The Influence of the Animal Age upon the Rate of Evolution of the Murine Leprosy

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It was pointed out that some differences concerning the rat susceptibility to the *M. lepraemurium* would be correlated to its age at the moment of inoculation. Young rats seems to be more susceptible than adults (MUÑOS RIVAS, 1954)⁷⁾.

In opposite, NAKAMURA & SHINGU (1953)⁸⁾ found the susceptibility of young mice to the *M. lepraemurium* to be lower in comparison with that of adult mice. This result, however, differs from that reported by KANDA *et al.* (1953)⁶⁾; according to it the susceptibility of suckling, young and adult mice to the *M. lepraemurium*, inoculated either subcutaneously or peritoneally, is similar. In parallel, FUJITA *et al.* (1956)²⁾ found no changes concerning the susceptibility of mice of the DD strain reliable to the animal age.

This disagreement shows that new experimental data in this connection are needed. As a contribution to this field, the correlation between the degree of susceptibility to *M. lepraemurium* and the age of mice and golden hamsters at the moment of inoculation will be studied. The results will be based either on the evolution of the leprosy lesions, histologically studied, or on the rate of evolution of the disease, estimated through the survival of the inoculated animals.

MATERIAL AND METHODS

Either suckling (3-6 days old) or young adult (60-70 daysold) rats of both sex were peritoneally inoculated with a *M. lepraemurium* suspension already rendered free from tissue particles with the aid of the HANKS (1951)⁵⁾ technique slightly modified. These animals were grouped according to the age and the size of inoculum (see Table 1).

Table 1 Experimental animals, dose of inoculum and animal age at the moment of inoculation.

Group	Animals		Dose of inoculum (mg)	Age at the moment of inoculation (days)	Body weight (g.)
	No.	species			
1	17	rat	1.0	3- 6	4- 6
2	17	rat	2.0	3- 6	4- 6
3	20	rat	2.6	60-70	90-120
4	20	rat	1.0	3- 6	4- 6
5	20	rat	2.0	3- 6	4- 6
6	20	rat	2.6	60-70	90-120
7	12	hamster	1.0	10-12	8-10
8	12	hamster	2.0	10-12	8-10

Suckling golden hamster (10-12 days old) of both sex were peritoneally inoculated with this same mycobacteria suspension (Table 1).

The rats from the groups 1, 2 and 3 were kept until the death to provide data for the survival study. The animals from the other groups were periodically killed (2 or 3 animals each 30 days), supplying material for the macroscopical and histological study of the leprosy lesions evolution.

The histological study was performed on pieces of omentum, spleen, liver, lymph nodes and lungs, already fixed in Bouin's fluid or in a 10% formalin solution, embedded in paraffin and stained by HE and the FARACO (1938)¹⁾ modification of ZIEHL-NEELSEN technic.

RESULTS

Leprosy lesions evolution 1-Rats- The animals of the group 6, inoculated as they were 60-70 days old, display resting leprosy lesions until the 60-90th day after inoculation. The resting lesions remain stationary and show a little amount of bacilli. Nevertheless, even within this period besides the resting lesions, that predominate, some other lesions do occur. These lesions differ from the former, since they seem to evolve slowly, as far as their histological characteristics is concerned.

In opposite, resting lesions could be found until the 120th day after inoculation in the suckling rats of groups 4 and 5. Only occasionally some leprosy lesions presenting the characters of slowly developing ones were found at the end of this time.

This result indicates that as a consequence of the animal age at the moment of inoculation the length of the resting phase of the leprosy lesion evolution becomes altered. Suckling rats display leprosy lesions where the resting phase is longer and more marked.

Concerning the fast evolving phase of the lesion evolution, that follows the stationary phase and where the leprosy lesions develop fastly and show a great amount of bacilli, there is no alteration reliable to the age influence.

