SPECIAL ARTICLE

Drug resistance in leprosy—a review

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Although the introduction of dapsone made possible the effective chemotherapy of leprosy, we have required more than 30 years to learn how to use the drug properly. From the beginning, dapsone was used as monotherapy in a variety of dosages, although, by the time the drug was in use worldwide, it had already been established that cavitary pulmonary tuberculosis could not be cured by monotherapy with any antituberculosis drug. As a result, drug resistance in leprosy has become a serious problem, and demands immediate action.

Dapsone resistance was first proved^{1, 2} soon after multiplication of *Mycobacterium leprae* in the mouse foot-pad had been described.^{3, 4} It was estimated that, during the period 1964–66, the prevalence of dapsone resistance was only 2 per 1000.⁵ However, the situation had changed by 1976 by which time it was apparent that dapsone resistance was already a significant problem.⁶ Since the establishment in 1977 of the Scientific Working Group on Chemotherapy of Leprosy (THELEP) of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases,

in a number of leprosy-endemic areas to assess the true magnitude of the threat to leprosy-control activities.⁷ Similar studies have also been carried out outside THELEP. As a result of these studies, not only is secondary dapsone resistance understood to be distributed worldwide, with rapidly increasing prevalence rates and alarming annual incidence rates in some areas;⁸ also, primary dapsone resistance has been detected with unexpectedly high prevalence rates.^{9, 10} In addition, secondary resistance to bactericidal drugs other than dapsone, i.e. rifampicin (RMP)^{11 13, 50}, ethionamide (ETH)^{14–16}, and clofazimine (CLO)¹⁷, to two bactericidal drugs, i.e. doubly resistant strains, have also been detected.^{11 13, 17, 50}

Basic knowledge and speculation regarding drug resistance in leprosy

DEFINITION OF DRUG RESISTANCE

Epidemiologically, there are two types of drug resistance. Secondary or acquired resistance, a result of inadequate chemotherapy, is usually accompanied by a classic history, i.e. initial improvement, followed by deterioration despite continued treatment.¹⁸ The other type is primary resistance, which occurs in patients who have not received chemotherapy, and results probably from infection with drug-resistant organisms that originated from another patient who had relapsed with secondary resistance.

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ETIOLOGY OF DRUG RESISTANCE

M. leprae still cannot be cultivated *in vitro*, and *M. leprae*-infection of the foot-pad of the immunologically normal mouse does not develop bacterial populations large enough to contain drug-resistant mutants. Thus, our knowledge of how drug resistant *M. leprae* develop is only fragmentary. However, drug resistance of *M. tuberculosis* has been extensively studied. The following observations on *M. tuberculosis* might be useful in understanding the etiology of drug resistance in leprosy.

- (i) Resistant bacilli are present in wild strains, i.e. bacterial populations that have never been exposed to an antituberculosis drug. This phenomenon was first demonstrated after the discovery of streptomycin, and was later found to be characteristic of other antituberculosis drugs as well. These resistant bacilli occur spontaneously as the result of mutational events. The frequency and degree of resistance of these mutants in a wild strain depend on many factors, such as the origin of the strain, the drug, its concentration, and the size of the bacterial population. The mutation frequency, which is closely related to the degree of resistance, is 10^{-6} to 10^{-7} for low-, and 10^{-8} to 10^{-9} for high-degree resistance. Therefore, among 10^9 *M. tuberculosis*, one may expect to find 100–1000 naturally occurring low-resistant mutants, 10–100 moderately resistant mutants, and 1–10 highly resistant mutants against any drug to which resistance occurs in step-wise rather than single-step fashion.^{19–23}
- (ii) The development of drug resistance during treatment is probably the result of a selective process. Whereas a majority of susceptible organisms are killed by the drug, the resistant mutants survive and multiply. Finally, they replace the susceptible organisms in the population. Drug resistance is not the result of a progressive adaptation of susceptible organisms to the drug to which they are exposed; no homogeneously resistant strain has ever reverted to susceptibility.^{19–21}

If these observations can be applied by analogy to *M. leprae*, drug resistance during monotherapy is more likely to occur in multibacillary (LL, BL and BB) leprosy. Only 10^8 *M. tuberculosis* are commonly found in pulmonary cavities before chemotherapy.²⁰ By conservative estimate, an untreated multibacillary patient has a total of 10^{11} organisms, 10% of which are viable.²⁴ Assuming the frequency of the spontaneously resistant mutants of *M. leprae* to be of the same order as for *M. tuberculosis*, one would expect the following outcomes:

