A Century of Progress in Experimental Leprosy R. J. W. Rees¹

The 28th February 1973 was chosen to celebrate the centenary of Gerhard Henrique Armauer Hansen's first claim to have observed microscopically bacillary bodies in the tissues from leprosy patients. No one today would doubt or deny that Hansen was able to consistently observe "little rods" in tissues from patients with "tuberous" (lepromatous) leprosy and thus provide, for the first time, strong evidence to support his theory that leprosy had a contagious or infectious etiology. However, in 1873 no human diseases had been shown to have an infectious etiology and therefore Hansen's observations were strongly opposed by all the then great authorities on the etiology of human diseases. To appreciate the climate of opinion at the time of Hansen's observation I quote from the history of Hansen by González Prendes (34): "Danielssen was annoved with a daring young doctor (Hansen) who had the temerity to discuss that which the bestendowed medical brains accepted without hesitation. 'This is insolence' said Danielssen."

Many of the problems and frustrations facing those working in the field of experimental leprosy during the past century were similar to those faced by Hansen once he had identified bacilli in tissues of leprosy patients. Hansen, however, had in addition to establish a case for an infectious etiology in opposition to Boeck and Danielssen "the fathers of modern leprology," all fellow Norwegians working in Bergen. It was at the ancient St. Jörgen Leprosy Hospital which had existed in Bergen since about 1300 that Danielssen began to work in 1839. He radically changed the scientific environment of the establishment, and from the languid and indifferent way in which it carried on its activities, succeeded in converting it into the world center of leprology. From the vast amount of clinical

and pathological material available to Danielssen at this hospital he, together with Boeck, presented a series of publications on which our current biological knowledge of leprosy is based. It was in 1868, when Danielssen was Director of St. Jörgen Hospital, that he appointed Hansen as Medical Officer to the hospital. Therefore, within five years, by hard work and careful observation Hansen was convinced that leprosy had an infectious etiology. While Hansen appreciated the honor he had been given in being appointed by Danielssen to the St. Jörgen Hospital and highly appreciative of Danielssen's great knowledge of leprosy, he was convinced that Danielssen was mistaken regarding the hereditary etiology of the disease. It was therefore against this background of master and student that Hansen eventually presented to the world his evidence for an infectious etiology (17). In addition to Danielssen treating Hansen's claims with contempt, he provided evidence in support of the hereditary theory by attempting to inoculate himself and some of his co-workers with material from patients with leprosy and showed that all the inoculations failed to take.

The scientific climate at the time when Hansen made his important observations are given in some detail in order to appreciate the scepticism and personal antagonism that he had to face. As Hansen wrote later in his memoirs, (19) "a teaching that bacteria caused disease was then in its infancy, and no chronic disease was known to be of bacterial nature." Therefore Hansen was well aware that evidence for an infectious agent based on microscopical observations in tissues would be significantly strengthened by the isolation of a "germ" on culture and the transmission of a leprosylike infection by inoculation of material from the tissues of leprosy patients. Gonzales Prendes (34) makes it clear that Hansen was well aware of the importance of confirming these criteria and that he delayed further publication while attempting

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to do so. It is revealing from Gonzáles Prendes' history of Hansen that he refers to the criteria which Hansen attempted to meet in order that a germ could be considered a specific causal agent of any disease, as those laid down then by Henle:

- to find the germ present always in the same disease.
- 2. to be able to make a culture of the germ and reproduce the disease by inoculation in experimental animals.

These Henle criteria were apparently formulated before the now famous postulates of Koch. In retrospect we must admire Hansen's insistence on accepting Henle's postulates as final proof of the "rods" as being the etiological agent of leprosy, since after a century of work *M. leprae* has still not been cultured and only since 1960 has an experimental infection been obtained in animals.

Hansen must have suffered great frustrations, for on the one hand as a scientist he accepted that however consistently he observed bacilli in the patient's tissues, final proof of their etiological significance must fit Henle's other criteria, and yet, on the other hand, Hansen's whole concept of a contagious etiology was being ridiculed by Danielssen and other authorities. Yet Hansen remained sincere, as it is clear from his publications (17, 18, 19) where he consistently referred to his attempts, though all negative, to culture or to infect animals with material from leprosy patients. Although advances in bacteriology are reviewed elsewhere in this FESTSKRIFT, Hansen also showed his insight into the problems of culturing M. leprae by choosing only those tissues which had not ulcerated in order to avoid the extraneous bacteria, but in spite of these precautions cultures were frequently overgrown by bacteria or moulds dissimilar to the organisms he observed in leprosy tissues. Hansen's abortive attempts to infect animals were confined to rabbits.

