 0.2 % in diet
Untreated	1	4/6	0/6	0/6
	2	9/10	1/12	3/12
	3	10/10	1/10	1/12
	4	17/18	1/6	2/10
	5	10/12	3/12	4/10
	6	8/12	0/12	1/10
		58/68	6/58	11/60
Treated A. Relapsed-sensitive	1	6/7	0/6	0/7
	2	10/10		0/10
	3	7/10	1/12	2/12
	4	10/12	1/12	4/12
	5	9/12	0/12	0/10
		42/51	2/42	6/50
B. Relapsed-resistant	1	10/10	5/8	6/8
	2	5/6	4/6	4/6
	3	10/12	6/10	5/12
		25/28	15/24	15/26

TABLE 7. *Concentrations of DDS (μgm./ml.) in the sera and tissues of mice fed different levels of drug in diet.*[a]

Dose of DDS		Liver	Kidney	Carcass	Serum
% in diet (gm.)	mgm./kgm. body weight				
0.1	200.0				10 – 15
0.025	50.0	1.72	0.57	1.43	3.33 ± 0.65
0.01	20.0	0.80	0.24	0.37	0.89 ± 0.51
0.006	12.0	0.38	<0.05	0.16	0.55 ± 0.16
0.001	2.0	0.095	<0.05	<0.05	0.15 ± 0.10
Man 100 mgm./day	2.0				1 – 5

[a] Bushby, S. R. M. and Rees, R. J. W. Unpublished data, 1967.

μgm./ml. in mice fed 0.001 per cent. From these correlations, which admittedly embrace many assumptions, it would be expected that the demarkation level for testing DDS resistance would be at a concentration of 0.01 per cent in the diet; those strains showing multiplication would be resistant, and those failing to multiply would be sensitive. The data presented from our current studies are consistent with these assumptions. In is of interest to note that, on this basis, even the strains of *M. leprae* with lower degrees of DDS resistance have a resistance ratio of 100.

In considering this field of DDS resistance of *M. leprae* it is reasonable to include the broader field of the antibacterial activity of the sulfonamides in general. Although this is one of the oldest fields of chemotherapy, and although sulfonamide resistance has been reported for many other species of bacteria, sulfonamides remain one of the more difficult chemotherapeutic agents to study *in vitro*, as compared with the majority of such agents that have been discovered subsequently. This is in part because of the presence of sulfonamide antagonists in commonly used culture media and the particular sensitivity of the antibacterial activity of sulfonamides to the size of inoculum of the organism. Because of these special problems with sulfonamides, which in fact are interrelated, I have been unable to find any definitive data on the pattern of emergence of resistance to these drugs, and in a particularly relevant review of this subject (14) it is pointed out that *in vivo* tests are more advantageous than *in vitro* studies on sulfonamides.

These considered opinions, therefore, add weight to the current experimental studies on *M. leprae*, which for other reasons have to be carried out *in vivo*. On the more relevant aspects of DDS resistance in *M. leprae*, there is the general belief based on studies *in vitro*, that it is difficult to induce DDS resistance in mycobacteria. Much of this negative evidence is suspect because in most of the methods large inocula have been used, completely disregarding the known sulfonamide-antagonistic effect of the bacilli *per se*. However, it is not without interest that highly DDS-resistant strains of BCG (6) and of *M. tuberculosis* (8) have been obtained *in vitro*. In contrast, and perhaps of even greater interest, has been the complete failure to show the emergence of DDS-resistant strains of *M. tuberculosis* in a large series of patients with pulmonary tuberculosis in chemotherapeutic trials carried out in East Africa (5), and in our own studies on five Chinese patients in Malaysia infected with both *M. tuberculosis* and *M. leprae* and receiving DDS (13). The DDS sensitivity of these five strains of *M. tuberculosis* was no different from that of six strains of *M. tuberculosis* from Chinese patients in Singapore who were not receiving DDS.

