EFFECT OF IONIZING RADIATIONS ON THE RATE OF EVOLUTION OF MURINE LEPROSY[1, 2]

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It has been assumed that the immunologic defense system is depressed as a result of total body irradiation ([1, 8, 12]). Whole body radiation inhibits the synthesis of antibodies, if carried out before the inoculation of the antigen. The inhibitor effect of ionizing radiations on antibody production has led to the belief that infective diseases could be modified as far as their evolution is concerned, if the host were previously subjected to radiation.

On the basis of these statements ionizing radiations have been used in experimental murine leprosy. Takayama ([11]) exposed rats to 150 or 500 r of x-radiation and afterward inoculated them with *M. lepraemurium;* according to his results such an experimental condition would influence the onset of murine leprosy lesions. Kelkar and Ranadive [9] exposed golden hamsters to 100-200 r of x-radiation with the purpose of producing a suitable condition favoring *M. leprae* growth.

On the other hand, normal mice subjected to ionizing radiations show a life shortening ([10, 13, 14]). The decrease of life span seemed to depend upon premature onset of diseases which frequently occur during mice senescence, since the effect cannot be attributed to any other special cause.

These results suggest that murine leprosy, especially its rate of evolution, could be influenced by the effect of such radiations. With the purpose of studying the eventual effect of radiations on murine leprosy, rats and mice previously subjected to ionizing radiations were inoculated with *M. lepraemurium* and the evolution of the disease was studied until death of the animals.

MATERIALS AND METHODS

Mice and rats of both sexes were used in this experiment. The mice, belonging to the C strain, were 7 weeks old at the beginning of the experiment. The rats (Wistar strain) were 6-7 weeks old.

The animals were exposed to beta-gamma radiations from a cesium-barium bomb for 137 minutes. Every animal (mouse or rat) was exposed to 500 r, only once, at the beginning of the experiment. To accomplish the radiation the animals were caged in special compartments arranged as a circle around the source of radiation, in such a way that each animal was kept at the same distance from it (28.7 cm.). The rats were placed

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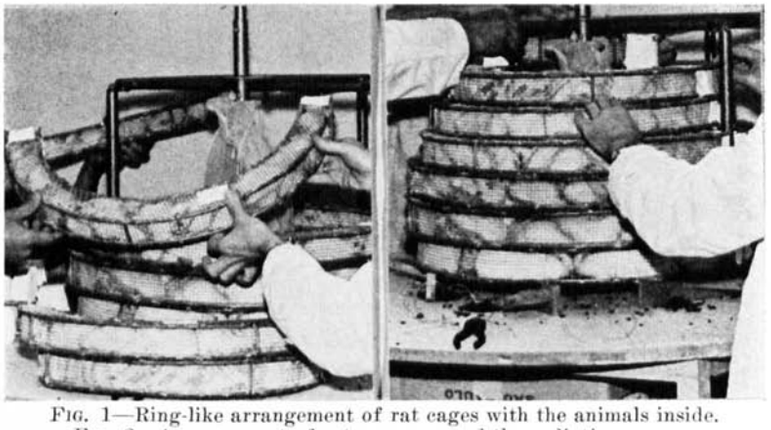

FIG. 1—Ring-like arrangement of rat cages with the animals inside.
FIG. 2—Arrangement of rat cages around the radiation source.

in ring-like cages (Figs. 1 and 2), with an 0.4 x 0.4 mm. mesh wire network. The mice were placed in plastic tubes with perforated walls (3.7 cm. in diameter).

The experimental animals were set in groups as indicated in Table 1.

