New Prospects for the Study of Leprosy in the Laboratory

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Although Mycobacterium leprae was identified earlier than Myco. tuberculosis, it has still not been cultured in vitro and only in 1960 was an infection obtained in laboratory animals. However, important advances have been made in the field of experimental leprosy in the last decade due to the development of new techniques and models for studying Myco. leprae in vivo, thus overcoming the limitations imposed by a non-cultivable mycobacterium. Quantitative techniques using Myco. lepraemurium provided the first model for developing an indirect method for distinguishing dead (non-infectious) from living (infectious) bacilli, based on morphological differences in organisms stained by the Ziehl–Neelsen method. However, the most important advances resulted from the limited and localized growth of Myco. leprae when inoculated into the foot pads of mice and, later, the more substantial and generalized multiplication of Myco. leprae in immunologically deficient mice (thymectomized and irradiated with a dose of 900 r). Moreover, in the immunologically deficient animals, the infection eventually resulted in a disease replicating that of lepromatous type leprosy in man, including the involvement of peripheral nerves.

The results from these studies and the future prospects for the study of leprosy in the laboratory are reviewed in this article.

Of the mycobacterial diseases affecting man, leprosy is second only to tuberculosis in presenting a world health problem affecting more than 10 million people, most of whom live in newly developing tropical and subtropical countries. Leprosy is a chronic infectious disease caused by Mycobacterium leprae, an obligatory intracellular parasite often giving rise to virtually no symptoms. Clinically, the disease manifests itself most commonly in the skin, nose, upper respiratory tract and peripheral nerves and its form is related to the capacity of the individual to destroy the organism. The effect on nerves is responsible for the serious disabilities and deformities of the feet, hands and face, and a Scientific Meeting on Rehabilitation in Leprosy (1961) estimated that in at least 25% of all cases there is some degree of deformity.

It is of interest that Myco. leprae is the only species of mycobacterium which affects nerves in either man or animals. This unique property deserves special study, for it is likely to increase our knowledge both of the organism and of peripheral nerve fibres.

Patients with leprosy present a wide range of symptoms and signs, ranging between one or other of the 2 polar forms of the disease; namely, tuberculous leprosy, in which there are few bacilli, and lepromatous leprosy, in which the number of bacilli is extremely high. Thus, a study of the pathogenesis of leprosy requires knowledge drawn from a wider range of scientific disciplines than for any of the other mycobacterial diseases. Hitherto, progress has been severely restricted because Myco. leprae cannot, as yet, be grown in vitro and has only been grown in vivo to a limited extent since 1960 (Shepard, 1960a, 1960b). This is unfortunate because the bacterium was one of the first to be linked specifically to a human disease.

It is obvious that, if the causative organism cannot be cultivated or the disease transmitted to experimental animals, neither the bacteriology nor the pathology of the infection can be studied in the laboratory. The lack of success with these techniques has seriously limited the scope of fundamental and
applied research in the leprosy field since the isolation and identification of causative organisms, and their subsequent cultivation in vitro and in vivo has resulted in the control of most of the bacterial diseases of man. Failure to cultivate and transmit leprosy in the laboratory has not been due to lack of effort. The first attempts were made by Hansen and since then by many bacteriologists and pathologists. From time to time, claims to success have been made but, until recently, none has been upheld although, between 1874 and 1930, all the other important human pathogens were successfully cultured by one means or another. Since 1960, however, techniques have been developed for the transmission of leprosy to animals, and the situation has been transformed.

This review is confined to a consideration of the experimental models developed during the last 10 years which have now reached a stage that permits Myco. leprae to be studied on a sound experimental basis. Although it is still impossible to grow the bacilli in vitro, these models have already contributed significantly to the study of leprosy in man and are likely to contribute even more during the next 10 years. Although the most important advances arose from the development in 1960 of techniques for transmitting leprosy to animals, it was a prerequisite that experimental models using other species of mycobacteria were developed before the laboratory study of human leprosy was started. The study of models, particularly those using Myco. lepraemurium, established the method for determining, indirectly, the infectivity of Myco. leprae and the techniques of tissue culture adapted to the cultivation of human leprosy bacilli.

MODELS BASED ON COMPARATIVE STUDIES WITH MYCO. LEPRAEMURlUM

An indirect method for determining the viability of leprosy bacilli

Most species of bacteria are completely destroyed when they die within an infected host, but mycobacteria are an exception since they retain both their bacillary form and the property of staining with carbol fuchsin when they are no longer alive. However, in a Myco. leprae infection it was not possible to determine directly whether bacilli in the patient were alive or dead because they could not be cultured. Therefore, indirect methods for measuring their viability had to be used. For these studies the closely related Myco. lepraemurium proved to be an ideal model since, although the rat leprosy bacillus also failed to grow in vitro, its viability could be assessed in terms of infectivity by determining its ability to produce disease following its reinoculation into animals. Early studies using electron microscopy showed that viable Myco. lepraemurium, that is, bacilli that infected animals, could readily be distinguished from non-viable bacilli, that is, bacilli that failed to infect animals (McFadzean & Valentine, 1959; Rees, Valentine & Wong, 1960). The essential differences were that the cell wall of the non-infectious (i.e., dead) bacillus, though still intact, was no longer uniformly filled but contained only electron-dense aggregates of disorganized protoplasmic material, whereas the infectious form was uniformly electron-dense. Evidence that this was a general phenomenon was provided by using an entirely different species of bacterium, Escherichia coli, which had the added advantage that viability could quickly be tested by means of conventional viable colony counts on solid media. The results of such studies showed that there was a close correlation between the decrease in the number of colonies and the increase in the proportion of degenerate forms of E. coli seen under the electron microscope. Furthermore, the degenerate changes which took place in E. coli resembled those seen in Myco. lepraemurium (Rees, Valentine & Wong, 1960).

Once it had been established that living and dead forms of bacilli could be distinguished, a new set of comparisons was undertaken to see how far the morphological changes seen under the electron microscope were correlated with those shown by the light microscope when Myco. lepraemurium was stained with carbol fuchsin using the routine Ziehl-Neelsen method. Rees & Valentine (1962) developed a new technique that allowed individually identified and stained bacilli to be examined first under the light microscope and then under the electron microscope. Close agreement was found between the proportion of degenerate forms of Myco. lepraemurium seen under the electron microscope and the proportion of bacilli showing irregular staining with carbol fuchsin under the light microscope. From these studies it appeared that the electron-dense material within the cell wall of the organism corresponded exactly to the part staining with carbol fuchsin. It was therefore concluded that all forms of Myco. lepraemurium showing irregular staining were dead and only those showing uniform or "solid" staining were likely to be viable. It was reasonable to expect that this assumption could be extended to the human leprosy bacillus since the
same morphological changes were likely to be shared by all species of mycobacteria. This expectation was fully confirmed when, using the same technique, suspensions of *Myco. leprae* were examined by both light and electron microscopy (Rees & Valentine, 1962). More recently, it has been possible to confirm this assumption by demonstrating experimentally that only the uniformly staining forms of *Myco. leprae* are capable of multiplying in the mouse foot pad (Rees, 1965b; Shepard & McRae, 1965).