- (i) when the treatment is regular and in full dosage, only the highly resistant mutants are likely to survive after a period of favourable response;
- (ii) when the treatment is only in a low dosage of the drug, even the low-resistant mutants will survive;
- (iii) if the compliance with treatment is poor, unfortunately a frequent occurrence,²⁵ the resistant mutants will survive, because drug concentrations in blood or tissues cannot always be maintained above certain critical levels. It is possible that, initially, there may exist only mutants with low-degree resistance, and that the mutants with higher degree resistance arise only as the result of further mutations.^{23, 26}

Once the surviving resistant mutants multiply to a certain level, clinical signs of relapse occur. Should the patient receive regular treatment in full dosage, relapse occurs quite late and mainly with high-degree resistance. On the other hand, should the patient receive treatment in low dosage or irregularly, relapse occurs earlier and mainly with low- or intermediate-degree resistance, as shown by the studies in Malaysia and Ethiopia.^{5, 6, 8, 18, 27, 28}

If these assumptions are true, treatment failure caused by drug resistance must be expected in every multibacillary patient under monotherapy. Thus far, however, no data have demonstrated a prevalence rate of secondary dapsone resistance greater than 30% in any area in which dapsone has been used as monotherapy for more than 30 years (see Table 1). This means that at least 50% of multibacillary patients do not develop drug resistance. It may be argued that, because of the exquisite susceptibility of *M. leprae* to dapsone, and also because of the very long generation time of

Table 1. Results of surveys of secondary dapsone resistance

Country	Number at risk*	Minimal prevalence (% per year)	Incidence (% per year)	Degree of resistance	Reference
Burundi	925	37	—	majority high	(29)
Costa Rica	200	68	1.0	majority high	(30)
China				majority high	(31)
(Jiangsu)	236	51	—	or intermediate	
(Shanghai)	777	86	—	majority high	(32)
Ethiopia	1500	100†	3.0	mostly intermediate or low	(5, 18)
India					
(Karigiri)	1580	95	—	majority high	(33–35)
(Chingleput)	660	29	—	majority high	(unpublished data)
Israel	100	37	—	intermediate	(36)
Malaysia					
(1964–66)	5000	2	0.1	high	(2, 5)
(1973)	5000	25	0.3	majority high	(37)
Mali	105	57	3.0	intermediate or high	(38, 39)
Upper Volta	355	70	—	majority high	(40)

* All multibacillary patients who began dapsone monotherapy at least 5 years ago and were still living during the survey.⁷

† About one-third of the resistance confirmed by mouse foot-pad test.

M. leprae, clinical signs of resistance will take many years to appear. However, it can hardly be imagined that the majority of them will ever relapse. The explanation for this is not evident. On the other hand, there is no indication that monotherapy can prevent the development of drug resistance; therefore, that not all patients treated with monotherapy relapse with drug resistance does not justify treatment of patients with monotherapy.

Proof of drug resistance

Drug resistance should be suspected in a multibacillary patient who has relapsed, either under treatment or after he has stopped treatment, or whose clinical response is less favourable than expected, or in a new patient who may have had an intimate contact with a secondary resistant case.

Two methods have been used to prove drug resistance.

CLINICAL TRIAL

This method, which can be easily carried out anywhere,²³ has been used mainly for patients with *prima facie* evidence of secondary drug resistance.^{18, 23, 27, 28} After the patient has been fully assessed clinically, histopathologically, and bacteriologically, he is given the tested drug in full dosage. The

full dosage of dapsone is 300–400 mg twice weekly by injection or 100 mg daily by mouth, either under supervision or while monitoring the urine to confirm the presence of sulphone. Clinical assessment, skin smears and skin biopsy are repeated regularly during the trial. If his strain of *M. leprae* is fully susceptible to the drug, the patient will respond to treatment at the same rate as other previously untreated and sensitive patients. In drug-resistant cases, several different patterns of response may be seen. Patients who have previously been on regular, full dosage treatment will show no, or only partial, response for a brief period, and then deteriorate. In other patients, especially those previously on low or irregular dosage, the initial response to the treatment will resemble that of drug-sensitive patients. However, after a time new, histopathologically active lesions appear with elevated MI, and old lesions become reactivated. Proof of resistance by clinical trial may require from 3 months to more than 5 years.²⁷

