THREE FACTORS WHICH MAY INFLUENCE THE EXPERIMENTAL TRANSMISSION OF LEPROSY

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Innumerable attempts to transmit human leprosy to animals have resulted in failure. The difficulties surrounding such undertakings is indicated by the even more remarkable fact that experimental transmission of this disease from man to man has never been accomplished with certainty. Much of the pertinent information on this subject has recently been summarized and discussed by Wade (7). When attempts to study transmission directly in the host species have failed so consistently, one must either become resigned to the fact that studies in that species are unlikely to provide an understanding of the factors involved in failures of transmission, or he must secure and weigh evidence obtained by other methods of approach.

The basis of the present approach through animal experimentation is a simple one, namely, that biological principles have a remarkably consistent manner of reasserting themselves in a variety of species and circumstances. The noncultivable mycobacteria, each within its natural host, are faced with the problem of parasitizing cells. Certain common factors may be expected to influence, either favorably or unfavorably, the outcome of this process. If one is to gain an inkling concerning the nature or the relative importance of any presumed factors, experiments must be made with material which afford freedom of experimental design. Provided this design permits a valid interpretation of the results in terms of the factors which prevent or which permit successful parasitism of cells, they cannot fail to provide implications which are pertinent to the general problem of transmission.

The three factors considered in the present work are: (*a*) the role of specific host adaptation; (*b*) the influence of cellular debris which accompanies the inoculated bacilli; and (*c*) the influence of acquired resistance. Other factors will be considered in future reports.

METHODS

The experiments were made with the Hawaiian strain of the murine leprosy bacillus. This strain was obtained from a wild

rat by Badger in 1935, and has since been passed by Badger or by Fite in rats or mice, depending on the requirements of their work.[1] When the present experiments were initiated these bacilli had been maintained in this laboratory by three successive passages in a subfamily of so-called "Swiss" white mice, while a separate line had been maintained by three passages in a subfamily of Wistar rats. The bacilli from these two sources will hereinafter be referred to as "mouse" and "rat" bacilli, respectively. Lepromata developing in sites inoculated with the respective bacilli will be designated "mouse" lesions and "rat" lesions, irrespective of the animal in which the bacilli were inoculated.

Three types of bacillary suspensions were used. (1) The type used except when otherwise specified was the "clarified supernate" type, derived from subcutaneous lepromata which had been homogenized in distilled water by means of a glass mill and then centrifuged for two minutes at 1000 RPM to remove gross particles and cells; cell extractives and small particles of cellular material remained in the supernatant suspensions. The bacillary content of these suspensions was determined by actual counts, and they were diluted with water to obtain the desired numbers of bacilli in 0.1 cc. of inoculum. (2) Suspensions prepared as above mixed with equal volumes of washed, packed blood cells from the rat or mouse immediately prior to injection, control aliquots being diluted with equal volumes of water. (3) Bacilli which had been washed by centrifugation and resuspension to remove tissue components as completely as possible prior to counting and diluting to the required numbers.

For the test of host adaptation, identical numbers of fresh bacilli from both rat and mouse sources were injected in multiple paired sites on the back of each test animal. All rats received either two or three injections of rat bacilli on the left side and corresponding injections of mouse bacilli on the right side. Mice were also inoculated in accordance with this pattern, except that only two sites were established on each side. One drop of Higgins india ink diluted 1:10 was added to each cubic centimeter of suspension to facilitate identification of the sites of inoculation. The use of multiple pairs of lesions in each animal

[1] I am indebted to Dr. George L. Fite, then at the Marine Hospital, Carville, Louisiana, for supplying a mouse infected with this strain; and also for the suggestion that, because of adaptation of the strain to that animal, transmission of the infection to rats might require a large dose of bacilli and that there might be a long delay in the development of lesions.

served to minimize the usual difficulties due to differences in individual susceptibility, and to permit close comparisons between the lesions resulting from the materials inoculated.

Results were judged by palpation for positive lesions at intervals of two or four weeks. All doubtful observations were assigned zero or negative values. The lepromata were rated for size by comparing them with a series of paraffin balls of known volumes which could be moulded into any desired form. Excised lepromata were weighed to provide more precise data.