**Rate of multiplication of leprosy bacilli in vivo**

One explanation for the chronicity of human leprosy might be that *Myco. leprae* divides more slowly than other bacteria or even other mycobacteria; this hypothesis could be tested in murine leprosy since the natural and experimental infections are known to be chronic. By applying quantitative techniques it has been possible to follow the total number of stained acid-fast bacilli in the tissues of animals during the infection and therefore to determine the rate of multiplication or the generation time of *Myco. lepraemurium*. Several different groups of workers (Hilson & Elek, 1957; Hobby et al., 1954; Rees, 1957) have found that even in susceptible animals, the bacilli divide only every 10–14 days. This very long generation time is unique for micro-organisms and even for other species of mycobacteria. It is most unlikely that *Myco. leprae* would have a shorter generation time than that of *Myco. lepraemurium*. The long generation time must be considered when claims of successful cultivation or transmission of *Myco. leprae* are being assessed particularly if the claim suggests that the organism is multiplying rapidly. It is even more important to accept a long generation time when methods for attempting to cultivate or transmit *Myco. leprae* are being planned. The model has been shown to be applicable by Shepard & McRae (1965), who demonstrated that *Myco. leprae* has a generation time of 13–25 days in the mouse foot pad (see p. 791).

**Time taken to kill leprosy bacilli and the fate of dead organisms following chemotherapy**

It is known that in patients receiving successful chemotherapy, in particular after treatment with dianodiphenylsulfone, a very long time elapses before bacilli disappear from the lesions. Whether this was due to the slow rate at which the drug killed the bacilli or to the inability of the host to dispose of the dead organisms could only be a matter of conjecture in the absence of direct or indirect evidence. However, when murine leprosy was used as a model to study the effect of chemotherapy on the rate of kill (as determined by the proportion of irregularly stained, "dead", bacilli) and the ability of the host to dispose of the bacilli killed by chemotherapy *in vivo* (Rees & Waters, 1963), indirect evidence on this point became available. Mice heavily infected with *Myco. lepraemurium* were treated with isoniazid and the total number of bacilli and the total numbers of viable (solidly staining bacilli) were determined in the treated and untreated groups at regular intervals for more than a year. The results showed that isoniazid was very effective since the untreated animals all died of gross infection within 100 days, whereas the treated mice lived for more than a year. Furthermore, 73% of the bacilli in the treated mice showed irregular staining after only 28 days and the proportion had reached 93% by the 63rd day of treatment. There was, therefore, a 90% kill within 50 days of treatment. On the other hand, the fall in the total number of stained bacilli in the tissues of the treated mice was much slower; it took 250 days for a 90% fall in numbers to occur (Fig. 1). Thus, although isoniazid was effectively bactericidal within a relatively short time, dead *Myco. lepraemurium*

![FIG. 1](image)

"Viable" and total populations of *myco. lepraemurium* in the liver and spleen of untreated and isoniazid-treated mice

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*a* All untreated mice died with gross infections by day 100.

*b* Reproduced, by permission, from Rees & Waters (1963).

**A** = Untreated, total count;

**B** = Untreated, viable count;

**C** = Isoniazid-treated, total count;

**D** = Isoniazid-treated, viable count.
were not nearly so quickly removed from the infected tissues. After continuous treatment with isoniazid for 1 year the proportion of solidly staining acid-fast bacilli started to rise again; in other words, viable organisms were reappearing. The total number of such organisms steadily increased and within 3 months the animals died of *Mycobacterium leprae*-murium infections. Bacilli recovered from animals that had relapsed under treatment with isoniazid were inoculated into healthy mice which were also treated with isoniazid. These animals showed no response to the drug and the infections took the same course as in untreated controls (Hart, Rees & Valentine, 1962). This indicated, without doubt, that the recurrence of active disease in the mice used in the first experiment was due to the appearance of isoniazid-resistant organisms and that the relapse was heralded by the reappearance of bacilli which were morphologically viable.

**Use of tissue culture systems for the cultivation of *Mycobacterium leprae*-murium in vitro**

*Mycobacterium leprae*-murium, like *Mycobacterium lepra*, is predominantly an intracellular parasite and so far neither organism has been cultured in bacteriological media. The dependence on an intracellular environment strongly suggested that tissue-culture methods might offer a means of growing *Mycobacterium leprae*-murium *in vitro*. The first successful claims of limited multiplication of *Mycobacterium leprae*-murium in tissue culture systems were made by Wallace, Elek & Hanks (1958) and by Rees & Wong (1958). Since then, techniques have been developed using an established strain of rat fibroblast cells (Rees & Garbutt, 1962) or cultures of mouse macrophages (Chang, 1960) in which indefinite and continuous multiplication of *Mycobacterium leprae*-murium has been obtained. The success of these tissue-culture methods for *Mycobacterium leprae*-murium provides a wealth of knowledge which can be applied directly to the problems concerned with the very similar, slow-growing organism *Mycobacterium lepra*. In particular, the studies have stressed the importance of using quantitative methods for determining the total number of acid-fast bacilli present at the beginning and at the end of each culture period, and therefore basing multiplication on absolute increases in the bacillary population. The value of examining the morphology of the bacilli as a sensitive means of determining their survival in tissue culture has also been made clear. It is significant that the characteristics of *Mycobacterium leprae*-murium maintained in continuous cultivation in tissue-culture systems for more than 3 years have not changed (Rees & Garbutt, 1962). *Mycobacterium leprae*-murium grown in tissue culture do not multiply in bacteriological media, still have a generation time of 10-12 days and retain their pathogenicity for mice and rats. These observations are of considerable importance since they confirm the generally accepted view of the stability of bacterial populations, which is in sharp contrast to so many of the acid-fast bacillary strains, claimed to be *Mycobacterium lepra*, which were isolated from leprosy patients. These strains had a wide and variable range of characteristics which were, unjustifiably, explained on the basis of adaptation or mutation.

**Application of the results of studies on murine leprosy to human leprosy**

*Mycobacterium leprae*-murium has been extensively used as a model for leprosy research, although more recently its value has been criticized. Such criticism is justifiable only when murine leprosy has been used uncritically as a model for the human infection—namely, for screening potential antileprosy drugs and in comparative pathological and histological studies. Where murine leprosy has been used to answer specific questions or to test hypotheses directly related to *Mycobacterium lepra*, these studies have provided valuable information. It is clear from the results of these studies that a new and valuable technique was available that could now be used in man to determine the viability of *Mycobacterium lepra* in patients and thus to assess precisely their probable response to chemotherapy, the infectivity of the various types of leprosy, and, by histological studies, the viability of *Mycobacterium lepra* in different tissues. Thus the morphological index (MI), that is, the percentage of solidly staining bacilli seen in smears or sections from leprosy patients, could now be used as the measure of viability.