TABLE 1.—*Grouping of animals with respect to radiation and treatment.*

Group	Animal species	No. of animals	Total radiation	Dose of *M. leprae murium*	DDS treatment
1	mice	30	500 r	1.43 mgm.	—
2	mice	30	500 r	0.35 mgm.	—
3	mice	30	500 r	0.175 mgm.	—
4	mice	30	500 r	(control)	—
5	mice	30	(control)	1.43 mgm.	—
6	rats	30	500 r	1.68 mgm.	—
7	rats	30	500 r	0.42 mgm.	—
8	rats	30	500 r	0.21 mgm.	—
9	rats	30	500 r	(control)	—
10	rats	30	(control)	1.68 mgm.	—
11	rats	30	500 r	1.68 mgm.	40 mgm./day
12	rats	30	(control)	1.68 mgm.	40 mgm./day

The inoculation of *M. lepraemurium* was performed intraperitoneally one day after the radiation. The animals received different amounts of the same suspensions of bacilli, whose concentration (in mgm. of bacilli/ml.) had been previously estimated through a technic previously described ([3]). This suspension was free from tissue particles and bacteriologically sterile.

The animals of groups 11 and 12 received 4,4'-diaminodiphenylsulfone (DDS) treatment, which was started 7 days after mycobacterial inoculation and carried out orally by mixing the DDS with the food. The DDS treatment was maintained unchanged throughout the experiment.

Every 30 days, until the 300th day after *M. lepraemurium* inoculation, one animal of each experimental group was killed by ether inhalation, to provide material for pathologic studies throughout the leprosy evolution. Pieces of liver, spleen, lymph nodes, omentum and lungs, were taken out, fixed in 10 per cent formalin solution, embedded in paraffin and stained by hematoxylin and eosin and Ziehl-Neelsen technics.

The animals of every group that died throughout the experiment were used for histologic purposes. However, mainly, these animals provided data for the study of

survival, which was taken as a mean in estimating the rate of murine leprosy evolution. The estimation was made through the value of the mean of survival. The comparison among the means was carried on through the analysis of variance.

RESULTS

The animals of groups 4 and 9 (exposed to radiation but not inoculated with *M. lepraemurium*, i.e., controls) showed at the 30th day after radiation some atrophy of the lymphoid organs and a few associated changes in their histologic structure; the other organs did not present microscopic alterations. The lymphoid tissue atrophy regressed progressively, in such a way that 60 days after radiation the histologic structure of these organs did not differ from the normal. These results indicate that radiations in the dose used produced only reversible and minor alterations of lymphoid organs.

The effects of radiation on the evolution of murine leprosy lesions were determined by comparison of radiated and nonradiated animals inoculated with equal doses of mycobacteria. Variations in the inoculum size supplied further information on the influence of the dose of bacilli on the rate of evolution of leprosy lesions.

Histologic study:—Nonirradiated mice and rats (groups 5 and 10) inoculated with a dose of *M. lepraemurium* suitable for the development of murine leprosy ([5]), showed lesions that evolved through an initial resting phase, where they progressed slowly, or remained stationary, and contained few bacilli. After this period the lesions evolved rapidly and showed progressively larger numbers of bacilli (latter or fast-evolving phase).

In determining the effect of radiation on murine leprosy lesions account must be taken of both the length of the resting phase and the rate of evolution of the latter phase. In the resting phase, the effect of radiation on mouse leprosy lesions could not be detected during the first 60 days at least following the mycobacterial inoculation. Later, however, the effect became manifest. Some rapidly evolving leprosy lesions could be found as soon as 90 days after the inoculation, at a time when nonirradiated control mice always showed resting lesions. This finding suggests that as a consequence of radiation the resting phase of mouse leprosy lesions is shortened.

A similar effect of radiation could be detected in rats inoculated with *M. lepraemurium*. In this animal species the differences between radiated and nonradiated groups appeared more consistent. In the former the resting phase often lasted less than 60 days and some lesions became of the fast-evolving type 30 days after inoculation. Many active fast-evolving lesions could be seen 60 days after inoculation in radiated rats, whereas nonradiated animals only occasionally showed active lesions at this time and these were few. As a consequence, in the radiated rats, 90 days after inoculation, the lesions became larger and more numerous and contained a greater amount of bacilli, than in nonradiated controls. The differences later became steadily more conspicuous and remained so until the animals died.

