EFFECT OF BCG VACCINATION, LEPROMIN TESTING AND NATURAL CAUSES IN INDUCING REACTIVITY TO LEPROMIN AND TO TUBERCULIN [1]

JAMES A. DOULL, M.D., RICARDO S. GUINTO, M.D., AND MARIO C. MABALAY, M.D.
Medical Director, Epidemiologist, and Assistant Epidemiologist, respectively
Leonard Wood Memorial (American Leprosy Foundation)

I. INTRODUCTION

The development of reactivity to lepromin following vaccination with BCG was first reported by Fernandez (5) in 1939. A number of workers have made similar observations with BCG administered intradermally, by scarification, or by the oral route. The finding is of practical importance because reactivity has been interpreted by many leprologists to be indicative of some degree of immunity to leprosy—sufficient at least to protect against the development of the lepromatous type. The observation is also of scientific interest; it is additional evidence that various species of Mycobacterium, in this instance the attenuated bovine strain known as BCG, possess antigens of a group nature that are capable of stimulating the animal body to produce antibodies against other members of the genus, in this case *M. leprae*. For a detailed discussion of this interesting subject reference may be made to a recent review by Souza Campos (16).

A second consideration is that reactivity to lepromin observed on repetition of the test may be due to the stimulus of the first test dose and not to any other possibly antigenic substance that may have been given. In 1940 Lara (11) stated that young children had become Mitsuda positive following injections of lepromin. In 1943 Fernandez (6) reported that, in a high proportion of persons, reactivity to lepromin had followed intradermal injection of a suspension of *M. leprae* or of *M. tuberculosis* killed by heat. In the use of *M. leprae* he was actually performing the lepromin test.

Rodriguez (13) found that dogs may react to lepromin. Wade (personal communication) stated that all dogs tested by him at Culion have shown reactivity to lepromin in the multiple doses used—2 to 12 or 14. The intensity of the reaction, however, was increased by repetition of the test. Feldman *et al.* (3, 4) reported that lepromin-negative dogs became reactive after repeated injections of lepromin. The animals also became reactive to a similar suspension of the rat-leprosy bacillus, and to cultures of a so-called leprosy bacillus and of a mycobacterium from soil, but not to Old Tuberculin (1/100).

A third pertinent and well-established fact is that healthy persons ap-

[1] This study was supported in part by a grant (E-778) from the National Institutes of Health, United States Public Health Service.

parently acquire reactivity to lepromin in some natural manner; that is, without receiving any prior artificial stimulus. In our previous studies (8, 10) in the Philippines, 20 per cent of children under 5 years of age and about two-thirds of school children 7 to 9 years of age showed reactivity of the Mitsuda type. The initial results in young children in the present study are discussed later. These findings, and those of others in many parts of the world, demonstrate not only that reactivity may be frequent in the general population but that in children its frequency may increase rapidly with age.

The suggestion has been made that the late lepromin reaction (Mitsuda) is the result of sensitization caused by the test dose itself. Wade (17) has stated: "It would seem (as noted by Rodriguez) that the elements that are directly concerned in the production of the reaction lesion may not be formed until after the antigen is introduced." At present, this hypothesis cannot be established or disproved; there is no method of determining whether or not these elements are present prior to testing the individual with lepromin.

If sensitization by the test dose is the major factor which causes the Mitsuda lesion to appear two or three weeks later, it is necessary to assume that capacity to respond must increase as children grow older. A simpler hypothesis is that age is not significantly related to capacity to respond and that these elements (or antibodies) occur as a result of mild or subclinical infections with one or another species of Mycobacterium but not necessarily with *M. leprae*. It cannot be denied, however, that the dose of lepromin may play a part in producing or mobilizing these antibodies. This effect would be indistinguishable from that attributed to "natural causes" in this and similar studies.

It is clear, therefore, that factors other than BCG may be operative in causing vaccinated children to become reactive to lepromin. Nevertheless, as far as we are aware, no report has appeared of any study designed to distinguish between the antigenic effect of BCG and that of other causes which may induce reactivity.

The island of Mactan, in the province of Cebu, Philippines, appeared to offer advantages for such a study. This island, which is separated from the city of Cebu by a narrow channel, has two municipalities: Opon with about 40,000 inhabitants, and Cordova with 7,200. Cordova has been the locus of intensive epidemiologic studies of leprosy by the Leonard Wood Memorial and the Department of Health of the Philippines since 1933. Special studies of a supplemental nature have been made in Opon. Leprosy prevalence is about equal in the two municipalities. From 1951 to 1955, inclusive, the Eversley Childs Sanitarium, which serves the area, received 40 bacteriologically positive patients from Opon and 10 from Cordova. Tuberculosis is thought to be highly prevalent, but reliable statistics are not available. Prior to this study no children of preschool age living on Mactan had been given BCG. A relatively small number had been tested

with lepromin in our previous studies, and records of these tests were on file. For greater convenience in the field work the area of this study was limited to Opon, and included the central and more thickly populated section (the poblacion) and eight other contiguous districts, or barrios, the whole covering approximately 17 square miles.

The field work required approximately seven months. Initial lepromin and tuberculin tests were made over a period of two weeks from November 21 to December 7, 1954. Vaccinations and control inoculations were given within the shortest possible space of time, from January 19 to January 29, 1955, 6 to 9 weeks after the initial tests. The final tests were made from April 20 to April 29, 1955, 90 to 100 days, and in most cases 90 to 95 days, after vaccination with BCG and control inoculations, and, on the average, 143 days after the initial testing.

Although the primary purpose was the study of the late lepromin or Mitsuda reaction, advantage was taken of the opportunity to make comparable observations on early lepromin reactivity of the Fernandez type and on the development of hypersensitivity to tuberculin.

II. MATERIALS AND METHODS

Sufficient amounts of the same lots of PPD-S and of lepromin were obtained for the preliminary and later tests. The lepromin was prepared by a modified Mitsuda-Hayashi method and was part of a lot supplied by the Memorial to the South Pacific Commission for testing in the Loyalty Islands. It was used in the usual dosage of 0.1 cc. The PPD-S was made at the Phipps Institute, University of Pennsylvania. It is the preparation adopted as the International Standard. It was used in freshly prepared dilutions containing 0.0001 mgm. (5 TU) in the test dosage of 0.1 cc. In no instance was a dilution used after 48 hours.

Preliminary lepromin tests were made on the left forearm and final tests on the right. Preliminary tuberculin tests were made on the right forearm and final tests on the left. The early (Fernandez) reaction was read at 48 hours; areas of erythema of a diameter of 10 mm. or larger were called positive. The late (Mitsuda) lepromin reaction was read on the 21st or 22nd day but, because it has been found that a few reactions become evident later, final readings were made at 6-8 weeks. Nodules with a diameter of 5 mm. or more were regarded as positive. Tuberculin test readings were made at 48 hours and graded as follows: 1+, erythema with definite edema 5-10 mm. in diameter; 2+, erythema with edema 11-20 mm.; and 3+, erythema with edema exceeding 20 mm.

