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The First Decade in Experimental Leprosy

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Considerable developments have occurred in the application of the method for growing Mycobacterium leprae *in the mouse foot-pad since it was first described about 10 years ago. The method has been used to study growth curves and histology in normal and in thymectomized irradiated mice, to identify supposed isolates of* Myco. leprae *that have been made in tissue-culture or in non-living media, to evaluate tests of experimental vaccines, to investigate applications to clinical investigations (the loss of infectivity during chemotherapy as a means of monitoring a drug trial, the demonstration of drug-resistance, and the clinical problem of the patient who responds poorly to therapy), and to study new drugs—e.g., dapsone, acedapsone, clofazimine, and rifampicin.*

It has been known since 1957 that *Mycobacterium leprae* will grow in the foot-pads of mice, but the conditions required for consistent results, particularly the relationship between time and the number of bacilli, were not clear until 1960 (Shepard, 1960a, 1960b). The observations have subsequently been confirmed in many laboratories (Rees, 1964; Pattyn & Janssens, 1965; Maeda & Nakamura, 1968; Levy, Murray & Shepard, 1970; etc.). Transmission has also been achieved in several other species of small rodent: white rats (Hilson, 1965), Syrian and Chinese hamsters (Binford, 1965; Waters & Niven, 1965), and a species of *Mystromys* (Binford, 1968). These other species have not been employed extensively in laboratory experiments, probably because the mouse is the most convenient and readily available laboratory animal and because in some of the species mentioned early bacillary growth (within the first 6 months or so) is less extensive than in the mouse.

The results in the normal mouse are very consistent. A recent review showed that in the laboratory of the Center for Disease Control *Myco. leprae* had been isolated from 404 specimens, representing 249 patients infected in more than 21 countries, and 725 passages (from mouse to mouse) had been successful. Several dozen strains are kept in continuous passage, and the oldest strain has been in mouse passage for 11 years.

Rees and his colleagues (Rees, 1966; Rees et al., 1967; Rees & Weddell, 1968) have shown that another useful model is thymectomized, irradiated mice. The *Myco. leprae* infection in the normal mouse does not faithfully reproduce many aspects of the natural human disease, and it has always been hoped that the experimental infection in one of the other species of rodent, or in some other experimental animal, might do so. In the thymectomized, irradiated mouse, however, leprosy infections develop that have many features of lepromatous disease in man (i.e., frequent bacillary invasion of nerves, foam cells, and large number of bacilli). The greater number of bacilli in thymectomized, irradiated mice is useful in work that has not previously been possible with infections by small numbers of bacilli (serology, vaccination, studies of bacillary metabolism, bacterial anatomy, etc.).

The disadvantages of using thymectomized, irradiated mice are however real: thoroughly inbred lines of mice must be available, facilities for accurate and uniform irradiation of the mice are needed, and the mice must be unusually free from other infections that would kill them in the first few days or months after thymectomy and irradiation, i.e., before the *Myco. leprae* could multiply to the desired extent. Probably when thymectomized, irradiated mice develop an extensive *Myco. leprae* infection, they should be kept in isolation since they shed leprosy bacilli from the nose (Rees, 1970). (In general, the risk to laboratory personnel increases in proportion to the concentration of infectious agent in the material being handled.)

MATERIALS AND METHODS

It has sometimes been stated that the transmission of *Myco. leprae* to mice requires exacting and meticulous technique, the force of the statement being that the technique is suitable for only a few labora-

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tories throughout the world. However, many laboratories (altogether about 20) have been able to demonstrate successful transmission.

A reproducible method of counting bacilli is required since it is multiplication that is being observed; several methods have been described (Hart & Rees, 1960; McRae & Shepard, 1968a). Bacterial counts are made on suspensions of tissue prepared in tissue disintegrators (Shepard, 1960b) or tissue grinders (Rees, 1964).