It is on the basis of all these failures to establish Henle's criteria that Hansen correctly stated in all his writings, even as late as 1895 (20) that in spite of the bacillus not having been experimentally identified he defined leprosy as a chronic disease produced by the leprosy bacillus. In retrospect

we can appreciate how much he must have personally suffered every time he added this proviso. Melsom (28) in his history of Hansen aptly summarized the situation, "... he was unfortunate in discovering a microorganism which up to this day no one has been able to cultivate." Melsom's statement still holds true in 1973 for cultivation in vitro. Although Hansen attended the First and Second International Congresses of Leprology in Berlin (1897) and in Bergen (1909), where his theory that leprosy was an infectious disease was fully endorsed, we can only assume that he died a frustrated man having failed to prove experimentally the existence of M. leprae-a fate to be shared by all those who for the next 48 years took up the challenge!

EXPERIMENTAL LEPROSY IN THE PERIOD 1873-1960

Hansen faced the difficulty of a precedent when in 1874 he claimed a bacillus as the causal agent of leprosy, or for that matter any infectious disease. However, this was short-lived since in the next 20 years a bacterial etiology for nearly all the infectious diseases in man were fully established. Therefore, continuing failure in this period to culture or transmit M. leprae by the then most knowlegeable bacteriologists was significant, albeit frustrating. For example, the renowned Neisser, who isolated the gonococcus, failed to cultivate or transmit M. leprae (30) as did other leading bacteriologists (22.46). On this basis it soon became apparent that M. leprae represented a special problem. Since no real advances were made until 1960 I will divide my FESTSKRIFT presentation into two periods, the "dark ages" from Hansen's work to 1960 and from 1960 to date in which experimental models in animals became available for the study of M. leprae.

In the period 1873-1905 rabbits and guinea pigs were inoculated with *M. leprae* by various routes, but particularly favoring the anterior chamber of the eyes because this route had proved successful for the transmission of tuberculosis.

The field of experimental human leprosy was further stimulated when in 1903 a new chronic granulomatous disease in rats was identified and shown to be caused by an acid-fast bacillus, *Mycobacterium lep*raemurium (^{11, 44}). These workers appreciated the similarities in the histopathology of rat and human leprosy, including failure to culture *M. lepraemurium in vitro*. However, because bacilli recovered from the granulomatous lesions reproduced the disease following reinoculation into rats, Dean (¹²) suggested that rats might prove to be a susceptible host for *M. leprae*. This interesting and reasoned suggestion, based on other pathogenic bacteria causing disease in man or rats, was not followed up until much later.

From the literature on attempts to transmit *M. leprae* to animals in the period from 1905 onwards it is very clear that many efforts were made and that the problems still appealed broadly to general bacteriologists. Moreover, on the one hand there was the direct attempt to produce leprosy in experimental animals, and on the other hand the indirect approach by bacteriologists who had supposedly cultured *M. leprae* from the tissues of leprosy patients and therefore needed to show that the cultivable isolates could reproduce the experimental disease when inoculated into animals.

A particularly good and critical review of the direct and indirect attempts to produce leprosy in experimental animals during this period was given by Muir (29). Thus he refers to lesions produced in Japanese dancing mice (13) and in monkeys (Macacus) (29, 31) following subcutaneous injection of suspensions or fragments of leprosy tissues. In a critical review of these studies Muir (29) pointed out that: 1) similar lesions were produced by the injection of suspensions of leprosy tissue which had been autoclaved, 2) macroscopic lesions and the persistence of acid-fast bacilli at the site of inoculation was not adequate evidence that the lesion was active or had resulted from the multiplication of M. leprae because Borrel, in unpublished experiments, had shown the formation of similar macro- and microscopic lesions following the injection of dead M. tuberculosis, and 3) where fragments of lepromatous tissues were inoculated, persistence of acid-fast

bacilli was inadequate evidence of their multiplication, because the explanted leprosy tissues contained very large numbers of bacilli which could have been redistributed without having multiplied.

