THE INFLUENCE OF REPEATED LEPROMIN TESTING ON THE MITSUDA REACTION IN HEALTHY PEOPLE [1]

LUIZ MARINO BECHELLI, M.D.

Professor of Dermatology
Ribeirão Preto Faculty of Medicine
University of São Paulo
São Paulo, Brazil

In the first phase of the investigation of the effects of BCG vaccination in leprosy, the experiments consisted in observing its influence in groups of persons who had given negative Mitsuda reactions to first injections of lepromin, to whom was then administered the vaccine and later a second lepromin injection. The positivization of reactivity obtained was usually regarded as due exclusively to the effects of the vaccination. However, observation of the effect of repeated lepromin tests, as well as the adoption of control groups in the studies of a group of which I was a member ([3, 11, 12]), and later by Silva and associates ([14]), showed that in persons who had not been vaccinated, or who had received a placebo instead of the vaccine, there was positivization of the lepromin reaction, previously negative after the first injection, in variable proportions of cases up to 80 per cent or more.

These facts were not new. Lepromin positivization after repeated tests was observed by Bargehr ([2]) and de Langen ([7]) in 1926, and more recently by Cerqueira ([4]), de Souza Campos ([15, 16]), Fernandez and others.

In 122 Mitsuda-negative children, de Souza Campos ([16]) observed positivity on the second test in 82 per cent. Furthermore, in 32 with 1+ reactions there was intensification to 2+ or 3+ in 69 per cent, and from 2+ to 3+ in 72 per cent of 65. Repeating the test in another group of 62 negative children, he observed persistence of negativity in 37 in the second test, in 16 in the third, in 11 in the fourth, and only in 2 in the fifth.

However, these facts were practically forgotten or not properly considered when the effect of BCG on lepromin conversion was studied. Paula Souza, Ferraz and Bechelli ([11]) for the first time insisted on the importance of these facts in experiments with that vaccine. They observed positivization of the Mitsuda reaction upon repeated lepromin testing in 70 to 80 per cent of healthy children. Also of 31 children who were 1+ positive, 48 per cent showed intensified reactions, 2+ or 3+ (Table 1).

[1] This paper was presented in the symposium on immunology at the VII International Congress of Leprology, held in Tokyo, Japan, November 12-19, 1958.

TABLE 1.—*Results of second lepromin testing, 11 months after the first tests.*[a]

First lepromin test	No. of cases	Second lepromin test			
		Negative	One-plus	Two-plus	Three-plus
Negative	34	10 (29.4%)	15 (44.1%)	8 (23.5%)	1 (2.9%)
One-plus	31	1 (3.2%)	15 (48.3%)	14 (45.2%)	1 (3.2%)
Two-plus	11	—	—	8 (72.7%)	3 (27.3%)
	—	—	—	—	—
Total	76	11	30	30	5

[a] Report of Paula Souza, Ferraz and Bechelli (1953).

In 1955 Paula Souza, Bechelli, Ferraz and Quagliato ([12]) confirmed their previous report. In this instance they investigated the results of vaccination with fresh BCG, BCG 15 days old, and heat-killed BCG, and for comparison a control group received no vaccine. The data are presented in Table 2.

TABLE 2.—*Results of second lepromin testing in children given fresh BCG, old (15 days) BCG, and heat-killed BCG, and in unvaccinated controls, the retesting done 30 days after vaccination and about 75 days after the first testing.*[a]

First lepromin test	Group	No. of cases	Second lepromin test				
			Negative [b]	One-plus	Two-plus	Three-plus	Increase [c]
Negative and doubtful	Fresh BCG	37	5 (13.1%)	13 (35.1%)	10 (27.0%)	9 (24.3%)	32 (86.5%)
	Old BCG	27	6 (22.2%)	13 (48.1%)	8 (29.6%)	0 (0.0%)	21 (77.8%)
	Killed BCG	29	9 (31.0%)	13 (44.8%)	3 (10.3%)	4 (10.3%)	20 (69.0%)
	Control	35	7 (48.6%)	17 (48.6%)	9 (25.7%)	2 (5.7%)	28 (80.0%)
One-plus	Fresh BCG	62	4 (6.4%)	22 (43.5%)	27 (43.5%)	9 (14.5%)	36 (58.1%)
	Old BCG	51	4 (7.8%)	20 (39.2%)	17 (33.3%)	10 (19.6%)	27 (52.9%)
	Killed BCG	51	3 (5.9%)	27 (52.9%)	14 (27.4%)	7 (13.7%)	21 (41.2%)
	Control	67	3 (4.5%)	31 (46.3%)	23 (34.3%)	10 (14.9%)	33 (49.2%)
Total		359	41	156	111	51	

[a] Report of Paula Souza, Bechelli, Ferraz and Quagliato (1955).
[b] Including doubtful-grade reactors.
[c] Total who became positive from negative, or increased from 1+ to 2+ or 3+.