The routine bacteriological examination of patients is based on smears prepared from diseased skin or a nasal scraping and, after staining the smear by the carbol fuchsin method, the density of bacilli (this is known as the bacteriological index (BI)) is scored, irrespective of the morphological appearance of the bacilli. The progress of the patient under treatment is then judged by the rate of disappearance of bacilli from these smears, that is, by the fall in the BI. It has long been considered that even the most active antileprosy drugs, including diaminodiphenylsulfone, leave much to be desired since in patients with the more severe lepromatous type of leprosy many years
elapse before negative smears give evidence of a "cure". Hitherto, it has been assumed that more active drugs are required to kill *Myco. leprae* more rapidly than diaminodiphenylsulfone, an assumption that was based on the fact that the BI falls so slowly. Now that it has been demonstrated conclusively that the viability of *Myco. leprae* can be assessed on the basis of their morphology rather than on the total number of the bacilli, it has been possible to show, in carefully controlled studies, that a very high proportion of bacilli are killed in 3 months in patients receiving standard treatment with diaminodiphenylsulfone (Waters & Rees, 1962). This observation suggests that persisting lesions and many of the manifestations of leprosy, including reactions of the erythema nodosum lepromatum type, that follow the initial phase of chemotherapy must be due, in part, to the presence of dead bacilli. It implies that a more rapid cure will be achieved only if other drugs or methods are found that could be used, after the initial killing of leprosy bacilli with standard antileprosy drugs, to enhance the host's ability to dispose of dead, but still intact, leprosy bacilli.

Thus the introduction of the MI as an index of viability has not only provided a rapid method for determining, within a period of only 3–6 months (Fig. 2), the activity of potential antileprosy drugs (Waters, Rees & Sutherland, 1967) but has led to an entirely new approach to the problem of leprosy chemotherapy. Furthermore, an increase in the MI during treatment provides a sensitive measure of the patient's deterioration, whether this is due to failure to take the drug or to the emergence of drug resistance (Pettit & Rees, 1964; Pettit, Rees & Ridley, 1966) (see p. 793).

**EXPERIMENTAL LEPROSY IN ANIMALS**

Before describing advances that have been made in the field of experimental transmission of leprosy to animals since 1960, it is pertinent to review briefly the general problems and methods used in animal transmission experiments and the criteria for assessing successful claims. These were defined in detail by the Technical Committee on Pathology and Experimental Transmission (1963) at the Eighth International Congress of Leprology in Rio de Janeiro, Brazil. Bacilli for inoculating laboratory animals should be obtained from patients with untreated leprosy and with a high MI and, because of the possible contamination of skin by other cultivable mycobacteria, biopsy specimens should not be taken from ulcerated lesions. Moreover, all suspensions prepared for purposes of inoculation should be cultured on a variety of media suitable for isolating mycobacteria. In addition to each group of animals inoculated with fresh suspensions of bacilli, there should be a group inoculated with heat-killed organisms and an uninoculated group of animals. Quantitative bacteriological methods should be used in order to determine the number of organisms inoculated and subsequently to determine the number of organisms present in the animals. These rigorous methods and checks were introduced in order to exclude as far as possible the inoculation and subsequent development of infections with contaminating strains of mycobacteria, and also the possibility that the animals themselves might be carriers of mycobacteria. This latter possibility has been demonstrated very clearly by the elegant work of Nishimura and his colleagues working at Osaka in Japan, showing that a proportion of apparently healthy mice and other rodents can be carriers of a murine leprosy-like infection (Nishimura et al., 1964). The criteria for assessing successful claims for transmission of leprosy were also rigorously defined. In addition to counting the number of bacilli isolated from the animal tissues, the organisms should be cultured, using again media suitable for growing mycobacteria. Successful transmission of an infection from one patient should be reproduced from others, using the same experimental conditions. A standard type lepromin should be prepared from the bacilli harvested from the animals and compared

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**FIG. 2**

**EFFECT OF TREATMENT WITH DIAMINODIPHENYL-SULFONE** for 6 MONTHS ON THE MORPHOLOGICAL INDEX (MI) IN 6, PREVIOUSLY UNTREATED, LEPROMATOUS PATIENTS

![Graph showing the effect of treatment with diaminodiphenylsulfone on the morphological index (MI) in 6, previously untreated, lepromatous patients](image)

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*a* 100 mg daily.
with a similarly prepared human lepromin from patients with tuberculoid and lepromatous leprosy; these tests should be carried out and read blindly. Finally, a histopathological examination should be made of the infected tissues including, in particular, a careful examination of the nerves, using the requisite staining methods.

Experimental leprosy in normal animals

Undoubtedly the most important direct contribution to the study of leprosy since the identification of *Myco. leprae* by Hansen has been the transmission of experimental leprosy to animals. This was achieved first by Shepard (1960a, 1960b), at the Communicable Disease Center, Atlanta, Ga., USA, who showed that a reproducible and limited infection could be produced when the foot pads of mice were inoculated with *Myco. leprae*. Moreover, an identical type of infection has now been produced in other centres throughout the world.

General features

The infection obtained in the mouse foot pad is a local one and is dependent upon the number of bacilli inoculated. Thus inocula of $5 \times 10^5$–$10^4$ *Myco. leprae* multiply 100-fold in 6–8 months, whereas larger inocula fail to give a higher yield, and inocula of $10^4$ bacilli fail to show any multiplication. Moreover, having multiplied as far as they are able, the bacilli gradually die (Rees, 1964). A similar type of infection has been obtained subsequently in the ears of mice and in the ears and foot pads of hamsters (Waters & Niven, 1965, 1966) and in the foot pad of the rat (Hilson, 1965). Although these experimental infections give only limited multiplication of *Myco. leprae*, they are reproducible and can be maintained indefinitely in the laboratory by passage and are adaptable to quantitative analysis. It is probable that more than 200 strains of *Myco. leprae* derived from individual patients with active disease originating from nearly every part of the world have produced an identical type of infection when inoculated into foot pads of mice. For example, in our own series we obtained successful transmission with all 89 strains of bacilli isolated from individual patients with active leprosy from different parts of the world and with different types of the disease (79 patients with lepromatous leprosy and 10 with borderline tuberculoid leprosy). The sources of the 89 strains is shown in the following tabulation.

<table>
<thead>
<tr>
<th>Origin</th>
<th>No. of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>7</td>
</tr>
<tr>
<td>Central</td>
<td>1</td>
</tr>
<tr>
<td>East</td>
<td>1</td>
</tr>
<tr>
<td>West</td>
<td>3</td>
</tr>
<tr>
<td>Burma</td>
<td>6</td>
</tr>
<tr>
<td>India</td>
<td>8</td>
</tr>
<tr>
<td>Malaysia</td>
<td>66</td>
</tr>
<tr>
<td>Malta</td>
<td>1</td>
</tr>
<tr>
<td>Pakistan</td>
<td>1</td>
</tr>
<tr>
<td>Samoa</td>
<td>1</td>
</tr>
<tr>
<td>West Indies</td>
<td>1</td>
</tr>
</tbody>
</table>

These results are important since they indicate that the virulence of strains of *Myco. leprae* for the mouse is similar, irrespective of whether the strains were derived from patients with the more tuberculoid or more lepromatous form of the disease, thus suggesting that the pattern of disease in man is determined by the host and not by the parasite. However, these findings do not exclude the possibility of strain difference in *Myco. leprae* of the type, for example, that has been shown with *Myco. tuberculosis* in animals where both *Myco. tuberculosis hominis* and *Myco. tuberculosis bvis* are virulent in the guinea-pig but only the latter is virulent in the rabbit.