Although in the period from Hansen to 1930 concerted efforts were made to establish infections in animals by leading bacteriologists and leprologists, their efforts were abortive or at the best inconclusive. However, because the results relied on the appearance of macroscopic lesions, on qualitative increases in acid-fast bacilli or on typical cellular responses in the tissues for evidence of successful transmission, it is clear from their writings that they were aware of the limitations of these methods. The limitations of these criteria were clearly demonstrated by inoculating "control" series of animals with autoclaved leprosy tissues or other species of live or autoclaved mycobacteria. At the same time they pointed out the possibility of tissues from leprosy patients being contaminated with other readily cultivable mycobacteria (29). It is disconcerting to realize how many of these criteria and vital controls insisted on by these earlier workers of excellence, were ignored by those who followed in the next 30 years. While up to 1930 only a limited range of animal species had been inoculated with M. leprae, because of the negative results it was suggested that more work should be done on anthropoids. Moroever, because leprosy occurred in man chiefly when his natural resistance had been lowered and when his diet was defective, Muir (29) suggested for the first time that animals, including monkeys and chimpanzees, with similar deficiencies, should be used. It is of interest that up to this period no mention had been made of nerve involvement or studies on "lepromins" prepared from lesions in animals inoculated with M. *leprae*, as criteria for identifying the experimental infections.

In the next 30 years (to 1960) an ever increasing number of workers, with equivalently variable expertise, inoculated *M. leprae* into an ever widening range of animal species using "normal" animals or animals made deficient in one way or another. Since none of the positive claims are further reported by the author or substantiated by other workers, I have decided that for this FESTSKRIFT a detailed review of this period would not contribute to progress in this field. However, some of the studies in this period must be recorded as they relate scientifically, and sometimes emotionally, to those who persisted in or had taken on for the first time, a challenge that had remained unsolved for 57-87 years! Failure by 1930 by prima donnas in the field of bacteriology and leprosy was in itself enough to divert younger medical scientists from entering this field, since there were so many other more profitable and exciting fields of bacteriology in which progress was more or less guaranteed. This special situation left the challenge open to the more clinically orientated workers in the field of leprosy, who appreciated the importance of establishing an experimental model in animals for advancing their subject, but who were now not experts in bacteriology or experimental pathology, and to those who, in other disciplines, were, from time to time, prepared to divert their energies and bacteriological expertise to the leprosy problem.

A number of examples are briefly reviewed in order to illustrate the principles which then were being used to overcome past failures. Thus Adler (1), a famous parasitologist, claimed the successful transmission of M. leprae to Syrian hamsters previously splenectomized. This new approach to reduce the immunological capacity of the host was repeated by others in hamsters and monkeys (6) without success. The publication by Adler illustrates one of the difficulties which those in the field of experimental leprosy, and indeed in other fields, have had to contend with when considering the relevance of a positive result claimed by an author in a single publication, but never rebutted or followed up by publication. Thus, when the writer interviewed Adler in 1957, the latter stated that "all subsequent attempts to infect splenectomized hamsters with M. leprae had failed." In addition to the use of splenectomy, the theme running through this period was concerned with the use of monkeys and manipulations which might

reduce their resistance to infection with M. leprae. Thus Collier (9) on the theory that the consumption of the tuber, Colocassia antiquorium, was an etiological factor in leprosy, claimed he had infected monkeys fed on the tuber. Success was attributed to destruction of the adrenal cortex by a sapotoxin contained in the tuber which lowered the resistance of the animal. Thus Cochrane (8) made repeated attempts to infect monkeys with M. leprae using animals fed with this tuber or following complete or partial splenectomy. While in general results were negative, in a few animals there was systemic spread of the infection from the site of inoculation, and nodules appeared in the skin containing acid-fast bacilli. However, even in these apparently positive animals, after a period of 9-12 months, the bacilli and nodules subsided. Cochrane was the first to use the lepromin test in animals inoculated with leprosy tissues and showed that while uninoculated monkeys were lepromin negative, a proportion of those inoculated became lepromin positive. Carpenter and Naylor-Foote (7) attempted to infect laboratory animals with human leprosy after exposure to various adverse conditions which might enhance their susceptibility. They included attempts to produce metabolic alterations by feeding dessicated thyroid, by interference with the production of humoral antibodies, by blockage of the reticulomechanical endothelial system with colloidal carbon particles, by administration of cortisone, by periods of starvation and by whole-body X-irradiation. They particularly claimed infections with M. leprae in rats and hamsters subjected to whole body X-irradiation prior to inoculation. Their studies are the first that this author has found where quantitative and comparative assessments were made of acid-fast bacilli at the site of inoculation in treated and untreated animals. However, Carpenter and Naylor-Foote were unable to repeat their findings with lepromatous tissues shipped from abroad. On the basis of later studies by Shepard and Rees it is possible that Carpenter and his colleagues were the first to record true multiplication of M. leprae in the tissues of hamsters and rats and that attempts to repeat the work with material shipped from abroad may have failed because the bacilli died en route.