From this review on the emergence of sulfonamide-resistant strains of bacteria in general, or the emergence of DDS-resistant strains of mycobacteria in particular, there are no generalizations that are directly applicable to the current findings on DDS resistance in *M. leprae*. This is unfortunate, because there is good evidence that the emergence of drug resistance in bacteria is related to the chemotherapeutic agent and not to the species of bacteria. However, from the present data there is good evidence that standard treatment with DDS in man results in levels of DDS far more in excess of the MIC for *M. leprae* than are achieved in the chemotherapy of other infections and therefore this special situation must be considered. It has been pointed out already that in man *M. leprae* may be exposed to concentrations of DDS between 100 and 500-fold more than the MIC, and that DDS-resistant strains of *M. leprae* isolated from man have resistant ratios of from 100 to more than 1,500.

From these data it is reasonable to compare the patterns of resistance found with many other types of chemotherapeutic agents, and in particular streptomycin with *M. tuberculosis*. Here resistance develops in single steps, and the streptomycin-resistant variants of *M. tuberculosis* segregate into groups with characteristic levels of resistance, the distribution of which is discontinuous (11). Of equal importance is the frequency of the single-stepped mutants within the population of tubercle bacilli.

On division of the variants into three groups of low, medium and high levels of resistance there emerged 1,000, 100 and 2 resistant mutants in the three groups respectively from a population of 10^9 *M. tuberculosis*. If the pattern of developing resistance of *M. leprae* to DDS is similar to that of *M. tuberculosis* to streptomycin, then, under standard DDS chemotherapy, or even considerably lower doses, the emergence of drug-resistant mutants would be extremely rare. These suggestions are entirely speculative, but require urgent investigation. Although the foot pad infection in normal mice is far too small for the study of resistance, the enhanced type of infection, in animals thymectomized and irradiated ([20]), which results in populations up to 10^9 *M. leprae*, should be adequate for study of the pattern of resistance to DDS by *M. leprae*.

Finally, it is possible that the infrequent occurrence of relapses under DDS is due, not to the rarity of DDS-resistant mutants, but rather to the extremely high concentrations of DDS obtained in the tissues by the regimens of DDS currently used, compared with the MIC of DDS against *M. leprae*. At present this is only a theoretic possibility, but it is truly a possibility and should be borne in mind in the proposed trials using lower doses of DDS and intermittent regimens. However, the fundamental and practical importance for carrying out such trials in man at present far outweigh the theoretic objections based on the possible emergence of drug resistance.

SUMMARY

Multiplication of *M. leprae* in the mouse foot pad infection is inhibited when mice are treated with known antileprosy drugs. This method has now been applied successfully to demonstrate the emergence of drug-resistant strains of *M. leprae* in a proportion of patients relapsing during treatment. Systemic studies on such patients have revealed 12 with DDS-resistant strains of *M. leprae*. Strains resistant to thiambutosine also have been demonstrated through use of this method. More detailed studies have shown that DDS-resistant strains of *M. leprae* multiply in the mouse foot pad as freely as sensitive strains, that DDS resistance persists on further passage in mice, and that strains resistant to DDS show cross resistance to other sulfonamides. Similarly, thiambutosine-resistant strains of *M. leprae* show cross resistance to thiacetazone.

For practical purposes in chemotherapy a drug-resistant organism is one that multiplies in the presence of a drug at concentrations above the maximum that can be achieved in the patient. In man on maximum standard tolerated doses of 100 mgm. DDS/day serum levels of 1-5 μgm./ml. are obtained. From more recent detailed studies in mice receiving different concentrations of DDS in their diet, it has been shown that animals fed 0.01 per cent DDS give serum levels of approximately 1 μgm./ml. Therefore, all strains of *M. leprae* multiplying in mice fed 0.01 per cent DDS or more in their diet can be considered resistant. It has been shown also, by use of the mouse foot pad infection, that strains of *M. leprae* are exquisitely sensitive to DDS; the estimated minimal inhibitory concentration (MIC) is between 0.01 and 0.03 μgm. DDS/ml. On the basis of the studies in mice it is probable that concentrations of DDS between 100 and 500-fold more than the MIC are achieved in man. Such favorable antibacterial ratios are unique in the field of chemotherapy. The relevance of these data to the emergence of DDS resistance in patients receiving different doses of the drug is discussed.