The evidence that the infection produced in the foot pads or ears of various rodents, including the mouse, rat and hamster, is overwhelmingly in favour of the infection being due to the human leprosy bacillus. Although this is now universally accepted, it is important to recapitulate the criteria which were used to establish this evidence and the care that was taken by those working in the field to satisfy all the criteria that are outlined in the following section. The criterion of bacterial multiplication was based on precise quantitative methods so that the number of bacilli inoculated could be compared with the number finally harvested in the foot pads of mice. The pattern and rate of multiplication were uniformly reproducible with all strains of *Myco. leprae* derived from untreated patients. All the inocula and harvests were cultured on media suitable for isolating mycobacteria, in order to exclude the possibility that the infection was caused by a cultivable organism. Lepromin prepared from bacilli harvested from the mouse foot-pad infections was compared with standard Mitsuda-type lepromin prepared from man in a series of patients with different types of leprosy and the pattern of response was identical (Shepard & Guinto, 1963). The histology of the foot-pad infection in mice was also studied and the pattern of response was shown to be similar for all strains of *Myco. leprae* and, although
the cellular changes were indeterminate and not characteristic of the polar types of leprosy as seen in man, they were not characteristic of those produced by any other known species of mycobacteria (Rees & Weddell, 1968). Moreover, in a proportion of the infected animals acid-fast bacilli were found, occasionally late on in the infection, within nerves of the foot pad or in the sciatic nerve (Wiersema et al., 1965; Rees & Weddell, 1968) and therefore showed a selectivity for peripheral nerves shared by no other species of mycobacteria.

Although the infection produced by the local inoculation of Myco. leprae into the ears or foot pads of rodents was a limited one and multiplication only occurred when the number of bacilli inoculated was less than 10^6, bacilli could be harvested from these infections and reinoculated into animals, where again they multiplied and reproduced the same bacteriological and histological patterns of response. Therefore, because the characteristics of Myco. leprae were not changed by serial passage, this method could be used for maintaining indefinitely experimental infections with Myco. leprae for study in the laboratory (Rees, 1965b; Shepard, 1965b). Moreover, a similar pattern of infection has been obtained in the many different strains of mice used although more detailed comparisons suggest that the CBA and BALB/C strains of mice are the most susceptible (Shepard & Habas, 1967).

**Recent Applications of Infections in the foot pads of mice for the study of Myco. leprae**

Once it was established that Myco. leprae could be transmitted to animals it was hoped that this experimental infection would provide the first opportunity for studying Myco. leprae in the laboratory. These hopes have been fully justified; within less than 10 years, despite the limited nature of experimental human leprosy in normal animals, this in vivo technique has provided new information of great importance concerning the properties of Myco. leprae. For practical reasons the mouse foot-pad infection with Myco. leprae has been chosen by most laboratories working in this field.

**Generation time of Myco. leprae**

During the logarithmic phase of multiplication in the mouse foot-pad a generation time of 13–25 days has been observed (Shepard & McRae, 1965). This very long generation time is consistent with the chronicity of the disease and the long incubation period observed in man and, although comparable to the generation time of Myco. lepraemurium it is otherwise unique, even for the slowly multiplying Mycobacteriaceae.

**Drug sensitivity testing**

The foot-pad infection, in spite of its limited nature, has been used successfully for testing drugs for antileprosy activity. Thus it has been shown that when Myco. leprae is injected into the foot pads of mice treated with known antileprosy drugs the organisms fail to multiply. Therefore, the mouse foot-pad infection provides, for the first time, a specific test for screening new antileprosy drugs and the search is no longer restricted to drugs that are known to be active against Myco. tuberculosis (Table 1). Hitherto, chemotherapy in leprosy had necessarily evolved from an entirely empirical basis and even the regimen of treatment with diaminodiphenylsulfone, which has been the standard used for leprosy since 1943, was established by trial and error. This was due to the fact that although diaminodiphenylsulfone has slight in vitro and in vivo activity against Myco. tuberculosis in guinea-pigs, it is ineffective against tuberculosis in man. Thus on an empirical basis it has been accepted that the standard dose of diaminodiphenylsulfone should be 100 mg daily; however, there are important practical and clinical advantages to be gained by modifying this regimen. For example, intermittent treatment with diaminodiphenylsulfone could more easily be supervised than daily treatment. Furthermore, leprologists are now tending to advocate smaller doses of diaminodiphenylsulfone because these appear to reduce the frequency and severity of acute reactions (exacerbation) with their attendant nerve and eye complications but are equally effective in controlling the disease. On account of these trends, the mouse foot-pad technique is used not only for screening drugs but has also been specifically applied to determine, for the first time, the minimal inhibitory concentration (MIC) of diaminodiphenylsulfone in vivo against strains of Myco. leprae from previously untreated patients (Shepard, 1967b; Rees, 1967a, 1967b). The sensitivities of 5 "wild" strains of Myco. leprae from patients in Malaysia were tested in this way using concentrations of 0.0001% and 0.00001% of diaminodiphenylsulfone in the diets of the mice. The results are shown in the following tabulation:
The MIC was determined by feeding groups of mice with diminishing concentrations of diaminodiphenylsulfone in their diet and determining the concentrations of diaminodiphenylsulfone in the sera of each group. The results of these studies are shown in Table 2, from which it is clear that the MIC for diaminodiphenylsulfone against wild strains of *Mycobacterium leprae* is approximately 0.015 μg/ml. Thus, *Mycobacterium leprae* in the mouse is exquisitely sensitive to diaminodiphenylsulfone; an unexpected finding, because all other species of mycobacteria are relatively insensitive to sulfones and even the diaminodiphenylsulfone-sensitive micro-organisms such as group A streptococci (Francis & Spinks, 1950) and *Plasmodium berghei* (Thompson, Oleszewski & Waitz, 1965) are 3–100 times less sensitive than *Mycobacterium leprae* to diaminodiphenylsulfone. The standard treatment of 100 mg of diaminodiphenylsulfone per day for man gives serum concentrations of approximately 1.5 μg/ml. Assuming that it is permissible to extrapolate these findings from mouse to man, they suggest that a daily dose of 1 mg of diaminodiphenyl-

