MITSUDA REACTIONS INDUCED BY REPEATED LEPROMIN TESTING IN CHILDREN REMOVED AT BIRTH FROM THEIR LEPROUS PARENTS

FAILURE OF BCG TO INDUCE STRONG REACTIVITY IN PERSISTENTLY MODERATE REACTORS[1]

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Regarding the significance of the Mitsuda phenomenon, its prognostic importance has gained more or less general recognition. It is widely accepted that a strong lepromin reaction in a patient denotes a favorable prognosis, although de Souza Lima and de Souza Campos (15) do not entirely agree with this view.[2] On the other hand, a negative reaction in a patient is interpreted as indicating weak or no resistance, and hence a poor prognosis.

As pointed out by Rotberg and associates (13), studies in endemic areas have demonstrated a high frequency of negative reactors among very young children. De Souza Campos (14), applying the test to children of leprosy patients, found that all those who were isolated at birth gave negative reactions, while among those who had lived with their parents there was a direct correlation between the frequency and strength of positive reactions and the duration of contact. He also found that children of bacteriologically negative nerve-type cases gave negative reactions, while children of nodular and mixed cases were positive. Likewise Lara (6, 7), in a study of 110 nonleprous children living with their leprous parents, found a direct correlation between the duration and constancy of exposure and the proportion of positive reactors in the first and subsequent tests. He also showed definitely that both increase of age as well as repeated testing increased the proportion of strongly positive reactions. Chiyuto (2), and Manalang (9) on the basis of Chiyuto's observations, both concluded that the practically uniform nonreactivity of very young children to the lepromin test indicates lack of resistance, hence susceptibility to leprosy infection.

The studies of Bargehr and of de Langen according to Rotberg (12),

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[2] In studies of the Mitsuda reaction in "incharacteristic" cases, these authors observed development into the lepromatous type even of some that were lepromin positive, although in a much lower proportion among those with strong reactions than among weak reactors. They also noted, in a few cases with previously negative reactions which became reactive to lepromin, that the strongly positive (3+) ones became clinically cured while three out of four with only weak (1+) or moderate (2+) reactions became lepromatous.

gave rise to the concept of the "positive reaction due to minimal and previous repeated infection producing immunizing antibodies," a concept accepted by some later workers including Rotberg himself. Other early studies by several workers, all cited by Rotberg, made on persons without previous known contact gave evidence that reactivity to lepromin does not necessarily indicate previous infection with *M. leprae*. However on reanalyzing the data presented by those workers and discounting those reactions below 0.5 cm., Rotberg arrived at an entirely different conclusion. He believed that nonreactivity may be due either to lack of the normal capacity to react (designated by him as "N factor") which exists in most individuals and is congenital and hereditary, or merely to absence of previous exposure to *M. leprae*.

More recent studies indicate that reactivity to the lepromin test may be subject to the influence of cross sensitization by an existing tuberculous infection (1, 3), or by vaccination with BCG (5, 11). The literature of the employment of BCG in connection with leprosy has recently become extensive. It is certain that BCG vaccination is usually very effective in converting lepromin-negative persons to positive reactors and many workers have high expectations of the value of reactivity so induced for protection against leprosy infection.

PRESENT STUDY

The present report concerns our observations on the stimulation of reactivity in nonreactive children, or of stronger reactivity in weakly reacting ones, by repeated testing with lepromin. This work was done on the assumption, as in studies of many others, that the stronger the reactivity, natural or induced, the greater the chance of avoiding infection. The purpose was to prepare the children, after the passage of the first few years, to be returned to their parents in the colony. There was no other place to send them under postwar conditions in the Philippines, and the limited facilities of the Balala nursery[3] were needed to give more recently born babies similar advantages of survival and protection. At the time this work was commenced BCG was not available in the Philippines, nor would we at that time have been willing to assume the responsibility for using it on these children, any mishap to whom would have aroused much unease and resentment in the colony.

