Recent Bacteriologic, Immunologic and Pathologic Studies on Experimental Human Leprosy in the Mouse Foot Pad

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The evidence for the transmission and multiplication of human leprosy bacilli in the foot pads of mice ([5, 6, 15, 19]) was formally accepted by a special committee of the VIIIth International Congress of Leprology in Rio de Janeiro in 1963 ([1]). This work represents an important advance since it provides, for the first time, an opportunity for studying the pathogenesis of leprosy in an experimental animal. It also provides an opportunity for studying the host-parasite interreactions in one of the most chronic of human infections caused by the slowest growing bacterium known. More recently it has been shown that an infection similar to the one obtained in the mouse foot pad can be produced also by the inoculation of *M. leprae* into the ears ([11, 25]) and foot pads ([25]) of hamsters, the ears of mice ([24]), and the foot pads of rats ([3, 4]). In this paper I will summarize our present results and briefly discuss some of our more recent studies on the bacteriologic, pathologic and immunologic aspects of food pad infection in mice.

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BACTERIOLOGIC STUDIES

General aspects. Quantitative analyses have shown that the multiplication of *M. leprae* is limited, is dependent on the size of the inoculum, and is confined to the foot pad. For example, inocula of 10^4 *M. leprae* (counted as acid-fast bacilli) yield 10^6 in 6-8 months, and although smaller inocula may eventually give the same yield, larger ones give no higher yields, and inocula of 10^6 or more fail to multiply significantly. Once the bacterial population in the foot pad has reached approximately 10^6 it remains steady for many months, although there is a gradual increase in the proportion of dead bacilli ([15]). *M. leprae* recovered from foot pads can be passaged, apparently indefinitely, their bacteriologic characteristics remaining unchanged. In our own studies we have successfully transmitted human leprosy to the foot pads of mice from 35 previously untreated patients from

different parts of the world: Malaysia ([27]), Burma ([6]), East Africa ([1]), and the West Indies ([1]). All 35 "strains" of *M. leprae* have produced an experimental infection; furthermore 29 of the strains came from patients with lepromatous type infection, 5 from patients with borderline and 1 from a patient with reactional tuberculoid leprosy. A summary of the results is shown in Table 1. The consistent reproducibility of all the characteristics of the foot pad infection by 35 strains of *M. leprae* obtained from patients from many different parts of the world and with different types of leprosy strongly suggests that human leprosy is produced by one strain of bacillus, but that the multitude of clinical manifestations seen in man result from different host responses. These results in the mouse foot pad do not support the hypothesis of Convit ([2]) that *M. leprae* from patients with borderline leprosy represent mutants better adapted for infecting experimental animals. Although multiplication is limited, several of our strains have been maintained successfully by passage from foot pad to foot pad and one strain has reached the 5th passage, giving to date a total bacillary increase of 5.8×10^{12}. Multiplication of *M. leprae* has been obtained in the foot pads of P, TO, CBA and Chatterjee strains of mice.

Viability of *M. leprae*. We set out several years ago to see if the viability of leprosy bacilli could be determined indirectly from their morphologic appearances. In our earlier studies we used *M. lepraemurium* as our model ([9, 18]), because a final check could be made on the viability (measured as infectivity) by putting the bacilli back into mice or rats and seeing if they produced disease. The results of those studies showed conclusively that living and dead *M. lepraemurium* could be identified by their different morphologic appearances and that these appearances could be seen in bacilli stained by the classic Ziehl-Neelsen method. All bacilli that showed irregular staining were incapable of producing disease in animals and therefore were considered dead. Because *M. leprae* showed identical morphologic differences ([16, 17]), we concluded that the same methods could be used for distinguishing dead and live *M. leprae*. At that time we could not put these observations to direct test with *M. leprae* because the organism could neither be cultured nor transmitted to experimental animals. Now, using the foot pad infection, Shepard and McRae ([23]) have elegantly confirmed our conclusions. Their detailed calculations show that, while all irregularly stained bacilli are dead (noninfectious), not all solidly staining bacilli are necessarily viable, since on a proportional basis solidly staining bacilli may give a 5 per cent overestimate of viability. We have studied this problem by determining the infectivity of *M. leprae* from 4 patients who had been on full treatment with diaminodiphenyl sulfone (DDS) for 12 to 16 months. The 5 strains of bacilli from these patients were inoculated into mouse foot pads in a dose of 10^4. The suspension contained very high proportions of degenerate bacilli, ranging from 94 to 99 per cent. The foot pads were harvested 7-18 months later and in only 4 of 60 was there multiplication. The 4 mice showing multiplication of bacilli were derived from the suspension containing only 94 per cent degenerate bacilli. The results shown in Table 2 confirm that irregularly stained *M. leprae* are nonviable and show clearly that a very high proportion of the bacilli in patients treated with DDS are killed in 12-16 months on treatment.