2- Hamsters- As a result of the animal age at the moment of *M. lepraemurium* inoculation, the initial phase of the disease evolution, which in adult inoculated hamsters remains as long as 150 days (HADLER & ZITI, 1956),³⁾ becomes shorter whenever the inoculation is carried on into suckling animals. In such animals the initial phase of the disease remains only until the 90-120th day after inoculation. Furthermore, when the inoculation is performed into suckling animals, during the course of the initial phase of the disease, besides the stationary initial lesions, some leprosy lesions displaying the histological characters of those found in the late phase of the disease do appear. The presence of such lesions, that show a progressive development and do contain a great amount of bacilli (HADLER & ZITI, 1956), distress the limits of the two phases of lesion evolution, in opposite to what happens with adult hamsters.

Survival of rats- The rate of evolution of the murine leprosy does not depend upon the animal age at the moment where they were inoculated (see Table 2). Suckling rats (3-6 days old) inoculated with *M. lepraemurium* show a mean of survival that does not differ significantly from that showed by rats that were 60-70 days old when the inoculation was carried on.

Table 2 shows further that in suckling rats there is no correlation between the size

Table 2 Influence of age at the moment of inoculation on the survival of rats peritoneally inoculated with *M. lepraemurium*.

	Age at the moment of inoculation		
	3-6 days	3-6 days	60-70 days
No. of animals	17	17	20
Dose of inoculum	1 mg	2 mg	2.6 mg
Mean of survival (days)	211.8±3.2	209.3±3.5	218.5±1.5

of inoculum and the mean of survival.

DISCUSSION

The dose of inoculum used differs according to the animal age at the moment of inoculation. However, the inoculum variation was not proportional to the animal weight. As a consequence some changes concerning the leprosy lesions evolution, which were ascribed to the influence of the age at inoculation, would depend upon the effect of the size of inoculum. This hypothesis seems to be unreliable since the increasing of the inoculum does from 1 to 2 mg does not produce any apparent change regarding the lesion evolution.

The animal age at the moment of inoculation influences the leprosy lesion evolution but does not produce any change concerning the rate of evolution of the rat leprosy. As the resting phase of the lesion evolution becomes larger when *M. lepraemurium* is inoculated into suckling rats, an increasing of animal survival would be expected. Nevertheless, the mean of survival does not increase.

On the other hand, in suckling rats it was not possible to show any correlation between the dose of inoculum and the mean of survival, in opposite to what happens with young adult rats. The inoculation of different doses of *M. lepraemurium* into young adult rats (90-120 g of body weight and 60-70 days old) shows a linear regression of the size of inoculum on the animal survival (HADLER & ZITI, 1958).⁴⁾ The factor reliable to the correlation between the size of inoculum and the animal survival seems to occur only in the adult age. Perhaps it would be related to the decreasing of the resting phase length that also occurs in that age.

Regarding the hamsters there is also an influence of the animal age at the moment of inoculation on the leprosy lesions development and evolution. As a consequence, in suckling hamsters inoculated with *M. lepraemurium* the initial phase of the lesion evolution is shorter and less marked than in adult animals. It seems that the lysis of bacilli in tissue cells, which occurs in the initial phase lesions of adult hamsters inoculated with *M. lepraemurium* (HADLER & ZITI, 1956) is less marked into the homologous lesions of suckling hamsters. In such instance, the late lesions, where there is no bacilli lysis and where the bacilli multiply actively, do appear earlier.

As far as the influence of the animal age at the moment of *M. lepraemurium* inoculation on the leprosy lesions evolution is concerned the rat and the hamster do not behave similarly. Whereas in the former species there is a longer lesion evolution in the latter the lesions evolves faster.

SUMMARY

The influence afforded by the animal age at the moment of inoculation upon the rate of evolution of the murine leprosy was studied in rats and hamsters, peritoneally inoculated with *M. lepraemurium*.