Clinical trial is suitable only for those patients who relapse with active, new lesions with high BI and MI. However, in field surveys, employing the susceptibility test in the mouse foot-pad, resistant strains have been isolated even from patients without clinical relapse. The clinical trial pays more attention to the change of clinical signs and MI. The former indicator seems to be too subjective, and the latter too difficult to standardize. More importantly, patients with low-degree resistance may require as long as 5 years for confirmation of resistance,^{23, 28} thus delaying combined therapy. For these reasons, clinical trial has generally been given resistance.⁸

DRUG SUSCEPTIBILITY TEST IN THE MOUSE FOOT-PAD

Because mouse inoculation is not possible unless sufficient organisms are recovered from skin biopsy specimens, such tests can be carried out only among multibacillary patients with a BI ≥ 3 in at least one skin lesion. The patient can be started on combined therapy immediately after the biopsy specimen, without awaiting the results of the study. Moreover, susceptibility of the *M. leprae* to a series of drugs can be tested simultaneously by this method.

Immunologically normal mice are inoculated with $5-10^3$ or 10^4 *M. leprae* into one or both hind foot-pads, and divided into several groups. One group is kept as untreated controls and fed normal diet; the rest are divided into drug-treated groups and fed special diets, into which have been incorporated different concentrations of drug. For example, 3 treated groups are fed diets containing 0.0001%, 0.001% or 0.01% dapsone. Usually 6–12 months after inoculation, mice are sacrificed and the acid-fast bacilli are harvested from the foot-pad tissue. If no multiplication of *M. leprae* is observed in the control group, the test is considered to have failed, possibly because the proportion of viable bacilli in the inoculum was too low. If multiplication of *M. leprae* is observed only in the control group, and not in the drug-treated groups, the strain is considered susceptible to the drug tested. If multiplication of *M. leprae* is observed in some or all drug-treated mice, the strain is resistant, and the degree of resistance is reflected by the highest concentration of drug in the diet that permits multiplication of *M. leprae*.

Drug susceptibility testing by the mouse foot-pad technique is quite reliable. However, the test is available in only a limited number of laboratories, and the majority of leprosy-control programmes and leprosaria do not have access to mouse foot-pad facilities. Therefore, setting up regional or national reference laboratories may be desirable.

Regarding drug-susceptibility testing by this technique, the following aspects need to be discussed, and some of them require further study.

The critical concentration of drug, and the critical proportion of resistant organisms as criteria of drug resistance

The only satisfactory definition of resistance is based on the behaviour of wild strains.⁴¹ In

tuberculosis, a critical concentration of drug and a critical proportion of resistant organisms had been set up as criteria of drug resistance.^{19, 21}

The critical concentrations of drugs for the diagnosis of resistance in the mouse foot-pad test are the concentrations of drug in the mouse diet, these being 0·0001% dapsone, 0·0001%–0·001% CLO, 0·003% RMP, and 0·01% ETH or prothionamide (PTH).

The more important problems appear to be in relation to bactericidal drugs other than dapsone. The numbers of wild strains fully titrated against these drugs have been small, and the criteria for the critical concentrations of these drugs are not reliable. Therefore, many more wild strains of *M. leprae* should be titrated for susceptibility to RMP, CLO, and ETH/PTH. These drugs have not yet been used in several endemic areas, so there remain good opportunities to carry out these crucial studies.

To determine whether a population of *M. tuberculosis* is drug resistant or not, it is not enough to establish the presence of resistant bacilli; all large bacillary populations contain some. Therefore, it is necessary to determine whether the proportion of resistant bacilli is abnormally large.^{19, 21} So far, there are no data regarding the proportion of resistant *M. leprae* in a bacillary population. It may be assumed that when the population of a wild strain of *M. leprae* is no larger than 10^6 , it will behave as a homogeneous population. As soon as the population becomes large, e.g. 10^{10} or more, it is no longer homogeneous for any drug, and has become a mixed population, because of the spontaneous occurrence of resistant mutants. However, the inoculum for the mouse foot-pad is usually of the order of 10^4 organisms, the majority of which are dead. As the frequency of the spontaneous drug-resistant mutants is very low, probably no larger than 10^{-6} , there is no possibility of detecting these resistant mutants before treatment. After several months' treatment with dapsone or several days' treatment with RMP, the majority of the viable drug-susceptible bacilli have been killed. But because the total number of bacilli does not change significantly, the proportion of viable bacilli decreases to such a level that their capacity to infect mice is lost.⁴²

The resistant mutants cannot be isolated unless they have multiplied to a certain level. By this time, the viable bacterial population has again become virtually homogeneous, because the number of susceptible bacilli has decreased to a low level as 'persisters'. Therefore, at no time does it appear possible to isolate in the mouse a mixed (drug-susceptible and drug-resistant) population of *M. leprae*. The isolation of drug-resistant bacilli indicates that the resistant mutants have multiplied, and that the patient is a resistant case.