Temperature control in the animal rooms is a matter of unknown importance. In the Center for Disease Control laboratories in Atlanta the air temperature of the animal quarters is maintained at about 20°C, but no comparisons have been made with animals kept in quarters that are heated in winter but whose temperature is not regulated in summer. Comparisons have been made between different constant temperatures (Shepard, 1965b); it was found that at constant air temperatures of 4°C and 35°C no growth of *Myco. leprae* occurred, and that at temperatures of 10°C and 30°C it was slower than in the optimal range of 15–25°C. At an air temperature of 20°C the average mouse footpad temperature is 30°C but it varies according to the activity of the mouse. In many areas endemic for leprosy the air temperatures may be as high as 35°C during the day, so the bacterial growth might be expected to be slower, and air-conditioning in animal houses would seem to be indicated. However, A. B. A. Karat (personal communication) has reported successful transmission at Vellore in South India with mice kept in quarters that were not airconditioned. (Man perspires at temperatures above about 30°C, thereby lowering the temperature of exposed skin; mice do not perspire, and their foot-pads are warmer at a given higher air temperature than human skin at sites of predilection for leprosy, e.g., nose, ears, and cheeks. Moreover, mice do not prosper at temperatures higher than about 25°C.) Some other information on the growth of *Myco. leprae* in footpads of mice is given in Table 1.

Table 1. Growth of *Myco. leprae* in the mouse foot-pad

skin biopsy specimens	most specimens from lepromatous patients have sufficient bacilli and, in the absence of erythema nodosum leprosum, the bacilli are consistently infective
most favourable size of inoculum	5×10^3 bacilli
minimal infectious dose	$5 \times 10^1 - 5 \times 10^2$ bacilli ($3 \times 10^0 - 4.0 \times 10^1$ solid bacilli)
usual harvest at plateau	$2 \times 10^5 - 2 \times 10^6$ bacilli, depending on the strain
usual time from inoculation to plateau phase	150–200 days
usual rate of growth	12–13 days per generation in the logarithmic phase, for the entire period from inoculation to the early plateau phase the average is 20–40 days
strain of mice	CFW, P, Balb C and CBA are most often used; C3H, DBA, and 101 are probably good also; ICR, C57BL, C57L, and A are less favourable
optimum air temperature	15–25°C, slower growth at 10°C and 30°C; no growth at 4°C and 35°C
preservation of infectivity of bacilli	at 0°C there is not much change for 7–14 days. Freezing causes much loss; glycerol prevents part of the loss during freezing; " cool-drying " is better than freeze-drying
destruction of infectivity	heating to 60°C for 1 hour removes infectivity; treatment with sodium hydroxide (2 % for 20 minutes at room temperature) does not alter infectivity, therefore it may be used to decontaminate specimens such as nasal washings, soil, and insects; digestion with trypsin (0.25 % of " 1 : 250 " enzyme at pH 7.6) is also without effect and may be used to purify tissue suspensions

The process of thymectomy and irradiation has been described by Miller (1963), Rees (1966), and Rees et al. (1967). Mice are thymectomized when they are 4–8 weeks old. After a week or so, a radiation dosage of 900–950 r is given, and within 24 hours about 5×10^6 synegeneic bone-marrow cells are transfused. Such mice have long-lasting immunological depression, especially of cellular immunity; they are distinctly slow in rejecting foreign skin grafts (Miller, 1963).

RESULTS

Growth curves and histology in normal and in thymectomized, irradiated mice

The growth curve of *Myco. leprae* in normal mice was given by Shepard & McRae (1965) and Shepard & Congdon (1968). With the usual inoculum of 5×10^3 bacilli, there are 3 phases to the growth curve: (1) the lag phase, which normally lasts about 90 days; (2) the logarithmic phase, during which the bacilli undergo regular binary fission; and (3) the plateau phase, in which the number of bacilli increases slowly or not at all.

The rate at which leprosy bacilli appear in the inoculated foot, and the level of the bacillary population in the plateau phase, vary somewhat with the strain of *Myco. leprae* employed. The median harvest of the most slowly growing strains in CFW mice is about 2×10^5 bacilli, while that of the fastest-growing strains is about 2×10^6. A chief difference between fast- and slow-growing strains is the point on the growth curve at which the infiltrate of macrophages and lymphocytes appears; this is later in fast-growing strains and seems thereby to allow a greater growth of bacilli (Shepard & McRae, 1971).

The histological events in the normal mouse are as follows. A cellular reaction to the injected fluid occurs and consists primarily of the polymorphonuclear infiltrate in the first 2 or 3 days; this is followed by a mononuclear infiltrate, which disappears in a week or two. During the remaining lag phase, and during the logarithmic phase, the bacilli are located in fibroblasts and histologically the foot does not differ from an uninoculated foot. The logarithmic phase is terminated abruptly by the appearance of a mixed infiltrate of macrophages and lymphocytes. Apparently, the local immune event is triggered only when the leprosy bacilli reach a critical concentration. The bacilli may be present only as single and small packets when first detected, but later globi are regularly present.