The results of histologic study indicated a striking increase in the rate of evolution of murine leprosy lesions as an effect of radiation. This effect seemed more pronounced in rats than in mice.

On the other hand, by comparing the size and the number of murine leprosy lesions, as well as the number of bacilli in each lesion, in animals inoculated with different doses of *M. lepraemurium,* it was possible to detect a relationship between the inoculated dose of mycobacteria and the rate of lesion evolution. This relationship, already demonstrated in nonradiated animals ([5]), is found also in radiated ones. Both in mice and rats previously radiated and inoculated with different doses of *M. lepraemurium,* the rate of evolution of the murine leprosy lesions is proportional in some measure to the inoculum size. The lesions appeared slightly larger and more numerous in animals injected with the larger doses of bacilli, even 30 days after inoculation. This difference becomes more readily apparent as the disease evolution progresses.

Survival study.—In studies on the effect of radiation on the survival time of animals inoculated with *M. lepraemurium,* in which radiated animals were compared with nonradiated ones, it was found that this procedure shortened the life span. This determination was based on differences observed among the means of survival-time and could be detected easily in both mice and rats (Table 2). The differences observed were statistically significant ($F < 0.05$) and indicated that in animals receiving equal doses of mycobacteria the disease evolved faster in those that had been subjected to radiation.

The increasing rate of evolution of murine leprosy noted as a consequence of radiation, reached the same level in both rats and mice. In the former species the rate of evolution increased 15.3 per cent and in the latter 17.6 per cent. This similarity is not in accord with the data provided by the histologic study, which suggest a more pro-

TABLE 2.—*Means of survival time of animals exposed to radiation and inoculated with different doses of* M. lepraemurium. *Comparison with nonradiated controls.*

	Nonradiated rats (controls)	Radiated rats		
Dose of inoculum in mgm.	1.68	1.68	0.42	0.21
Mean of survival time in days	218.5 ± 3.3	185.0 ± 4.2	261.3 ± 4.8	261.9 ± 4.4
	Nonradiated mice (controls)	Radiated mice		
Dose of inoculum in mgm.	1.40	1.40	0.35	0.175
Mean of survival time in days	185.2 ± 10.6	156.5 ± 9.5	184.1 ± 7.3	192.9 ± 11.7

TABLE 3.—*Regression in means of survival time in relation to size of inoculum in mice inoculated intraperitoneally with three different doses of* M. lepraemurium.

Size of inoculum in mgm.	No. of animals	Mean survival times in days	
		Obtained values	Theoretical values
1 (0.175)	20	192.9	190.2
2 (0.350)	20	184.1	176.5
4 (1.400)	20	156.5	162.8

Regression coefficient: b = −13.7

nounced effect of radiation on rat than on mouse lesions. This disagreement will be discussed later.

There is a close correlation between the dose of inoculum and the mean survival time of animals subjected to radiation and afterward inoculated with *M. lepraemurium*. This correlation is similar to one found in nonradiated animals ([5]). In each instance the regression of the logarithm of inoculum size in relation to the mean animal survival time (Table 3) is linear, as far as the mouse is concerned. However, in radiated rats this regression displays such large deviation from linearity that it cannot be considered as linear. There are striking reasons for belief that the smaller dose of bacilli used to inoculate rats was too small, lying outside the straight-line limits of the dose-survival regression line. In such way, the correlation between dose of inoculum and mean survival time could not be considered as suitably traced in our radiated rats (Table 4).

TABLE 4.—*Regression in means of survival time in relation to size of inoculum in rats inoculated intraperitoneally with three different doses of* M. lepraemurium.