Fresh BCG was obtained from the Alabang BCG Laboratory of the Department of Health of the Philippines, was refrigerated until the day of use, and was used within 11 days. It was given in a single intradermal injection of 0.1 cc. containing 0.1 mgm. of BCG. Lyophilized BCG was obtained from the Tice Research Laboratory, Chicago. Fresh dilutions were made daily as required and it was given intradermally in a single injection of 0.1 cc. containing 0.12-0.16 mgm. of BCG. It was considered highly desirable to study the effectiveness of this product in inducing positivity to lepromin because of its superior keeping qualities. Diphtheria toxoid was obtained from the National Drug Company and injected subcutaneously in a dosage of 0.5 cc.[2] Saline injections were made intradermally in a dosage of 0.1 cc.

---

[2] Daudén Valls *et al.* (2) have reported that vaccines other than BCG induced lepromin positivity.

In giving the lepromin and tuberculin tests and BCG vaccinations great care was taken to produce a definite wheal at least 8 mm. in diameter. In a few instances this was achieved with difficulty on account of thinness of the skin in infants and their tendency to struggle during the injections.

For initial tests, vaccinations and retests, the staff was divided into three teams: R.S.G. and assistant; M.C.M. and assistant; and Mr. Roman Ponce and assistant. All tuberculin and lepromin readings were made by two of us (R.S.G. and M.C.M.) in approximately equal proportions. The first 200 initial lepromin and tuberculin readings were read by R.S.G. and M.C.M. working together and, as far as was practicable, initial tests and retests in the same child were measured by the same examiner. Before engaging in the present study the leaders of these teams, working together, had made several thousand lepromin and tuberculin tests in the course of a large-scale program of BCG vaccination of school children on Mactan Island, for which they were responsible.

Selection of children.—A total of 550 apparently healthy children between the ages of 6 and 35 months, living in their homes, were selected. None was known to have lived in household association with a patient suffering from leprosy.

To provide a control group that would not be subject to the possible stimulus of the lepromin test, but for which a base line of reactivity could be estimated, an artificial arrangement of 110 sets of 5 children each was set up, the sets being numbered consecutively as they would be encountered in the field work. For each set, four white marbles and a red one were shaken well and dropped from a container in succession, the order of the red one being recorded. This might have been No. 2 in the first set, No. 4 in the second, etc. The children represented by the red marbles constituted the *basic controls*. Presumably they were representative of the whole in all respects, including potential reactivity to lepromin.

All children were then tested with PPD-S, and all *except the basic controls* also with lepromin. Nothing more was done at this time to the basic controls or to those children of the remaining four-fifths who were positive to PPD-S or showed lepromin reactivity of the Mitsuda type. The remaining children, negative to both tests were arranged into quartets on a geographical basis, as they would be encountered in systematic visiting of their homes. Each child of each quartet was then assigned to an inoculation subgroup from a listing prepared from a book of random numbers. Children of Subgroup A were given fresh BCG; those of B, lyophilized BCG; those of C, diphtheria toxoid; and those of D, saline. Ninety to 100 days after these inoculations were given, and on the average 143 days after the initial tests, all available children were tested with lepromin and PPD-S, including the basic controls and those originally positive to either lepromin or PPD-S.

Children lost from the study.—Six children died during the course of the study, 40 removed from Mactan or were temporarily absent, and in 21 instances the parents withdrew their consent. Thus, a total of 67 were dropped and 483 completed all required inoculations and tests. As the groups were constituted at the end, however, they were remarkably alike as regards distribution throughout the nine districts of the community (Appendix, Table A). The age distribution of the basic controls showed a slightly lower proportion in the age group 2 to 3 years, and a compensatory higher proportion under 1 year, in comparison with the total of all other children completing the study. The children of the four inoculation subgroups were very close to one another in age distribution (Appendix, Table B).

III. RESULTS OF INITIAL TESTS

For the 483 children who completed the study, the results of the initial Mitsuda and PPD-S tests are shown in Table 1. It will be noticed that 92, or 23.2 per cent, of the 396 tested children showed the Mitsuda type of

reactivity to lepromin on initial testing. Of 81 children 6 to 11 months of age who were tested, 4.9 per cent were positive; of 145 one year of age, 17.9 per cent, and of 170 two years of age, 36.5 per cent.

TABLE 1.—*Results of preliminary lepromin and tuberculin tests of the 483 children who completed the study.*

Result	Number of children	
Positive to both tests	5	
Positive to lepromin only	87	
Positive to PPD-S only	4	
Negative to both tests	300	396
Basic controls		87
TOTAL		483

The early lepromin (Fernandez) reaction was positive in only 13 children of the 396 tested, or 3.3 per cent. In 7 of these 13 the initial Mitsuda result was also positive. In 6 the initial Mitsuda and PPD-S tests were negative, and these were included in the inoculation subgroups as follows: 2 were allotted to Subgroup A, 1 to B, and 3 to D. Among these 6 children there were 3 with small papules below the size accepted as indicating reactivity of the Mitsuda type, and 3 with no palpable nodules whatever.

The proportion reacting to PPD-S was also very small. Only 9 reactors were found among 396 children, or 2.3 per cent. One additional reactor was found in the basic controls.

IV. RETESTS OF CHILDREN ORIGINALLY NEGATIVE WHO WERE GIVEN BCG, OR DIPHTHERIA TOXOID OR SALINE

The results of retesting of children of the inoculation subgroups are shown in Table 2. There is seen no evidence of material difference in the percentages positive on final test as between Subgroup A, which was given fresh BCG, and Subgroup B, which received the lyophilized product. These products were about equally efficacious in causing reactivity to lepromin and to tuberculin, and the statistics for these subgroups have therefore been combined in the remainder of the analysis. Similarly, Subgroups C and D show no significant difference. An injection of saline can have no conceivable antigenic effect, and it was concluded that diphtheria toxoid has likewise no capacity to produce reactivity to the antigens under study. Consequently, Subgroups C and D were also studied as a single unit.