Statistical analyses were made by calculating the standard deviation of the differences between percentages of positive lesions, employing denominators of *n-1* for all comparisons involving less than 30 observations. The value *k* indicates how many times the observed differences exceeded the standard deviation. The corresponding *P* values were converted into the percentage probability that the observed differences did not arise by chance, and in the tables are designated "P level." A level of 95 per cent was regarded as significant, and levels above 98 per cent as highly significant.

RESULTS

HOST ADAPTATION

Experiment 1.—In the first experiment two sites were established on the right side of each of eleven rats by inoculation of $1x10^8$ mouse bacilli into one site and of $1x10^7$ into the other.

TABLE 1.—*Cumulative lesions positive in rats inoculated with the same numbers of murine leprosy bacilli form rat and mouse sources (Experiment 1).*[a]

Number of bacilli inoculated	Weeks	"Rat" bacilli, lesions positive	"Mouse" bacilli, lesions positive	Difference in percentages	k	P level
10^8	16	10/11 (91%)	6/11 (55%)	36	1.99	95%
	20	10/10 (100%)[b]	3/10 (30%)	70	4.57	99%
	32	9/ 9 (100%)[b]	4/ 9 (45%)	55	3.12	99%
10^7	16	1/11 (9%)	0/11 (0%)	9	------	------
	20	4/10 (40%)	2/10 (20%)	20	0.95	66%
	32	5/ 9 (56%)	2/ 9 (22%)	34	1.47	86%

[a] In the fractions the numerators represent positive lesions, and the denominators represent sites inoculated.

[b] One rat died between the 16th and 20th weeks, and one was lost during operation between the 20th and 32nd weeks.

Nine days later similar sites were established on the left side of these animals with the corresponding numbers of rat bacilli. The record of weeks elapsed in the experiment was based on the date of inoculation of the mouse bacilli. The results are shown in Table 1.

It will be seen that 10^8 rat bacilli produced a significantly higher proportion of positive lesions in these rats than did 10^8 mouse bacilli after 16, 20 and 32 weeks. With the small group of animals and the low incidence of early positive lesions, the results with 10^7 microorganisms fail to show significant differences between the rat and mouse bacilli. It may be noted, however, that the differences in percentages increased at each time interval due to more continual and successful production of lesions by the rat bacilli. After both 20 and 32 weeks the 10^7 rat bacilli provided more positive lesions than the 10^8 mouse bacilli. If the total number of palpable lesions produced by both doses of rat bacilli are combined for comparison with the total number of lesions produced by mouse bacilli at each of the time intervals, the differences between the rat and mouse bacilli are highly significant.

A second interesting difference between the rat and mouse bacilli under these competitive circumstances is the fact that two well-established mouse lesions regressed between the 16th and 20th weeks, while another regressed between the 20th and 30th weeks. Furthermore, the two mouse lesions which appeared between the 20th and 32nd weeks occurred in two rats from which both lesions due to rat bacilli had been removed surgically lest they become ulcerated.[2]

A further comparison of the relative success of the bacilli from the rat and the mouse sources is provided by the data, summarized in Table 2, on the weights of the lepromata which were excised from time to time, either for the purpose of making this comparison or to remove those which might soften and ulcerate. The rat bacilli produced during the course of the experiment 10 lesions which required removal, while only 5 mouse lepromata became large enough to be removed for weighing. A

[2] It is perhaps because of the removal of the rat-bacillus lesions that the two mouse sites became positive. In the third experiment no lesions were biopsied until after the situation with respect to the two types of bacilli had become more stabilized, and all the mouse lesions regressed. A later communication will present evidence that the presence of well-developed lesions tends to inhibit the growth in other sites, and that removal of the larger lesions has a favorable influence on the development of small or latent lesions.

total of 18.9 gm. of lepromа tissue was harvested from the rat sites, while only 2.2 gm. was recovered from the sites inoculated with mouse bacilli.