In the control group of 35 children Mitsuda negative or doubtful to the first lepromin test, the second test made about 75 days after the

TABLE 3.—*Results of second testing of Dutch and Brazilian children, BCG-tested and controls.*[a]

First lepromin test	Group	No. cases	Second lepromin test			
			Negative	One-plus	Two-plus	Three-plus
Dutch children (5-14 years old)						
Neg. and ±	BCG	28	6 (21.4%)	13 (46.4%)	9 (32.1%)	0 (0.0%)
				(78.6%)		
	Control	31	18 (58.1%)	11 (35.5%)	2 (6.4%)	0 (0.0%)
				(41.9%)		
1+	BCG	16	2 (12.5%)	10 (62.5%)	3 (18.7%)	1 (6.2%)
					(25.0%)	
	Control	11	1 (9.1%)	8 (72.7%)	0 (0.0%)	2 (18.2%)
					(18.2%)	
Brazilian children (3-14 years old)						
Neg. and ±	BCG	9	1 (11.1%)	7 (77.8%)	1 (11.1%)	0 (0.0%)
				(88.9%)		
	Control	22	9 (40.9%)	12 (54.5%)	1 (4.5%)	0 (0.0%)
				(59.1%)		
1+	BCG	20	2 (10.0%)	17 (85.0%)	0 (0.0%)	1 (5.0%)
					(5.0%)	
	Control	33	2 (6.0%)	27 (81.8%)	1 (3.0%)	3 (9.1%)
					(12.1%)	

[a] Report of Bechelli, Quagliato and Nassif (1953).

first injection of lepromin, showed 80 per cent with some degree of positivization of the reaction, a percentage similar to that obtained with BCG. There was also intensification of previously weakly-positive Mitsuda reactions in 67 control children to 2+ and 3+ in 49.2 per cent.

Bechelli, Quagliato and Nassif ([3]) observed positivization of the Mitsuda reaction after repeated lepromin testing in groups of Dutch and Brazilian children, as well as intensification of 1+ reactions (Table 3).

The placebo-treated controls of the Dutch children showed 41.9 per cent positivization, while after the BCG vaccination there was positivization in 78.6 per cent. Of the Brazilian control children who received the placebo, positivization occurred in 59.1 per cent, while those who received BCG showed 88.9 per cent positivization.

Ignacio, Palafox and Jose ([6]) repeated the lepromin test at short intervals in children from 3 to 18 months of age. In the first test (September-October 1949) only 22 per cent were positive. In the second test (November 1949) the positivity went up to 74 per cent. On the third test (May 1950), the Mitsuda reaction was positive in 96 per cent. All of the cases were positive in the fourth test (September-October 1950).

Silva, Rabelo Neto and Castro ([14]) obtained results similar to those of Paula Souza, Bechelli, Quagliato, Ferraz and Nassif. In three age groups up to 14 years, there were more positives in the BCG-vaccinated individuals than in the unvaccinated controls, but the reverse was found in the 15-21 years group; the percentages of positives in those 22 or more years old were virtually identical. Of the first-test negatives in the control group, only 19.2 per cent were negative to the second test.

Paula Souza and Bechelli, in an abstract sent to the Tokyo congress, reported that in 41 very young children, 0-4 years of age, positivization (1+ only) of the Mitsuda reaction in a second lepromin test made 40 days after the first one had been observed in 11, or 44 per cent, of the 25 original negatives (Table 4). Statistical analysis by W. Galvão showed an interval of confidence from 24.5 to 61.5 per cent. This means that in 100 groups composed of 25 children negative to the first lepromin test, 95 out of them will have Mitsuda's positivization between 24.5 and 61.5 per cent.

Bechelli, in another Tokyo congress abstract, reported that in a group of children aged from 8 days to 11½ months, there was positivization (only 1+) of 8.6 per cent of 34 first-test negatives. In another group of 26 children aged from 16 days to 5½ months, 1-plus positivization occurred in 7.0 per cent. In a third group of 17 children, from 14 days to 5 months old, none showed positivization, although 7 of them had received BCG twice (0.2 gm. oral), 9 and 15 days after the lepromin was injected. On the other hand, Paula Souza *et al.* ([12]) and Bechelli *et al.* ([3]) observed that, on retesting 4.5 per cent of 1+ reactors were

TABLE 4.—*Results of second testing of 41 young children.*[a]

First lepromin test	No. cases	Second lepromin test			
		Negative	One-plus	Two-plus	Three-plus
Neg.	25	14	11	0	0
1+	8	—	7	1	0
2+	8	—	3	4	1
Total	41	14	21	5	1

[a] Report of Paula Souza and Bechelli, Tokyo congress abstract.

negative or doubtful. This also occurred in a group that had received BCG in similar proportion, 6.4 per cent.