In the next few months a similar histological picture may be seen, or one that resembles borderline leprosy in which there are collections of macrophages with abundant cytoplasm with surrounding zones of smaller macrophages and lymphocytes. The number of viable bacilli drops during the first few months of the plateau phase but secondary waves of growth may follow (Shepard & McRae, 1965).

A year or so after inoculation, the bacilli have often decreased to low levels, but usually the granulomatous reaction persists. Rees & Weddell (1969) have described the appearance, about 20 months after inoculation, of borderline or borderline–lepromatous type lesions in the inoculated foot and in the other hairless regions, i. e., the nose, the ears, and the other feet. After intravenous inoculation, the lesions in the same peripheral hairless regions appear earlier; they have a greater tendency to lepromatous histopathology and contain higher numbers of bacilli.

In the thymectomized, irradiated mice the events are the same as in normal mice until the end of the logarithmic phase. Apparently, the same proportion of bacilli in the inoculum is able to initiate growth, and the bacilli grow at the same rate (Shepard & Congdon, 1968; Rees & Weddell, 1969; Rees, Pearson & Waters, 1970). At the end of the logarithmic phase the local immune response is so attenuated, however, that bacterial multiplication can continue, although at a reduced rate. A local disease eventually develops that mimics lepromatous disease with foam cells, heavy nerve invasion, etc. Metastasis occurs to the other hairless sites, with the development there of lepromatous-type lesions. After intravenous or intraperitoneal inoculation such involvement is seen earlier.

In the early stages of infection in the normal mouse, the experimental disease does not produce superficially visible signs. Nerve invasion is not frequent at the level of the inoculation—not more than 6% according to Shepard (1963)—but is common farther up the nerve a year or more after inoculation (Wiersema, 1965). About 20 months after the inoculation, sciatic nerve damage is frequent and many mice develop a " foot drop ", at least in the CBA line (Rees & Weddell, 1969). In the thymectomized, irradiated mouse, the inoculated foot may develop distinct swelling 12 months or so after infection. Sciatic nerve damage and " foot drop " are observed as late signs (Rees & Weddell, 1969).

Identification of supposed isolates of Myco. leprae

Claims that *Myco. leprae* have been cultivated in tissue culture or in artificial media have appeared

regularly in the literature. In many cases the mycobacterium isolated is a contaminating organism rather than *Myco. leprae*, and use should be made of the mouse foot-pad model to establish the identity of the isolate. Most mycobacteria do not grow in the mouse foot-pad, and most of those that do, e.g., *Myco. marinum* and *Myco. ulcerans*, have an entirely different time scale and histological reaction. *Myco. lepraemurium* has a similar histological reaction but it grows faster than *Myco. leprae*, does not form globi, and metastasizes to the regional and abdominal lymph nodes after 6 months or so.

In some claims to have cultivated *Myco. leprae*, the question has been whether actual multiplication occurred. Here also, inoculation into foot-pads of mice may be helpful because the viability of *Myco. leprae* is lost after 2 months in many media. The apparent increase in these cases is presumably a technical artefact of counting (McRae & Shepard, 1970).

Correlation of morphological changes with infectivity

Evidence has been provided that viability, as measured by infectivity for mice, is confined to bacilli that stain solidly, i.e., darkly and uniformly (Shepard & McRae, 1965; McRae & Shepard, 1968b).

Tests of experimental vaccines against leprosy

Vaccine protection against experimental infection, as evidenced by a lowering in the number of *Myco. leprae* growing in the infected foot-pad, is provided by a number of mycobacterial species, especially tubercle bacilli, when infected as heat-killed suspensions (Shepard, 1965a). Live BCG is perhaps the most effective vaccine tried, and vaccines made from several strains of BCG and several commercial products were effective (Shepard, 1965a, 1968). BCG was active even during the incubation period, after the mice had been inoculated with *Myco. leprae* (Shepard, 1966). Experimental vaccines prepared from oil-treated BCG cell walls have been found to provide about as much protection as live BCG (Shepard & Ribi, 1968).

Application to clinical investigation

Skin biopsy specimens from most lepromatous patients provide sufficient bacilli for the inoculation of mice; in the absence of erythema nodosum leprosum, the bacilli are consistently infective. It is not necessary for the laboratory to be located in an endemic area since the bacilli survive well at 0°C for several days and specimens may be shipped anywhere in the world if convenient air transport is available.

