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Lepromin Retesting as a Factor of Lepromin Test Positivation[1, 2]

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Much has been discussed not only about the nature of the lepromin reaction, but also about the induction of its positivity by previous contact with *M. leprae* and other mycobacteria.

The objective of this paper is to study the reaction of guinea pigs to successive inoculations of lepromin, keeping in mind that the experiment on a laboratory animal permits a better control than on a man. Some authors, among which are Vasquez (8), Souza Campos (7), Lara (4, 5) and Bechelli (1) have shown that in the human being repeated lepromin applications induce positivity. Studies were made on dogs by Wade (9), Feldman *et al* (2, 3) and Olmos de Castro *et al* (6), making it evident that repeated injections of lepromin not only induce positivity of the reaction but also increase its intensity.

We decided to use the guinea pig not only because of its being more easy to manipulate, but also to be able to more easily arrange a greater quantity of animals.

MATERIALS AND METHODS

In this experiment 25 guinea pigs were used, each three to four months old, weighing an average of 350 gm. The animals were inoculated intradermally monthly with 0.1 ml of lepromin containing 1.6×10^8 bacilli per milliliter. Each animal was subjected to a total of seven inoculations. Readings were taken 2, 7, 14, and 21 days after each inoculation; consequently, the total number of readings was 28 for each guinea pig. The reactions were measured in their greatest diameter, in millimeters, only the infiltrated area being considered. The duration of the experiment was seven months, after which time only 22 guinea pigs remained alive.

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[2] Work conducted at the Instituto of Leprology, Rio de Janeiro, Brasil, and presented at the Tenth International Leprosy Congress, 17 August 1973, Bergen, Norway.

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RESULTS

We avoided the criteria of positivity as established in the readings in the human. The reasons are varied. There was difficulty in determining what would be a positive reaction in quantitative terms, since it was found that inoculation of lepromin into nonsensitized animals resulted in negative readings (0 mm) at 2, 14, and 21 days. At the seventh day, however, reactions appeared that varied from 1 mm (5 guinea pigs) to 3 mm (one guinea pig) in six guinea pigs of the 22 observed (27.27%). The average reaction in these six guinea pigs was 1.33 millimeters.

The chronological criteria also did not seem good in that in response to the first inoculation (unsensitized animals) there was a reaction exclusively on the seventh day, while for the rest of the inoculations (previously sensitized animals) the reactions appeared chronologically different from those in the human. There was a tendency toward diminution and even disappearance of the reactions, progressively, in the readings at 14 and 21 days.

Due to these difficulties two criteria were adopted which would permit judging the sensitizing capacity of repeated lepromin tests on qualitative and quantitative bases.

Qualitative criteria. Any guinea pig that presented any infiltration, no matter how small (1 mm was the lower limit) at the point of the lepromin inoculation was regarded as a positive reaction. Table 1 shows the results of this reactivity. Though in the six reactive guinea pigs of the first test the reactions were only one millimeter diameter in almost all of them, this reactivity even though quantitatively minimal seems to be due to a beginning of sensitization during these seven days. All of the six following tests presented intense reactions demonstrating that there undoubtedly was a sensitization to the antigen due to successive reinoculations. This reactivity was most pronounced in the readings at days 2 and 7; there was decreasing

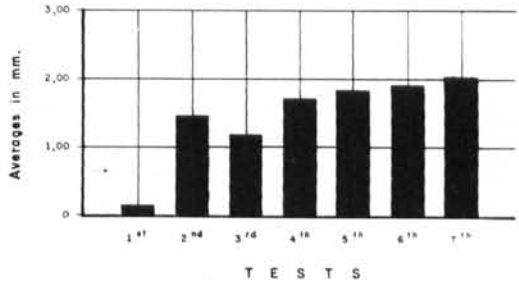
TABLE 1. *Lepromin tests in 22 guinea pigs. Averages in mm of the reactions.*

READINGS / TESTS	AVERAGES IN MM.			
	2 days	7 days	14 days	21 days
1 st	0,00	0,36	0,00	0,00
2 nd	1,50	2,19	1,48	0,86
3 rd	1,52	2,38	0,86	0,09
4 th	3,05	2,43	1,00	0,33
5 th	3,48	2,40	1,00	0,42
6 th	3,11	2,72	0,83	0,00
7 th	3,75	3,44	1,38	0,06