TABLE 1. *Transmission of experimental human leprosy to the mouse foot pad.*

Source of *M. leprae* (untreated patients)		No. of successful transmissions	Type of leprosy	
			Lepromatous	Borderline/ tuberculoid
Malaysia 27 Burma 6 E. Africa 1 W. Indies 1	35	35	29	6

TABLE 2. *Infectivity (viability) in the mouse foot pad of* M. leprae *from patients treated with DDS for one or more years.*

Strain No.	Treatment (months)	Degenerate bacilli (per cent)	Proportion of foot pads showing multiplication of *M. leprae*	Time of examination (months)
14693	12	99 (894/903)	0/12	7-18
14642	12	97 (876/902)	0/12	7-18
14642	16	96 (600/627)	0/12	8-14
14806	12	99 (216/219)	0/12	8-12
14808	12	94 (470/500)	4/12	8-12

Chemotherapeutic studies. Although the foot pad infection is a limited one, it is sufficient for screening antileprosy drugs ([14, 22]). The results of our studies are summarized in Table 3. The results show that multiplication of *M. leprae* in the mouse foot pad is significantly inhibited by diaminodiphenyl sulfone (DDS), phenazine (B.663), CIBA 1906, thiosemicarbazone (TB1) and two long-acting sulfonamides (Fanasil and Madribon). It is of particular interest that multiplication of *M. leprae* is inhibited by DDS, since this drug is the most active drug against leprosy in man, but has little or no therapeutic activity *in vitro* or *in vivo* against any other known species of mycobacteria. All together 9 strains of *M. leprae* have been tested against DDS, fed at between 0.1 and 0.025 per cent in the diet to mice in groups of 6 to 12, and, although in several strains of *M. leprae* multiplication has been inhibited completely, the overall result on nearly 100 foot pads has been complete inhibition in only 82 per cent. We have obtained much greater inhibition with CIBA 1906 and thiosemicarbazone than has been obtained by Shepard and Chang ([22]). In the same table the results are given for CIBA 1906 and TB1 against 4 strains of *M. leprae* obtained from patients receiving two or more years' treatment with CIBA 1906. A much lower proportion (9/24) of the foot pad infections were inhibited by CIBA 1906 from these patients than *M. leprae* derived from previously untreated patients. It is also of interest that only 11/25 of the foot pad infections from these 4 patients were inhibited by thiosemicarbazone, because of the known cross resistance existing between

TABLE 3. *Chemotherapeutic studies using the mouse foot pad test.*

Drug-regimen (per cent daily)	Previous treatment of patient	Strains tested (number)	Activity in foot pads (Multiplication) inhibited: proportion)
DDS (0.1)	Nil	9	{ 82-100%
DDS (0.025)	Nil	2	
Phenazine: B.663 (0.006)	Nil	1	10/10
Ciba 1906 (0.1)	Nil	3	15/17
TB 1 (0.2)	Nil	2	11/12
Madribon (0.1)	Nil	2	18/21
Fanasil (0.04)	Nil	1	9/11
Fanasil (0.1 thrice weekly)	Nil	1	9/11
Ciba 1906 (0.1)	Ciba 1906[a]	4	9/24
TB 1 (0.2)	Ciba 1906[a]	4	11/25

[a]Two or more years of treatment.