The percentages of the combinations of subgroups becoming reactive, with their standard errors, are shown in Table 3. Although the proportion acquiring Mitsuda reactivity (71.2%) is not so great as that which has

been reported by others, it is nevertheless substantial. The much smaller increase in the proportion showing the Fernandez reaction (32.7%) suggests that BCG is much less potent in this respect. The proportion acquiring hypersensitivity to PPD-S, 59.0 per cent, is lower than was expected

TABLE 2.—*Numbers and percentages of children becoming reactive to lepromin and to PPD-S, according to the nature of the inoculum.*

Subgroup	Inoculum	No. of children[a]	Positive on final test					
			Mitsuda		Fernandez[a]		PPD-S	
			No.	Per cent	No.	Per cent	No.	Per cent
A	Fresh BCG	76	53	69.7	20	27.0	43	56.6
B	Lyophilized BCG	80	58	72.5	30	38.0	49	61.3
C	Diphtheria toxoid	71	17	23.9	6	8.5	5	7.0
D	Saline	73	22	30.1	9	12.9	5	6.8
TOTAL, all subgroups		300	150	50.0	65	22.1	102	34.0

a Six children showing an initial Fernandez reaction were excluded in calculating the percentages on retesting.

even with the low test dose of 5 TU. Aronson *et al.* (1), however, using the same test dosage (0.0001 mgm.) of standard PPD-S, found on retesting after 2 to 3 months that the proportions of conversions varied from 42.9 to 68.4 per cent.

TABLE 3.—*Percentages of originally negative children reacting to lepromin and to PPD-S 90 to 100 days after inoculation; for those receiving BCG (A and B) and for those given toxoid or saline (C and D).*

Reaction	BCG Subgroups A & B (156) Positive on retest		Toxoid or saline Subgroups C & D (144) Positive on retest		Difference (A & B)–(C & D)	
	Per cent	S.E.	Per cent	S.E.	Per cent	S.E.
Mitsuda	71.2	3.6	27.1	3.7	44.1	5.2
Fernandez	32.7	3.8	10.6	2.6	22.1	4.6
PPD-S	59.0	3.9	6.9	2.1	52.1	4.5

The gain in reactivity among the children of the toxoid and saline subgroups (C and D) is of great interest. The responsible factors, in theory, were some unknown natural cause or causes and the original test-

ng with lepromin.[3] At this point there is no basis on which to separate these factors. It is clear, however, that taken together they constituted an important element in causing reactivity to lepromin, and an appreciable but much less important one in causing hypersensitivity to PPD-S. It will be observed that the proportions exhibiting reactivity of all three types, Mitsuda, Fernandez and PPD-S, are large in relation to their standard errors. When the effect of these factors is removed, by subtraction of the results for the toxoid and saline subgroups from those for the BCG subgroups, the part attributable to BCG is reduced to the following percentages: for Mitsuda reactivity, 44.1; for Fernandez, 22.1; and for PPD-S, 52.1.

It is possible that the proportion of children becoming hypersensitive to PPD-S was lowered by exclusion from the inoculated subgroups of 87 children who were positive to lepromin but negative to PPD-S (Table 1). These children might have responded better to BCG than those who were negative to lepromin. The following argument shows that the results would not be greatly changed if these children had been included among the negatives and distributed proportionally among the four inoculation subgroups. To allow the maximum possible effect of BCG, suppose that all such children given BCG had become positive, and that 21.8 per cent of those given toxoid or saline had done likewise (as shown in Table 6). Under these assumptions it is found that the increase attributable to BCG would be raised from 52.1 per cent to an estimated 58.0 per cent. There is, however, no evidence of which we are aware that BCG is more effective in producing hypersensitivity to tuberculin lepromin-positive children than in others.

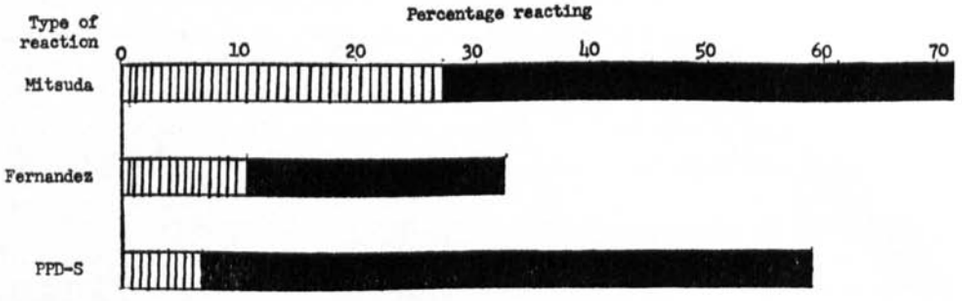
The increases in reactivity of the three types are illustrated in Text-fig. 1.

V. ESTIMATION OF RELATIVE IMPORTANCE OF NATURAL CAUSES, LEPROMIN TESTING AND BCG VACCINATION

Having established the fact that causes other than BCG vaccination played a substantial part in inducing reactivity to lepromin and to tuberculin in the young children enrolled in this study, an attempt was next made to estimate the respective roles played by natural causes, lepromin testing, and BCG. It is obviously desirable to obtain estimates that will apply to an unselected sample of the children (i.e. one like the basic controls) rather than to groups of children known to be initially negative to PPD-S and lepromin, like those in Groups A to D. Consequently, in the

[3] It is assumed throughout this study that the PPD-S had no antigenic effect. The low proportion of reactors, 5.7 per cent, in the Basic Control Group at the end of the study supports this opinion. Seibert and Glenn (15) found that repeated injections of 10 times the regular second dose used in the skin test in man did not lead to significant sensitization in guinea-pigs. They did find, however, that PPD-S produced precipitins in rabbits injected repeatedly, and also induced the Arthus phenomenon in these animals.

subsequent calculations the results for Groups A and B, and for Groups C and D, will be adjusted by adding to these groups the results for an appropriate quota of children who were initially positive to lepromin, PPD-S, or both.



TEXT-FIG. 1. Percentages of children, originally negative to lepromin and PPD-S, reacting to these antigens 90-100 days after inoculation. Full lengths of bars: Subgroups A and B, given BCG. Open portions: Subgroups C and D, given diphtheria toxoid or saline. Solid portions: Effect of BCG.

The computation is easily understood by considering three groups of children, comparable to each other in all respects except as regards differences attributable to the inoculations and tests that they were given. These groups are:

(1) ABR. This group consists of the original Subgroups A and B, given BCG, together with their appropriate quota of children (R) who were positive, on the initial testing, to lepromin (Mitsuda), PPD-S, or both. The children of ABR presumably were exposed to whatever natural stimuli were operative in the area, as well as to the effect of the lepromin test and of the BCG vaccination.

(2) CDR. This group consists of the original Subgroups C and D, reconstituted to include their appropriate quota of positives (R). The children of CDR presumably were exposed to natural stimuli as well as to the effect of the lepromin test. This group did not receive BCG.

(3) Basic Controls. This group, as noted above, was composed of children drawn at random from the total before any tests were applied. They were not given a lepromin test or any other inoculations, except a test dose of PPD-S. If they had been tested with lepromin at the outset, presumably they would have shown the same proportion of reactors as the other four-fifths of the children who were tested; that is, 23.2 per cent for reactivity of the Mitsuda type, and 3.3 per cent for that of the Fernandez type. Although only one child of the basic controls was positive to PPD-S at the outset, the same proportion that was observed in the other four-fifths of the children (2.3%) is assumed to apply, as for the Fernandez and Mitsuda reactions.