Size of inoculum in mgm.	No. of animals	Mean survival times in days
1 (0.21)	20	261.9
2 (0.42)	20	261.3
4 (1.68)	20	185.0

On the basis of the linear regression between the means of survival time and doses of inoculum, the generation time average for *M. lepraemurium* from mice inoculated intraperitoneally could be estimated in radiated animals. The procedure used to estimate this value was identical with that reported in another paper ([5]) concerned with nonradiated rats. From the mean survival time for the smallest dose of inoculum (192.9 days) the mean of survival-time for the largest dose (156.5 days) was subtracted. This difference (36.4 days) results as a consequence of variation in inoculum size (see Table 3). The variation corresponds to three mycobacterial generations. The mean of generation time, therefore, is 12.1 days. By comparing this result with one obtained from nonradiated mice (Table 5), when the same method was used,[6] the effect of radiation on the bacillary generation time can be inferred. A striking shortening of generation time is apparent as an effect of radiation.

In the case of the radiated rats the effect of regression of inoculum size on the mean of survival time is not linear for the doses used. This lack of linearity makes it difficult to estimate the generation time for *M. lepraemurium* in this animal species. As an approach on the supposition that the two larger doses of inoculum would be confined within the straight limits of the regression line, the generation time from radiated and introperitoneally inoculated rats was estimated as 38.5 days. In rats also radiation seems to affect the generation time for *M. lepraemurium* (Table 5).

TABLE 5.—*Means of generation time for* M. lepraemurium *from normal and radiated mice and rats. Results obtained by the same method.*

Mice		Rats	
Radiated	Nonradiated	Radiated	Nonradiated
12.1 ± 2.6 days	31.6 ± 1.5 [a] days	38.5 ± 2.1 [b] days	55.1 ± 4.2 [c] days

[a] Hadler and Ziti (6).
[b] This result must be considered as an approach.
[c] Hadler and Ziti (5).

These results indicate that in mice, and perhaps in rats, exposure to radiation prior to inoculation induced great changes in the generation time of *M. lepraemurium*. The generation time became less than half of the original value, in mice exposed to radiation (Table 5).

The results of DDS treatment of radiated rats inoculated with *M. lepraemurium*, analyzed on the basis of evolution of lesions and of survival time, show lack of effect of this drug. On comparing the leprosy lesions no appreciable difference was found between DDS-treated and nontreated radiated rats, either macroscopically or histologically throughout all the disease evolution. The leprosy lesions of radiated rats did not regress as did the lesions of nonradiated animals when DDS was given in a suitable dose and for a long time (2). On the other hand, comparison of the means of survival-time of DDS-treated rats, only one group of which had been subjected to radiation (Table 6), showed a statistically significant difference. It appears that as an effect of radiation DDS treatment proved unable to influence the rate of evolution of the disease, in contrast to the result in nonradiated rats.

TABLE 6.—*Effect of DDS treatment on the mean time of survival of radiated and nonradiated rats. DDS dose 40 mgm./day; inoculum dose 1.68 mg.*

	Radiated	Nonradiated
Number of animals	15	20
Mean survival time in days	226.5 ± 8.13	397.2 ± 9.8

DISCUSSION

As has been stated previously (4, 5) the rate of evolution of murine leprosy is regular and uniform if the animals are inoculated intraperi-

toneally with a suitable dose of *M. lepraemurium*. This fact serves as the basis of a very profitable procedure for detecting the influence of any factor on the evolution of murine leprosy. It was also stated that, corresponding to changes in the rate of evolution of the murine leprosy lesions a parallel alteration in the survival time of inoculated animals takes place. In this connection the means of survival-time appear as important and useful data ([3]), since they are easy to detect and can be expressed numerically. The results of this paper will be discussed on this basis.

Exposure to ionizing radiation prior to *M. lepraemurium* inoculation, influences the rate of evolution of murine leprosy, decreasing the mean survival time of radiated animals as compared with that of non-radiated ones. This effect was found in both mice and rats. Histologic study supported the belief that as a consequence of the effect of radiation a change occurred in the resting phase of murine leprosy lesions which was responsible for the rise in the evolutive rate of the disease.