Nevertheless, in some drug-susceptibility tests in mice, only a small proportion, e.g. 1/10 or even 1/20, of animals treated with the lowest concentration (0·0001%) of dapsone demonstrate multiplication of *M. leprae*, whereas the majority of animals in the control group show multiplication. One explanation of such results is that the population of *M. leprae* is mixed, with the majority susceptible to 0·0001% dapsone, and a minority resistant. There are alternative explanations.

To test the possibility that the patient's population of *M. leprae* is mixed, and, more importantly, to evaluate the relationship between a resistant strain and a resistant patient, the following experiment is proposed. From those tests in which only a small proportion of animals fed 0·0001% dapsone demonstrate multiplication of *M. leprae*, bacilli should be recovered from drug-treated mice, and designated inoculum A. Bacilli should also be recovered from control animals and designated inoculum B. The bacilli should be passaged to groups of control mice and to animals administered 0·0001% dapsone in diet. If, at harvest, a majority of the treated animals inoculated with inoculum A and a small proportion of the treated animals inoculated with inoculum B demonstrate multiplication of *M. leprae*, then there is a mixed population. If, on the other hand, a majority of animals from both treated groups show multiplication, the population is homogeneous, and probably there was some technical error in the original experiment. Finally, if both inocula behave similarly in both control and treated mice, reproducing the results noted in the original test, one should consider the possibility of phenotypic variation of susceptibility to dapsone among members of a single clone of *M. leprae*.

Standardization of the drug-susceptibility test in the mouse foot-pad

Although the mouse foot-pad technique has been basically standardized through workshops and some documents,⁴³ the following aspects of the susceptibility test also require standardizing.

Criteria of multiplication of M. leprae. The smallest number of *M. leprae* detected by present counting methods is rather large. It can hardly be concluded that multiplication of *M. leprae* has occurred if 2×10^4 AFB per foot-pad are harvested after inoculation of 5×10^3 or 10^4 AFB; a count of 2×10^4 AFB per foot-pad indicates that only 2 or 3 AFB were observed during counting, and these could be merely the inoculated bacilli. It would be better to define 10^5 AFB per foot-pad as the criterion of multiplication of *M. leprae*.

Mouse diet. The drug-containing mouse diets should be analysed regularly by standard methods, to ensure that the proper concentrations of drugs are in the diets. Some drugs, such as RMP and other rifamycin derivatives, are unstable, especially after they have been incorporated into the mouse diet. Also, the procedures for preparing and preserving the diets should be standardized.

Minimal number of animals per group. In some biopsy specimens, the proportion of viable bacilli is quite low. The more animals inoculated and harvested, the greater the possibility of detecting multiplication of *M. leprae*. After multiplication of *M. leprae* has been confirmed in the control group, each treated group should have at least 4 mice. When manpower is available, harvesting of mice should be done individually. If multiplication of *M. leprae* has occurred in only a small proportion of mice, it may be masked by pooling the foot-pad tissues from several mice.

Main observations on drug resistance in leprosy**RESISTANCE TO DAPSONE**

With respect to drug resistance, dapsone is the most important of the antileprosy drugs, apparently because it has been widely used as monotherapy for more than 30 years. It is still the only drug available for most leprosy patients in developing countries.

Dapsone is extremely effective in inhibiting the multiplication of *M. leprae*. This has been confirmed both in experimental studies in the mouse⁴⁴⁻⁴⁶ and in small scale clinical trials.⁴⁷ Dapsone-resistance is identified when bacilli obtained from patients multiply in mice receiving 0.0001% or more dapsone in the diet. The degree of resistance is defined as low, intermediate or high, depending upon the strain's ability to multiply in mice administered 0.0001%, 0.001%, or 0.01% dapsone.⁸ Because the degree of resistance varies remarkably among strains, and because the risk of emergence of dapsone-resistant infection differs between patients treated initially with low dose sulphone and those treated initially with dapsone in full dose,³⁷ the mutation that produces dapsone resistance has been identified as 'step-wise' rather than 'single-step'.