CIBA 1906 and thiosemicarbazone against *M. tuberculosis*. Although our results would indicate that partial resistance to CIBA 1906 had occurred in *M. leprae* derived from our 4 patients receiving this drug, and that they were also showing cross resistance to thiosemicarbazone, the results are not conclusive, since Shepard and Chang obtained little or no activity with thiosemicarbazone and CIBA 1906 respectively. On the other hand, the foot pad infection has shown beyond doubt that strains of *M. leprae* can become resistant to DDS ([14]). Seven patients were selected who, despite 13-15 years of sulfone therapy, still showed active disease and a high Bacteriologic Index with a high proportion of solidly staining bacilli (Morphologic Index) (Table 4.) The 7 patients were taken into our research wards and each one biopsied, partly for obtaining bacilli for inoculating mouse foot pads. All patients were then treated with 300 mgm. DDS by injection twice weekly for 6 months, and then examined clinically, bacteriologically and histologically. The results show clearly that the 4 strains from the patients who responded to the test period on DDS were all completely inhibited by DDS (0.1 per cent in the diet), whereas the 3 strains from the patients showing no response were not inhibited by DDS in the mouse foot pad infection (Table 5). Thus there was a complete correlation between the studies in man and in animals, and for the first time we can definitely say that in 3 patients their failure to respond to DDS was due to the presence of DDS-resistant strains of *M. leprae*.

PATHOLOGIC STUDIES

The successful transmission of *M. leprae* to animals offers for the first time an oppor-

TABLE 4. *Bacteriologic status at time of selection and response after a six month test period on DDS.*

	Bacteriologic assessment of skin smears (average of six sites)				
	At time of selection		After six months		Assessment of improvement
Patient No.	Bacteriologic index	Morphologic index	Bacteriologic index	Morphologic index	
5075	4.8	37	4.5	12	+1
9055	4.2	42	3.6	4	+2
9386	3.7	38	3.0	32	0
10458	3.9	43	4.6	49	0
10607	4.7	53	4.3	1	+2
10735	4.3	36	4.8	31	0
10755	4.0	48	3.6	4	+2

TABLE 5. *DDS sensitivity using the mouse foot pad infection of* M. leprae *from 7 patients with* prima facie *evidence of resistance.*

	Multiplication of *M. leprae* in foot pads		
Patient No.	Untreated mice	DDS-treated mice (0.1%) DDS in diet	Results of sensitivity tests
5075	+	0	Sensitive
9055	+	0	Sensitive
9386	+	+	Resistant
10458	+	+	Resistant
10607	+	0	Sensitive
10735	+	+	Resistant
10755	+	0	Sensitive

tunity for examining human leprosy experimentally. Since one of the most consistent manifestations of leprosy in man is the predilection of *M. leprae* for nerves, a systematic histologic analysis of all the tissues of infected foot pads was started. Thus far, 20 foot pads have been examined from batches of mice that were infected 3-23 months previously with *M. leprae* (11 directly from patients and 4 from mice in first passage) obtained from 11 patients. In 17 of the 20 foot pads a few isolated irregularly stained (degenerate) acid-fast bacilli were found in macrophages situated in the epineurium of cutaneous nerve bundles and in scanty cellular infiltrates in the region of dermal neurovascular bundles. A few macrophages, containing sometimes as many as 15 irregularly stained bacilli, were also found in the connective tissue sheaths of the muscles. Occasionally irregularly stained organisms could be seen in perineurial cells of cutaneous nerve bundles, but, surprisingly, none in Schwann cells within the perineurium. Moreover, none was found in relation to motor nerves.