Doull, Guinto and Mabalay ([5]) observed positivization in 34.7 per cent of the children studied by them. It should be noted, however, that they regarded as positive "nodules with a diameter of 5 mm. or more," whereas the usual custom is to grade 3 mm. nodules in the 1+ positive class, as recommended by the Madrid congress.

DISCUSSION

From the reports that have been reviewed it would appear that:

1. Positivization of the Mitsuda reaction in healthy people may occur after repeated lepromin testing, in a high percentage of them on the second test. This occurrence is independent of sensitivity to tuberculin, having been observed in children negative to 1:1,000 and 1:10 test doses of OT.

2. Intensification of weakly positive (1+) Mitsuda reactions in healthy people may be seen on repeated testing, in a relatively high proportion of cases.

3. It is also a fact that the frequency of such changes depends to a considerable degree upon age, being least frequent in very young children.

Some specialists, as Azulay ([1]) and Rosemberg, Souza Campos and Aun ([13]) have not obtained positivization after two lepromin injections in healthy children which served as controls in their experiments.

Loss of weak reactivity may sometimes be seen. In our material we have observed that an average of 10 per cent of healthy persons with 1+ Mitsuda reactions in the first test became negative in the second one. The same thing has also been observed in subjects who had received BCG.

The experiments of the authors cited, and those of our own group, make it difficult to evaluate the effect of sensitization by the Koch bacillus and of the action of BCG vaccination, considering only the Mitsuda reaction. The fact is that in these experiments a first lepromin test is done to select subjects who react negatively; BCG is then administered, and later on a new test is performed. What would have occurred without the BCG vaccination, only on repeating the test? What does BCG do in addition to the effect of repeated testing? It is indispensable to have a control group, which the majority of experiments have not had. Even when BCG is administered at the same time as lepromin is injected, it is difficult to dissociate what is due to the vaccination and to the injection of the leprosy antigen. Here, too, the inclusion of a control group is essential for the comparison.

What is the action of lepromin? Does it act as a sensitizer? Does it have the capacity to create or to increase a state of resistance or immunity against leprosy? I believe that it acts as a sensitizer, in an organism that will answer in a positive or a negative way according to its capacity of defense.

In many persons this sensitization and positivization of the reaction occur within 30 days, and in others after 50, 70 or more days after repeated testing; and, finally, there are persons who will not react to lepromin. Thus lepromin acts as sensitizer and an indicator of the capacity of resistance of the individuals tested. According to this hypothesis, persons with resistance against leprosy await contact with *Mycobacterium leprae*—alive from a contagious patient, or dead in the lepromin—to exhibit their capacity of defense.

I do not know whether lepromin by itself may or may not increase resistance. This question has not yet been answered, and demands further research.

Nevertheless, some authors who believe in this effect of lepromin have used it to that end, chiefly when the Mitsuda reaction began to be studied. Recently, Lara and Nolasco ([10]) reviewed the results of experiments which Lara had been carrying on since before 1940 ([6,8,9]). In that year 110 children from 2 weeks to 18 months of age received 3 injections of lepromin at intervals of 4 months. Another group of 110 similar children served as the control. Both groups of children continued to live with their leprous parents. By 1946, 40 cases of leprosy had developed in the group which received lepromin, and 51 in the control group. Ten years later there was complete involution of the disease in 97 per cent of those who had had lepromin, and in 80 per cent of the control group. It was concluded:

"Our more recent observations again support the earlier findings which suggested a beneficial action of lepromin-test injections in very young exposed children."

This refers to children who had been under the age of six months when the injections were given.

Other points have to be considered with respect to the influence of repeated lepromin tests: Is the Mitsuda reaction obtained after repeated testing really positive? What is the percentage of this positivization? The histologic examination should be used to determine the real value of positivizations by lepromin as well as of those obtained after administration of BCG.

CONCLUSION

Repeated injections of lepromin may cause positivization of the Mitsuda reaction, or intensification of the reactions in weakly-positive reactors. It is therefore indispensable to have a control group in experiments with BCG or other vaccines administered for the purpose of increasing resistance. As yet there are not enough data to prove that repeated lepromin injections may increase resistance against leprosy.

RESUMEN

Inyecciones repetidas de lepromina pueden ocasionar positivización de la reacción de Mitsuda o intensificación de la misma en reactores débilmente positivos. Resulta por lo tanto indispensable tener un grupo testigo en los experimentos con BCG u otras vacunas administradas a fin de acrecentar la resistencia. No hay todavía suficientes datos para demostrar que las inyecciones repetidas de lepromina acrecienten la resistencia contra la lepra.

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