Binford and Feldman suggested the importance of using sites of inoculation (skin or testis) where the tissue temperatures were lower on the basis that M. leprae in man particularly invaded sites where the temperature was less than 37°C. Therefore, Binford launched a large-scale study using golden hamsters inoculated in the ear or testis with M. leprae and some of the animals received corticosteroids. The studies which were based entirely on clinical and histological assessments showed the development of gross lesions consisting of histocytic granulomas containing many acidfast bacilli in a significant proportion of inoculated animals (3). Later, when bacteriological assessments were made, it was shown that cultivable mycobacteria could be isolated from the hamster lesions and also identical and cultivable mycobacteria were present in homogenates from some biopsies of skin obtained from leprosy patients in a similar area in the Philippines from where the original tissues had been obtained (4). The omission of bacteriological surveillance for a study of this magnitude initiated in the late 1950's would now seem inexplicable, since Hansen and those that followed him had already experienced contamination of biopsies of skin by cultivable strains of mycobacteria.

Before completing a review of studies on the experimental transmission of leprosy in the period up to 1960 it is relevant to include the history of the inoculation of man with leprosy. While in other infectious diseases inoculation of man has been rare and usually accidental, and not of great import, in leprosy they have attracted more attention presumably because of the universal failure to infect animals. Thus in leprosy there have been made deliberate attempts to infect man as well as more detailed reporting of accidental inoculations. Deliberate attempts to inoculate man were first recorded by Danielssen and Boeck (10) and were negative, as were others at that time. However, Rogers and Muir (40) accepted as valid the accidental inoculation by a finger prick reported by

Marchoux (27), a similar accidental inoculation reported by Langen (26) and the deliberate self inoculation by Lagoudaky $(^{25})$. The most recent and particularly well documented and convincing evidence of accidental transmission to man was reported by Porritt and Olsen (33). Two young men from Michigan, where leprosy is not and never has been endemic, who as members of the U.S. Marine Corps were tattooed one after the other in Melbourne in 1943. In 1946, when the two men were living in different parts of the United States, lesions appeared in both of them at the sites of the tattooing, which were shown to be leprosy.

The conclusions from these observations on deliberate or accidental inoculation of man are two-fold: 1) the high proportion of failures, indicating that in general man is insusceptible or the route of inoculation has been unfavorable, and 2) the positive association between the intradermal route of inoculation with the development of leprosy lesions.

EXPERIMENTAL LEPROSY IN THE PERIOD 1960-1973

This positive era which has transformed the whole field of experimental leprosy was heralded by two publications by Shepard in 1960 (41, 42). Shepard was new to the field of leprosy research, but as a bacteriologist he based his evidence for successful transmission of M. leprae to mice on a significant quantitative increase in the number of acid-fast bacilli harvested from the site of inoculation (footpad) compared with the number of bacilli inoculated. He chose the mouse footpad as a potential site for the multiplication of M. leprae, based on the work of Fenner (15) who had shown that two strains of mycobacteria M. ulcerans and M. balnei, with optimal growth temperatures of less than 37°C, multiplied locally when inoculated into the mouse footpad but failed to multiply in the internal organs of mice inoculated intravenously. Shepard's first two publications provided significant quantitative evidence that by six months a high proportion of samples of M. leprae derived from nasal washings of 22 patients with lepromatous

leprosy and bacilli from biopsies of skin from 16 lepromatous patients, multipled when inoculated into the footpads of mice. The inocula from all these patients contained approximately 5,000 acid-fast bacilli, and the increases at six months were approximately in the range of 50 to 1,000fold. While the reproducibility of these increases were overwhelming, their detection required very accurate quantitative technics, which had not hitherto been applied to studies on the transmission of M. leprae to experimental animals. Shepard's publications also included full bacteriological surveillance of both the inocula and harvests, to exclude the possibility that the acid-fast bacteriologic increases were due to cultivable strains of mycobacteria.

The carefully planned approach and the impressive reproducible data presented by Shepard provided *prima facie* evidence of the transmission of M. leprae to an experimental animal. Thus after 87 years of failures to establish a reproducible experimental model for the transmission of M. leprae, Shepard's mouse footpad heralded the long awaited opportunity for a method by which M. leprae could be studied in the laboratory. While, Shepard's data was so much more impressive than any other prior claims, their true significance has depended upon subsequent reproducibility in other laboratories, first established by Rees (35) and then by laboratories throughout the world.