Loss of infectivity during effective chemotherapy. When lepromatous patients are treated with standard doses of dapsone, the number of bacilli infective for mice decreases to undetectable levels in less than 100 days (Shepard, Levy & Fasal, 1968, and unpublished data). The mouse is sufficiently sensitive for about 99% of the loss in infectivity to be measured. By comparison, observations of the morphological index can indicate only about 90% of the loss of viability, and they give much less precise information near the end point. Consequently, a drug trial that is monitored by mouse inoculation provides more rigorous answers with fewer patients than one monitored by morphological index observations only.

Objections to the use of mice to measure changes in viability of leprosy bacilli in clinical trials have been made on the grounds that normal mice are more resistant than lepromatous patients, as indicated by the inability of the normal mouse to develop lepromatous-type disease. However, the immune mechanisms in the normal mouse do not appear to be exerted until the growth of the bacilli has reached plateau levels and is already detectable. Rees, Pearson & Waters (1970) have recently reported that thymectomized, irradiated mice, which are of course capable of developing lepromatous disease, were not more sensitive than normal mice in their ability to detect viability of leprosy bacilli from two patients, one being treated with dapsone and the other with rifampicin.

Another source of confusion arises from this use of mice. As already mentioned, with the techniques in use mouse inoculations can measure a loss of only about 99% of the pre-treatment infectivity and a negative result, therefore, is not proof that all the bacilli in the patient are dead. There are about 10^{11}–10^{12} viable *Myco. leprae* in the average lepromatous patient, and a reduction of 99% lowers the total to 10^{9}–10^{10}. Presumably, all the bacilli must be killed before treatment can be stopped without fear of bacteriological relapse. There are no microbiological techniques in leprosy, or in any other infectious disease, that can be used to test for the death of the last organisms. The only operation that can be carried out is to stop treatment and see if the patient relapses. A direct approach of this sort has been used to test therapeutic regimens in pulmonary tuberculosis.

Demonstration of drug-resistance. Strains of *Myco. leprae* from untreated patients are sensitive to 0.0001% of dapsone in the diet (Shepard, McRae & Habas, 1966; Rees, 1967; Shepard, Levy & Fasal, 1969).

In a few patients, dapsone resistant strains of *Myco. leprae* eventually appear and the patients no longer respond to therapy (Pettit & Rees, 1964; Pettit, Rees & Ridley, 1966; Shepard, Levy & Fasal, 1969). Demonstration in mice is the only way of proving drug resistance and is an important factor in the management of the patient; one-third to one-half of the patients who have apparent dapsone-resistance are in fact not taking the drug and their bacilli are completely sensitive.

Assistance in clinical problems. In an established clinic a frequent problem is the patient who has been on treatment for a number of years and who does not seem to be responding. The lesions may seem to be progressing, and dapsone-resistance is considered possible. In such cases mouse inoculation has been found very helpful. If the patient's bacilli will not multiply in mice, it can be considered that they are responding properly. If bacilli can be isolated, the patient is either not taking the dapsone, or dapsone-resistant strains have appeared; tests of dapsone-sensitivity will distinguish between these alternatives. If erythema nodosum leprosum has appeared during treatment the response of the patient's bacilli may be assumed to be satisfactory, since the bacilli will probably be non-infectious at that stage. In patients who have erythema nodosum leprosum before treatment is started, the proportion of viable bacilli is so low that their presence can be demonstrated only irregularly. However, we have observed one instance of dapsone-resistant bacilli in a patient who had erythema nodosum leprosum before treatment and continued to have it during several years of therapy that was irregular and discontinuous because of the reactions.

Our general practice, in the case of the poorly responding patient who has a reasonable history of sulfone therapy for several years, is to test for dapsone-sensitivity on first isolation in mice. An answer will usually be available in 4–7 months. However, if there are not sufficient bacilli to provide an inoculum of at least 1×10^3 bacilli, or if the patient has erythema nodosum leprosum, dapsone-sensitivity is tested at the next passage if the first inoculation is successful.

Studies of new drugs

Perhaps the greatest use of mice in leprosy research today is in the study of new drugs. The three most important drugs in the treatment of leprosy are discussed briefly to illustrate how the peculiarities of the action of each drug in mice reflect its action in man. Knowledge of the minimum effective dosage, of the blood and tissue levels at the minimum effective dosage and at acceptable dosages in man, of the duration of persistence of the drug after its administration, and of the rate of bactericidal activity is necessary for the rational design of therapeutic regimens that are applicable in leprosy-endemic areas, many of which have limited medical resources.