Secondary dapsone resistance

At the moment, secondary dapsone resistance has been detected in more than 25 countries.⁸ Pearson reviewed the available data on secondary resistance to dapsone in 1981.⁵ Since then, sporadic instances of secondary dapsone resistance proved in the mouse foot-pad have also been reported from Guadeloupe,¹³ Martinique,¹³ Indonesia,⁴⁸ Nepal⁴⁹ and New Caledonia.⁵⁰

The results of surveys for secondary dapsone resistance are summarized in Table 1. Most of the recent surveys have followed the THELEP Protocol.⁷ Four studies followed-up the same population for varying durations and, therefore, were able to estimate the incidence rates. Currently, 2 more formal surveys are being carried out in Burma and in India. Resistant cases have been detected in all these areas, but the data are incomplete. Based on clinical examinations, the

prevalence and incidence rates in Burma appear to be the highest—of the order of 200 per 1000 and 30 per 1000 per year⁵¹ respectively.

Based on the available data, it is clear that secondary dapsone resistance is now a worldwide phenomenon. A majority of the resistant strains of *M. leprae* are of intermediate- or high-degree resistance. Generally speaking, some study areas listed in Table 1 have quite effective leprosy control programmes. For example, in Malaysia, many patients have been treated for many years as in-patients. Their treatment has been reasonably regular and well supervised. In most cases, dapsone had been used in full dosage. Therefore, the Malaysian figures for prevalence and incidence of dapsone-resistant leprosy can be taken as the best results that can be achieved by dapsone monotherapy.⁵ The poorer the quality of the control programme, the more likely is the occurrence of dapsone resistance and its rapid increase. Recently, the alarming figure of a 3% annual incidence rate has been reported from both Ethiopia¹⁸ and Mali,³⁹ and this is probably also true in Burma.⁵¹ This is a clear indication of the seriousness of the situation.

Among the dapsone-resistant patients, the duration of treatment before relapse is much longer than in drug resistance in tuberculosis, perhaps because of the very long generation time of *M. leprae*. This duration varies remarkably, ranging from 2¹⁸ to 24 years,²⁷ and appears to correlate with the dosage of dapsone and regularity of treatment. If resistance develops among the patients receiving low dose dapsone or irregular treatment, it usually develops within fewer than 10 years of treatment, and frequently with low or intermediate levels of resistance.^{23, 27}

Primary dapsone resistance

Five primary dapsone-resistant patients, all proved in the mouse foot-pad, were reported in 1977.⁹ Subsequently, several surveys were carried out. The available data are summarized in Table 2. In

Table 2. Results of surveys of primary dapsone resistance

Country	Number of patients		Prevalence (per 1000)	Degree of resistance	Reference
	Total	Resistant			
China	20	10	500	low or intermediate	(Ji <i>et al.</i> , unpublished data)
Ethiopia	29	16	550	majority low	(9)
India					
(Chingleput)	56	21	375	majority low	(10)
(Karigiri)	12	5	420	low or high	(52)
Korea	18	4	222	majority low	(56)
Mali	40	14	350	majority low	(10)
Nepal	15	13	870	mostly high	(53)
Philippines	55	2	36	low or intermediate	(57)
Malaysia	22	6	270	majority high	(5)
Martinique & Guadeloupe	17	12	700	low or intermediate	(54)
USA					
(Carville)	93	18	190	majority low	(16)
(San Francisco)	54	1	18	low	(55)

addition, sporadic cases have also been reported from Burundi,²⁹ India⁶⁹ and Indonesia.⁴⁸ Although the available data are still limited, primary dapsone resistance appears to have become ubiquitous. Until now, it has gone unrecognized in many places, perhaps because it has not been sought.