The most striking findings were the presence of small foci of densely packed bacilli, having the appearance of "microcolonies," situated in and closely adjacent to striated muscle fibers, and the absence of any cellular infiltrate [13]. The number and density of bacilli within such colonies varied from animal to animal and muscle to muscle, but they were always easy to find in specimens where macrophages, containing irregularly stained organisms, were present. Where individual bacilli could be identified within the "microcolonies" they always appeared to be uniformly stained with carbol fuchsin (viable). The muscle fibers in these specimens had been cut both longitudinally and transversely, and it was thus possible to determine the position of these bacilli quite easily. The larger "microcolonies" lay in vacuoles within the sarcolemma, often in close relationship to a darkly stained nucleus morphologically quite distinct from those of the muscle fibers themselves. Others lay in relation to similar darkly stained nuclei that appeared to have squeezed themselves between the muscle fibers and adjacent capillaries, being partly inside and partly outside the limiting sarcolemma. In addition, smaller colonies and sometimes evenly stained single organisms were seen inside single muscle fibers. Many of these were lying at some distance from the surface of the fiber.

The presence of relatively large numbers of healthy acid-fast bacilli in muscle fibers in 14 of 20 foot pads was quite unexpected, particularly in view of the previous description of the histology of *M. leprae* in the mouse food pad [20]. Moreover, so far as we are aware, comparable nests of viable bacilli have not been reported in the muscles of patients with leprosy or other mycobacterial infections. On the other hand, it is significant that bacilli have been observed in striated muscle in the ears of hamsters inoculated with *M. leprae* [11,25].

The majority of viable organisms lay either free in muscle fibers or in "satellite cells" within the fibers. The latter may either reside within the basement membrane of muscle fibers or reach them from without, and it is of considerable interest that the existence of cells of this type in frog muscle [10] and in patients with progressive muscular dystrophy [7] has recently been reported.

These observations suggest that the limited multiplication of *M. leprae* in the mouse foot pad occurs predominantly in striated muscle fibers rather than in association with nerve fibers or in cells of the reticulo-endothelial system. It has been suggested that *M. leprae* multiply in the mouse foot pad because the temperature is low, and it is assumed that *M. leprae*, like other known cultivable species of mycobacteria [8,12] affecting the skin, multiply only at temperatures below 37°C. The fact that the majority of healthy *M. leprae* lie not in the skin or subcutaneous connective tissue but in striated muscle fibers must throw doubt on this hypothesis, for the temperature will be high, not low, in this particular tissue. Doubt concerning the low-temperature hypothesis is greatly strengthened by finding that the inoculation of *M. leprae* into mouse thigh muscles also results in their multiplication and localization as in the foot pad (Table 6).

At present we have no explanation for the attraction of muscle fibers and satellite cells of muscle fibers for *M. leprae* in the

TABLE 6. *Increase in the number of bacteria in thigh muscles of mice inoculated with fresh or passage strain of* M. leprae.

	Mouse No.	Time harvested (months)	Increase
Passage strain 839 (A3) i Dose/thigh muscle 4.7×10^3	718	8	Histology
	719	10	282
	720	10	0[a]
	721	11	102
	722	11	Histology
	723	11	0[a]
Fresh strain 963 Dose/thigh muscles 10^4	326	6	Histology
	327	8	440[b]
	328	8	0[a]
	332	6	Histology
	333	8	286[b]
	334	8	0[a]

[a]Harvest less than 2.25×10^4.
[b]From pool of left and right thigh muscles.

mouse foot pad. But it is not without interest that, in man, Schwann cells have an exquisite predilection for *M. leprae* and a functional role in relation to nerve fibers, which satellite cells may well have for muscle fibers. Further, comparative investigations in the mouse foot pad and in thigh muscles with several other species of mycobacteria (strains Chatterjee, marianum, Charbotier, BCG and *M. lepraemurium* and *M. ulcerans*) have demonstrated that only *M. leprae* has a specific predilection for striated muscle fibers. None of the other species is taken up by, or multiply in, muscle fibers or satellite cells.