TABLE 2.—*Numbers and weights of lepromata harvested after inoculating equal numbers of murine leprosy bacilli of rat and mouse origins (Experiment 1).*

Weeks	Lepromata caused by "rat" bacilli		Lepromata caused by "mouse" bacilli	
	Number	Weight (grams)	Number	Weight (grams)
16	3	1.2	3	0.4
		1.4		0.8
		1.5		0.4
20	2	1.5	1	0.3
		2.0		0.0
24	4	1.5	1	0.0
		1.8		0 0
		2.0		0.0
		4.0		0.3
32	1	2.0	0	0.0
Totals	10	18.9	5	2.2
Average weight:		1.89 ± 0.6		0.22 ± 0.5
Difference in weight:		1.67 ± 0.56; k = 5.9; P level = 99%		

For purposes of calculating the standard deviation among the rat lesions and among the corresponding mouse lesions at each date of removal, it was necessary to assign zero as the weight of the mouse lepromata if no lesion could be palpated. From the weights shown in the table it is apparent that these values did not in any case overlap, and that the difference in the weights of the lepromata produced by rat and mouse bacilli was highly significant.

ROLE OF TISSUE COMPONENTS

Experiment 2.—This experiment was a pilot one in which doses of 10^7, 10^6 and 10^5 rat bacilli were inoculated as controls into sites on the left side of four rats, while 10^7 rat bacilli suspended in rat blood cells were inoculated in three sites on the right side of the back. The lesions produced by bacilli with blood

cells were either delayed in appearance or smaller in size than the controls produced by the same dose (10^7) of bacilli alone. By the end of five months they resembled most closely, on the average, the lesions developing from 10^6 bacilli alone. By the end of eight months the lesions due to 10^7 and 10^6 bacilli alone had been removed, and those produced by 10^7 bacilli with blood cells were comparable to those produced by 10^5 bacilli alone. In short, the lesions resulting from 10^7 bacilli with blood cells developed poorly, remained approximately stationary in size, and were overtaken by those induced by 1/100th the number of bacilli alone.

EVALUATION OF HOST ADAPTATION AND EFFECT OF TISSUE COMPONENTS

The inoculation of rat and mouse bacilli into a single animal species, as in Experiment 1, is open to the objection that, although the numbers of bacilli were similar, it was not shown that the bacilli from the rat and the mouse sources were equivalent in viability and virulence. Furthermore, there remained the possibility that mouse bacilli might infect rats more successfully if unaccompanied by small cell particles and tissue extractives from the mouse. In other words, the inoculation of washed bacilli might destroy the apparently valid evidence of host adaptation.

Since Experiment 2 provided evidence that accompanying cells from the homologous host interfered with the development of lesions in rats infected with rat bacilli, it became also of interest to inquire whether an excess of heterologous cells accompanying heterologous bacilli would even more decisively handicap interspecies transmission.

Experiment 3.—Because of the foregoing considerations, this experiment was factorial in design. That is, an attempt was made to determine whether the influence of host adaptation and of the presence or absence of tissue elements could be demonstrated within one experiment which tested each of these factors under several circumstances. To that end both rats and mice were inoculated, on opposite sides of the back, with both rat and mouse bacilli. The bacilli used had then been passed four times through the respective host species in this laboratory. The $3x10^8$ bacilli inoculated into the uppermost pair of sites in each of 18 female rats and eight mice represented, respectively, 0.1 cc. of rat and of mouse bacilli from the clarified supernates of approximately 5 per cent tissue suspensions. The second pair of sites in the rats and mice received the same number of rat and mouse

bacilli with an equal volume of packed homologous blood cells as a tissue substitute. The lower pair of sites, in rats only, also received $3x10^8$ washed bacilli from the respective sources. The inoculation pattern in the rats is illustrated in Text-fig. 1, A.