Normally, during the resting phase of murine leprosy as shown by a variety of evidence ([6]), most murine leprosy lesions remain stationary and the bacilli grow very slowly. This phase appears to be influenced rather by factors related to the host organism, since it is not affected by changes in the source of *M. lepraemurium* ([6]). Several pieces of evidence lead to the belief that the resting phase is a period of lesion evolution in which some defense mechanism of the host organism attempts to inhibit the growth of the bacilli.

The effect of radiation in decreasing the length of the resting phase and consequently increasing the rate of evolution of murine leprosy probably is derived from a disorder of the host defense system. In such a disorder the *M. lepraemurium* could grow more freely. This hypothesis agrees with the histologic results.

In addition, radiation produced another effect leading to an increase in the rate of evolution of murine leprosy. Probably this effect also was a result of an alteration in the defense system of the host organism; it became apparent as a change in the generation time of *M. lepraemurium*. The hypothesis that excludes bacterial cell participation in the change in generation time is supported by the fact that the mean of generation time for *M. lepraemurium* became markedly shorter in both mice and rats, although at different levels, in animals subjected to radiation prior to inoculation.

These results suggest that the effect of irradiation, either on the resting phase of the lesion or on the generation time of *M. lepraemurium* depends upon its influence on unknown host factors that inhibit the mycobacterial growth. Although the radiation effect in each instance appears to result from a host defense system disorder, the action on the resting phase of the lesion can be distinguished from one modifying the generation time of the bacilli. Each effect seems to involve a different mechanism of the defense system, and so they would

have different meanings. This hypothesis is supported by the following results, which indicate a lack of parallelism between these two reported effects: in mice inoculated with *M. lepraemurium* the effect of radiation is only slightly apparent in the resting phase of the lesion but strikingly so on the generation time of the bacilli. In contrast in rat leprosy a more intense effect leads to changes in the resting phase, while the generation time of the bacilli seems less affected. Each of these effects occurs in the absence of any alteration in the microscopic aspects of the murine leprosy lesions.

Furthermore, an additional change of the host reaction against *M. lepraemurium* seems to occur as another effect of radiation. This one, like those previously reported, is not associated with any histologic alteration of the inflammatory reaction. It becomes manifest on comparison of the degree of correlation between the size of inoculum and the rate of evolution of murine leprosy in radiated and nonradiated animals. This correlation can be studied through the regression in the mean survival time in relation to size of inoculum, the regression coefficient being used to estimate it.

The regression coefficient of survival in relation to size of inoculum in radiated mice is −13.7 (Table 3). This value is significantly different from that obtained for normal mice (−36.3), when the same experimental conditions were maintained ([6]). The different behavior of radiated mice, which show a smaller influence of the dose of mycobacteria on the rate of evolution of murine leprosy, also indicated that radiation disturbs the defense system against *M. lepraemurium*.

Since the inflammatory reaction in murine leprosy remains unchanged in spite of the radiation effects, the host defense system disorder produced by radiation must be ascribed to other factors rather than to some alteration concerned with tissue reaction. Indeed, the histologic structure of the murine leprosy lesions both in mice and rats is always lepromatous in type, where the mycobacteria are not lysed by the host cells. In this instance a change in the rate of lysis of bacilli by inflammatory cells cannot account for the alteration in the evolution of the lesion. A disorder of the humoral defense mechanism can be considered responsible.

We must take into account the fact that Craddock and Lawrence ([1]), Jacobsen *et al* ([8]) and Taliaferro *et al.* ([12]) have shown that the inhibiting effect of radiation on the formation of antibody remains for 2-3 weeks only. In the studies here reported the effect of radiation on murine leprosy, which extends for a much longer time, cannot be interpreted as a consequence only of a decrease in antibody formation.

Besides the effects which involved changes in the rate of evolution of murine leprosy, another interesting radiation effect could be detected. This one was concerned with the influence of radiation on the activity of DDS on murine leprosy. As DDS became inactive in all radiated animals so treated, it is probable that this drug acts indirectly

on the *M. lepraemurium,* perhaps through the mediation of the host organism. In the case of such treatment, the DDS would accomplish its chemotherapeutic effect only when the host was able to play its own role, which seems to be altered as an effect of radiation. This alteration would be also related to disorder of the host defense system.

