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Hereditary Characteristic that Varies Among Isolates of *Mycobacterium leprae*

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Isolates of *Mycobacterium leprae* in mouse foot pads were found to differ in two related properties, the average rate of growth between inoculation and harvest (G) and the number of bacilli in the harvest (H). For "fast" strains the median values for G were less than 25 days per generation, and the median values for H were above $10^{6.1}$. For "slow" strains the median values for G were above 30, and the median values for H were below $10^{5.6}$. The G and H values for the 59 isolates for which data were available formed a continuous spectrum between the two extremes; there was no correlation with dapsone resistance. The fastness characteristic was stable; it did not change on passage in mice and was in agreement when more than one isolate had been made from the same patient. No important differences were apparent according to geographic origin of the infection of the patient. Histological studies showed that fast strains grew to a higher level without inducing the infiltrate of lymphocytes and macrophages that appears at the end of the logarithmic phase of growth in mouse foot pads. Although fast strains often had higher ratios of solidly staining (and presumably viable) bacilli in the inoculum, the fast-slow difference was not accounted for by the solid ratio. Slow strains differed from fast by having longer times until harvest and by having fewer generations of growth, even when their frequently lower solid ratios were taken into account.

In our laboratory, several dozen isolates of *Mycobacterium leprae* are kept in continuous mouse foot pad passage, and some of them have been in passage for many years. One isolate, NSa, attracted our attention because it was taking less time per passage than most of the others. Then a study of the correlation between solid ratio and infectivity (1) led us to speculate that fast strains, such as NSa, might be characterized by high solid ratios and fast growth in a way that would account for at least part of the correlation between solid ratio and average generation time. Consequently, a systematic study of the recorded data was made, and it showed that isolates of *M. leprae* differed in their rates of growth and in the number of bacilli that characterized the plateau stage of the infection.

MATERIALS AND METHODS

The general methods used are given in the preceding paper (1), as are the definitions of G, the average generation time between inoculation and harvest; G_S, the average generation time calculated on the assumption that all the solid bacilli (and only the solid bacilli) are infective for mice; $S/(N + S)$, the proportion of solidly staining bacilli in the inoculum; H, the number of *M. leprae* harvested for transfer; and F_S, the number of solid *M. leprae* inoculated into the foot. G_{med} and H_{med} are the median G and H values of each isolate.

The study includes all 59 strains with acceptable data for five passages or more. The data were accepted for the study if the inocula into mice were 10^4 or less *M. leprae*, if histological sections were done regularly, and if CFW mice were used. The techniques for measuring dapsone (DDS) sensitivity have been described (2).

RESULTS

The principal finding was that isolates could be arranged on a fast-slow scale. In Fig. 1, G is given for each passage of the five fastest and the five slowest strains. The five fast strains have clustered values below 25, whereas the five slow strains have variable values with medians above 30. Examination of the data shows that G had no tendency to change on passage, either with these strains or with others.

In Figure 2, H is given for each passage of the same isolates. The fast ones have clustered values with medians above $10^{6.1}$, whereas the slow strains have variable values with medians below $10^{5.6}$. The value of H, with these and the other 49 isolates, had no tendency to change on passage.

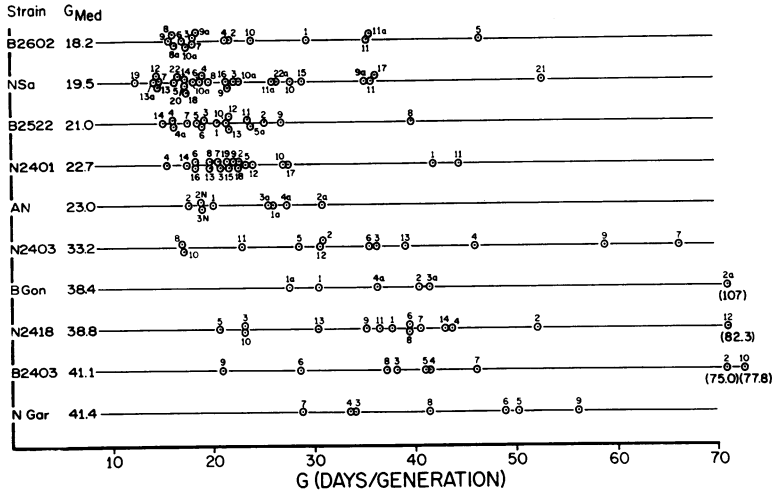

FIG. 1. *Average generation time (G) at each passage of the five fastest and five slowest strains. G_{med} is the median value. The number of the passage is indicated at each point (the letters refer to parallel passages).*