Acknowledgments. The leprosy tissues on which the main part of these studies were undertaken, were provided particularly by my colleagues Drs. J. M. H. Pearson and J. H. S. Pettit of the Leprosy Research Unit, Malaysia, Dr. A. B. A. Karat (Karigiri, S. India), S. G. Browne (Uzuakoli, E. Nigeria), and many other leprologists throughout the world, receipt of which I acknowledge with gratitude. I am also indebted for supply of pure drugs to Dr. J. M. Mungavin, Imperial Chemical Industries Ltd. (dapsone and thiacetazone), Dr. J. Garrod, Roche Products, Ltd. (sulphadimethoxine and sulphormethoxine), and Mr. A. B. Tattersal, Ciba Laboratories Ltd. (thiambutosine).

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DISCUSSION

Dr. Thompson. I was interested to see that strains of *M. leprae* that showed resistance to DDS gave somewhat lower yields of bacilli in the mouse foot pads of animals receiving DDS than untreated animals; this same feature applies to our sulfonamide-resistant strains of malaria parasites when studied in the mouse.

We have found that resistance to DDS develops stepwise and that it is possible to have lines with varying degrees of resistance. I wonder if you have observed evidence of a similar situation in the resistance of *M. leprae* to DDS.

Dr. Rees. Yes, I believe we have. We certainly have resistant strains, isolated from relapsed patients with varying degrees of resistance, viz., from 0.01 to 0.1 μg/ml. (see Table 3). However, our data are too limited at the moment to be sure that this truly represents the "single step" mutants that arise with *M. tuberculosis* against streptomycin. It is because our evidence to date is suggestive that I asked you earlier whether you had such evidence with sulfonamides against malaria parasites.

Dr. Chang. You mentioned that two of your five originally resistant strains became sensitive to DDS after two passages in untreated mice, i.e., in a little over one year. I would like to know if this type of resistance could be considered as phenotypic rather than genotypic. According to the definition of drug resistance I quoted yesterday[1] this type of resistance may be the result of physiologic adaptation instead of a genetic change of the microorganism.

Dr. Rees. The short answer to your question is that from the mass of detailed data accumulated on the genetics of the development of drug resistance in bacteria we can say that "reversion" can be and often is a genotypic phenomenon. Therefore, the results of our tests to determine the stability of DDS resistance are not inconsistent with the concept that DDS resistance in *M. leprae* is genotypic in nature. In fact the limited information available from our studies is in favor of a genotypic rather than a phenotypic change, because three of the five strains have retained their resistance when passaged at least three times through untreated mice. The latter passages represent a period of at least 30 months and a total increase of not less than 10^6. Of course it is impossible, with an organism that cannot be grown *in vitro*, to carry out the complex design of experiments that are necessary to exclude completely the possibility that no phenotypic type changes can occur in *M. leprae* that result in resistance to DDS.

Dr. Binford. Employing the well established Shepard foot pad method for measuring the efficacy of drugs against *M. leprae*, Dr. Rees, by precise technics in hundreds of mice, has presented convincing evidence that high resistance of *M. leprae* to DDS can be demonstrated experimentally. Fortunately in lepromatous leprosy this resistance is rare. He estimates that it may occur in one of 250 patients. Even with this low incidence, such cases gradually accumulate in leprosy clinics and hospitals, to become problems of greater concern, not only to themselves and their physicians but also to other patients, because, as ever present examples of treatment failures, they tend to cause other patients to become less enthusiastic about persevering in an extended treatment course that may have to continue for many years. Hopefully, objective data on DDS-resistant strains of *M. leprae* will lead to a better method for controlling the disease in the patients who are infected. It is especially significant that several strains of *M. leprae* were shown to be resistant not only to DDS but to other sulfonamides and to Ciba 1906 and TB1.

[1] DAVIS, B. D. *In* Bacterial and Mycotic Infections of Man. R. J. Dubos, Ed., Philadelphia, Lippincott, 3rd ed., 1958, p. 680.

Dr. Rees and his colleagues have now introduced a promising method for enhancing the growth of *M. leprae* in thymectomized and irradiated mice. I hope that the resistant strains of *M. leprae* will be further investigated by this new method. I hope also that Dr. Rees will not only assess the growth of the resistant bacilli by counting, but also, by using histopathologic methods, will determine the pathogenicity of the resistant bacilli in the immunologically paralyzed mice.