IMMUNOLOGIC STUDIES

One of the most interesting aspects of experimental leprosy in animals and tuberculoid type leprosy in man, requiring elucidation, is the factor or factors responsible for limiting the infection in respect both to site and multiplication of the bacilli. Localization to the foot pad or ear could be explained if *M. leprae*, like other known cultivable species of mycobacteria affecting the skin, such as *M. balnei* [12] and *M. ulcerans* [8], multiply only at temperatures below 37°C. On the other hand, multiplication of *M. balnei* and *M. ulcerans* at the site of inoculation is not restricted to the same low ceiling as in the case of *M. leprae*. These two cultivable species of bacilli spread from the local site of inoculation and settle in cooler superficial sites where they multiply freely and produce satellite lesions. Therefore the very limited infection produced by *M. leprae* is not likely to be explained on a temperature basis, particularly since recent observations strongly suggest the main site of multiplication of leprosy bacilli in the mouse foot pad is in striated muscle fibers, which are most unlikely to be at a temperature lower than 37°C.[13] It is most tempting in both experimental leprosy and tuberculoid leprosy in man to suggest that the limiting factor is an immunologic one resulting from the infection. This suggestion is strengthened by the observation of Shepard [21] that prior vaccination with BCG, given either locally into the foot pad or at other sites in the body, more or less completely inhibits the multiplication of *M. leprae* inoculated subsequently into the mouse foot pad. Cortisone [23] and Suramin [15], compounds that have been shown to enhance infection significantly with other species of mycobacteria, have no effect on infections with *M. leprae* in the mouse foot pad. Therefore, in the present study, we set out to test whether or not the limiting factor had an immunologic basis by exposure of the mice to thymectomy and total body irradiation. We chose these combined procedures because of their known powerful immunologic depressive effect in the

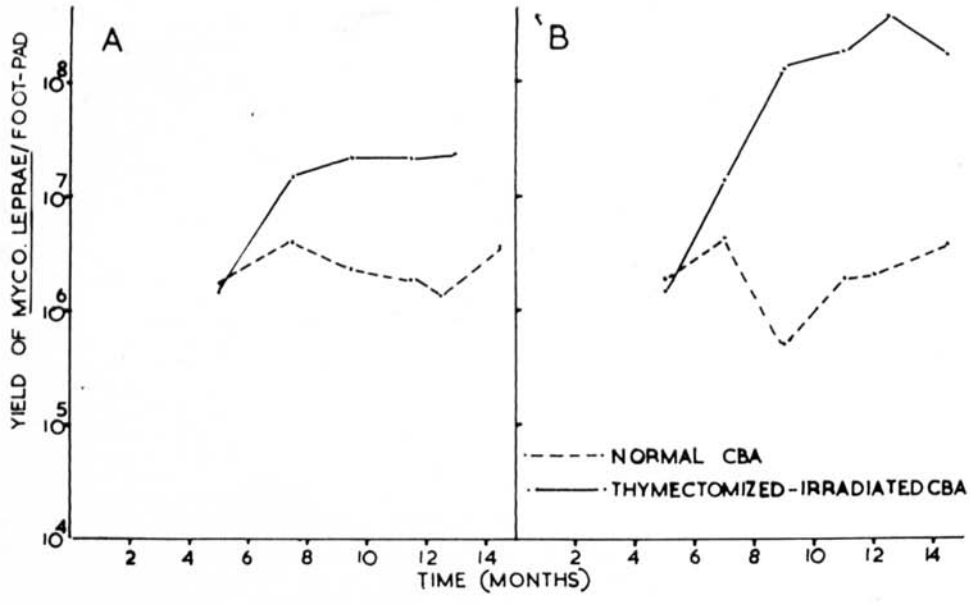

FIG. 1. Growth curves of *M. leprae* in the foot pads of thymectomized, irradiated and normal CBA mice inoculated with 10^4 new (A) or passage (B) strain of bacteria.

homograft reaction and antibody production. The results in two experiments with *M. leprae* showed clearly (Table 7 and Fig. 1) that significantly greater multiplication of bacilli occurred in the animals exposed to thymectomy plus total body irradiation (900r), and therefore a part of the limiting factor could be an immunologic one. This conclusion was strengthened by the observation in a subsidiary experiment that an infection with *M. tuberculosis* was also enhanced by thymectomy plus total body irradiation (Table 8). The present studies have particular interest since they represent the first deliberate attempt to use thymectomy (plus irradiation) to depress the development of immunity in experimental infections with *M. leprae* and *M. tuberculosis*.