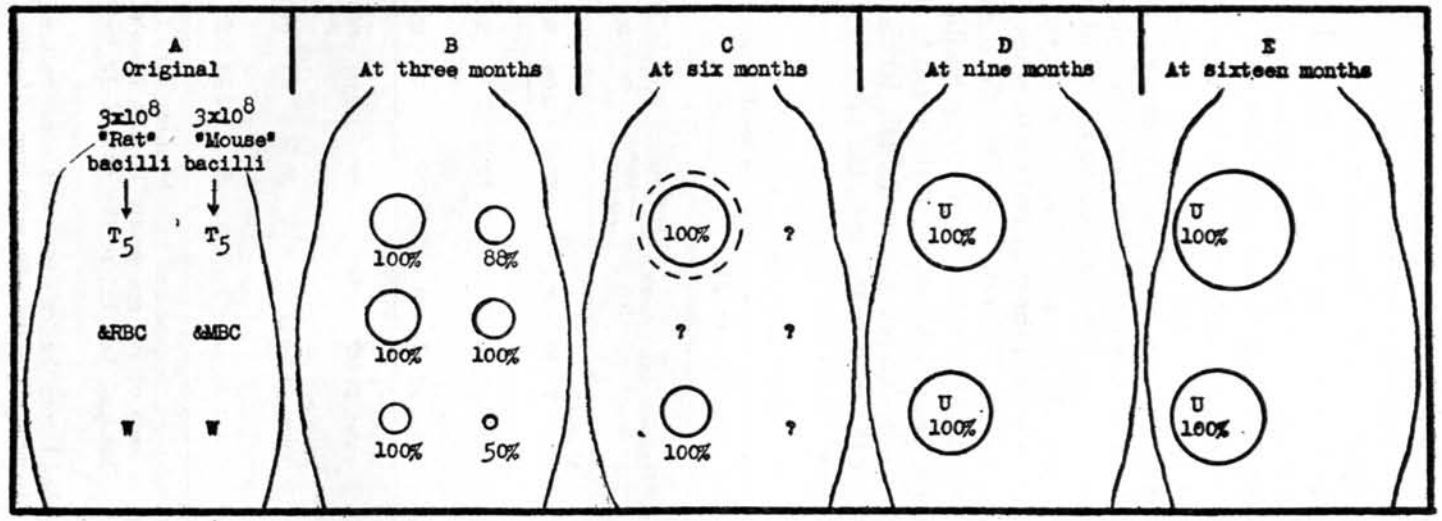
To consider first the question of host adaptation, the total number and the proportion of lesions found positive in both rats and mice after 8 and 12 weeks are summarized in Table 3 (see also Text-fig. 1, B). In rats, the rat bacilli again produced significantly more lesions than the mouse bacilli, whereas in the mice the mouse bacilli produced the higher incidence of lesions. Since only five mice with 10 sites for each source of bacilli remained after 12 weeks, the 20 per cent higher incidence of lesions due to mouse bacilli provided the only failure to reach the 95 per cent level of significance. The combined data for homologous transmission of rat bacilli to rats and of mouse bacilli to mice indicate that the evidence for adaptation of the bacili to the hosts from which they were derived attained high levels of significance, irrespective of the modifications in the preparation of the bacillary suspensions.

TABLE 3.—*Incidence of positive lesions when both rats and mice were inoculated with identical numbers of murine leprosy bacilli from rat and from mouse sources (Experiment 2).*

Weeks	Homologous infection, lesions positive	Heterologous infection, lesions positive	Difference in percentages	k	P level
8	"Rat" in rats: 35/45 (78%)[a]	"Mouse" in rats: 26/45 (58%)[a]	20	2.08	96%
	"Mouse" in mice: 11/12 (92%)[b]	"Rat" in mice: 6/12 (50%)	42	2.46	99%
	Combined data: 46/57 (81%)	Combined data: 32/57 (56%)	25	3.38	99%
12	"Rat" in rats: 35/42 (83%)	"Mouse" in rats: 27/42 (64%)	19	2.00	95%
	"Mouse" in mice: 10/10 (100%)	"Rat" in mice: 8/10 (80%)	20	1.50	87%
	Combined data: 45/52 (87%)	Combined data: 35/42 (67%)	20	2.39	98%

[a] Three rats failed to develop any lesions and have been excluded from the tabulation. Three sites in each of 15 rats equals 45 sites inoculated with each type of bacillus. One rat died before the end of 12 weeks, leaving 42 sites.

[b] Two of the eight mice died before the 8th week and another before the 12th, leaving only 12 and 10 lesions inoculated with each type of bacillus.