If these assumptions are accepted, the proportions of ABR and of CDR that would show reactivity of the various types at the end of the

study can readily be obtained for comparison with one another and with the actual results observed in the Basic Control Group. The change in the basic controls can be estimated by comparison of the actual results with the estimated status at the outset. When these estimates are made the antigenic value of each of the respective factors will be as follows:

For BCG.—The excess, in the estimated proportions reacting at the end of the study, of ABR over CDR. These groups differed only in that ABR received BCG, while CDR received toxoid or saline.

For lepromin.—The excess of the estimated proportion of CDR reacting at the end of the study over the actual proportion for the basic controls. These groups differed only in that CDR received initial and final lepromin tests and injections of toxoid or saline, whereas the basic controls received only the final lepromin test.

For natural causes.—The excess of the actual proportion of the basic controls reacting at the end over the estimated proportion at the beginning of the study. As noted in the Introduction, any reactivity caused by the final test is of necessity included in this increase.

A. INFLUENCE OF EACH FACTOR ON REACTIVITY OF THE MITSUDA TYPE

The basis for calculation of the proportions of ABR and of CDR expected to show the Mitsuda type of reactivity at the end of the study is given in Table 4.

TABLE 4.—*Numbers and proportions of children showing the late (Mitsuda) lepromin reaction on final testing, classified according to the results of initial lepromin test and inoculation subgroup; exclusive of basic controls.*

Original lepromin classification (Mitsuda)	Children		Positive on final test (Mitsuda)	
	No.	Proportion of total	No.	Proportion of original class
Positive: (Mitsuda)	92	.232	81	.880[a]
Negative:				
Positive to PPD-S	4	.010	4	1.000[b]
A and B (BCG)	156	.757	111	.712
C and D (toxoid or saline)	144		39	.271

a Of 92 children originally positive to lepromin (Mitsuda), 11 were graded as negative on the final test. In all of these, there were small nodules present: 3 x 4 mm. in 2, and 4 x 4 mm. in 9.

b Although there were only 4 in this group, it is of interest that all because Mitsuda-positive.

In computing the estimated percentages of children showing lepromin reactivity of the Mitsuda type at the end of the study, each of the reconstituted groups ABR and CDR is assumed to have been composed, at the

outset, of the same proportions of children who were (*a*) positive to lepromin, or to both lepromin and PPD-S, that is 0.232 or 23.2 per cent; (*b*) negative to lepromin but positive to PPD-S, 1.0 per cent; and (*c*) negative to both, 75.7 per cent. These are shown in Table 4 as proportions of the total. The originally reactive children (R), prorated respectively to ABR and CDR, are likewise assumed to have shown at the end the average frequency of reactivity for all positive children, namely, 88.0 per cent for those positive to lepromin or to both lepromin and PPD-S, and 100.0 per cent for those positive only to PPD-S. For children who were negative at the outset, the proportions becoming positive and used in the computations of Table 5 are those shown for the Mitsuda reaction in Table 3, that is, 71.2 per cent for those of ABR, the BCG group, and 27.1 per cent for those of CDR, the toxoid and saline groups.

By applying the specific reactivity rates for each component part of each group, ABR and CDR, to the proportion which this part was of the total at the outset, products are obtained which represent the amount that each component would contribute to the final rate for the group. The sum of these products is the weighted estimate of the proportion reacting at the end of the study.

For ABR: (.232) (.880) + (.010) (1.000) + (.757) (.712) = .753, or 75.3 per cent

For CDR: (.232) (.880) + (.010) (1.000) + (.757) (.271) = .419, or 41.9 per cent

Effect of BCG.—The estimated proportion of children developing reactivity of the Mitsuda type because they were given BCG is the percentage for ABR minus that for CDR. This is 75.3 per cent minus 41.9 per cent, or 33.4 per cent, with a standard error of 4.1 per cent. It may be emphasized that this is a proportion of the total population; that is, of all children including the original positives.

Effect of lepromin.—At the end of the study, when the lepromin test was applied to the children of the Basic Control Group, 34.7 per cent [4] showed reactivity of the Mitsuda type. The estimated percentage developing reactivity of the Mitsuda type because they received a lepromin test is, therefore, 41.9 per cent for CDR minus 34.7 per cent for the basic controls, or 7.2 per cent. This difference is small in relation to its standard error (5.9%) and cannot be stressed, other than to say that it is in the direction expected on the basis of the results reported by Lara (11) and Fernandez (6).

Effect of natural causes.—This is taken as the difference between the per cent of the basic controls reacting at the end, or 34.7 per cent, and the estimated proportion at the beginning, 23.2 per cent.The apparent gain is

[4] See Appendix, Table C. The actual percentage was 32.1. Adjustment for slight differences in age constitution between basic controls and other children raises this to 34.7. In the case of the Fernandez and PPD-S reactions the actual results in the basic controls are taken because adjustment makes no difference in the rates.

thus 11.5 per cent. It is remarkable that such a gain occurred in 143 days; that is, in less than five months. The standard error of this difference is 5.5, which indicates a level of significance a little better than .05.

B. INFLUENCE ON LEPROMIN REACTIVITY OF THE FERNANDEZ TYPE

In a similar manner estimates have been made of the percentages expected to show the early (Fernandez) type of reactivity to lepromin for Groups ABR and CDR. The data for these estimates are given in Table 5.

TABLE 5.—*Numbers and proportions of children showing an early (Fernandez) lepromin reaction on final testing, classified according to presence or absence of the early reaction on initial testing and inoculation subgroup; exclusive of basic controls.*

Original classification (Fernandez)	Children		Positive on final test (Fernandez)	
	No.	Proportion of total	No.	Proportion of original class
Positive: Mitsuda or PPD-S		.018		
Positive Fernandez	7	.225	6	.857
Negative Fernandez	89[a]		39	.438
Negative: Mitsuda and PPD-S		.015		
Positive Fernandez A & B	3		3	1.000
Positive Fernandez C & D	3		0	—
Negative Fernandez A & B	153	.742	50	.327
Negative Fernandez C & D	141		15	.106

a Of these 89 children, 85 were Mitsuda positive on initial testing; 4 were negative but positive to PPD-S. Of the 85, 39 or 45.9 per cent became Fernandez positive, a remarkably high proportion in comparison with that for the negatives of Subgroups C and D (10.6%). None of the 4 Mitsuda negatives became Fernandez positive.

The proportions expected to show reactivity of the Fernandez type at the end would be as follows:

For ABR: (.018) (.857) + (.225) (.438) + (.015) (1.000) + (.742) (.327) = .372, or 37.2 per cent

For CDR: (.018) (.857) + (.225) (.438) + (.015) (0) + (.742) (.106) = .193, or 19.3 per cent.