### Table 1

**Tests of Activity of Drugs Against *Mycobacterium leprae* Using the Mouse Foot-Pad Technique**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (% in diet)</th>
<th>No. of strains tested</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaminodiphenylsulfone</td>
<td>0.1-0.0001</td>
<td></td>
<td>+</td>
<td>Rees (1965b), Shepard (1964)</td>
</tr>
<tr>
<td>Sulfadimethoxine</td>
<td>0.1</td>
<td>4</td>
<td>+</td>
<td>Rees (1965b)</td>
</tr>
<tr>
<td>Sulformethoxine</td>
<td>0.04</td>
<td>2</td>
<td>+</td>
<td>Rees (1965b)</td>
</tr>
<tr>
<td>Sulformethoxine</td>
<td>0.1 (3 times weekly)</td>
<td>1</td>
<td>+</td>
<td>Rees (1965b)</td>
</tr>
<tr>
<td>Diphenylthioureas</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiambutosine</td>
<td>0.1</td>
<td>2</td>
<td>0</td>
<td>Shepard &amp; Chang (1964)</td>
</tr>
<tr>
<td>Thiambutosine</td>
<td>0.1</td>
<td>6</td>
<td>+</td>
<td>Rees (1965b)</td>
</tr>
<tr>
<td>Ba-22'330 b</td>
<td>0.1</td>
<td>1</td>
<td>+</td>
<td>Rees (1967a)</td>
</tr>
<tr>
<td>Ba-36'223 c</td>
<td>0.1</td>
<td>1</td>
<td>+</td>
<td>Rees (1967a)</td>
</tr>
<tr>
<td>SU-2079 d</td>
<td>0.01</td>
<td>1</td>
<td>+</td>
<td>Rees (1967a)</td>
</tr>
<tr>
<td>Thioacetazone</td>
<td>0.1</td>
<td></td>
<td>P</td>
<td>Shepard &amp; Chang (1964)</td>
</tr>
<tr>
<td>Thioacetazone</td>
<td>0.2</td>
<td>6</td>
<td>+</td>
<td>Rees (1965b)</td>
</tr>
<tr>
<td>Clofazimine</td>
<td>0.01</td>
<td>1</td>
<td>+</td>
<td>Shepard &amp; Chang (1964)</td>
</tr>
<tr>
<td>Clofazimine</td>
<td>0.006</td>
<td></td>
<td>+</td>
<td>Rees (1965b)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>2 mg/day e</td>
<td>1</td>
<td>+</td>
<td>Shepard &amp; Chang (1964)</td>
</tr>
<tr>
<td>Isoniazid</td>
<td>0.01</td>
<td></td>
<td>+</td>
<td>Shepard &amp; Chang (1962, 1964)</td>
</tr>
<tr>
<td>p-Aminosalicylic acid</td>
<td>0.1</td>
<td></td>
<td>+</td>
<td>Shepard &amp; Chang (1962, 1964)</td>
</tr>
<tr>
<td>Cycloserine</td>
<td>0.5</td>
<td></td>
<td>P</td>
<td>Shepard &amp; Chang (1962, 1964)</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>0.25</td>
<td></td>
<td>0</td>
<td>Shepard &amp; Chang (1964)</td>
</tr>
<tr>
<td>Ditophal</td>
<td>0.5</td>
<td></td>
<td>0</td>
<td>Shepard &amp; Chang (1964)</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>0.5</td>
<td></td>
<td>0</td>
<td>Shepard &amp; Chang (1964)</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>10 mg/day f</td>
<td>1</td>
<td>+</td>
<td>Shepard (1964)</td>
</tr>
</tbody>
</table>

* a = Full activity; P = partial activity; 0 = inactive.
* Ba-22'330 = 4-(3-carboxypropoxy)-4'-dimethylamino-diphenylthiourea
* Ba-36'223 = 4-dimethylamino-4'-(4-hydroxybutoxy)-diphenylthiourea
* SU-2079 = 4-butoxy-4'-diethylaminoethoxy-diphenylthiourea
* Once-daily injections.
sulfone would be effective in man. This means that there is strong support from the animal studies for encouraging trials, using lower doses of diamino-
diphenylsulfone in man, to reduce the incidence of reactions without the fear of diminishing the therapeutic efficacy of the drug.

**Drug-resistant strains of Myco. leprae**

The result which has emerged from the systematic investigation of foot-pad infections has been direct proof of the existence of drug-resistant strains of Myco. leprae. Carefully controlled investigations on specially selected patients who showed active disease despite treatment with diamino-
diphenylsulfone for at least 10 years, combined with studies on the diamino-
diphenylsulfone sensitivity of bacilli from these patients using the mouse foot-pad technique, have shown that a proportion of such patients are infected with diamino-
diphenylsulfone-resistant strains of bacilli (Pettit & Rees, 1964; Pettit, Rees & Ridley, 1966; Adams & Waters, 1966; Rees, 1967b). To date, 19 diamino-
diphenylsulfone-resistant strains from individual patients in different parts of the world have been detected using the mouse foot-pad test (Table 3). From the data presented in Table 2 on the serum levels obtained in patients on regimens of 100 mg of diamino-
diphenylsulfone daily (1.5 μg/ml), and from the MIC of diamino-
diphenylsulfone for wild strains of Myco. leprae calculated from the foot-pad test (0.015 μg/ ml), relapses under such doses of diamino-
diphenylsulfone in man due to the emergence of drug-resistant strains, gives a resistance ratio of 100. The results from Table 3 show clearly that the degree of resis-
tance developed by all 19 strains had a resistance ratio of not less than 100. The relevant data on resistance studies which show that the correlation between studies in man and mouse are satisfactory, are summarized below:

**TABLE 2**

**CONCENTRATION OF DIAMINODIPHENYSULFONE IN THE SERA OF MICE FED DIFFERENT LEVELS OF DRUG IN THE DIET**

<table>
<thead>
<tr>
<th>Dose of diaminodiphenylsulfone</th>
<th>Concentration of diaminodiphenylsulfone in serum or plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage in diet, mg per kg</td>
<td>Concentration of diaminodiphenylsulfone in serum or plasma</td>
</tr>
<tr>
<td>of body-weight.</td>
<td>mg</td>
</tr>
<tr>
<td>0.1</td>
<td>200.0</td>
</tr>
<tr>
<td>0.025</td>
<td>50.0</td>
</tr>
<tr>
<td>0.01</td>
<td>20.0</td>
</tr>
<tr>
<td>(Man: 100 mg/day)</td>
<td>(2.0)</td>
</tr>
<tr>
<td>0.001</td>
<td>2.0</td>
</tr>
<tr>
<td>0.0001</td>
<td>0.2</td>
</tr>
<tr>
<td>(Man: 1 mg/day)</td>
<td>(0.02)</td>
</tr>
<tr>
<td>0.00001</td>
<td>0.002</td>
</tr>
</tbody>
</table>

a g/100 g.
b Estimated by Glazko's method.
c Calculated concentration. Serum concentration varies linearly with doses between 0.1% and 0.0001%. Actual value is below level of detectability.

Man 100 mg of diaminodiphenylsulfone/day; serum level = 1.5 μg/ml
Mouse MIC of diaminodiphenylsulfone; serum level = (0.015 μg/ml)
Man 1 mg of diaminodiphenylsulfone/day; serum level = (0.018 μg/ml)
Man/mouse (100 mg of diaminodiphenylsulfone/day; serum level in man) / (MIC in mouse) = "therapeutic ra-
tio" = 1.5/0.015 = 100
Man/mouse Similarly, strains of Myco. leprae resistant to treatment with 100 mg of diaminodiphenylsulfone/day in man would be expected to have a resistance ratio of not less than 100
Mouse/man This expectation was confirmed: diaminodiphenylsulfone-resistant strains of Myco. leprae from patients receiving 100 mg of diaminodiphenylsulfone/day multiplied in mice fed doses of diaminodiphenylsulfone resulting in serum levels from 1 μg–12.5 μg of diaminodiphenylsulfone ml, giving a resistance ratio of 100–1250
TABLE 3
SENSITIVITY OF STRAINS OF MYCO. LEPRAE FROM RELAPSED DIAMINODIPHENYLSULFONE-TREATED PATIENTS a TO DIAMINODIPHENYLSULFONE b

<table>
<thead>
<tr>
<th>Strain</th>
<th>Country of origin</th>
<th>Sensitivity to diaminodiphenylsulfone: c percentage of drug in the diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.1</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>Malaysia</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>6</td>
<td>India</td>
<td>R</td>
</tr>
<tr>
<td>7</td>
<td>Malaysia</td>
<td>R</td>
</tr>
<tr>
<td>8</td>
<td>India</td>
<td>S</td>
</tr>
<tr>
<td>9</td>
<td>West Africa</td>
<td>R</td>
</tr>
<tr>
<td>10</td>
<td>India</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>15</td>
<td>Malaysia</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>S</td>
</tr>
</tbody>
</table>

a All these patients failed to respond bacteriologically, histologically or clinically to a rigorously controlled period of not less than 6 months on a supervised dose of 600 mg of diaminodiphenylsulfone per week.

b A dosage of 0.01% of diaminodiphenylsulfone in the diet of mice gives a serum concentration of 1 μg/ml and is therefore of the same order as the concentration in man on a regimen of 100 mg/day. Thus the level of resistance in the 19 strains of Myco. leprae determined in mice is consistent with the therapeutic failure of diaminodiphenylsulfone in the patients.

c S = Sensitive; R = resistant.