TEXT-FIG. 1. Incidence and size of lepromata during evolution of the infection in the eight most susceptible rats.

A. Original sites and experimental modifications. "T_5" = bacilli from clarified 5 per cent tissue suspension; "& RCB" and "& MBC" = the same suspension with rat and mouse blood cells, respectively; "W" = washed bacilli.

B. Incidence and size after 3 months. The areas of the circles are proportional to the weights (averages of estimates) of lepromata. (The incidence and weight values for these eight rats are higher than for the whole group inoculated.)

C. The broken outer circle signifies removal of the lepromata after six months.

D. Note recurrence of the lesions at the sites of those which had been removed. U = ulceration.

E. Final pattern after 16 months. No evidence of the regressed lesions could be found.

The influence of host adaptation was again substantiated by the data on the sizes of the lesions at both 8 and 12 weeks. Those produced by homologous transmission attained, after these intervals, about 5 times the average size of the lesions produced by the heterologous bacilli.

The next question is whether washing the bacilli would remove any factors to which an apparent host adaptation could be attributed. It must first be noted that after 8 and 12 weeks the washed bacilli (rat and mouse combined) had produced in the rats—the only animals inoculated with this material—only one-half as many palpable lesions as the unwashed control bacilli. Washing, therefore, was apparently unfavorable. Although the washed bacilli were slow in producing lesions, it is of great interest that the rat bacilli produced 53 per cent and the mouse bacilli only 20 per cent of positive lesions after 8 weeks; and after 12 weeks the former produced 64 per cent and the latter 29 per cent of positive lesions. At both intervals the rat bacilli provided significantly more positive lesions than the mouse bacilli. It is evident that host adaptation can be demonstrated with washed bacilli.

A further question concerns the influence of the debris from injected blood cells on homologous and heterologous transmission. In the mice, this extraneous material failed to influence either the incidence or the size of positive lesions produced by either type of bacilli after 8 and 12 weeks. These animals proved to be more susceptible than the rats, and their positive lesions tended to ulcerate very early. Since removal of lepromata was not permitted by the plan of this experiment, it was decided to dispose of the mice at the end of 14 weeks. In the rats the inclusion of blood cells lowered, but not to a significant degree, the incidence of positive lesions caused by both rat and mouse bacilli after 8 and 12 weeks. At this stage of the infection the inclusion of blood did not modify the average size of those lesions which were palpable.

The fact that the presence of debris from blood cells influenced both homologous and heterologous transmission in the same way affords further evidence that the bacilli were not handicapped in the heterologous transmissions because of tissue components which accompanied the bacilli, but that the differences measured were due to host adaptation of the bacilli themselves.

The final question is whether the presence of added tissue debris was capable of preventing persistently successful para-

sitism. It is fortunate that eight of the most susceptible rats were retained as stock for inbreeding, for by the fifth and sixth months the development and regression of certain groups of lesions had added decidedly to the significance of the experiment (see Text-fig. 1, C). Regression of lesions had occurred not only in the mouse bacillus sites on the right side of the animals, but also in the sites which had been established with rat bacilli accompanied by blood cells.

At the end of the sixth month the lepromata were removed from the upper left site in each animal. After nine months the same pattern of lesion distribution persisted (Text-fig. 1, D). Not one of the lesions caused by rat bacilli combined with blood cells could be located by palpation. These rats were retained for another seven months—i.e., until the end of 16 months after inoculation—when all the rats were sacrificed for microscopic examination. During this period the picture remained unchanged. The average weight of lesions from the upper left site was 2.8 gm., while those from the lower left site averaged 1.7 gm. In the sites of the regressed lesions it was not possible to find any traces of residual lesions or of carbon. The veins below the skin and in the subcutaneous tissues ran in their normal orderly fashion when crossing the previously positive lesion sites. From the unmistakable evidence of regression of lesions due to rat bacilli with rat blood cells, it may be seen that the presence of cell debris interfered with the successful and continued parasitism of otherwise susceptible tissues.