Dapsone and acedapsone. As already mentioned, strains of *Myco. leprae* from untreated patients are susceptible to 0.0001% of dapsone in the diet, a dosage that produces about 10 ng/ml in the blood of mice (Ozawa, Shepard & Karat, 1970). Since dapsone is fairly evenly distributed in the blood and non-hepatic tissues, 10 ng/ml may be taken as the minimum inhibitory concentration (MIC) of dapsone for *Myco. leprae*. Standard dosages in man, 50–100 mg daily, produce 1000–2000 ng/ml of blood, or 100–200 times the MIC. The repository sulfone acedapsone, given in an injection of 225 mg every 75 days, releases an average of 2.4 mg of dapsone daily (Shepard, Tolentino & McRae, 1968) and produces blood levels that average 50 ng/ml, or at least 5 times the MIC (Ozawa, Shepard & Karat, 1970). The mouse results thus indicate that acedapsone should be therapeutically active in man, and clinical trials showed that this was the case (Shepard, Tolentino & McRae, 1968). Further experience with acedapsone is needed before it can be seen whether dapsone-resistance will be more of a problem with acedapsone than with dapsone itself; blood levels of dapsone are low but they are steady and one can be certain there is no surreptitious avoidance of drug intake. In a trial of acedapsone in New Guinea in 406 patients, including 28 lepromatous cases, with sufficient bacilli for bacteriological follow-up, the reponse was still favourable after 750 days of treatment (Russell et al., 1970). The applicability of the findings in mice to the disease in man was further confirmed in a short-term trial in lepromatous leprosy in which 1 mg of dapsone administered orally was found to be active therapeutically (Waters, Rees & Ellard, 1968). This dosage provided an average serum concentration of 18 ng/ml (Ellard, Rees & Waters, personal communication). Under ordinary circumstances, except perhaps in the presence of erythema nodusum leprosum, there appears to be no advantage in lowering the dosage of dapsone itself from the standard level.

Clofazimine. This drug is ordinarily administered to man in dosages of 100–300 mg daily; the red

pigmentation of the skin it causes is objectionable for light-skinned patients; however, the minimum effective dosage of clofazimine in mice is 0.0001–0.001% (Shepard, 1969) and these levels produce little pigmentation. It is possible that the usual human dosage could be lowered without losing therapeutic effectiveness. An important, but yet unanswered, question is how frequently the drug should be administered. It is excreted very slowly and a daily schedule would appear to have no therapeutic advantage over a less frequent schedule with the same total intake; the drug is deposited in the tissues of both the mouse and man, so a repository effect exists throughout the body. Measurements of the blood or tissue clofazimine content do not provide estimates of the MIC in the way they do for dapsone, because the distribution in the body is so uneven. It is not even certain that a lower intake will result in lower concentrations of the drug in the immediate environment of the organism. The quantitative answer to these questions can probably not be provided by mouse experimentation, and will have to be obtained by clinical trials in man. Nevertheless, mouse experiments in progress confirm the expectations that infrequent ingestion or injection is effective (Shepard, unpublished data); for example, the drug is active when 0.01% is administered in the diet for 2 days every 4 weeks, or when 1 mg is injected intraperitoneally on the day of infection.

The bactericidal rate in the mouse cannot be worked out by the " kinetic " method because of the persistance of clofazimine in the tissues (Shepard, 1969) but measurements have been made of the rate in man. In patients with dapsone-resistant *Myco. leprae* who received 100–300 mg of clofazimine daily, mouse inoculation showed that the viability of the *Myco. leprae* declined rather more slowly than in new patients treated with dapsone (Levy, Shepard & Fasal, unpublished data). For 50 days, there was not much change in infectivity but the infectivity then decreased at about the rate seen in dapsone-treated patients, until it was no longer demonstrable after 150 days of treatment.

Rifampicin. The unique feature of rifampicin is its rapid bactericidal action. The administration of 0.03% in the diet of mice for only 2 days appears to kill most of the organisms. By comparison, dapsone must be administered for 60–90 days to produce the same bactericidal effect (Shepard, unpublished data).