Although the mouse foot-pad test has provided the first direct evidence of the emergence of resistance to diaminodiphenylsulfone, the phenomenon appears to be rare, and may have resulted from the use of excessively high doses of diaminodiphenylsulfone. It has to be admitted that the use of smaller doses of diaminodiphenylsulfone, resulting in concentration of diaminodiphenylsulfone in the serum and tissues nearer to the MIC of diaminodiphenylsulfone for Myco. leprae, could lead to the emergence of a greater number of resistant strains. This possibility must be weighed against the advantages to be gained by a significant diminution in the incidence of serious reactions that are believed to be directly related to high doses of diaminodiphenylsulfone.

Similar but less extensive studies have demonstrated the emergence of thiambutosine-resistant strains of Myco. leprae (Rees, 1967a, 1967b); such strains show cross-resistance to thioacetzone, a feature shared by thiambutosine-resistant strains of Myco. tuberculosis (Konopka et al., 1955).
Effect of BCG vaccination against Myco. leprae

The foot-pad infection technique provides an experimental model for investigating the prophylactic effects of various vaccines and has already demonstrated that vaccination by BCG significantly inhibits the multiplication of Myco. leprae (Shepard, 1965a, 1966). This finding is important although it is not unique since it has already been shown experimentally that vaccination by BCG can produce protective immunity against species of mycobacteria other than Myco. tuberculosis (Fenner, 1957). Still more recently, Shepard & Ribi (1968) have shown that vaccination with the cell-wall fraction of BCG incorporated in an oily base is as protective as living BCG, weight for weight, against infections with Myco. leprae in the foot pads of mice. Thus, mouse foot-pad infections with Myco. leprae provide an important model for investigating the value of prophylactic vaccinations against human leprosy which, if they could eventually be applied to man, would be expected to play a major role in the eradication of the disease. The current importance of these experimental observations that BCG prevents the multiplication of Myco. leprae in the mouse foot-pad is that they support the preliminary results in man that BCG vaccination significantly reduces the incidence of early type leprosy in child contacts in Uganda (Brown & Stone, 1966; Brown, Stone & Sutherland, 1968).

EXPERIMENTAL LEPROSY IN MICE
WITH REDUCED IMMUNOLOGICAL CAPACITY

The successful transmission of human leprosy to animals in 1960 provided the first and only means of studying Myco. leprae in the laboratory. Although this experimental model has, in less than a decade, provided more information on the human leprosy bacillus than was available previously, progress in the field of leprosy research was still restricted by the limited nature of the infection. Clearly, the next step was to determine the factor or factors preventing Myco. leprae from multiplying freely in mice or in other rodents. On the assumption that the infection was limited by the development of immunity, various methods for reducing the immunological capacity of mice were investigated in an attempt to enhance the infection. The assumption was confirmed when it was demonstrated that enhanced infections with Myco. leprae could be obtained in mice following thymectomy and whole-body irradiation (with 900 r) as a means of reducing their immunological capacity.

General features of the infection in thymectomized, irradiated mice inoculated with Myco. leprae

It has now been established (Rees, 1965a, 1965b; Rees & Weddell, 1968; Rees et al., 1967) and confirmed (Gaugas, 1967; Shepard & Congdon, 1968) that when the immunological capacity of mice is reduced by thymectomy plus irradiation (with 900 r), Myco. leprae inoculated locally into the foot pads or ears multiply more freely and yield 100–1000 times more bacilli per site than in normal animals (Fig. 3), and that in due course the infection spreads to other sites. Moreover, similarly treated mice become heavily infected in specific sites when inoculated intravenously with Myco. leprae (Rees & Weddell, 1968; Rees et al., 1967). Although, in these animals, the generation time is not reduced, the bacilli continue to multiply for a longer period. The spread of infection in locally inoculated animals is also highly selective. The sites of predilection are in the skin of the ears, hind and fore paws, the tail and also the nose (Table 4).

The same rigorously controlled criteria have been used to identify Myco. leprae in the enhanced infec-

![Growth curves of Myco. leprae in the foot pads of normal and thymectomized, irradiated mice inoculated with 10⁴ bacilli](image)

**FIG. 3**

**GROWTH CURVES OF MYCO. LEPRAE IN THE FOOT PADS OF NORMAL AND THYMECTOMIZED, IRRADIATED a MICE INOCULATED WITH 10⁴ BACILLI b**

---

a T + 1; dose, 900 r.
b Data taken, by permission, from Rees et al. (1967).

- - - Total number of Myco. leprae.
- - - - Number of viable Myco. leprae.
TABLE 4
LOCALIZATION AND YIELD OF MYCO. LEPROE IN A THYMECTOMIZED, IRRADIATED \( \text{a} \) MOUSE 19 MONTHS AFTER THE INTRAVENOUS INJECTION OF \( 3 \times 10^7 \) BACILLI

<table>
<thead>
<tr>
<th>Organ or tissue</th>
<th>Estimated yield of bacilli (( \times 10^6 ))</th>
<th>Percentage of total yield</th>
<th>Degenerate bacilli (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot pads:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hind</td>
<td>1 740</td>
<td>18</td>
<td>56</td>
</tr>
<tr>
<td>Fore</td>
<td>960</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>2 700</td>
<td>28</td>
<td>95</td>
</tr>
<tr>
<td>Ears</td>
<td>4 800</td>
<td>49</td>
<td>50</td>
</tr>
<tr>
<td>Nose</td>
<td>1 800</td>
<td>18</td>
<td>61</td>
</tr>
<tr>
<td>Muscle of leg</td>
<td>76</td>
<td>0.8</td>
<td>30</td>
</tr>
<tr>
<td>Muscle of body</td>
<td>160</td>
<td>1.6</td>
<td>31</td>
</tr>
<tr>
<td>Skin of tail</td>
<td>21</td>
<td>0.2</td>
<td>89</td>
</tr>
<tr>
<td>Skin of body</td>
<td>5</td>
<td>0.05</td>
<td>55</td>
</tr>
<tr>
<td>Liver</td>
<td>130</td>
<td>1.3</td>
<td>82</td>
</tr>
<tr>
<td>Spleen</td>
<td>53</td>
<td>5.0</td>
<td>87</td>
</tr>
<tr>
<td>Lung</td>
<td>10</td>
<td>0.1</td>
<td>70</td>
</tr>
</tbody>
</table>

Total \( 9 755 \)

\( \text{a} \) Dosage: 900 r.

tions that were used in normal animals and have included testing the sensitivity of the organisms to diaminodiphenylsulfone (Rees & Weddell, 1968; Rees et al., 1967) and the production of lepromin (Draper, Rees & Waters, 1968).