The subjects were 50 young children who, with one exception, had been separated at birth from their leprous parents.[4] The previous reports from

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[3] Balala is the name of the "clean" residential section of Culion. For essential aid in the re-establishment of the nursery, thanks are due to the U. S. Public Health Service-Philippine Department of Health joint health relief program. The former entity, under Dr. Howard F. Smith, U. S. P. H. S., contributed equipment and personnel.

[4] The exceptional one was born a few hours before admission in a small watercraft that was bringing the mother to the hospital for delivery. This child gave a negative reaction at the first test.

Culion referred to (6, 7) dealt with children who had had varying degrees of exposure.

Of the first four test injections given to the entire group, the first was in September-October 1949; the second test was made soon afterward, in November; the third one was six months later, in May 1950; and the fourth was after another

TABLE 1.—*Mitsuda reaction in 50 children of leprous parents, removed at birth and living in the Balala nursery.*

Reaction	Age group						Total
	To 3 mos.	3 to 6 mos.	6 to 12 mos.	12 to 18 mos.	18 to 24 mos.	2-3 yrs.	
First lepromin test (September-October 1949)							
Neg.	16	8	10	5	—	—	39 (78%)
1+	2	1	3	4	—	—	10 (20%)
2+	—	—	—	1	—	—	1 (2%)
3+	—	—	—	—	—	—	—
Total	18	9	13	10	—	—	50
Second lepromin test (November 1949)							
Neg.	1	5	4	3	—	—	13 (26%)
1+	5	8	7	6	—	—	26 (52%)
2+	1	3	1	6	—	—	11 (22%)
3+	—	—	—	—	—	—	—
Total	7	16	12	15	—	—	50
Third lepromin test (May 1950)							
Neg.	—	—	1	—	1	—	2 (4%)
1+	—	—	5	—	2	—	7 (14%)
2+	—	1	16	7	7	—	31 (62%)
3+	—	—	4	3	3	—	10 (20%)
Total	—	1	26	10	13	—	50
Fourth lepromin test (September-October 1950)							
Neg.	—	—	—	—	—	—	—
1+	—	—	—	—	1	—	1 (2%)
2+	—	—	1	15	9	7	32 (64%)
3+	—	—	1	12	2	2	17 (34%)
Total	—	—	2	27	12	9	50

four months or so, in September and October 1950. The first of two special subsequent tests, the fifth of the series, was made on a selected group in March 1951, and the final test of the series was applied to the whole group in December of that year.

The lepromin used was prepared by the Hayashi technique, and the same stock was used in the first four tests. The criteria of grading the reactions were also the same as Hayashi's, namely: a reaction of 3 to 5 mm. was considered weakly positive (1+), 6 to 10 mm. moderately positive (2+), and above 10 mm. strongly positive (3+). Only the readings made on the third week and subsequently were considered in grading the reactions. After the first test, observations were also made of the sites of the preceding tests, with reference to the occurrence of flare-up or activation apparently provoked by later tests. This observation was suggested by Dr. C. B. Lara, who had previously noticed the flare-up of previously nonreacting or weakly reacting sites upon repeated testing, using new sites. The arm and forearm were used except in the last test, when the lower part of the thigh was used.

RESULTS

The results of the first four tests are given in Table 1. At the outset 78 per cent of the 50 children were negative, and only one gave a 2+ reaction. The second test gave radically different results: only one-fourth of the group remained negative, and nearly one-fourth were now of 2+ degree. No 3+ reaction was seen until the third test, at which time only two individuals remained nonreactive. At the fourth test those two had also become positive, and only one child remained as weakly responsive as 1+; by that time a full one-third were strong reactors.

Further tests.—The results of the two further tests of this series are shown in Tables 2 and 3.

Fifth test: This test, made in March 1951, five to six months after the fourth one, was applied only to the 32 children with 2+ reactions, to see if they would be stronger then. Only 3 of them increased to the 3+ grade, the other 29 being still only moderately reactive; in none was the reaction weaker than the previous 2+.