ROLE OF INCREASED RESISTANCE

The mice employed in the present work proved to be more susceptible than the rats. Although it has been shown that mouse-adapted bacilli are superior to bacilli from rats for the rapid production of early lesions in these mice, inoculation of the latter strain into single sites or as many as six sites in each mouse has never failed to produce lesions eventually. Such lesions have never been observed to regress. In Experiment 3 the discrimination between bacilli of rat and mouse origin appeared to be declining by the end of 14 weeks. This result might be expected if resistance did not increase at all, or not rapidly enough to interfere with readaptation of rat-passed bacilli to the mouse.

When rats have been inoculated in single sites with mouse bacilli, including the original transfer from the Fite mouse to Wistar rats, a small proportion of the rats has usually failed to develop lesions; but no palpable lesion which has developed has

ever been observed to regress. Nevertheless, with the multiple-site inoculation patterns employed in this study, there have been observed repeatedly indications that the infected rats acquire an increasingly effective resistance. Three instances of regression of mouse lesions were observed in Experiment 1, in which the larger rat lepromata were continuously being removed. Although the rats of Experiment 2, inoculated with rat bacilli and blood cells, were unable to prevent the development of lesions in such sites, they obviously acquired during the course of the infection a means of holding in check the further development of those lesions containing blood cell debris. In Experiment 3 the mouse bacilli had established one or more lesions in each of the eight most susceptible rats by the end of three months, but by the end of six months all had regressed (Text-fig. 1, C). Persistent local lesions were produced under only two of the six circumstances in this experiment, namely (*a*) with bacilli of homologous origin and (*b*) with these bacilli used directly from clarified supernates of leproma suspension or washed free of tissue components.

DISCUSSION

Before discussing the possible bearing of the factors here studied on the experimental transmission of human leprosy to animals or to other humans, it is necessary to consider certain advantages and limitations of the conditions under which the observations were made. It is not certain that a similar discrimination between these factors could have been achieved by inoculating single sites in the large numbers of rats or mice which would have been required in each of 18 or more experimental groups. The attempt to rule out the influence of varying resistance by inoculating two or more pairs of sites in each animal provided a fortunate experimental design, since the immunization which evidently resulted from the introduction of large numbers of bacilli into multiple sites tended to inhibit competitively those lesions in which the bacilli were for any reason at a disadvantage. Although this method proved to be admirably suited for the immediate purpose, the observations are subject to the limitation that they were made under conditions quite different from those which exist in natural infection.

Much of the work on experimental transmission of leprosy has involved attempts to modify animal resistance, which implies that the difficulty lies in the natural resistance of the species to which transfer is attempted. Following only three serial

passages of the murine bacilli through rats and through mice, it was found that the microorganisms were handicapped when transferred in large numbers to the alternate susceptible species. It would appear that the factor of prolonged host adaptation may be an important impediment to the transmission of human leprosy to animals. Such adaptation may also be one of the reasons why the mycobacteria of human and murine leprosy are so strictly limited to their respective host species under natural endemic conditions.

The genetic differences between rats and mice are too great to permit suggesting that adaptation of the bacilli to different strains of mice or rats might influence the ability of the organisms to effect cross transmission within a single species. Remarkable genetic differences, however, do exist between different families of inbred mice with respect to their acceptance of transmissible tumors, and their susceptibility to tubercle bacilli (4). It is not impossible that passage of murine bacilli through appropriately selected mouse families would permit demonstration of family adaptation, and that by such means one could obtain a clue that the familial tendency in human leprosy involves among other things an adaptation which handicaps the microorganisms when they are transmitted to persons of materially different genetic constitution.

