SURVIVAL TIME AS A MEAN TO ESTIMATE THE RATE OF EVOLUTION OF MOUSE LEPROSY[1, 2]

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Early studies led to reports that the mouse is not susceptible to infection with *M. lepraemurium* ([16]), but more recent investigations have established the fact that this animal species develops progressive and lethal disease when inoculated with a suitable dose of this mycobacterium ([13, 17, 18, 23, 24, 25]). The mouse has been considered more susceptible than the rat ([12]).

Since the mouse has proved very useful for experimental studies on the chemotherapy of murine leprosy the development and evolution of localized lesions induced by *M. lepraemurium* have been investigated in this animal. The development and evolution of corneal lesions have been followed throughout their course in several investigations ([19, 20]). The rate of growth of some lesions could be measured indirectly, either through the increase in number of bacilli within a single organ, such as the spleen, liver, testis or peritoneum ([1, 2, 14, 15]), or through the increase in weight of one organ, such as the spleen ([26]).

Several features in the pathogenesis of mouse leprosy, however, have been overlooked. The rate of evolution of the disease, from the time of inoculation until the death of the inoculated animal, has not yet been investigated properly. These features should be considered as important with reference at least to assays of experimental chemotherapy. The main purpose of this paper is to investigate some features of mouse leprosy, such as its rate of evolution and the survival rate of mice inoculated intraperitoneally with different doses of *M. lepraemurium*. Similarities and differences between mouse and rat leprosy will be indicated.

MATERIALS AND METHODS

Mice and rats of both sexes, weighing respectively 22-25 gm. and 100-200 gm., were inoculated intraperitoneally with *M. lepraemurium*. The inoculum used was a bacillus suspension freed from tissue particles by the aid of a slight modification of Hanks' technic ([11]). In order to estimate the number of bacilli in 1 ml. of inoculum, a sample of the latter was weighed after it had been dried to constant weight.

Variations were tested, both in the size of inoculum and the source of bacilli, in order to obtain results in addition to those related to the rate of evolution of the disease.

Size of inoculum.—The relationship between dose of inoculum and rate of evolution of mouse leprosy was studied by comparing the results from three different doses of inoculum. Three groups of mice, composed of 30 animals in each group, were inoculated with doses respectively of 0.40, 0.80 and 1.60 mgm. These doses were

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derived from different volumes of the same bacterial suspension. The mycobacteria used were taken from lesions of a rat inoculated 6 months previously. One group of 30 rats inoculated with 1.60 mgm. of *M. lepraemurium* from the same source, served as a control.

Source of inoculum.—In order to investigate the possibility that *M. lepraemurium,* on becoming adapted to the mouse organism, would be able to influence the rate of evolution of mouse leprosy, two sources of bacilli were used, viz., bacilli taken from rat lesions and bacilli recovered from mouse lesions. The means of survival time of groups of mice inoculated with these two kinds of inoculum were compared.

Three groups of 25 mice each were inoculated intraperitoneally with 1.60 mgm. of bacilli obtained as follows: (1) bacilli taken from lesions of a rat inoculated six months previously with *M. lepraemurium* maintained by rat passage for 15 years; (2) bacilli recovered from a mouse of group 1 after the first passage; and (3) bacilli recovered on second passage from a mouse of group 2.

The mice were compared also with a group of 30 rats inoculated intraperitoneally with 1.60 mgm. of bacilli from rat lesions.

Of the animals from each experimental group of mice and rats, 15 to 20 were kept until death. They supplied data for survival studies based on the mean of survival. The comparison among groups was made by analysis of variance among the means. Of the remaining 10 animals of each group, 2 were killed every 30 days: these provided material for study of the development and evolution of murine leprosy lesions. Additional information in these respects was obtained from necropsies on animals dying from disease during the course of the experiment.

The development and evolution of murine leprosy lesions were estimated roughly on the basis of macroscopic and histologic study of specimens of spleen, liver, lymph nodes, omentum and lung. Pieces of these organs were fixed in formalin, embedded in paraffin, sectioned, and stained with the hematoxylin-eosin and Ziehl-Neelsen staining procedures.

RESULTS

Evolution of mouse leprosy lesions.—Thirty days after inoculation mouse leprosy lesions were found only in the peritoneum and peritracheal lymph nodes, where commonly they reached microscopic size only. The histologic picture permitted their identification as active or developing lesions according to criteria used previously in the classification of rat leprosy lesions ([4, 5]).

Both peritoneal and lymph node lesions undergo regressive histologic changes 60 to 90 days after inoculation. Such changes indicate that although some lesions regress and wane most remain stationary. The proportion of regressive lesions depends on the size of the inoculum; it is greater in those animals inoculated with the smaller doses of bacilli. The liver, spleen and lung lesions, which were few and small, displayed similar changes.