TABLE 2.—*Fifth test (March 1951), of 32 children giving moderate (2+) reactions on the fourth test.*

Reaction	Age group			Total
	12 to 18 months	18 mos. to 2 years	2-3 years	
2+	1	12	16	29 (91%)
3+	—	2	1	3 (9%)
Total	1	14	17	32

Final test: This test, made in December 1951, was applied to all of the 47 children of the original group still available.[5] It was only the

[5] Three children had been released to healthy relatives outside. One, then only 1+ (the only one less than 2+ in the fourth test), had been released before the fifth test. Two others, persistently 2+, had been released after that test.

fifth one for the 17 that had been omitted from the fifth test because they had given strong reactions before. They, and the 3 strong reactors of the fifth test, were—with one inadvertent exception—given only one-half of the usual dose, i. e., 0.05 cc.

TABLE 3.—*Final test (December 1951), of 47 children, sixth for the 30 remaining subjects of the fifth test, fifth for the 17 strong reactors of the fourth test.*[a]

Reaction	Age group				Total
	2 to 3 years		Over 3 years		
	Full dose	Half dose	Full dose	Half dose	
2+	5	1[b]	7	—	13 (28%)
3+	11	15	5	3	34 (72%)
Total	16	16	12	3	47

a All previous strong reactors, with one inadvertent exception, were given only one-half of the usual dose.

b This child was previously 3+, but reacted only moderately to the half dose given.

This time there was a marked preponderance of strong reactors, 72 per cent, compared with 34 per cent at the fourth test. This was a little more than two years after this experiment was begun.[6]

COMPARISON WITH LARA'S 1939 REPORT

Lara's group of 110 nonleprous children, not removed from their leprous parents, who were subjected to the Mitsuda test three times at intervals of four months (6, 7), are compared in Table 4 with those obtained in the first four tests of the present group of children, unexposed to leprosy since birth. The latter group had a strikingly low proportion of positive reactions at the first test, only 22 per cent against 72.7 per cent in Lara's group. In both cases, with repetition of the testing there was a progressive increase in the positive reactors so that at the third test there were 96 per cent positives in our group and 99 per cent in Lara's group. At the fourth test our group was 100 per cent positive.

It may also be mentioned that in the present study many of the children showed distinctly positive reactions in the retests while still below one year of age. This confirmed Lara's observations, which to our knowledge were the first to be made, of positive reactions in extremely young children. Further, the five oldest children in our series gave only moderately strong (2+) reactions at the fourth test, as likewise did about 76 per cent of all children in the two oldest age groups; yet nearly 45 per

[6] To avoid complicating this report unduly, we do not go into the matter of ulceration in the stronger reactions observed in this work. In the most severe of them the ulcer would attain a size of 10 mm. in diameter, or even more, with corresponding depth and suppuration; and on healing they left conspicuous scars of considerable size.

cent of the younger children (6 to 18 months) showed strong (3+) reactions that time, some of them while only 7 or 8 months old (see

TABLE 4.—*Comparison of the present nursery group and Lara's children not separated from their leprous parents.*

Reaction	Present group		Lara's group[a]	
	Number	Per cent	Number	Per cent
First lepromin test				
Neg.	39	78	30	27.3
1+	10	20	65	59.1
2+	1	2	15	13.6
3+	—	—	—	—
Total	50		110	
Second lepromin test				
Neg.	13	26	3	2.9
1+	26	52	32	30.5
2+	11	22	46	43.8
3+	—	—	24	22.8
Total	50		105	
Third lepromin test				
Neg.	2	4	1	0.97
1+	7	14	16	15.5
2+	31	62	54	52.4
3+	10	20	32	31.1
Total	50		103	
Fourth lepromin test				
Neg.	—	—		
1+	1	2		
2+	32	64		
3+	17	34		
Total	50			

a No fourth test of this group.

Table 1). As has been said, the same stock of lepromin was used in all these four tests of our children.

Only 3 (approximately 10%) of the 32 persistently moderate reactors attained the 3+ grade at the fifth test. And again, at the last test, the five oldest children—about three and one-half years of age—still gave only the moderate-grade response.