The role of excessive amounts of tissue derived from the respective hosts of origin was investigated on the physiological premise that if large amounts of tissue were to accompany the inoculated bacilli there should occur in such lesion sites larger numbers of phagocytic cells and more persistent population of histiocytes than in lesions where no cell detritus existed. Furthermore, a large population of mobile cells should provide a higher ratio of cells to bacilli, fewer bacilli should be captured by each phagocytic cell, and there should be a lower probability that the bacilli could find circumstances compatible with multiplication. Although the inclusion of packed blood cells with the bacilli failed to prevent the occurrence of positive lesions in rats and mice after 8 to 12 weeks, it must be borne in mind that the palpation of lesions or the weighing of lepromata affords no information concerning the numbers of viable bacilli present, or concerning the proportions of the mass which are due to cells and to bacilli. The positive lepromin response to killed bacilli in humans, and the "nodules" which result from the inoculation of large numbers of leprosy bacilli into animals without inciting progressive lesions, serve to illustrate the inadequacy of methods

which do not measure directly the viability of the bacilli or the increase or decrease in their numbers. The essential fact is that lesions containing cell detritus, though established by rat bacilli, disappeared in each of the 8 most susceptible rats among 18, while the smaller and more latent lesions produced by washed rat bacilli continued to develop in spite of increasing resistance of the animals.

It is well known that the many attempts to transmit human leprosy to noninfected persons by injection of crude leproma suspensions have failed, whereas the surgical accident (2), the failure to sterilize a needle (1), and tattooing (5) have resulted in the establishment of local leprous lesions. Rogers and Muir (6) cite other examples of accidental transmission by means which appear unlikely to transfer large amounts of tissue. Certainly one of the outstanding differences between the experimental attempts and the accidental transmission has been the smaller numbers of bacilli introduced in the latter instances, and particularly the absence of large amounts of accompanying tissue components. On the basis of the present evidence it is suggested that the use of crude tissue suspensions has not been favorable to experimental transmission of human leprous infection.

Prior to the present experiments I had assumed that the success or failure of local leprous infection was not likely to be modified by the presence or absence of lesions elsewhere. The remarkable fact that, in rats infected in multiple sites with large numbers of bacilli, there may be regression of palpable lesions under the circumstances described further supports the suggestion (see also 7) that the transfer of large numbers of bacilli is not necessarily a favorable factor as regards transmission, under circumstances where host resistance may be stimulated. In this connection, it may be noted that Meikeljohn (3) observed that very large doses of encephalomyelitis virus were capable of immunizing horses prior to the production of recognizable infection, while smaller doses produced clinical disease.

Fortunately, there exists with respect to humans considerable information which shows that one or more injections of lepromin may transform lepromin negative to lepromin positive reactors. Since human lepromata tend to contain on the average the general order of 2½ billion bacilli per gram of nodule, it is apparent that the 0.1 cc. of 5 per cent tissue suspension employed as lepromin may provide perhaps 10 million heat-killed bacilli, and that this number of bacilli in a single dose is capable of altering the response of certain individuals. The use of 1 cc. of

fresh 10 per cent leproma suspension in attempted transmission would provide 20 times the number of bacilli in the lepromin dose, and would probably excite immunological stimulation in an even greater proportion of the recipients.

SUMMARY AND CONCLUSIONS

The foregoing experiments reveal that at least three factors influence the success or failure of experimental transmission of murine leprosy. These factors are: (*a*) the influence of host adaptation, (*b*) the adverse effect of an added tissue component, and (*c*) the role of the resistance which, under certain circumstances, may be acquired by the animals during the course of infection.

Following three serial passages of the Hawaiian strain of leprosy bacillus (obtained in a mouse) through a subfamily of Swiss mice and, separately, through a subfamily of Wistar rats, it was found that the bacilli from either source were handicapped when transmitted to the heterologous species.

When the inoculated bacilli were accompanied with blood cells as a tissue substitute, the lesions produced were delayed and failed to develop normally. They eventually were equalled or surpassed by lesions established with 1/100th or 1/10th the same number of bacilli without added cellular material. Under other circumstances the inclusion of blood cells was shown to contribute to the regression of established lesions.

Infected rats can acquire a remarkable degree of resistance during the course of an infection established by the inoculation of large numbers of bacilli into multiple cutaneous sites. Under certain circumstances this resistance is sufficient to cause regresion of well-established lesions when the bacilli are handicapped by the two factors mentioned above.

The implications of these findings in connection with transmission of human leprosy to animals and to other humans have been discussed. It would appear that such transmission would be more likely to succeed with bacilli which had been freed of excess tissue debris, by the inoculation of only one site per recipient, and by employing moderate rather than large doses of bacilli.

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