On the basis of the histologic picture this period of evolution of mouse leprosy could be regarded as a stationary one. It characterizes the resting phase in the evolution of the lesion. Throughout this phase there was suggestive bacterioscopic evidence that the inoculated *M. lepraemurium* was not growing. It could be inferred from histologic study that the number of bacilli in the lesions remained constant or decreased slightly.

The length of the resting phase was related closely to the size of

the inoculum. In mice inoculated with 0.4 mgm. of bacilli it lasted up to 150 days, while in animals receiving 0.8 and 1.6 mgm. of mycobacteria lesions in the resting phase were found only up to 120 and 90 days respectively.

Comparison between mouse and rat lesions showed the resting phase to be more striking and longer lasting in the former. In rats, as a rule, the resting phase lasted less than 90 days and whereas some lesions remained stationary, there was some evidence of slow development in others. Regressive lesions were never found.

In both animal species the resting phase is followed by a rapidly evolving one in which lesions increase steadily in size, and bacilli within the lesions become numerous. Histologic study suggests that the bacilli multiply rapidly. Lesions of the omentum and lymph nodes, as well as those of liver, lungs and spleen, soon reach macroscopic size in both the mouse and the rat. Histologically there was no evidence suggesting that the size of the inoculum influenced the rate of evolution of the phase of rapid development in both mouse and rat lesions.

Rate of evolution of mouse leprosy.—The mean survival time of mice inoculated with *M. lepraemurium* does not differ significantly from that of rats inoculated with a similar dose of bacilli (Table 1). This result is surprising since the resting phase is longer in mice than in rats, and a slower rate of evolution of the disease might therefore be expected in the former animal species.

TABLE 1.—*Means of survival time in mice and rats inoculated intraperitoneally with M. lepraemurium. Influence of the size of inoculum on the rate of evolution of mouse leprosy.*

Mice	Size of inoculum in mgm.		
	0.40	0.80	1.60
No. of animals	15	15	15
Mean of survival (in days)	258.2 ± 6.5	227.8 ± 2.0	195.0 ± 1.5
Rats			
No. of animals	—	—	20
Mean of survival (in days)	—	—	218.5 ± 1.5

Table 1 shows in addition an appreciable difference among the means of survival, resulting from variations in the size of inoculum. The differences, which depend upon the regression of the size of inoculum on the mean of survival time, are statistically significant ($F<0.05$). Statistical analysis of this regression (based on the logarithm of the doses) indicates that it is linear (Table 2).

The linear regression of the mean of survival time on the dose of bacilli permits biologic dosage of the inoculum, in both mice and rats (9). On the other hand, the results indicate that mice inoculated intraperitoneally with *M. lepraemurium* in ranges between 1 and 2

mgm. develop a disease with a predictable rate of evolution. In this case the mean of survival time reaches approximately 200 days.

On the basis of the linear regression of the mean of survival time on the dose of inoculum, it was possible to estimate the generation time for *M. lepraemurium* whenever this mycobacterium was inoculated in mice. Generation time was estimated as follows: The difference in effect between 0.4 mgm. and 1.6 mgm., respectively, which represents two generations, is 258.2 — 195.0 or 63.2 days. The generation time for *M. lepraemurium* may be calculated as half of this figure, or 31.6 ± 1.5 days.

Influence of the source of inoculum.—The source of the inoculum in these studies was not found to play any role in the evolution of mouse leprosy. The results shown in Table 3 show only insignificant differences among the means of survival time among mice inoculated with bacilli from either rats or mice. There does not seem to be any evidence supporting the belief that *M. lepraemurium* could be adapted to the mouse organism.

In addition, no relation was found between the length of the resting phase in mouse lesions and the source of the inoculum. Mice inoculated with bacilli recovered from mice did not differ, as far as the length of the resting phase was concerned, from mice injected with mycobacteria obtained from rats.

TABLE 2.—*Regression of means of survival time on the size of inoculum. Mice inoculated intraperitoneally with three different doses of M. lepraemurium.*

Size of inoculum	No. of animals	Mean of survival time (in days)	
		Obtained values	Theoretical values
1(0.4 mgm)	20	258.2	263.6
2(0.8 mgm)	20	227.8	227.0
3(1.6 mgm)	20	195.0	190.4

Regression coefficient: b = −36.6

TABLE 3.—*Influence of the source of inoculum on the survival time of mice inoculated intraperitoneally with 1.60 mgm. of bacilli.*

	Bacilli from rats	Bacilli from mice	
		First pass	Second pass
No. of animals	15	20	20
Mean of survival (in days)	195 ± 1.5	203.1 ± 3.2	189.4 ± 2.2

DISCUSSION

The evolution of mouse leprosy appears to be uniform, like that of rat leprosy ([7]). However, some differences in evolution are evident when the lesions in the two diseases are compared. Throughout their evolution mouse leprosy lesions display two distinct (resting and rapidly evolving) phases, which cannot be distinguished so clearly in the lesions of rat leprosy.