This rapid bactericidal effect is observed also in man (Rees, Pearson & Waters, 1970). In lepromatous patients treated with 600 mg daily, the morphological index fell to the base-line level in 4 weeks, as compared with 19 weeks in treatment with dapsone. The progress of one patient was followed by mouse inoculations; his bacilli were no longer infectious after 24 days of treatment with 600 mg of rifampicin per day. (The results were no different when the bacilli were inoculated into thymectomized, irradiated mice.) One control patient treated with dapsone still had a few infective bacilli after 69 days of treatment and this is within the range seen in various series of dapsone treatments (Shepard, Levy, & Fasal, 1968, and unpublished data).

RÉSUMÉ

BILAN DES DIX PREMIÈRES ANNÉES D'ÉTUDES EXPÉRIMENTALES SUR LA LÈPRE

C'est il y a dix ans qu'a été décrite pour la première fois la méthode permettant d'obtenir de façon régulière le développement de *Mycobacterium leprae* dans les coussinets plantaires de la souris. Aujourd'hui, quelque 20 laboratoires dans le monde sont parvenus à réaliser la transmission du bacille. Les progrès considérables accomplis durant ces dix années dans l'application de cette méthode sont décrits par l'auteur sous les rubriques suivantes: *a) Courbes de croissance bacillaire et histologie chez des souris normales et chez des souris irradiées et thymectomisées.* Le début de la courbe de croissance bacillaire (jusqu'à la fin de la phase logarithmique) paraît être le même chez les souris normales et chez les souris irradiées et thymectomisées, mais ensuite, *Myco. leprae* continue de croître chez ces dernières et, histologiquement, les infections finissent par ressembler à la lèpre lépromateuse humaine. Les souris irradiées et thymectomisées sont donc très utiles pour les études sur la pathogenèse des infections lépromateuses et pour celles qui exigent de grands nombres de *Myco. leprae.* Toutefois, nombre d'études expérimentales ne portent que sur la première partie de la courbe de croissance bacillaire, aussi continuera-t-on à utiliser fréquemment des souris normales. *b) Identification d'isolats présumés de* Myco. leprae. Dans le passé, il est arrivé fréquemment que l'on déclare à tort avoir isolé *Myco. leprae,* à la suite d'une confusion entre un contaminant mycobactérien et le bacille, ou de la persistance — alors qu'il n'était plus viable — de l'inoculum original; l'inoculation de coussinets plantaires de souris permet de distinguer ces deux sources possibles d'erreur. *c) Essais de vaccins expérimentaux contre la lèpre,* en particulier comparaison entre le BCG vivant et de nouveaux types de vaccins. *d) Application à la recherche clinique.* Les

inoculations à la souris fournissent un moyen sensible et précis de contrôler les essais de médicaments, qui permet mieux que tout autre de suivre la diminution du pouvoir infectant du bacille sous l'effet du traitement. D'autre part, on ne dispose d'aucune autre méthode pour prouver que les bacilles d'un malade sont résistants aux médicaments. Les inoculations à la souris aident aussi à mettre en évidence certains problèmes cliniques: les cas, par exemple, où le malade ne paraît guère réagir à la chimiothérapie. *e*) L'*étude des médicaments antilépreux* est devenue un domaine trop vaste pour qu'il soit possible de l'étudier dans le présent article, mais l'auteur donne un aperçu des recherches entreprises en faisant la corrélation entre les principales observations effectuées sur la souris et les effets observés sur l'homme de ce que l'on peut considérer comme les principaux médicaments antilépreux, pour le présent et le proche avenir, à savoir la dapsone et l'acédapsone, la clofazimine et la rifampicine. L'une des principales caractéristiques de la dapsone, chez la souris, est sa concentration inhibitrice minimale très faible, 10 ng/ml ou moins. L'acédapsone, qui est une préparation dépôt, maintient continuellement une concentration sanguine de sulfone plusieurs fois supérieure à ce taux et les premiers résultats thérapeutiques et chimioprophylactiques chez l'homme sont prometteurs. L'une des caractéristiques importantes de la clofazimine, chez les souris et chez l'homme, est qu'elle se dépose dans les tissus et que son excrétion est très lente. Elle s'est révélée efficace chez la souris à doses espacées et il faudrait essayer chez l'homme cette forme d'administration qui est plus pratique que la dose quotidienne. Quant à la rifampicine, sa caractéristique la plus intéressante est la rapidité avec laquelle elle détruit *Myco. leprae* chez la souris, rapidité d'action qui se retrouve aussi chez l'homme.

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