In order to demonstrate beyond doubt an immunologic basis for the enhanced infection following thymectomy plus irradiation it will be necessary to show that the enhancement can be neutralized by lymphocyte replacement. Because only a partial enhancement of infection has been obtained at present following thymectomy and irradiation, it is not possible to exclude other limiting factors. Further experiments must be undertaken in thymectomized and irradiated mice inoculated with doses of 10^6 *M. leprae* into the foot pad and also inoculated systemically.

SUMMARY

It has been conclusively demonstrated that human leprosy can be transmitted to the mouse foot pad, within which limited multiplication of *M. leprae* occurs. Multiplication of *M. leprae* is dependent on the size of the inoculum and is confined to the foot pad. Identical infections have so far been produced with 35 "strains" of *M. leprae* obtained from previously untreated patients from different parts of the world: Malaysia (27), Burma (6), East Africa (1) and the West Indies (1). Of these strains 29 were derived from patients with lepromatous type infection and 5 from patients with borderline and 1 from a patient with reactional tuberculoid leprosy. On the other hand, bacilli derived from patients treated for 12 to 16 months with DDS nearly always failed to multiply in the mouse foot pad. A very high proportion of bacilli from these patients were degenerate (showed irregular staining with carbolfuchsin), thus confirming the nonviability of these forms of bacilli. The foot pad infection can be inhibited by DDS, phenazine

TABLE 7. *Yield and proportion of degenerate bacteria in the foot pads of thymectomized-irradiated CBA and normal CBA and P strain mice inoculated with 10^4 new (A) or passage strain (B) of* M. leprae.

		Mouse strain					
		Normal P[a]		Normal CBA		Thymectomized-irradiated CBA	
Experiment	Harvest time (months)	Yield AFB[b] ($\times 10^6$)	Degenerate AFB[b] (per cent)	Yield AFB[b] ($\times 10^6$)	Degenerate AFB[b] (per cent)	Yield AFB[b] ($\times 10^6$)	Degenerate AFB[b] (per cent)
A	5	0.8	(0/7)	2.1	(1/18)	2.0	36
	7.5	5.1	26	6.0	56	16.7	17
	8					[c]	
	9.5	2.5	72	3.8	57	33.1	15
	10	0.9	(5/8)				
	11.5	1.8	78	2.6	73	33.3[d]	28
	12.5			1.2	(6/10)		
	13					35.7	41
	14.5			5.5	61		
B	5	0.8	(0/7)	2.7[d]	(0/23)	1.8	(0/15)
	7	0.5	(1/4)	6.4	52	11.7[d]	5
	9	1.3	72	0.7	(9/16)	108.0[d]	23
	11	5.8	40	2.6	(19/22)	261.7[d]	30
	12			2.9	58		
	12.5	1.8	69			583.0[d]	48
	14.5	8.8	48	5.7	54	304.6[d]	32

[a]One died at 3 months.
[b]Acid-fast bacilli.
[c]Sick mouse killed for histology.
[d]Inoculated into the foot pads of further mice.