It has been reported that rats inoculated intraperitoneally show lesions that are stationary until the 4th or 5th week after inoculation, and that after the 6th week ([3]) rapidly evolving lesions appear. Our results, however, show that some stationary lesions may be found up to 3 months after inoculation in rats. The resting phase is still longer in the mouse leprosy lesions, which in this respect may be considered as similar to leprosy lesions in the golden hamster. In the latter animal, and the mouse as well, the lesions show a distinct and long initial phase ([8]).

The uniform course of mouse leprosy may be influenced by the size of the inoculum. Small doses favored individual variations in animal survival. If a suitable dose of mycobacteria was inoculated, however, the standard error of the mean survival time was similar for mice and rats. For this reason these animal species serve equally well in providing data for survival studies.

There is some evidence that the resting phase of the lesions of mouse leprosy can vary in length in different organs of the body. After injections into the cornea in mice rapidly evolving lesions are apparent 6 weeks after inoculation ([22]). The contrast between this finding and that in our experience in mice inoculated intraperitoneally, indicates that local conditions may have a conspicuous effect on the length of the resting phase in lesions.

The resting phase appears to depend upon host conditions rather than mycobacterial factors. This statement is borne out by the effect of radiation on murine leprosy. Mice and rats subjected to radiation show a decrease in the length of the resting phase ([10]). However, variations in inoculum size can also modify the length of the resting phase in mice inoculated intraperitoneally, a fact in agreement with observations on mice injected in the cornea ([2]). These results indicated that either host factors or inoculum size may influence the onset of rapidly evolving lesions.

When the length of the resting phase is taken into account, the mean of survival time after inoculation should be longer in mice than in rats. A great similarity is seen, however, between the means of survival time when the two animal species are compared. This fact is due probably to the shorter generation time for *M. lepraemurium* from the mouse. The generation time for this mycobacterium was estimated as 31.6 ± 1.5 days in bacilli from the mouse and 55.1 ± 1.4 days in bacilli from the rat ([9]) when the same method was used. The faster rate of growth of the bacilli would compensate for the longer resting phase of the mouse lesions.

The length of the resting phase of the lesions of mouse leprosy must be taken into account in the design of experimental studies on chemotherapy in mouse leprosy. The length of treatment cannot be shorter than the resting phase.

The relationship between the size of the inoculum and the mean

of survival time in mice inoculated with *M. lepraemurium* indicates that the rate of evolution of mouse leprosy is a linear function of the injected dose of bacilli. In this connection, however, it is more nearly correct to consider the mean of survival time as a linear function of the product of the dose of inoculum and the proportion of viable bacilli. This fact must be kept in mind when the results from inoculation of different suspensions of bacilli are compared.

The mean generation time for *M. lepraemurium* from mice inoculated intraperitoneally, estimated on the basis of a correlation of inoculum size and mean of survival time, differs strikingly from values obtained with the aid of other methods. From determination of the number of bacilli in lesions in the testis, made in successive periods in their evolution in animals inoculated intratesticularly, Hilson and Elek ([14]) estimated the mean generation time for *M. lepraemurium* as 7—8 days for rats and 10 days for mice. By a similar method the mean generation time for this mycobacterium was estimated as 20 days in mice injected in the cornea ([2]), 12.6 days in mice injected intravenously ([21]), and 10.7 days in mice injected intraperitoneally ([1]). In spite of the large differences among the results provided by the bacillary content of leprosy lesions they differ markedly from the ones reported in this paper. Account must be taken of the fact that our result was inferred from a linear regression of means of survival time observed in 3 groups of 20 animals each. On the other hand it was not subject to errors due to failure of technics for counting bacilli.

Some disagreement among the means for generation time may be due to variation in the site of inoculation. The rate at which *M. lepraemurium* multiplies may depend on the organ where it was injected. This view is supported by results reported by Chang ([1]), who observed that the regression of the number of bacilli on time, although always linear, showed a slope varying from one organ to another, a fact indicating that local conditions can influence the bacilliary growth rate.