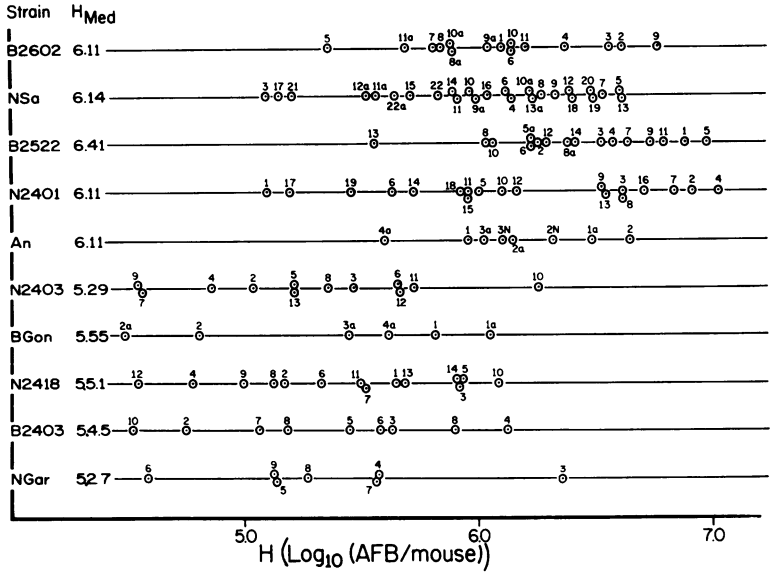

FIG. 2. *Number of bacilli harvested (H) at each passage of the five fastest and five slowest strains. H_{med} is the median value. The number of the passage is indicated.*

In Fig. 3, the median *G* is plotted against median *H* for the 59 strains with five or more passages. Results with four other strains with three or four passages are also included because their DDS sensitivities had been measured (three sensitive to 0.0001% DDS and one resistant to 0.001% DDS). High *H* is correlated with short *G*, and there is a continuous spectrum of values. Among the 24 strains that had been characterized for DDS sensitivity (4), there was no correlation between that property and fastness.

Sometimes two isolates had been obtained from the same patient. In each of these instances, isolations had been made from nasal washings and skin biopsy specimens taken at the same time. If fastness is a stable genetic quality of a strain when it grows in the patient from the original infecting quantum of the patient, the two isolates from the same patient should have the same *G* and *H* values. Figures 4 and 5 show that they did have, within experimental variability.

In Table 1, the median *G* and *H* values for the

strains are given according to the country or state in which the infection was probably acquired. When two isolates had been made from the same patient, the results were combined. The range of values for any one region of origin, i.e., the Philippines, appears to be complete; other differences appear to arise from sampling variation.

Most of the multiplication of *M. leprae* in normal mice occurs logarithmically and does so in the fibroblasts that are normally present in the foot. When the bacterial population reaches a sufficient level, lymphocytes and macrophages quickly appear in the area of the bacilli, and logarithmic growth is terminated. It seemed possible that slow strains were those that triggered the histological response at a relatively low bacterial population or perhaps initiated a different kind of cellular response.

The histological sections had all been saved, so they were reviewed for five of the fastest and five of the slowest strains. To review briefly, bacilli are usually not detectable in the early

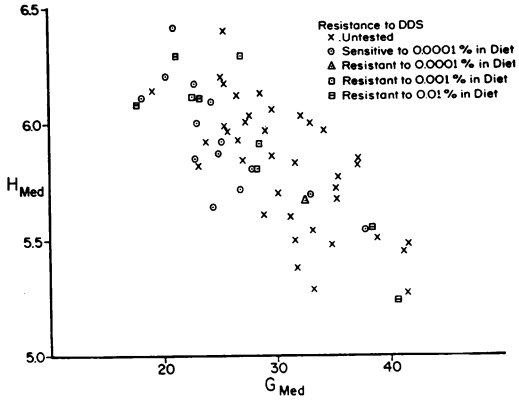

FIG. 3. *Median harvest (H_{med}) and median generation time (G_{med}) of each strain. Where known, the sensitivity to DDS is also indicated.*