SUMMARY

The effect of beta-gamma radiations on murine leprosy was studied in mice and rats exposed to a single dose of 500 r at the beginning of the experiment. The results were evaluated on the basis of either differences in evolution of the murine leprosy lesions or in the length of animal survival.

Histologic study showed that as a consequence of radiation the resting phase of murine leprosy lesions became shorter and so the lesions evolved more rapidly. Correspondingly there was an increase in the rate of evolution of the disease, which was demonstrated by comparison of the mean length of survival of radiated and nonradiated animals inoculated with the same dose of bacilli.

On the other hand, studies of the generation time average for *M. lepraemurium,* estimated from mice and rats, showed that radiation induced a striking shortening of generation time. DDS treatment appeared devoid of any effect in radiated rats.

These results suggest that radiation disturbs the defense system against murine leprosy. The fact that this effect occurs in the absence of any observed alteration in the histologic structure of the murine leprosy lesions, indicated that a disturbance of the humoral defense mechanism had taken place. A defense system alteration would also account for the lack of effect of DDS in radiated animals.

RESUMEN

Fué estudiado el efecto de radiaciones beta-gama de la lepra murina en ratones y rates expuestas a una sola dosis de 500 r al principio del experimento. Los resultados fueron evaluados sobre la base de: sea las diferencias en evolución de las lesiones de la lepra murina, sea en la duracion de sobrevida del animal.

El estudio histológico demonstró que como consecuencia de las radiaciones, la fase de resposo de las lesiones leprosas murinas se hacen mas cortas, y asi las lesiones de evolución de la enfermedad, lo cual fué demonstrado por comparación del término medio evolucionan mas rapidamente. Correspondientemente, hubo un aumento en el grado de sobrevida de los animales irradiados y no irradiados, inoculados con las mismas dosis de bacilos.

Por otro lado, los estudios de los tiempos promedios de generación para el *M. lepraemurium,* estimados para los ratones y ratas, mostraron que la irradiacion induce un extraordinario acortamiento del tiempo de generación. El tratamiento con DDT pareció carente de ningun efecto en las ratas irradiadas.

Estos resultados suqieren que la radiación áltera el sistema de defensa contra la lepra murina. El hecho de que este efecto ocurra en la ausencia de alguna alteración observada en la estructura histológica de las lesiones leprosas murinas, indícan que ha ocurrido un disturbio del mecanismo de defensa humoral. Una alteración del sistema de defensa puede ser también responsable por la falta de efecto del DDT en los animales irradiados.

RESUMÉ

L'effet des radiations bêta-gamma sur la lèpre murine a été étudié sur des souris et des rats exposés à une simple dose initiale de 500 r. Les résultats ont été évaluès sur la base des différences relevées dans l'évolution des lésions de lépre murine et dans la durée de survie des animaux.

L'étude histologique a montré que suite à l'irradiation la phase de latence des lésions de lépre murine était écourtée et que les lésions apparaissaient plus rapidement. De même une accélération fut notée dans l'évolution de la maladie, ainsi qui'il est démontré par la comparaison de la durée moyenne de survie d'animaux irradiés et non-irradiés ayant été inoculés avec la même dose de bacilles.

D'autre part, des études sur la période moyenne de reproduction de *M. lepraemurium*, d'aprés des estimations obtenues chez des souris et des rats, ont montré que l'irradiation a entraîné une réduction trés marquée de la durée de cette période. Le traitement par la DDS s'est révélé inefficace chez des rats irradiés.

Ces résultats suggérent que l'irradiation bouleverse le systéme de défense contre la lèpre murine. Le fait que cet effet survient en l'absence de toute altération observable de la structure histologique des lésions de lépre murine indique qu'une perturbation du mécanisme humoral de défense pourrait également rendre compte de l'absence d'effet de la DDS chez des animaux irradiés.

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