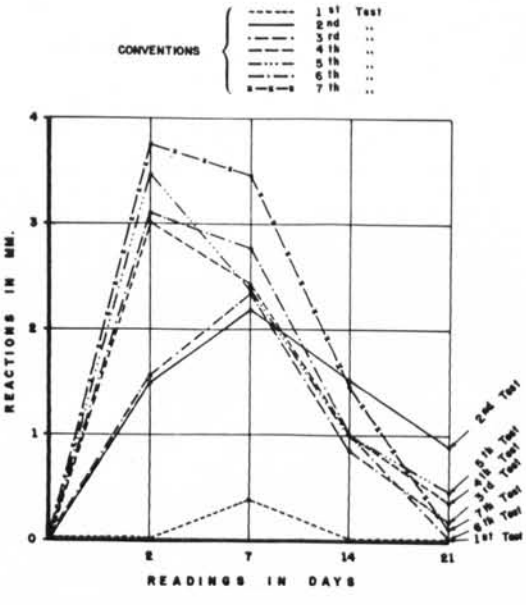
TABLE 2. *Results of the lepromin tests in guinea pigs. Averages in mm of four readings (2, 7, 14 and 21 days) of seven monthly successive tests.*

TESTS	AVERAGES IN MM.
1 st	0,09
2 nd	1,49
3 rd	1,20
4 th	1,70
5 th	1,84
6 th	1,96
7 th	2,12

GRAPH 1. *Results of the lepromin test in guinea pigs. Averages in mm of four readings (2, 7, 14 and 21 days) of seven monthly successive tests.*



GRAPH 2. *Lepromin tests in 22 guinea pigs. Averages in mm of the readings.*



percentage of reactivity in the readings at 14 and 21 days with a gradual disappearance of those reactions. Thus, in the guinea pig the initiation of reactivity took place in the first week, with histopathologic granuloma formation and necrosis. This differs from that which occurs in the human where the peak response is at 21 days. Thus, in the guinea pig there is a shortened Mitsuda phenomenon.

Quantitative criteria. All the reactions for each test were averaged in terms of millimeter reaction. Table 2 and Graph 1 show these results. The average diameter of the reactions was 0.09 mm in the first experiment (unsensitized animals), while it was 2.12 mm in the seventh experiment which is 23.5 times greater. This clearly shows sensitization of the animals.

The average diameter of the reactions increased gradually with repeated testing. This progressive increase in the average size of the reactions shows that the degree of sensitization increases with the number of repeated applications. We tried to determine the average diameters of the reactions of each reading as is seen in Graph 2. It is evident that the peak of each reading occurred at the second and seventh day with a tendency toward a disappearance of the reactions in the readings at 14 and 21 days, thus confirming that which already has been stated. All of the 22 guinea pigs tested and observed for seven months presented a certain degree of reactivity in one or more readings of any one of the tests. The fact seems to show the

inexistence of the "Rotberg anergic margin"[4] in guinea pigs.

SUMMARY

Repeated lepromin applications in guinea pigs induce a sensitization demonstrated by the progressive intensity of the reactions in subsequent tests.

The peak of the lepromin reaction in guinea pigs is reached between two and seven days, sooner than that which occurs in man (peak at 21 to 30 days). The lepromin reaction in guinea pigs is, therefore, shortened.

[4]Rotberg postulated (Rev. Bras. Leprol. **5** [1937] 45) that about 5% of the population does not have the "N" factor and therefore about 5% of the population will never react to the lepromin test, irregardless of procedures employed. This is the "anergic margin."

RESUMEN

El test de lepromina, usado en repetidas aplicaciónes en puercos de guinea, induce a una sensibilizacion demostrada por la progressiva intensidad de las reaccióones en los tests subsecuentes.

El apogeo de la lepromina reacción, en puercos de guinea, es alcanzado entre el segundo a septimo día; mas temprano de lo que ocurre en el hombre (apogeo entre 21 a 30 dias). La reacción lepromínica en puercos de guinea es mucho mas corta.

RÉSUMÉ

L'administration répétée de lépromine à des cobayes produit une sensibilisation, ainsi qu'en témoigne une augmentation progressive de l'intensité des réactions lors d'épreuves successives.

Le maximum de la réaction à la lépromine chez le cobaye est atteinte après deux à sept jours, plus précocement donc que celle qui se produit chez l'homme (maximum entre 21 et 30 jours). La réaction à la lépromine chez les cobayes, en est dès lors abrégée.

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