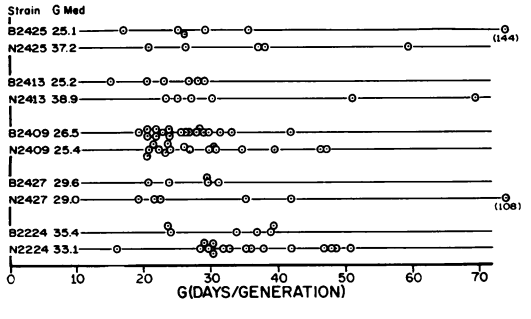

FIG. 4. *Average generation time (G) at each passage in cases where two isolates had been made from the same patient. (B) indicates skin biopsy specimen and (N) indicates nasal washing.*

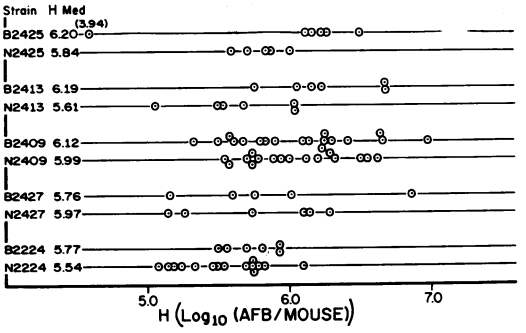

FIG. 5. *Number of bacilli harvested (H) at each passage in cases where two isolates had been made from the same patient. (B) indicates skin biopsy specimen and (N) indicates nasal washing.*

months after inoculation although a few are infrequently encountered by the section. Later, "significant" changes are observed, i.e., more than 30 infected cells in the section or the appearance of an infected infiltrate of lymphocytes and macrophages occupying more than 0.08 mm² (one-fourth of a 12.5 by 45× field of a Reichert Zetopan), and these changes are taken as a signal that significant bacterial multiplication has occurred and the incubation period has passed. A harvest is then scheduled, and it is performed about a month after the mouse is taken for the positive sections.

The principal finding of the histological study was that fast and slow strains differed in the particular sections that represented the first significant lesion (Table 2); fast strains usually did not have an infiltrate around the bacilli, whereas slow strains usually did. The few bacilli occasionally encountered before the first significant lesion were only infrequently associated with infiltrates. In sections taken during the months after the first significant lesion, infiltrates were usually associated with bacilli of both fast and slow strains, although fast strains had a somewhat lower incidence of infiltrates. When present, the infiltrate of fast and slow strains was the same. In the early months, it was composed of a mixed population of lymphocytes and macrophages. Later it was sometimes a more organized granuloma in which there was a peripheral zone in which lymphocytes predominated, and a central zone of macrophages with abundant cytoplasm that sometimes showed early "foamy" changes and frequently contained acid-fast bacilli.

In the accompanying paper we have reported that, when all the strains are considered together, the average generation time G for a mouse foot pad infection varies according to the proportion of solidly staining bacilli (solid ratio) in the inoculum and that G_S is constant at different solid

ratios. This is the result expected on the hypothesis that solid bacilli (and only solid bacilli) are viable. However, it seemed possible that such a result might be produced by strain differences, if fast strains routinely had higher solid ratios. For example, fast and slow strains might have the same average G_S values if the time to harvest was the same for both, but the average solid ratios differed by the same factor as the average harvests. Consequently, the analysis was repeated with the strains arranged in six categories according to median G. Solid ratios were not determined on every inoculum, but enough had been done to provide the data in Fig. 6, 7, and 8.

TABLE 1. *Median generation time of strains according to geographic origin of strain*

Place infection acquired	Median generation time (days/generation)
Puerto Rico	18.2, 21.2, 23.8, 25.8, 28.3, 34.8
Other Caribbean	22.7, 33.8, 38.4, 41.5
Louisiana	23.1, 26.4, 33.0
Texas	23.1, 26.4, 27.2, 29.4, 35.2
Mexico	22.8, 24.4, 27.3, 27.9, 34.2, 37.3, 37.6, 38.8
Other Latin America	29.6, 32.6
Hawaii	26.6, 28.5
SW Pacific	26.6, 31.2
Philippines	19.5, 23.0, 24.3, 25.2, 25.4, 27.0, 28.6, 31.8, 35.2, 37.2, 41.4
SE Asia	17.7, 21.0, 24.9, 27.6, 32.6
Other Asia and Africa	32.6, 31.7, 32.2, 20.3, 22.8

The faster strains had more high solid ratios in their inocula, but, nevertheless, within each category, G_S appeared to be constant at various solid ratios. Thus the hypothesis that only solid bacilli are viable was confirmed, even within fastness categories. The G_S values were found to progress through the categories, averaging about 14 days per generation for the fastest strains and 19 for the slowest.