TABLE 8. *Survival time and extent of tuberculosis in thymectomized-irradiated CBA and normal CBA and P strain mice inoculated intravenously with 0.02 mgm.* M. tuberculosis *(strain H37Rv).*

	Mouse strain: treatment		
	P: Untreated	CBA: Untreated	CBA: Thymectomized-irradiated
No. of mice	6	6	6
Day of death[a]	21: 22: 22 23: 25: 26		15: 15: 15 16: 17: 19
Mean survival time (days)	22	39[b]	15
Condition at time of death (D) or sacrifice (S)	D: Extensive tuberculosis of lungs only	S: Few pinpoint tuberculosis lesions of lungs only	D: Extensive miliary tuberculosis of lungs, livers and spleens

[a]Number of days after infection.
[b]All mice alive and well when sacrificed on day 39.

(B.663), Fanasil and Madribon. Almost complete inhibition has been obtained with Ciba 1906 and thiosemicarbazone (TB1) Using the mouse foot pad infections there is suggestive evidence that some strains of *M. leprae* derived from patients on two years or more of treatment with Ciba 1906 are partially resistant to this drug and show cross-resistance to thiosemicarbazone. There has been definite confirmation that, in a very few patients with progressive leprosy, despite many years of DDS treatment, these patients are infected with DDS-resistant strains of *M. leprae.*

Detailed histologic analysis of infected foot pads has revealed that a very high proportion of healthy-looking bacilli are situated within striated muscle fibers, appearing as "microcolonies." It is suggested that this is the main site of multiplication of *M. leprae* within the mouse foot pad. Following this observation it has been shown that *M. leprae* inoculated into the mouse thigh muscle can produce a similar picture with multiplication of the bacilli within striated muscle fibers. Comparative studies with several other strains of mycobacteria, including *M. lepraemurium*, have shown no predilection for muscle fibers.

On the assumption that the most likely factor resulting in limited multiplication of *M. leprae* within the mouse foot pad is an immunologic one, attempts have been made to enhance the infection by diminishing the immunologic response. Preliminary results indicate that significant enhancement can be obtained in previously thymectomized and irradiated CBA mice. It is hoped that this technic will eventually lead to a much more progressive and generalized infection with *M. leprae* in the mouse.

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DISCUSSION

Dr. Mason. Dr. Rees' paper is open for discussion.

Dr. Cochrane. I have been interested in the matter of *M. leprae* in nonstriated muscles for many years and have said that in looking through a section in which you cannot find acid-fast bacilli you should look at the nerves first and then at the muscles, because you not infrequently find small collections of acid-fast bacilli in the arrectores pilorum muscles.

Dr. Shepard. I think I can take care of most of these points when my turn comes later this afternoon. Now, as to the possible conflict over Ciba 1906. The work I referred to was done with Dr. Chang. I thought we used a little higher concentration than they did. Do you remember, Dr. Chang? We really should come to these meetings with our laboratory notebooks.

Dr. Rees. We both used 0.1 per cent Ciba 1906.

Dr. Shepard. Thank you. As regards the muscle location, we look at a good many sections as part of our routine. By now we have seen between five hundred and a thousand. We have found quite a bit of variation from one batch of mice and one experiment to the next. Sometimes we have seen frequent location in muscles and sometimes in subcutaneous tissue, but I do not know how to control it. We have tried to put the injection in the muscle and ended up with it in subcutaneous tissue and the other way around. I have been impressed, on a number of occasions, though, by the fact that organisms that were "plastered" right on the muscle bundles appeared to be in better condition than those elsewhere in the foot. I am not enough of an anatomist to know their pre-

cise location with respect to the muscle bundle. As regards the spread of *M. marinum* after foot pad injection, this varies among lines of mice; in many lines of mice the infection does not spread from one foot to other parts of the body. We have studied about 10 to 12 lines by now. Also in the lines we have studied there is a definite ceiling effect, if you want to call it that. The bacterial population comes up to a level of the high 10^6, or low 10^7, and holds there for a period of a couple of months at least, and resembles the *M. leprae* growth curve a great deal, except for the height of the ceiling plateau and the time scale.