On the basis of these figures the weights to be given to BCG, to lepromin, and to natural causes, respectively, as factors in inducing lepromin reactivity of the Fernandez type, are as follows:

Effect of BCG.—The estimated proportion of children developing reactivity of the Fernandez type because they were given BCG is 37.2 per cent minus 19.3 per cent, or 17.9 per cent, with a standard error of 3.9 per cent.

Effect of lepromin.—Again the Basic Control Group provides the third figure necessary to the calculation. At the end of the study the proportion of the basic controls giving a Fernandez reaction was 11.5 per cent. The estimated proportion of Group CDR that would show the Fernandez type of reactivity at the end of the study is 19.3 per cent. The difference, 7.8 per cent, is our estimate of the proportion becoming reactive because of the antigenicity of the lepromin test itself. In this case the standard error is 4.1 per cent, making the level of significance a little less than .05.

Effect of natural causes.—To estimate the effect of the third factor, it is necessary, as in the case of the Mitsuda reaction, to assume that if the Basic Control Group had been lepromin tested at the outset the proportion showing reactivity of the Fernandez type would have been the same as for the children who were tested; namely, 3.3 per cent. At the end of the study, as noted above, 11.5 per cent of the basic controls reacted. An apparent gain of 8.2 per cent took place which is attributed to natural causes. The standard error of this gain is 3.5 per cent, and the significance level is about .02.

As indicated in Table 5, the children who at the outset were positive either to the Mitsuda or PPD-S test, but negative to the Fernandez, contributed heavily to the estimated increase. It is impossible, from available data, to tell whether these conversions were due to natural causes, the lepromin test, or both. For ABR, they contributed 26.5 per cent of the estimated proportion reacting at the end: [(.225) (.438) ÷ (.372) = .265]; and for CDR, 51.1 per cent: [(.225) (.438) ÷ (.193) = .511]. Assuming that they were present in the same proportion (22.5%) in the basic controls, and that natural causes were responsible, in part at least, such children may have contributed substantially to the final rate for that group.

C. INFLUENCE OF HYPERSENSITIVITY TO PPD-S

Estimates of the proportions of Groups ABR and CDR expected to be positive at the end were made in the same manner for tuberculin as for each type of reactivity to lepromin. The basic data necessary for the calculation are given in Table 6.

The proportions expected to be hypersensitive at the end are:

For ABR: (.023) (.778) + (.220) (.218) + (.757) (.590) = .513, or 51.3 per cent

For CDR: (.023) (.778) + (.220) (.218) + (.757) (.069) = .118, or 11.8 per cent

On the basis of these estimates, as before, the weights to be given to BCG, lepromin, and natural causes are as follows:

Effect of BCG.—This is 51.3 per cent for ABR minus 11.8 per cent for CDR, or 39.5 per cent. It may be repeated that the test dose of PPD-S was small, and that possibly the excluded children who were positive to lepromin but negative to PPD-S at the beginning might have proved better reactors than those who were negative to both lepromin and PPD-S.

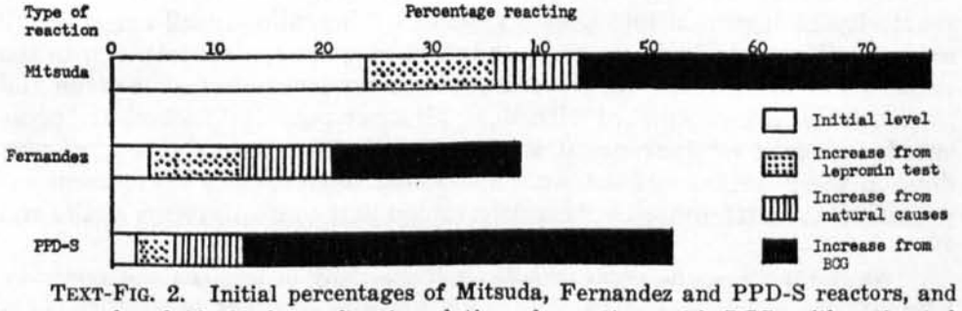
TABLE 6.—*Numbers and proportions of children reacting to PPD-S on final testing, classified according to results of initial tests with PPD-S; exclusive of basic controls.*

Original classification (PPD-S)	Children		Positive on final test (PPD-S)	
	No.	Proportion of total	No.	Proportion of original class
Positive:	9	.023	7	.778
Negative:				
Positive to lepromin (Mitsuda)	87	.220	19	.218[a]
A and B (BCG)	156	.757	92	.590
C and D (toxoid or saline)	144		10	.069

a Attention is drawn to the apparently greater tendency of the negatives who were Mitsuda positive than of the negatives of Subgroups C and D to become "spontaneously" positive to PPD-S.

Effect of lepromin.—At the end of the study only 5.7 per cent of the children of the Basic Control Group reacted to PPD-S. With respect to hypersensitivity to PPD-S, therefore, the estimate (11.8%) for the reconstructed Group CDR exceeds the actual figure for the basic controls by 6.1 per cent. The standard error of this difference is 3.1 per cent, and its level of significance about .05. This brings to mind the experiments of Melsom (12), who found that guinea-pigs, in the skin of which nodules from leprosy patients were implanted, became reactive to tuberculin; and of Fernandez and Cabanillas (7), who reported that 7 of 18 children became tuberculin positive six or seven weeks after receiving 1 to 3 intradermal injections of lepromin, but that the resulting allergy was weak and of short duration.

Effect of natural causes.—The proportion of the basic controls reacting to PPD-S at the outset is taken as 2.3, the average for the other four-fifths of the children. During the interval between the tests there occurred



TEXT-FIG. 2. Initial percentages of Mitsuda, Fernandez and PPD-S reactors, and increases found 90-100 days after inoculation of negatives with BCG, with estimated percentages attributable to natural causes, lepromin and BCG.

an apparent increase of 3.4 per cent; that is, to a terminal point of 5.7 per cent. This increase is small in relation to its standard error of 2.6 per cent.

The estimated percentages of children gaining reactivity of each type from each cause are illustrated in Text-fig. 2 and shown, with their standard errors, in the Appendix, Tables C, D, and E.

VI. DISCUSSION

The existence of reactivity to lepromin,[5] of unknown origin, in a variable proportion of persons is a well-established fact. When BCG is given without a preliminary lepromin test, this base line of reactivity is unknown. If there is an unvaccinated but otherwise comparable control group, the difference between the proportions of each group reacting at the end of observation will obviously indicate the effect of BCG. The final level that is reached in the control group will represent the initial proportion of reactors (base line), provided that all have remained reactors, whatever increase occurred from natural causes during the period of observation, and the effect, if any, of the lepromin test itself. The final level in the BCG group will represent the influence of all these factors, supplemented by the effect of vaccination with BCG.