These differences in G_S in the different categories were looked at more closely by plotting the numerator (t = number of days from inoculation to harvest) against the denominator [$\log_2 (H/F_S)$ = the number of generations of increase, calculated on the basis that only solid bacilli are viable] (Fig. 9–11). Slow strains were found to differ from fast strains by having both longer times to harvest and fewer generations of growth per passage even when their frequently lower solid ratios were taken into account. However, it seems possible that the longer times before harvest in slow strains are a result of the difficulty in detecting bacterial growth at the plateau values characteristic of slow strains, so it may be that all of the difference between fast and slow strains is caused by the difference in the numbers of organisms needed to evoke an inflammatory infiltrate.

Important features of the fast-slow difference in mice, lymphocytic infiltration, and number of bacilli suggest the lepromatous-borderline differ-

TABLE 2. *Incidence of cellular infiltrate in the presence of acid-fast bacteria in foot pads of mice inoculated with five "fast" and five "slow" strains*

Strain	G_{med}	H_{med}	Infiltrate associated with AFB[a]			
			Before FSL (No./total[b])	After FSL (No./total)	In FSL	
					No./total	Per cent
Fast strains						
B2602	18.2	1.1×10^6	0/1	0/0	1/10	10
NSa	19.5	1.4×10^6	0/6	9/14	4/22	18
B2522	21.5	2.6×10^6	1/2	1/1	5/11	45
N2401	22.7	1.3×10^6	0/2	3/9	1/12	8
An	23.0	1.3×10^6	0/3	1/1	1/6	17
Total			1/14	14/25	12/61	20
Slow strains						
N2403	33.2	2.0×10^5	1/3	4/4	8/12	67
BGon	38.4	3.5×10^5	0/3	0/1	4/4	100
N2418	38.8	3.2×10^5	0/6	4/7	9/12	75
N2403	41.1	2.8×10^5	0/2	1/1	5/9	55
NGar	41.4	1.9×10^5	0/3	2/4	5/7	71
Total			1/17	11/16	31/44	70

[a] Abbreviations: AFB = acid-fast strains; FSL = first significant lesion (*see* text).
[b] Number with infiltrate and AFB/number with AFB.

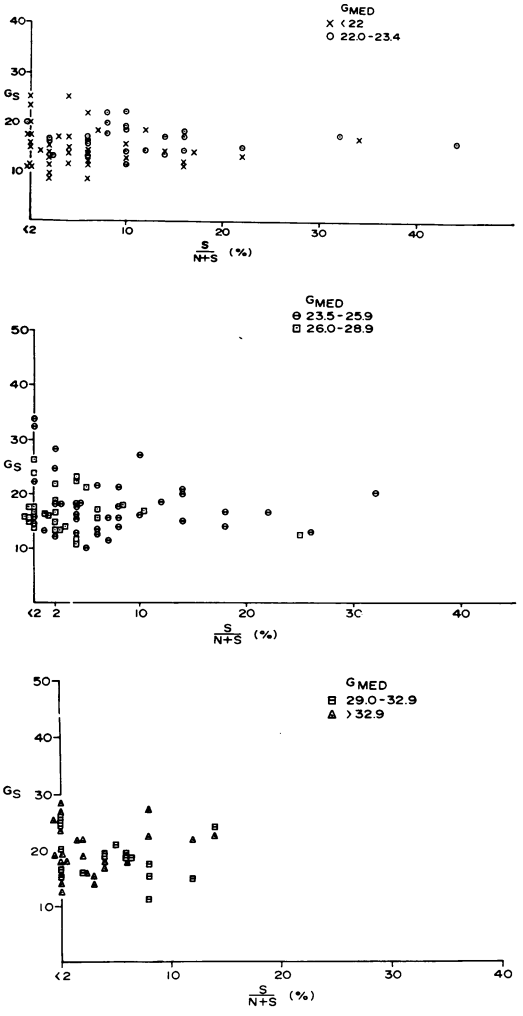

FIG. 6–8. *Generation times calculated on the basis that only solid bacilli are viable (G_S), according to the proportion of solidly staining bacilli [$S/(N + S)$] in the inoculum. The results are broken down according to the median generation time (G_{med}) of the strain.*