In tuberculosis, it is not easy to differentiate between primary resistance and undiscovered secondary resistance. The term 'initial drug resistance' has been used for resistance among newly discovered patients, when it is impossible to obtain a reliable history.²² One may question whether or not the so-called primary dapsone-resistant patients had in fact received prior dapsone treatment. Of course, this possibility must be kept constantly in mind. In certain areas with poor registration systems, some undiscovered secondary dapsone-resistant cases might be included as primary resistant patients; however, such a possibility should be much less likely in leprosy than in tuberculosis. In most developing countries, leprosy patients can receive treatment only from certain clinics, and usually their records are kept in these clinics. In addition, relapsed cases usually have some old lesions, and might be distinguished clinically from the new patients. The data on primary resistance from India and Mali, shown in Table 2, were obtained from THELEP controlled clinical trials.¹⁰ Considerable efforts, including urine analysis for dapsone, were undertaken to ascertain that the patients admitted to these trials had not been previously treated. The prevalence rates of primary resistance in India and Mali are not significantly lower than the figures obtained elsewhere.

If one compares the data in Tables 1 and 2, one may see that, with the exception of the Philippines, the prevalence rate of primary dapsone resistance is much higher than that for secondary resistance. Such a result, although unexpected, may be explained. The calculations for prevalence rates of secondary and primary dapsone resistance are entirely different. The denominator for secondary resistance is the total number of patients at risk, regardless of their skin-smear status. But most of these patients have been negative. The denominator for primary resistance is the total number of tested patients, all of whom are untreated multibacillary patients having lesions with BI ≥ 3 . In Shanghai, for instance,³² the number of patients at risk of secondary resistance was 777; of these, 92 (11.8% of the total) were skin-smear positive, and most likely the main sources of infection in the community. Sixty-seven of these 92 patients, representing 73% of the skin-smear positive group, had at least one lesion with BI ≥ 3 , and the proportion found to have resistance by mouse foot-pad was no smaller than 62%. Even ignoring the infectivity of patients with BI < 3 , the proportion of patients with resistance among the main sources of infection, *i.e.* all skin-smear positive multibacillary patients, should be greater than 45% ($62\% \times 73\%$). This could explain the high rate of prevalence of primary dapsone resistance.

By definition, primary dapsone-resistant patients are those infected with organisms from resistant patients. Therefore, it must be assumed that primary resistance, unlike the secondary resistance, occurs in at least as high a proportion of paucibacillary as that of multibacillary patients. One cannot demonstrate that such patients have resistance by inoculation of mice, because too few organisms can be recovered from the skin-biopsy specimens. If the primary dapsone-resistant paucibacillary patients are treated with dapsone monotherapy, some of them may undergo serious deterioration, and even downgrade towards the lepromatous end of the spectrum.

Because drug resistance is an inherited trait, the mutants isolated from the primary resistant patients should have the same degree of resistance as the source of infection, *i.e.* secondary resistant patients. In tuberculosis, reversion of resistant strains of *M. tuberculosis* to susceptible has never been observed.¹⁹⁻²¹ Very few data regarding the stability of resistance of *M. leprae* can be found in the literature, but there are a few resistant strains which remained dapsone-resistant for several years after the patients had changed their drugs.²³ The reason that most strains of *M. leprae* isolated from primary dapsone-resistant patients were of low-degree resistance, whereas the majority of strains isolated from secondary dapsone resistant patients have shown intermediate- or high-degree resistance, is still unknown. Perhaps the explanation lies in the long incubation period of multibacillary leprosy. Most patients recognized with primary resistance today were infected 10 years ago or more, at which time the characteristics of strains from secondary resistant patients may have been different from those of today. Because of the low degree of resistance, the majority of

primary dapsone-resistant patients may be expected to respond to treatment with dapsone in full dosage. However, dapsone must be combined with other drugs; otherwise the patients are very likely to relapse with high-degree resistance in the course of time.

RESISTANCE TO OTHER BACTERICIDAL DRUGS

Not much data on resistance to other bactericidal drugs can be found in the literature because these drugs have not been used as widely as dapsone and because resistance to them has not been systematically sought. However, during the past 10 years, a number of patients have been treated with these other drugs, after they have relapsed under dapsone monotherapy. Unfortunately, many patients had either been kept on monotherapy, or were given in addition such bacteriostatic drugs as thiambutosine (DPT). Because of high cost (of RMP) and side-effects (pigmentation in the case of CLO, poor tolerance in the case of ETH/PTH), the regularity of treatment with these other bactericidal drugs may have been even poorer than that with dapsone. One may anticipate that leprosy patients resistant to these drugs will appear in the near future, unless they are changed to combined therapy.