In addition to the higher yields of bacilli from such animals it has been shown that later in the infection there is frequently nodular swelling of the foot pads (Fig. 4) and the histology of the lesions replicates that seen in patients with lepromatous or borderline type leprosy (Fig. 5) (Rees & Weddell, 1968; Rees et al., 1967). Thus there is heavy infection of the peripheral nerves in the skin sites referred to (Fig. 6). Positive smears can be obtained from nasal swabs and the histological picture shows typical foam cells and degenerative changes in some of the infected peripheral nerves (Rees & Weddell, 1968). There is increasing evidence that these changes occur with the slow and partial recovery of the immunological capacity of the animals and can be enhanced in animals with established infections by the donation of immunologically competent syngeneic lymphoid cells from normal mice (Fig. 7) (Rees & Weddell, 1968).

**FUTURE PROSPECTS**

The successful transmission of human leprosy to animals (with the prospect of maintaining the infec-

**FIG. 4**

NODULAR SWELLING OF HIND FOOT PAD OF A THYMECTOMIZED, IRRADIATED MOUSE INOCULATED LOCALLY WITH \( 10^7 \) MYCO. LEPROE 9 MONTHS PREVIOUSLY
FIG. 5
SKIN FROM THE NODULAR, SWOLLEN FOOT PAD OF A THYMECTOMIZED, IRRADIATED MOUSE INOCULATED 8.5 MONTHS PREVIOUSLY WITH $10^4$ MYCO. LEPRAE $^a$

$^a$ Globi loaded with bacilli and foam (Virchow's cells) are seen in the dermis that is separated by a clear zone from the epidermis. These features replicate those seen in lepromatous leprosy in man. Stained: haematoxylin and cold carbol fuchsin; magnification: $\times2400$.

FIG. 6
MEDIAL PLANTAR NERVE FROM THE SAME MOUSE AS IN FIG. 5 TO SHOW SCHWANN CELLS INFECTED WITH MYCO. LEPRAE $^a$

$^a$ Stained: haematoxylin and cold carbol fuchsin; magnification: $\times5000$. 
FIG. 7

ELECTRON PHOTOMICROGRAPH OF DERMIS FROM EAR OF THYMECTOMIZED, IRRADIATED MOUSE
INOCULATED WITH 10^7 MYCO. LEPROE LOCALLY IN BOTH EARS AND FOOT PADS 15 MONTHS
PREVIOUSLY AND GIVEN SYNGENEIC LYMPHOID CELLS FROM A NORMAL MOUSE 5 MONTHS
BEFORE THIS PREPARATION WAS MADE.

The degeneration of nerve axons, myelin rings, and Schwann cells in 2 myelinated nerve fibres can be seen: x 45000.
tion indefinitely by serial passage) and the reproduction of some of the major characteristics of the human disease in laboratory animals by reducing their immunological capacity, provide, for the first time, a means of studying *Mycobacterium leprae* in the laboratory.

**Chemotherapeutic studies**

The enhanced infection provides a more rapid method for screening new drugs against *Mycobacterium leprae* and for applying the more quantitative and kinetic methods already developed in normal mice for studying the action of antileprosy drugs (Shepard, 1967a). In particular, the larger infections obtained in thymectomized, irradiated mice will provide a more sensitive method for determining and comparing the bacteriostatic, as well as the bactericidal, effects of drugs on *Mycobacterium leprae*. Although the application of the MI to chemotherapeutic studies in man indicates that a very high proportion of bacilli in the skin are killed, by diaminodiphenylsulfone, for example, within a period of 3–6 months, there is strong clinical evidence that patients relapse unless diaminodiphenylsulfone treatment is maintained for several years (Quagliato, Berquo & Leser, 1961). One possible explanation is that there are specific sites in the body in which diaminodiphenylsulfone and other antileprosy drugs are ineffective, or less effective, and that such sites provide a source of viable organisms. There are suggestions from histological studies on human tissues that arrectores pili muscles and Schwann cells may still harbour healthy bacilli when those in surrounding tissues are very degenerate. The heavy and generalized infection which can be obtained in intravenously inoculated thymectomized, irradiated mice provides an ideal model for investigating these possibilities since the cellular pattern of infection exactly mimics that seen in man, including parasitization of both muscle and Schwann cells. By treating such animals with antileprosy drugs, including drugs labelled with radioactive isotopes, it should be possible to determine both the distribution of the drugs and of degenerate and normal bacilli, at an intracellular level.

**Emergence of drug-resistant strains of Mycobacterium leprae**

The foot-pad infection technique in normal and thymectomized, irradiated mice is the only method available at present for detecting the emergence of drug-resistant strains of *Mycobacterium leprae* and the method could now be used to survey the importance of drug resistance on a world-wide basis. Because of the increasing use of low-dose regimens of diaminodiphenylsulfone, there is obviously a danger of diaminodiphenylsulfone resistance emerging. Under field conditions, however, if such resistance emerged it might remain undetected by the routine clinical and bacteriological methods until it had reached serious proportions. Because diaminodiphenylsulfone is the standard form of treatment throughout the world, such an occurrence would be disastrous to the control of leprosy. Every effort should therefore be made now to devise suitable experimental models which might be used to predict the rate of emergence of diaminodiphenylsulfone-resistant strains of *Mycobacterium leprae* in animals receiving decreasing doses of the drug. The bacterial populations in an established infection in intravenously inoculated, thymectomized, irradiated mice, would be large enough to detect resistance since such mice have a bacterial population of $10^{10}$ and drug-resistant mutants could be expected in a proportion of 1 : $10^7$.

**Routes of infection**

The routes of infection for leprosy in man are still unknown and the much more susceptible, thymectomized, irradiated mice provide an experimental model for investigating this important problem. The nose, upper respiratory tract, alimentary tract and skin are all routes of infection that should be studied. Moreover, because it is known that thymectomized, irradiated mice heavily infected with *Mycobacterium leprae* excrete bacilli from their nasal mucosa (Rees & Weddell, 1968), they could be used as “open cases” to determine their infectiousness for highly susceptible but non-infected thymectomized, irradiated mice, housed in the same cages.

**Sources of infection other than man**

There has been much discussion on whether vectors, either insect or mammalian, could be involved in the transmission of leprosy from man to man and whether any domestic or wild animals can be infected with *Mycobacterium leprae* and therefore be a source of bacilli to infect man. The successful transmission of human leprosy to normal or thymectomized, irradiated mice might be used in 2 ways to study these possibilities. The mouse foot-pad technique could provide, for the first time, a reliable method of identifying as *Mycobacterium leprae* any non-cultivable, acid-fast bacilli isolated from potential vectors. On the other hand, and perhaps even more
important, there is the observation that the intra-
venous inoculation of Myco. leprae into normal mice
can produce, towards the end of their life, an in-
fec tion of the nose and the paw skin. Because the
inoculation of Myco. leprae into the foot pads of
rats results in a pattern of infection similar to that
in mice, it is likely that both species are equally
susceptible to the human leprosy bacillus. It is
possible, therefore, that wild mice and rats in leprosy
endemic areas could be infected with Myco. leprae
and thus be a source of human infection. Since both
rats and mice are present in large numbers in all
epidemic and endemic leprosy areas throughout the
world, it is suggested that this possibility should be
investigated by sample surveying.