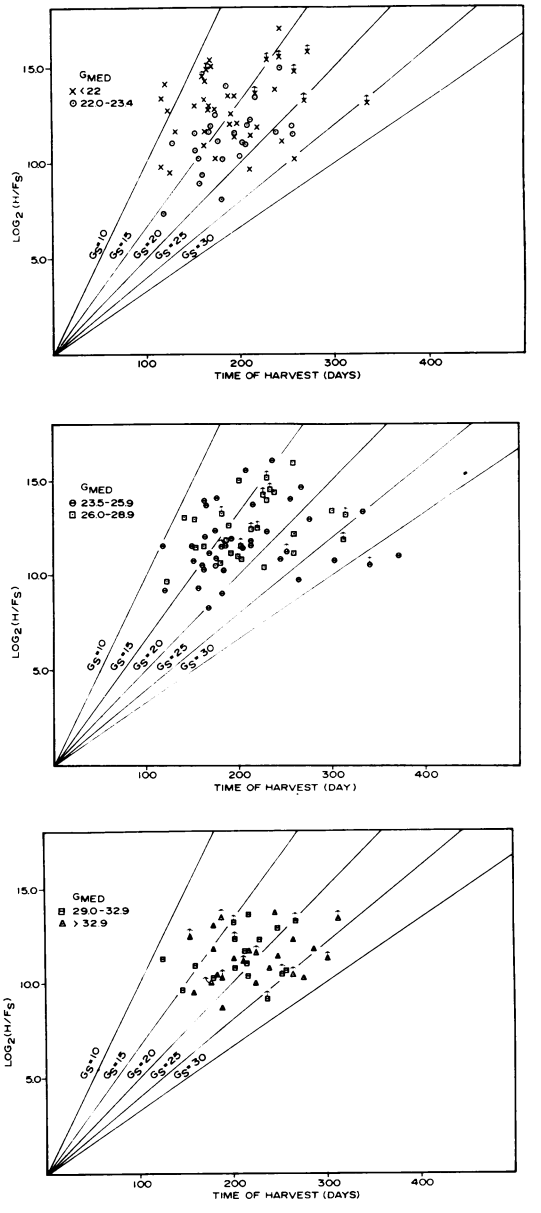

FIG. 9–11. *The two components of the generation time, G_S: number of generations of bacillary multiplication calculated on the basis that only solid bacilli are viable [$\log_2(H/F_S)$] and the time elapsed before the harvest was carried out. The points marked by arrows are minimal estimates in instances where the solid ratio was "0%" (no bacilli were scored solid during the examination).*

ence seen histologically in the human patient. The strains had come from many different clinical facilities, and we did not have systematic histopathological or clinical classification of the patients' disease. Probably all of the patients were LL or BL on the Ridley and Jopling scale (2). However, for some patients more complete information was available. Among these were several patients with diffuse LL disease from whom slow isolates had been made and three patients with BL disease from whom isolates had been made that fell in the middle range. Furthermore, the position of the patient on the scale was likely to be reflected by the number of bacilli present in the skin biopsy specimen and, especially, in the nasal washing of the patient (3). Analysis showed, however, that there was no significant relationship between the number

of bacilli in the biopsy or nasal washing and the median *G* or *H* of the isolate. Thus, the available evidence failed to support the notion that the fast-slow characteristic of the infecting strain determines the position of the patient in the lepromatous-tuberculoid spectrum. Further studies of this important point are planned.

DISCUSSION

The results show that there are clear differences among isolates of *M. leprae* as shown by the characteristic ways they grow in mice. The fastness characteristic did not change detectably on passage in mice, and the evidence suggests that it was stable even during bacillary multiplication in humans.

Whether the characteristic plays a role in human disease is, of course, of great importance, but the evidence is not conclusive. The fast-slow difference in mice certainly suggests the lepromatous-borderline difference in man. However, no correlation was observed between the number of bacilli in the original clinical specimen and the fastness characteristic. This point deserves more careful study, however, e.g., by a systematic retrospective histopathological classification of the disease in the patients or by a titration of the reaction produced in man by lepromins of the same bacillary content made from fast and slow strains. Nevertheless, the present findings suggest caution in the interpretation of certain genetic studies in which uniformity of disease within a family could have resulted from infection with a single strain.

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