On the basis of Shepard's original bacteriological observations and those confirmed by Rees (35) it was apparent that M. leprae from any bacilliferous and active case of leprosy multiplied within the mouse footpad, but their multiplication was limited to a total footpad yield of approximately a million organisms and by the size of the inocula. Thus significant multiplication only occurred when inocula contained less than a million acid-fast bacilli. From this data Rees assumed that these limitations of bacterial multiplication were determined by the development of infection immunity in the mouse. On this assumption he applied the mouse footpad technic to animals made immunologically incompetent by prior exposure to adolescent thymectomy followed by total body irradiation (900R). In these immunologically suppressed animals *M. leprae* continued to multiply, beyond the level of one million organisms, and were no longer restricted by the number of *M. leprae* inoculated into the footpad ($^{36}, ^{38}$).

These basic observations by Shepard and his colleagues and by Rees and his colleagues have fully established the mouse as a model for studying the behavior of M. leprae under in vivo conditions. Moreover, from other studies by Hilson (21) and by Fieldsteel and McIntosh (16), there is now evidence that normal and immunologically suppressed rats, respectively, behave similarly to mice when inoculated with M. leprae. Thus, mice and rats have been applied during the last 13 years as experimental models for investigating the behavior of M. laprae and for studying the pathology, pathogenesis and immunopathology of the host response to infections with M. leprae. Since there are still no technics for culturing M. leprae in vitro, these in vivo models have been fully exploited for investigating the characteristics of M. leprae, which would for other bacteria have been studied in vitro. However, the in vivo models established in normal and immunologically suppressed mice have reproduced so many of the characteristics of leprosy in man, that these models have also been exploited with great advantage.

While it was anticipated that once M. leprae was successfully transmitted to an experimental animal host, methods would become available for studying M. leprae under experimental conditions, none could have foreseen that these various in vivo models would be so advantageous for studying the pathology and immunopathology of the human disease. The bacteriological aspects of M. leprae have been reviewed by Shepard (43), while the pathogenesis, pathology and immunopathology using various mouse models have been reviewed by Rees (37). These models have shown that in normal mice, inoculated locally with M. leprae, there develops locally and systemically an infection resembling the borderline or borderline-tuberculoid type leprosy seen in man (³⁹). Whereas in

immunologically deficient mice (T/900R), M. leprae inoculation results in an infection resembling lepromatous type leprosy in man (37, 38). Furthermore, in these immunologically suppressed animals, their lepromatous state can be reversed by the transfusion of immunologically competent lymphocytes from syngeneic, normal mice (³⁸). Thus there is important experimental evidence that the type of leprosy is determined by the immunological capacity of the host. In all these experimental models there is good evidence that in both normal and T/900R mice, local inoculation of M. *leprae* is followed by a systemic infection via the blood stream (37). Finally, and most importantly, in all mice inoculated with M. leprae there is eventually, twelve months or later, infection of peripheral nerves, replicating one of the most characteristic features of human leprosy. This feature has provided a very important experimental model, namely a means of studying the pathogenesis of leprosy neuropathy at a very early stage. Preliminary studies indicate that leprosy neuropathy in mice is associated with damage to the peripheral nerve vessels and the perineurium, thus weakening the essential barriers which are normally responsible for maintaining the integrity of the endoneurium on which nerve conduction depends (5).

One warning on the use of mice for routine studies of M. leprae, arising from the observations by Nishimura and his colleagues (32), is that laboratory strains of mice can carry infections with Mycobacterium lepraemurium-like organisms. If these observations, which are based on strains of mice in Japan, are applicable to strains of laboratory mice throughout the world, then the greatest care must be given to the surveillance of the pathology and the rate of growth of acid-fast bacilli at all times in mice inoculated with M. leprae. Such surveillance must never be relaxed, because of the possibility that material from man may be contaminated with cultivable strains of mycobacteria and also, from Nishimura's work, mice may be harboring strains of Mycobacterium lepraemurium.

As well as the major exploitation of the mouse footpad technic since 1960, other

animal species have been studied for their susceptibility to M. leprae. In this field the most important observation has been the susceptibility of the armadillo (23, 24, 45). It has been shown that the nine-banded armadillo (Dasypus novemcinctus Linn.) can be exquisitely sensitive to the inoculation of M. leprae. Preliminary data indicates that when wild strains of armadillo are inoculated with M. leprae some develop leprosy within a period of 15 months. This evidence shows that the armadillo can be susceptible to M. leprae and can, without immunological manipulation, develop lepromatoid leprosy. The armadillo was chosen because it has a lower body temperature (32-35°C), has a long life-span (12-15 years) and as a primitive mammal, regularly produces litters of monozygous quadruplets. The full significance of these findings await further investigation. Howeven an animal of this size developing lepromatous-like leprosy would be a source of very large numbers of M. leprae and if it proves possible to breed this species under captivity, then it should be possible to select for and breed highly susceptible substrains of armadillos for studying the differences between susceptible and nonsusceptible animals.

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