If the rate of natural increase is high and the period of observation sufficiently long, any effect that the BCG has may be obliterated; that is, the frequency of reactivity in the control group will gradually approach that in the vaccinated. The latter, however, would gain not only an immediate but a permanent advantage in the theoretical case in which BCG has the capacity to cause reactivity in individuals not affected by natural causes. In the hypothesis of Rotberg (14) persons are assumed to exist who lack a quality that he calls Factor N, because of which they are *unresponsive* to the stimulus of natural causes (or of BCG.) If such persons exist on Mactan they must be very uncommon. In our studies (9) 95 per cent of persons of 20 years and over, not known to have lived in household association with leprosy, showed lepromin reactivity of the Mitsuda type.

It is not so well established that the lepromin test itself may induce reactivity to lepromin and possibly also to tuberculin. In all cases where an initial lepromin test is given the tested persons are subject also to the influence of natural causes. When a preliminary lepromin test is given and the negatives are vaccinated with BCG, all three potential factors are present. If one part of these negatives is selected as a control group, as was done in the selection of the toxoid and saline subgroups of the present experiment, the difference between vaccinated and control groups at the end

---

[5] As stated above, the usual definitions of reactivity to lepromin and tuberculin were accepted in this study. The children are classified according to diameter of the Mitsuda nodule in the Appendix, Table F, and of the edema of the tuberculin reaction in Table G.

of observation will indicate the effect of BCG. The difference between initial and final levels in the control group, however, will reflect the increase due to stimulation of natural causes plus that of the lepromin test. The difference between vaccinated and control groups will again be expected to diminish as natural causes continue to operate, until ultimately it disappears, unless BCG has the capacity to convert individuals resistant to the combined influence of the lepromin test and of natural causes.

In the first example, in which there is no preliminary test, there is no way of determining the increase due to natural causes in a given period of time. In the second case, in which the base line of reactivity has been obtained, the total effect of the lepromin test and natural causes can be determined, but these two factors cannot be distinguished one from the other.

In the present study, the control subgroups of original negatives (C and D) increased from their initial base lines in a period of 90 to 100 days following inoculation with saline or toxoid, and of 143 days from the original testing, 27.1 per cent in frequency of reactivity of the Mitsuda type, 10.6 per cent for that of the Fernandez type, and 6.9 per cent for hypersensitivity to tuberculin. The combined effect of the lepromin test and of natural causes is therefore of importance in all three types of reactivity, but especially so in the Mitsuda type. It must be taken into account in that form of experiment in which individuals are tested with lepromin and with tuberculin and then, if negative to both, vaccinated with BCG.

If the differences between vaccinated and control groups in the present experiment represent the correct picture, the effectiveness of BCG is much less than is commonly supposed. For the period of time mentioned, and for children of 6 to 35 months of age living in a specified environment in the Philippines, it was only 44.1 per cent for reactivity of the Mitsuda type, 22.1 per cent for that of the Fernandez type, and 52.1 per cent for hypersensitivity to PPD-S at the 5 TU level.

To estimate the respective roles of each of the three factors under consideration the device was adopted of establishing three groups of children comparable to each other at the outset in all respects as far as known. In one of these, the basic controls, there was no artificial stimulus given; in the second, the children received a lepromin test plus diphtheria toxoid or saline; and in the third, a lepromin test and BCG. Measurement of the lepromin and tuberculin status of these three groups may not have given precise and absolute values for each of the factors, but it certainly has yielded at least an approximation to their antigenic potentials. To obtain more reliable figures as to the effectiveness of the lepromin test itself, larger numbers of children would be required, especially in the Basic Control Group.

The most interesting question that arises relates to the natural cause or causes giving rise to reactivity to lepromin. That reactivity can occur

in the absence of specific infection with *M. leprae* seems beyond question. Reactors are frequent in areas where leprosy is very rare, and in endemic areas their frequency is not significantly and positively associated with prevalence of the disease. In the area of the present study the chance of contact with an infectious case of leprosy undoubtedy exists, but it is small even for those whose movements throughout the area are uninhibited. For children of 3 years of age and younger it must be much smaller; in fact, it is almost inconceivable that such children would come into effective contact with leprosy at the rate of 2 per cent per month.

Much has been written on the positive association between tuberculization and reactivity to lepromin, and there is no doubt that the two are frequently highly correlated, especially when tuberculization is defined as reactivity to high doses of tuberculin. This does not necessarily mean a causal relationship; it indicates only that reactivity to some component of tuberculin is present. The origin of this reactivity probably lies in prior infection with one or another species of Mycobacterium but not necessarily with *M. tuberculosis.*

This type of association was observed in the present study. At the end, 43.7 per cent of all Mitsuda positives reacted to PPD-S (5 TU), but only 8.2 per cent of Mitsuda negatives. A similar relationship was observed between the Fernandez and the tuberculin reactions. At the end, 63.4 per cent of all Fernandez positives reacted to PPD-S, but only 15.3 per cent of Fernandez negatives. The correlation is obviously high, but more than one-half of the Mitsuda reactors and more than one-third of the Fernandez positives apparently owed their lepromin reactivity to some cause other than infection with the tubercle bacillus.

If one were to seek a population free from tuberculosis in which to study the results of the lepromin test, it is unlikely that one would be found more satisfactory in this respect than the young children included in this study. If failure to react to small doses of tuberculin is acceptable evidence of freedom from infection, then at the beginning only 2.3 per cent of the children had been infected with *M. tuberculosis.* Nevertheless, 23.2 per cent showed reactivity of the Mitsuda type. At the end of the study, of the children in the Basic Control Group, removed from the effect of the initial lepromin test and of BCG, only 5.7 per cent reacted to PPD-S as compared to 34.7 per cent who showed lepromin reactivity of the Mitsuda type.

In our opinion, therefore, prior infection either with *M. leprae* or with *M. tuberculosis* does not offer an adequate explanation for the occurrence of natural reactivity to lepromin in this area. Here we are dealing with a phenomenon that is of unknown origin.

VI. SUMMARY

1. A report is made of an effort to determine the relative importance of natural causes, initial lepromin testing, and vaccination with BCG in

producing reactivity to lepromin and to tuberculin. The possibility is admitted that the final lepromin test may itself cause reactivity of the Mitsuda type but there is no available means of separating this effect from that of natural causes.

2. The subjects were 550 apparently healthy children, 6 months to 35 months of age, having no known contact with leprosy, living in their homes on Mactan Island, Cebu, Philippines, of whom 483 completed all requirements of the study.

3. A random sample of one-fifth constituted a basic control group, given initially only a tuberculin test (PPD-S, 0.0001 mgm.). The remainder were tested with lepromin and PPD-S, and those negative to both were divided at random into four subgroups. Two were vaccinated intradermally with BCG, one with a fresh preparation and the other with a lyophilized one. The other subgroups were given diphtheria toxoid and saline, respectively. Ninety to 100 days after the BCG and other inoculations, and, on the average, 143 days after the initial tests, all children including the basic controls were tested with lepromin and PPD-S of the same lots, and in the same dosages, as those used at the outset.