The results based on the evolution of the leprosy lesions and on the rate of evolution of the disease show, as a consequence of the animal age at the moment of inoculation: 1) In rats the length of the resting phase of the lesion evolution becomes altered; It is longer in suckling than in adult rats; 2) The initial phase of the disease evolution is shorter in suckling than adults hamsters.

The rate of evolution of the murine leprosy is similar both in suckling and in young adult rats, showing that it is not influenced by the age at the moment of inoculation.

In suckling rats, in opposite to what happens to young adult ones, there is no correlation between the size of inoculum and the mean of survival.

BIBLIOGRAPHY

- 1) FARACO, J. Bacilos de Hansen e cortes de parafina: método complementar para pesquisa de bacilos de Hansen em cortes de material incluído em parafina. Rev. Brasil. Leprol. **6**, 177-186, (1938)
- 2) FUJITA, Y., ICHIHARA, T. & ICHIHARA, T. Studies on the susceptibility of mice to murine leprosy. Repot II. The influence of sex and age of the DD strain mouse on susceptibility to murine leprosy. La lepro **25**, 40-44. (1956)
- 3) HADLER, W.A. & ZITI, L.M. Histological reactions produced by experimental inoculation of *Mycobacterium lepraemurium* into the golden Hamster (*Cricetus auratus*). Internat. J. Leprosy **24**, 297-306, (1956)
- 4) HADLER, W.A. & ZITI, L.M. Relação entre a dose de *M. lepraemurium* experimentalmente inoculada e a sobrevivência de ratos tratados e não tratados pela 4,4'-diaminodifenilsulfona. Rev. Brasil. Leprol. **26**, 19-25, (1958)
- 5) HANKS, J.H. Bacteriology of leprosy. Ann. N. York Acad. Sci. **54**, 1219, (1951)
- 6) KANDA, Y., NAKAGAWA, Y. & NAKAMURA, M. On the susceptibility of infant and adult mice to rat leprosy. La Lepro **22**, 135-137, (1953)
- 7) MUNOZ-RIVAS, G. Transmisión indirecta, de la lepra murina por las pulgas. An. Soc. Biol. Bogotá, **6**, 181-198, (1954)
- 8) NAKAMURA, M. & SHINGU, M. On the susceptibility of young mice and hamsters to the murine leprosy bacillus and the influence of hyaluronidase upon the onset of murine leprosy. La lepro, **22**: 97-101, (1953.)

動物日齢の鼠癩発症に及ぼす影響

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動物の日齢と鼠癩菌感受性の関係については既にマウス及びラットを用いた研究が報告されているが、成績の一致をみることができない。そこでこれに関連した新たな知見を得るために、ラットとハムスターについて次のような実験を行なった。

ラットは生後3～6日の哺乳仔と60～70日の成熟鼠およびゴールデン・ハムスターの哺乳仔(10～12日)を用い、鼠癩菌液の腹腔内注射後経時的な剖検(肉眼的並びに組織学的検索)と動物の生存日数を調べ比較検討した。

結果として、ラットの鼠癩病巣の進展は、哺乳仔と成熟鼠とでは病巣発現までの静止相(resting phase)の長さに差がみられた。即ち成熟ラットの静止相期間は菌接

種後60～90日であり、哺乳仔では120日であった。発症率は哺乳仔、成熟鼠共に日齢に影響されず、更に動物生存日数も両者に有意差を認めえなかった。また哺乳仔は接種菌量ともなら関連性を示めさない。

一方ハムスターは、成熟したものに接種した場合にみられる初期相(initial phase)が150日もの長い間存続するのに比べ、哺乳仔では短かく90～120日であった。これは成熟ハムスターの初期病巣の組織細胞中と違い、哺乳仔の細胞では菌の溶解があまり著明ではないからと考える。

ラット及びハムスターにおける鼠癩病巣の進展は、哺乳仔の場合その態度が異り、ラットは長く、ハムスターではわりあいに早い。