RMP

RMP kills *M. leprae* with exceptional speed, as proved in both experimental studies and clinical trials.^{58, 60} Within a few days after a single dose of 600 or 1500 mg RMP, 99.9% of *M. leprae* from an untreated patient have been shown to be killed.⁶¹ Such a rapid bactericidal effect has deeply impressed both the clinicians and the patients. Therefore, since the mid 1970s, RMP has been increasingly used despite its high cost. One might have expected that, after the experience of dapsone monotherapy, medical personnel would have rejected the use of RMP as monotherapy. Unfortunately, this has not been the case. As early as 1976, two RMP-resistant patients had been reported.¹¹ So far, 4 of 16 patients, who had been treated with RMP monotherapy in a dosage of either 300 mg or 600 mg daily, have developed resistance within 3–5 years.¹⁶ This indicates that, despite its rapid bactericidal effect, the development of secondary resistance to RMP, used as monotherapy, occurs earlier than in the case of dapsone monotherapy. Recently, 9 more resistant strains have been reported.^{13, 50} However, this regrettable situation does not deny the effectiveness of RMP. It is still the most powerful weapon we have. Even without any new powerful drugs, one might still expect that leprosy can be completely treated by regimens containing RMP, provided RMP is always combined with other antileprosy drugs capable of preventing the development of resistance to it.

CLO

The bactericidal effect of CLO to *M. leprae* has been confirmed in the mouse⁶² as well as in clinical trial.⁶³ Because of its anti-inflammatory effect, it can also be used to prevent or control lepra reactions. The drug was introduced into the treatment of leprosy in the 1960s, but it was not until 1982 that the first CLO-resistant leprosy patient was reported¹⁷ [this strain has since been found susceptible to 0.001% CLO, and resistant to 0.0001% CLO in another laboratory (L Levy, personal communication)], despite many patients having been treated with CLO monotherapy during the intervening years. The repository character of the drug appears to have delayed the appearance of resistance, as had been observed before with regular, full dosage dapsone monotherapy. 'One way' cross-resistance between CLO and RMP had been demonstrated in *Mycobacterium* sp. 607 by Morrison, i.e. the RMP-resistant strain was fully susceptible to CLO, but the CLO-resistant strain was also resistant to RMP.⁶⁴ The presence of this phenomenon should be tested in *M. leprae* as soon as possible.

ETH and PTH

Prima facie evidence of ETH-resistance was reported¹⁴ in 7 of 102 patients treated with 500 mg ETH as monotherapy; 4 of these were later confirmed as resistant by mouse foot-pad test.¹⁵ In addition, one of the three patients treated with ETH 500 mg daily as monotherapy in Carville developed ETH-resistance.¹⁶

In leprosy, the potential of cross-resistance between thioamides and thiacetazone (TBI) as well as DPT is a serious concern. All of these compounds have in common the group $-\text{CH}-\text{NH}_2$. Cross-resistance between TBI and DPT in *M. leprae* has been demonstrated, although not in every strain^{65, 66} (Ji *et al.*, unpublished data). Cross-resistance between TBI and ETH in *M. tuberculosis* has also been reported.²¹ Cross-resistance between ETH and PTH is to be expected, because the parts of the molecules responsible for antibacterial activity are identical; this has been confirmed in *M. leprae*.⁶⁶ The problem is that both TBI and DPT were quite widely used during the 1960s, and TBI is still being used as a component of combined therapy. Therefore, some leprosy patients must be harbouring mutants resistant to these 2 drugs. If cross-resistance between the thioamides and TBI as well as DPT exists in *M. leprae*, not only will this decrease the effectiveness of the thioamides, but it will also seriously threaten the effectiveness of multidrug regimens which contain thioamides. Pattyn and Colston reported that 2 ETH-resistant strains were also resistant to TBI and DPT, but that 3 DPT-resistant strains were susceptible to PTH.⁶⁶ Although this result is reassuring, the number of strains studied for cross-resistance is insufficient, and no TBI-resistant strain was included in the reported study. Therefore, there is urgent need for further evaluation of cross-resistance among TBI, DPT and ETH or PTH. Until the situation has been clarified, it would be preferable not to treat with multidrug regimens containing PTH patients who previously had been treated with either TBI or DPT for more than 2 years.

In conclusion, it seems unnecessary to conduct additional surveys for secondary dapsone-resistant leprosy, but it is necessary to collect more data with respect to resistance to RMP, CLO and PTH, in an attempt to estimate the magnitude of the threat to the prospects of combined therapy.