4. The two BCG preparations gave almost the same results, and this was true also of the toxoid and saline. Combining these pairs of subgroups, the percentages of *negatives* becoming reactive were: For BCG, 71.2 per cent for lepromin reactivity of the Mitsuda type, 32.7 per cent for that of the Fernandez type, and 59.0 per cent for hypersensitivity to PPD-S; for toxoid and saline, 27.1 per cent for Mitsuda, 10.6 per cent for Fernandez, and 6.9 per cent for PPD-S. The conversions in the toxoid and saline subgroups are attributed to the combined effect of natural causes and the lepromin test. Subtracting these percentages from those for the BCG subgroups, on the assumption that the vaccinated children were exposed, to the same extent as the others, to natural causes and that they reacted equally to whatever effect the lepromin may have had, the percentages credited to BCG are reduced to 44.1 for Mitsuda, 22.1 for Fernandez, and 52.1 for PPD-S.

5. Estimates have been made of the effect of natural causes, lepromin and BCG, on the assumption that there were originally three comparable groups of children. These were the basic controls; the negatives given toxoid or saline, with their appropriate quota of children who were positive on the initial testing; and the negatives given BCG, with their quota of original positives. The basic controls presumably were affected only by natural causes, the reconstructed toxoid and saline group by natural causes and the lepromin test, and the reconstructed BCG group by natural causes, the lepromin test, and BCG. The original status of the basic controls was taken as that of the other four-fifths of the children; the final status was known. The final position of the reconstructed groups was computed by taking into account the final status of original positives and negatives belonging to each group, and the proportion that each of these classes was

of the total group. The estimates of the proportions of all children becoming reactive from each cause, as derived from these calculations, are:

From natural causes.—Mitsuda, 11.5 per cent; Fernandez, 8.2 per cent; and PPD-S, 3.4 per cent. For PPD-S the gain is small in relation to its standard error. For the Mitsuda test, it is twice its standard error, representing a significance level of about .05. For the Fernandez, the gain is 2.3 times its standard error, or a significance level of about .02.

From the lepromin test.—Mitsuda, 7.2 per cent; Fernandez, 7.8 per cent; and PPD-S, 6.1 per cent. In the case of PPD-S the difference is twice its standard error, which suggests that lepromin caused a small proportion of the children to become hypersensitive. Further study of this question would be of interest. In the other cases the apparent gain is suggestive only.

From BCG vaccination.—Mitsuda, 33.4 per cent; Fernandez, 17.9 per cent; and PPD-S, 39.5 per cent. All of these increases are highly significant in the statistical sense.

6. The conclusion is reached that increase in frequency of reactivity to lepromin in persons vaccinated with BCG cannot be attributed to the vaccination alone. If no preliminary test is given, natural causes will contribute; if a preliminary lepromin test is given, both natural causes and the test. Admittedly, as far as the lepromin test is concerned the present study is suggestive only. More precise and reliable results would have been obtained if the groups, and especially that designated as the Basic Control Group, had been larger. The evidence presented, however, is in agreement with other reports in this respect.

7. The origin of natural reactivity to lepromin is a matter of great interest. In the subjects of this study, the increase that was observed could not have been due to infection with *M. leprae*. Infection with the tubercle bacillus is also an inadequate explanation, if failure to react to PPD-S in the dosage used signifies freedom from infection. Only 2.3 per cent reacted to PPD-S at the outset, as compared to 23.2 per cent who showed reactivity of the Mitsuda type. At the end of the study, only 5.7 per cent of the basic controls reacted to PPD-S, but 34.7 per cent were Mitsuda-positive.

ACKNOWLEDGMENTS

Grateful acknowledgment is made of assistance given by Mr. Roman L. Ponce and Miss Dionisia Potot of the Leonard Wood Memorial in the field work. We are also indebted to Dr. H. W. Wade of the Memorial for the lepromin; to Dr. Esmond R. Long and Dr. Florence B. Seibert of the Henry Phipps Institute, University of Pennsylvania, for the PPD-S; to Dr. J. S. Sumpaico of the Alabang BCG Laboratory of the Philippines Department of Health for the fresh BCG; and to Dr. Sol Rosenthal of the Tice Research Laboratory, Chicago, for the lyophilized product. Prof. William G. Cochran, of the Johns Hopkins University, generously advised us in the statistical procedures, and Dr. Morton Kramer, of the National Institutes of Health, prepared the listing of random numbers for assignment of children to inoculation groups.

RESUMEN

1. Preséntase la reseña de un esfuerzo encaminado a determinar la relativa importancia de las causas naturales, la comprobación con lepromina y la vacunación con BCG en la producción de reactividad a la lepromina y la tuberculina.

2. Los sujetos fueron 550 miños aparentemente sanos, de 6 a 35 meses de edad, que no habían tenido contacto conocido con la lepra y que vivian en sus hogares en la Isla de Mactan, Cebú, Filipinas, 483 de los cuales cumplieron todas las condiciones del estudio.

3. Una muestra tomada al azar, compuesta de un quinto del grupo, constituyó un grupo testigo básico, que recibió al principio únicamente una prueba con tuberculina (PPD-S, 0.0001 mgm.) Los demás fueron comprobados con lepromina y PPD, dividiéndose al azar los negativos a ambos en cuatro subgrupos. Dos grupos fueron vacunados intradérmicamente con BCG, uno con una preparación reciente y el otro con una liofilizada. Los otros grupos recibieron toxoide diftérico y solución salina, respectivamente. De 90 a 100 días después de las inoculaciones de BCG y otras, y, en conjunto, 143 días después de las pruebas iniciales, todos los niños, los testigos básicos inclusive, fueron comprobados con lepromina y PPD-S de los mismos lotes, y a las mismas dosis, que los usados al principio.

4. Las dos preparaciones de BCG dieron resultados casi idénticos, y esto fué también un hecho para el toxoide y la solución salina. Combinando estos pares de subgrupos, los porcentajes de *negativos* que se volvieron reactores fueron: para BCG, 71.2 por ciento para reactividad a la lepromina del tipo Mitsuda, 32.7 por ciento para la del tipo Fernández y 59.0 por ciento para hipersensibilidad a PPD-S; para el toxoide y la solución salina, 27.1 por ciento para la Mitsuda, 10.6 por ciento para la Fernández y 6.9 por ciento para PPD-S. Los virajes observados en los subgrupos del toxoide y de la solución salina se atribuyen al efecto combinado de las causas naturales y la prueba de la lepromina. Restando estos porcentajes de los de los subgrupos de BCG, partiendo de la suposición de que los niños vacunados estuvieron expuestos en la misma proporción que los demás a las causas naturales y que reaccionaron igualmente a cualquier efecto ejercido por la lepromina, los porcentajes asignados al BCG se reducen a 44.1 para la Mitsuda, 22.1 para la Fernández y 52.1 para el PPD-S.