ACTIVATION OF PREVIOUS SITES

Another interesting observation was the activation of previously negative or poorly reacting sites, apparently provoked by subsequent injection of lepromin into another site or area. This phenomenon has been seen repeatedly since the earliest studies of this reaction. A recent example is in a report by Rosemberg and associates (11), who observed an activation, or "remote positivization," of the Mitsuda test in 93 per cent of 27 children between the 70th and 112th day, induced by oral doses of BCG started 40 days after the lepromin was injected. In our study there were indications that the third injection caused a flare-up or intensification of the poorly-reacting second injection sites only in a small proportion (less than 10%) of the children who received the further injection within 11 weeks after the second. There was, however, a much higher proportion (over 70%) of such activations of the first injection sites after the second injections, most of which were given within only 9 weeks after the first.

BCG VACCINATION OF REFRACTORY CASES

The sixth test of the above series left a group of 12 relatively refractory children whose reactions had not increased beyond the moderate (2+) grade, this number being 40 per cent of the 30 involved in the fifth test and still available. In the hope of intensifying these reactions, these children were vaccinated with BCG as a part of a more extensive study to be reported on later.

The BCG used was manufactured by the Alabang Laboratories of the Department of Health and distributed by the National Chest Center in Manila. On receipt at Culion—at most a two-day voyage—it was used promptly, always within the expiration date. The refractory group (not all 12 every time) was vaccinated four times. Twice the multipuncture method was used, as done at the National Chest Center where one of us (C. A. P.) trained for this work; after that the BCG was administered by intradermal injection. Ten of these children were tuberculin negative before these vaccinations were begun; the other two were weakly but definitely positive.

The first vaccination (10 children, not including the two tuberculin positives) was in April 1952, five months after the sixth lepromin test; three months later only one of the ten reacted to tuberculin. In July 1952 the second multipuncture vaccination was done (all 12 children); two months later there was no new tuberculin reactor, 9 remaining negative. The group was then (September 1952) given its seventh lepromin test. Not one of them had changed from the 2+ grade, in spite of the

two BCG vaccinations. The existence of tuberculin sensitivity in 3 of them had no influence on the Mitsuda reaction.

BCG vaccination was resumed, now intradermally. In January 1953 one dose was given to each of the 11 children remaining (one had been discharged); only 4 of them still remained tuberculin negative when tested some two months later. The final vaccine injection, in June 1953, was given to 9 children (again passing up the two not given the first vaccination); none remained tuberculin negative after that. The eighth lepromin test was made in September 1953, and now 2 of the 11 proved to be 3+, while the other 9 remained of the moderate grade.

For a final check, these 11 children were given a ninth lepromin test after another eight months, in May 1954. The results were as they had been the last time; two gave 3+ and nine gave 2+ reactions.

The outcome, in short, has been that not less than 9, or 18 per cent, of the 50 original children could not be made strongly reactive by multiple lepromin injections plus repeated BCG vaccination, and this figure may have been larger had not 4 of them—none a strong reactor—been released during the long period of the experiment.[7] These refractory children were only *relatively* so, but the fact that they could not be made strongly reactive is the more significant because of the extraordinary measures used in the attempt to make them so. The fact that it took repeated BCG vaccinations to render them all reactive even to tuberculin is not without interest.

DISCUSSION

It cannot be denied that contact with a leprous environment is an important factor in inducing reactivity to lepromin, as in children allowed to grow up in the care of their leprous parents. In our study, where all the children—with one exception—had been isolated immediately after birth, the factor of exposure thereafter may be discounted.

The preponderance of negative reactions in the first test in our non-contact children as compared with the preponderance of positives in Lara's parent-contact group (Table 4) is significant and may be attributed to the lack of the exposure factor. Subsequent injections of lepromin, however, caused an ever-increasing proportion of positive reactions. At the last test (Table 3) all reacted positively, 34 of the 47 strongly so. Since these children had practically no contact with patients, it follows that their lepromin reactivity was induced by the repeated injections of the antigen, with possibly some but obviously limited effect of aging in the two-year period.

It may be significant, too, that in Lara's exposed group 73 per cent reacted positively to the first test, while in our unexposed group 74 per cent were positive at the second test, after sensitization with one injection

[7] Of these four, one was only 1+ after the 4th test, the only one of the 50 so weak at that time; two were 2+ after the 5th test; and 1 was 2+ after the 7th test.

of the antigen. Conditioning by previous testing is apparently of the same order as conditioning by exposure. The results indicate a high efficacy of administering even a single standard dose of antigen (lepromin), an indication further confirmed by the continued marked increase of positive reactors in the subsequent tests.