Prevention and treatment of drug resistance

Prevention of resistance is more important than treatment from the epidemiological point of view, and is also less expensive than treatment of drug-resistant patients. As mentioned previously, many patients will relapse with drug resistance if they are treated with bactericidal antileprosy drugs as monotherapy.

By analogy with the treatment of tuberculosis, to prevent the development of drug resistance, every multibacillary leprosy patient should be given combined therapy from the very first day of treatment. The spontaneous mutants resistant to any one drug should be fully susceptible to the other drugs. When the proportions of mutants resistant to any 2 of the drugs are 10^{-m} and 10^{-n} respectively, the proportion of doubly resistant mutants in a previously untreated bacterial population will be $10^{-(m+n)}$.²¹ Assuming that the total number of organisms in an untreated multibacillary leprosy patient is 10^{11} , and that 10% of them are viable, the population must consist of 3 fractions: the largest fraction, more or less 10^{10} , is composed of the organisms susceptible to both dapsone and a second bactericidal drug, e.g. RMP; a second fraction includes about 10^4 mutants resistant to dapsone; and the third fraction includes the same number of mutants resistant to RMP. Because the proportion of mutants resistant to either dapsone or RMP is 10^{-6} , the frequency of a doubly resistant mutant is 10^{-12} , and the possibility of finding an individual organism resistant to both dapsone and RMP is practically nil. If the patient is treated with dapsone plus RMP, RMP will kill the mutants resistant to dapsone, and, reciprocally, dapsone will kill the mutants resistant to RMP. Thus, drug resistance is prevented.

However, it should be emphasized that this description is valid only if the bacterial population

is normally susceptible to these 2 drugs, and treatment with the 2 drugs is started simultaneously. If the patient is treated with dapsone monotherapy for a certain period, and then RMP is added, the situation is no longer the same as that just described. Because of the period of dapsone monotherapy, the proportion of dapsone-resistant mutants might have increased by selection. Once the dapsone-resistant mutants have multiplied to a certain extent, e.g. to $\geq 10^6$, a certain number of RMP-resistant, i.e. doubly resistant-mutants will have appeared among the dapsone-resistant mutants, leading thereby to failure of combined therapy. Moreover, as mentioned earlier (p. 272), primary dapsone resistance is now quite common among untreated patients; therefore, the *M. leprae* of an untreated patient may no longer be fully susceptible to dapsone. Because the proportion of dapsone-resistant mutants is large even before treatment, and because the absolute number of the dapsone-resistant mutants is large enough to include a few RMP-resistant mutants, combined chemotherapy with dapsone plus RMP, even if started simultaneously, may fail, as RMP cannot kill the doubly resistant mutants. In order to prevent multiplication of the doubly resistant mutants, a third bactericidal drug must be included in the multidrug regimen. This is the reason for recommending, at this stage, that all multibacillary patients be treated with a combined regimen including 3 bactericidal drugs.⁸

Until now, most secondary-dapsone resistant patients have been treated with CLO or RMP,^{67, 68} usually as monotherapy or combined with a bacteriostatic drug. Although the presence of dapsone resistance has not altered the rate of initial response to the other drug, it is still possible for resistance to the second drug to develop, as if that drug had been given as monotherapy for a long period. Thus, the organisms become doubly resistant. In tuberculosis, double resistance is frequently observed after successive monotherapy.²¹ At least 12 doubly resistant strains of *M. leprae* have been reported—11 strains resistant to dapsone and RMP,^{11 13, 50} and 1 strain to dapsone and CLO.¹⁷ Because only 4 bactericidal antileprosy drugs with different mechanisms are currently available, the patient cannot receive proper treatment once a doubly resistant strain develops; moreover, he will also become a most dangerous source of infection. Therefore, prevention of such mutants is extremely important.

There have been a certain number of dapsone-resistant patients who had earlier been skin-smear negative for varying periods under dapsone monotherapy and had subsequently relapsed.^{18, 23, 27, 28} In order to prevent relapse caused by drug resistance, smear-negative multibacillary patients never previously treated with a multidrug regimen should also be retreated with multidrug regimens for fixed periods.

In short, all categories of multibacillary leprosy patients should receive combined therapy with bactericidal drugs. The regimen recommended for treatment of multibacillary leprosy by the WHO Study Group on Chemotherapy of Leprosy for Control Programmes⁸ has been designed to be effective in such patients.

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