Dr. Binford. With reference to the invasion of the striated muscle in the foot pads, I have been impressed, in looking at most foot pads histologically, by the fact that frequently there are pools of bacilli in the muscle cells. I have not given this any particular attention, as Dr. Rees has done, but I have noted these large masses in cells in the muscles in a number of slides, and I am interested that Dr. Rees has gone into this point more thoroughly. With reference to the results, Dr. Rees, in your thymectomized and irradiated animals, with rather distinct increase in numbers of bacilli that you found, did you note any increase in disease? Is there any more spread as seen histologically, or any other evidence of increased disease?

Dr. Rees. We had no evidence that there was any more spread of the infection in the thymectomized and irradiated mice than in the normals, but there was increased local infection as reflected in more foci of bacilli in the muscles. Could I ask you, Dr. Binford? Have you looked at your histology with the thought that the yield was coming particularly from bacilli within the muscle, and have you, like Shepard, thought these bacilli looked in particularly good condition?

Dr. Binford. As a histologist, not a bacteriologist, I have been looking at the effects of the bacilli on tissue rather than at the numbers of bacilli that could be counted, but I think I am correct in saying that they usually appeared very well preserved. Dr. Wiersema may have comments on the appearance of the bacilli in the muscles. With reference to Dr. Cochrane's mentioning of the smooth muscle in the skin, I think we have seen leprosy affecting striated muscle. We had one example of a nerve lesion, a great auricular nerve removed surgically as a tumor, in which we were able to see the spread of the granulomatous lesion into the tissue adjacent to the nerve. The striated muscle was invaded by small epithelioid granulomatous cell clusters, which we could identify as being around the nerves that branched into the muscle tissue.

Dr. Pattyn. In connection with the Ciba 1906 experiments, I was wondering if somebody could give us some information about the eventual stability of the product. We have run two experiments with 1906, with contradictory results. In one group of mice infection showed up approximately as in the controls, and in the other one it was apparently suppressed. I wonder if varying stability of the product could be responsible.

Dr. Wayne. I would like to ask Dr. Rees when the marrow was given to the irradiated animals, and if he believes that it has had any effect on the establishment of the new ceiling?

Dr. Rees. The marrow, Dr. Wayne, was given within half an hour of irradiation (900x) and is necessary to provide the animals with blood-forming cells. The experts tell me that the contribution of immunologically competent cells from the marrow would be expected to be very small. But I suppose it might have been sufficient; I am not sure. As to the stability of Ciba 1906—I thought it was a stable drug; certainly in tuberculosis in mice the results are unimpeachable. I was careful, as you notice, not to claim with certainty that the results of our experiments from patients previously treated with Ciba 1906 necessarily indicated any degree of resistance, because undoubtedly Dr. Shepard has found no effect with the drug against two fresh strains of *M. leprae*. Could this not all be due, not so much to a labile drug, but to one that is rather borderline

in chemotherapeutic concentration within the foot pad, leading to rather variable results? This can only be answered by testing more fresh strains of *M. leprae* from untreated patients.

Dr. Browne. I would like to add a clinical footnote to Dr. Binford's observation. There is a rare condition known as leprous myositis in which discrete foci of leprosy infiltration occur in the muscles, particularly of the thigh and the forearms, and there is also a chronic and more diffuse type of leprous myositis in which whole groups of muscles eventually become transformed into a fibrous mass.

Dr. Binford. Dr. Browne, have you made studies of the histology of any of these lesions?

Dr. Browne. There were reports in the INTERNATONAL JOURNAL OF LEPROSY some years ago in which it was noted that the original condition was actual lepromatous infiltration of the striated muscle and the final result fibrosis following autolysis.

Dr. Rees. The paper that I can recall was by Convit *et al.* (*Internat. J. Leprosy* 28 (.1960) 417-422). I was looking for evidence of *M. leprae* within muscle fibers in man, but as far as I recall Convit found predominantly evidence of cellular infiltration of striated muscle or bacilli between the fibers.

Dr. Mason. Thank you, Dr. Rees, for your interesting contribution. We shall proceed to the next report, by Dr. Shepard, on practical applications of mouse foot pad inoculations in leprosy research.