Some experimental results suggest that the mean for generation time for *M. lepraemurium* could be greater when the bacillus is injected intraperitoneally. Chang ([1, 2]) has observed that the maximum length of individual *M. lepraemurium* in mice inoculated intraperitoneally, is reached 5 to 7 weeks after the injection. As the maximum elongation of bacilli is known to precede cellular division, Chang's result ([1, 2]) affords good support for our report of the mean generation time of *M. lepraemurium*.

SUMMARY

The rate of evolution of mouse leprosy and the survival time of mice inoculated intraperitoneally with different doses of *M. lepraemurium* were studied in comparison with corresponding features in rat leprosy.

A resting phase, followed by a rapidly evolving phase, could be observed readily in the lesions of mouse leprosy. Evidence in several respects supported the view that *M. lepraemurium* does not grow during the resting phase.

The rates of evolution of mouse and rat leprosy are similar, in spite of the fact that the resting phase is longer in mice.

The evolution of mouse leprosy is uniform and well defined, but is influenced by the size of the inoculum used. There is a close connection between the logarithm of the dose of inoculum and the mean survival time in mice inoculated with *M. lepraemurium*. Statistical analysis of this correlation shows that it is a linear regression.

On the basis of this linear regression the generation time for *M. lepraemurium* in mice was estimated as 31.6 ± 1.5 days. This time is shorter than that observed in rats in studies by the same method.

As far as mean survival time is concerned, *M. lepraemurium* taken from rats and inoculated in mice showed no difference from bacilli taken from mice and inoculated in other mice. This result indicates that this mycobacterium does not become adapted to the mouse organism.

RESUMEN

Fueron estudiados el grado de evolución de la lepra del ratón y el tiempo de sobrevida de los ratones inoculados intraperitonealmente con diferentes dosis de *M. lepraemurium*, en comparación con las correspondientes figuras en la lepra de la rata.

Una faz de reposo, seguida por una rápida faz evolutiva puede ser observada fácilmente en las lesiones de la lepra de los ratones. Evidencias en varios aspectos, sostienen el punto de vista de que el *M. lepraemurium* no crece durante la faz de reposo.

Los grados de evolución de la lepra de los ratones y de las ratas son similares, a pesar del hecho de que la faz de reposo es mas larga en los ratones.

La evolución de la lepra del raton es uniforme y bien definida, pero es influída por la dimensión del inoculante usado. Existe una estrecha conexion entre el logaritmo de la dosis de lo inoculado con el término medio de sobrevida en los ratones inoculados con *M. lepraemurium*. El analisis estadístico de esta correlación muestra que es una regresión linear.

Sobre la base de esta regresión linear, el tiempo de generación para el *M. lepraemurium* en los ratones fué estimado como 31.6 ± 1.5 dias. Este tiempo es mas corto que el observado en ratas estudiadas con el mismo método.

En lo que concierne al tiempo medio de sobrevida, los *M. lepraemurium* tomados de las ratas e inoculados en ratones no mostraron diferencias con los bacilos tomados de ratones e inoculados en otros ratones. Este resultado indica que esta micobacteria no se adapta al organismo del ratón.

RESUMÉ

La vitesse d'évolution de lèpre de la souris et la temps de survie de souris inoculées intrá-péritonéalement avec différentes doses de *M. lepraemurium* ont été étudiés et comparés aux observations correspondantes faites dans la lèpre du rat.

Une phase de repos, suivie d'une phase d'évolution rapide, a pu être aisément observée dans les lésions de la lèpre de la souris. D'évidences convergentes il ressort que *M. lepraemurium* ne se multiplie pas durant la phase de repos.

La rapidité d'évolution de lèpre chez le rat et chez la souris est similaire, malgré que la phase de repos soit plus prolongée chez la souris. L'évolution de la

lèpre chez la souris est uniforme et bien défine, mais est influencée par la quantité inoculée. Il existe une relation étroite entre le logarithme de la dose inoculée et la moyenne du temps de survie chez les souris inoculées avec *M. lepraemurium*. L'analyse statistique de cette corrélation montre qu'il s'agit d'une régression linéaire.

Sur la base de la regression linéaire, le temps de génération de *M. lepraemurium* chez les souris a été estimé à 31.6 ± 1.5 jours. Ce temps est plus court que celui observé chez des rats dans des études menées par la même méthode.

En ce qui concerne la durée de survie moyenne, des bacilles *M. lepraemurium* obtenus chez des rats et inoculés à des souris ne se sont pas comportés d' une façon differente de bacilles obtenus chez de souris et inoculés à d'autres souris. Ce résultat indique que cette espèce de mycobactéries ne subit pas d'adaptation dans l'organisme de la souris.

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