There still remains the possibility of another, inherent, factor (besides aging, exposure, lepromin injection, and BCG vaccination) that is essential to the development of a strong positive reaction. This would be what Rotberg called the N factor. When this factor is present, exposure to a leprous environment or repeated testing with lepromin would probably induce a strong reaction in an originally nonreacting or poorly-reacting child. If absent or deficient, even repeated injections of lepromin or other antigens such as BCG will not suffice to evoke reactivity, or to convert weak reactors to strong ones—even in children above three years of age, as in our experiment. As said by Fernandez (4), "Unfortunately it is not [always] possible to provoke this supposedly protective allergy, since it is dependent on an unknown factor which is constitutional. When this factor is lacking, nothing can be gained by the intradermal injection of the antigen."

It seems quite probable that this inherent factor is responsible for the apparent freedom from manifestations of disease of many children of leprous parents living under conditions of constant exposure. Furthermore, even when lesions develop at an early age, a quite high proportion of the cases clear up and become free from further manifestations, at least for a considerable length of time (8, 10). This factor, obviously, is of basic importance in resistance to the disease, and the lepromin reaction is only an indicator of its presence in the individual.

It would seem appropriate, however, to lay equal emphasis on the very high proportion of our children in whom a distinctly positive (2+ or 3+) reaction could be induced by repeated lepromin injections, as possibly indicating the usual proportion of individuals (in this region) possessing relatively adequate resistance, although not necessarily absolute immunity, to leprosy infection. At the same time there is probable significance in the fact that, of the small group of relatively refractory children who remained only 2+ after the sixth test, only a minority underwent augmentation to 3+ reactivity after repeated BCG vaccination and the further lepromin testing that was involved.

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RESÚMEN

Preséntase una reseña de la reactividad de Mitsuda provocada por la comprobación repetida con lepromina. Los sujetos fueron 50 niños pequeños residentes en la

Leprosería de Culión que, con una excepción, habían sido separados al nacer de los padres leprosos. La excepción era un niño que no había estado expuesto a la madre más que algunas horas. En todos se usó el mismo repuesto de lepromina. Como positiva se consideró una reacción de 3 a 5 mm. o mayor a partir de la tercera semana. Se hicieron cuatro pruebas en todo el grupo: septiembre-octubre, 1949; noviembre, 1949; mayo, 1950; y septiembre-octubre, 1950. La quinta comprobación se hizo en marzo de 1951, pero solamente en 32 niños, todos con reacciones de 2+, para ver si podían inducirse respuestas más intensas. La prueba final se hizo en diciembre de 1951, unos dos años y cuarto después de la primera prueba, en los 47 niños todavía accesibles.

En la primera prueba, sólo 22 por ciento resultaron positivos, comparado con 72.7 por ciento observado por Lara hace algunos años en Culión en 110 niños pequeños que no habían sido separados de los padres leprosos. Al repetirse las pruebas, lo mismo que sucedió en la serie de Lara, hubo un aumento gradual en la proporción que reaccionaba, de modo que en la tercera prueba 96 por ciento reseultaron reactores, comparado con 99 por ciento en el grupo de Lara. En la cuarta prueba, reaccionaron todos los niños de la serie actual. Muchos de los niños obtuvieron reactividad y mostraron reacciones intensas, aunque tenían menos de un año de edad. Se observó, según ya han descrito otros, activación de sitios antes negativos o de poca reacción.

Después de la sexta prueba con lepromina, restaban 12 niños cuyas reacciones no habían pasado del grado de 2+. Diez de éstos, que eran negativos a la tuberculina, fueron vacunados con BCG en abril de 1952, y todos los 12 en julio de 1952, con la técnica de la multipunción. Dos meses después, al aplicarse una séptima prueba de la lepromina a estos niños, la reacción no había aumentado a más de 2+ en ninguno de estos niños.

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