*Application of enhanced infection for studying the
pathogenesis of human leprosy*

Leprosy in man presents a wide clinical spectrum
ranging from the tuberculoid type, where there are
few bacilli and the patient has a high degree of
immunity, to the lepromatous form, where there are
many bacilli and the patient has little or no resistance.
Superimposed on these very variable clinical forms
is the common feature that peripheral nerves are
infected. *Myco. leprae* is the only species of myco-
bacterium known to infect nerves in either man or
animals and the extent of damage to the infected
nerves appears to depend on the immunological
capacity of the host. Thus, in tuberculoid type
leprosy infection of nerves results in the destruction
of axons, whereas in lepromatous leprosy, the nerves
can be heavily infected with bacilli without damage
to the axons. The “target cell” for parasitization
by *Myco. leprae* within nerves is the Schwann cell.
In addition to the variable clinical picture “reak-
tional” episodes may occur and on these occasions
the existing lesions, or new ones, present as sites of
acute inflammation. These episodes are likely to
have an immunological basis; certainly, an increase
in the immunological capacity of the patient must
play a major role in one type of reaction since it is
followed by a shift in the clinical picture from the
lepromatous towards the tuberculoid form of the
disease. The term “reversal reaction” has been
applied, to such a shift.

The importance of nerve involvement in all forms
of leprosy, together with the very wide range of
clinical, bacteriological and histological forms, and
the way in which each seems to be dependent upon
fine differences in the immunological capacity of the
patient, have been stressed in order to illustrate the
complexity of leprosy in man. On account of the
chronicity and complex nature of the disease in
man, it is probable that the final elucidation of
the pathogenesis of human leprosy will be achieved only
if contributions are made from the experimental
studies and such contributions can only be made if
the human disease can be duplicated in experimental
animals. This prerequisite seems likely to be
achieved because already the inoculation of *Myco.
leprae* into mice subjected to thymectomy and total
body irradiation has reproduced completely the
lepromatous type of disease seen in man (Rees &
Weddell, 1968).

These two areas of immunological research in
leprosy are, of course, importantly related. Mice
have been shown to develop lepromatous-type
infection when they are subjected to procedures
(thymectomy and irradiation) that produce a pro-
found and long-lasting immunological depression,
and the immunological depression in lepromatous
patients has been made clear by well tried immuno-
logical procedures (Int. J. Leprosy, 1968). Fortunate-
ly, it is possible to increase the immunological
capacity of treated mice at will (by the intraperi-
toneal inoculation of syngeneic lymphoid cells from
normal mice) and already preliminary results of
such manipulations show that the progressive form
of lepromatous leprosy can be halted or that reversal
reactions can be produced which result in a shift
from the lepromatous form of the disease to the
tuberculoid form seen in man (Rees & Weddell,
1968). Therefore, there is every reason to believe
that these models can and should be developed in
order to provide means to study the pathogenesis of
the different forms of human leprosy, to study the
etiology of nerve involvement and damage and to
study the role of immunology in these processes.
RÉSUMÉ

On n’est pas encore parvenu à cultiver Mycobacterium leprae in vitro et il a fallu recourir à l’expérimentation in vivo afin de pouvoir étudier l’infection lépreuse. Par ailleurs, ce n’est qu’en 1960 qu’on a réussi à transmettre la lèpre à des animaux. Cela explique que les progrès de nos connaissances relatives à cette affection soient restés bien en deçà de ceux obtenus dans d’autres secteurs de la pathologie et que l’essentialité de la recherche en matière de lèpre ait porté sur l’obtention de cultures du micro-organisme in vitro et in vivo.

Le problème a pu être abordé indirectement grâce à l’élaboration de modèles expérimentaux. Une des premières réalisations de ce genre a été, il y a une dizaine d’années, l’application de techniques quantitatives à l’étude de Myco. lepraemurium qui a permis de définir une méthode indirecte pour évaluer la vitalité des bacilles de la lèpre en l’absence de cultures in vitro. On parvient actuellement à distinguer les bacilles vivants des bacilles morts, sur la base de critères morphologiques, après une simple coloration par la méthode courante de Ziehl-Neelsen. Cette découverte a beaucoup facilité l’étude de l’évolution de l’infection lépreuse chez l’homme et de l’infection expérimentale chez l’animal. L’indice mesurant la vitalité des bacilles, appelé « indice morphologique » fournit un moyen très sensible d’apprécier la réponse des malades à la chimiothérapie. C’est vers la même époque qu’on a obtenu pour la première fois la croissance de Myco. lepraemurium en cultures cellulaires et on peut espérer que ce mode de culture in vitro pourra ultérieurement être appliqué à Myco. leprae.

Un nouveau progrès, plus important encore, a été réalisé en 1960 lorsqu’on a démontré que Myco. lepra pouvait se multiplier dans le coussinet plante de la souris. Cette infection expérimentale a permis de calculer le temps de génération du bacille de la lèpre (13-25 jours), d’évaluer l’efficacité des médicaments antilépreux, de mesurer la sensibilité des souches de Myco. leprae d’origine humaine à la diamino-4,4’-diphenylsulfone (DDS), d’identifier les mutants résistants à la DDS ou à d’autres médicaments en cas de rechute en cours de traitement, et enfin d’étudier l’effet protecteur de la vaccination par le BCG.

Ce matériel d’étude, bien qu’ayant permis d’élargir notre connaissance de la maladie, ne donnait pas entière satisfaction, car l’infection expérimentale ainsi réalisée restait limitée et localisée et ne pouvait prétendre reproduire tous les aspects de la lèpre humaine, et notamment l’atteinte nerveuse. Cette lacune a été récemment comblée: on a montré qu’une infection progressive et généralisée succède à l’inoculation intraplantaire ou intraveineuse de Myco. leprae chez la souris dont les défenses immunitaires ont été affaiblies par thymectomie et irradiation totale de l’organisme. Bien plus, lorsque l’infection ainsi favorisée est bien établie, dès le 9e mois, l’évolution de la maladie chez l’animal présente des caractéristiques histologiques identiques à celles de la lèpre lépromateuse chez l’homme, avec infection et lésion des nerfs périphériques.

Les résultats obtenus grâce à ces modèles expérimentaux sont particulièrement importants, car pour la première fois on dispose de méthodes permettant l’étude directe de Myco. leprae en laboratoire. On doit naturellement continuer à mettre tout en œuvre pour obtenir la multiplication de Myco. leprae in vitro. En attendant, il n’est pas impossible que les progrès de nos connaissances concernant la pathogénie de la lèpre humaine dépassent nos espérances, maintenant que de nombreux aspects de la maladie peuvent être reproduits expérimentalement chez la souris privée de ses défenses immunitaires.

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