5. Se han hecho estimaciones del efecto de las causas naturales, de la lepromina y del BCG, presuponiendo que había al principio tres grupos comparables de niños, a saber: los testigos básicos; los negativos que recibieron toxoide o solución salina, con sus cuotas apropiadas de niños que resultaron positivos en la comprobación inicial; y los negativos que recibieron BCG, con su cuota de positivos primitivamente. Los testigos básicos fueron presuntamente afectados sólo por causas naturales, el grupo reconstruído de toxoide y solución salina por causas naturales y la prueba de la lepromina y el grupo del BCG reconstruído por causas naturales, la lepromina y el BCG. Se consideró que el estado primitivo de los testigos básicos era el mismo que el de los otros cuatro quintos de los niños; el estado final era conocido. Se computó la posición final de los grupos reconstruídos tomando en cuenta el estado final de los primitivos positivos y negativos pertenecientes a cada grupo y la proporción que correspondía a cada una de estas clases en el grupo total. A base de estos cálculos, he aquí las estimaciones de las probabilidades que tienen todos los niños de volverse reactores por efecto de cada causa:

Por causas naturales.—Mitsuda, 11.5 por ciento; Fernández, 8.2 por ciento; y PPD-S, 3.4 por ciento. Para el PPD-S, el aumento es pequeño en relación con su error aceptado. Para la prueba de Mitsuda, es el doble de su error aceptado, representando un valor de unos 0.05 de importancia. Para la Fernández, el aumento es de 2.3 veces su error aceptado, o sea un valor de unos 0.02 de importancia.

Para la prueba de la lepromina.—Mitsuda, 7.2 por ciento; Fernández, 7.8 por ciento; y PPD-S, 6.1 por ciento. Con respecto al PPD-S, la diferencia es el doble de su

error aceptado, lo cual sugiere que la lepromina hizo que una pequeña proporción de los niños se volviera hipersensible. El estudio ulterior de este punto resultaría interesante. En los otros casos, el aumento aparente no es más que indicativo.

Para la vacunación con BCG.—Mitsuda, 33.4 por ciento; Fernández, 17.9 por ciento; PPD-S, 39.5 por ciento. Todos estos aumentos son de la mayor importancia estadísticamente.

6. Se saca la conclusión de que no cabe atribuir solamente a la vacunación el aumento de la frecuencia de la reactividad a la lepromina en las personas vacunadas con BCG. Si no se ejecuta comprobación preliminar, las causas naturales participarán; si se ejecuta una prueba preliminar con lepromina, participarán tanto las causas naturales como la prueba. Reconocidamente, en lo tocante a la prueba de la lepromina, el estudio actual no es más que indicativo. Se hubieran obtenido resultados mas precisos y fidedignos si hubieren sido más grandes los grupos, y en particular el denominado Grupo Testigo Básico. No obstante, los datos presentados convienen en este sentido con otras comunicaciones.

7. El origen de la reactividad natural a la lepromina es un punto de mucho interés. En los sujetos de este estudio, el aumento observado no podía deberse a infección con *M. leprae.* Una infección por el bacilo tuberculoso también constituría una explicación inadecuada, si la falta de reacción al PPD-S a la dosis usada denota indemnidad de la infección. Solamente 2.3 por ciento reaccionaren al PPD-S en la inicación, comparado con 23.2 por ciento que mostraron reactividad de forma Mitsuda. Al final del estudio, solamente 5.7 por ciento de los testigos básicos reaccionaban al PPD-S, pero 34.7 por ciento eran Mitsuda-positivos.

REFERENCES

1. Aronson, J. D., Taylor, H. C. and McGettigan, M. T.The comparison of some tuberculins in BCG-vaccinated and unvaccinated persons. American Rev. Tuberc. **70** (1954) 71-90.
2. Daudén Valls, F., Mora Comas, J. and Daudén Sala, C. Influencia de la B.C.G. y otras vacunas en la leprominorreacción. Acta Dermo-Sif. **42** (1950-1951) 505-523.
3. Feldman, W. H., Karlson, A. G., and Grindlay, J. H. The Mitsuda reaction in dogs from a non-leprous area. Mem. V Congr. Internac. Lepra, Havana, 1948; Havana, 1949, pp. 621-622.
4. Feldman, W. H., Karlson, A. G. and Grindlay, J. H. Lepromin: Mitsuda's reaction with experimental observations in dogs. Ann. New York Acad. Sci. **54** (1951) 53-72.
5. Fernandez, J. M. M. Estudio comparativo de la reacción de Mitsuda con las reacciones tuberculínicas. Rev. argentina Dermatosif. **23** (1939) 425-453.
6. Fernandez, J. M. M. Sensitization to lepromin in presumably non-leprous individuals. Internat. J. Leprosy **11** (1943) 15-22.
7. Fernandez, J. M. M. and Cabanillas, L. Sensitization to tuberculin induced by lepromin. Leprosy Rev. **26** (1955) 163-167.
8. Guinto, R. S., Doull, J. A. and Mabalay, E. B. Tuberculization and reactivity to lepromin; association between lepromin and tuberculin reactions in school children in Cordova and Opon, Cebu, Philippines. Internat. J. Leprosy **23** (1955) 32-47.
9. Guinto, R. S., Doull, J. A. and Mabalay, E. B. A note on the lepromin reaction in males and females of the general population of Cordova, Mactan Island, Cebu, Philippines. Internat. J. Leprosy **23** (1955) 131-134.
10. Guinto, R. S., Doull, J. A. and Mabalay, E. B. The Mitsuda reaction in persons with and without household exposure to leprosy. Internat. J. Leprosy **23** (1955) 135-138.

11. LARA, C. B. Mitsuda's skin reaction (lepromin test) in children of leprous parents. II. Observations on newly-born to eighteen-month-old children. Internat. J. Leprosy **8** (1940) 15-28.
12. MELSOM, R. Experiments in inoculating guinea-pigs with leprous material. Acta dermato-venereol. **20** (1939) 451-462.
13. RODRIGUEZ, J. N. Observations on the leprolin (Mitsuda) reaction. Internat. J. Leprosy **6** (1938) 11-31.
14. ROTBERG, A. "Factor N" of resistance to leprosy and relationship with lepromin and tuberculin reactivity; doubtful value of BCG in antileprous immunization. (Abstract and conclusion.) Mem. VI Congr. Internac. Leprol., Madrid, 1953; Madrid, 1954, pp. 656-666.
15. SEIBERT, F. B. and GLENN, F. T. Tuberculin purified protein derivatives; preparation and analyses of large quantity for standard. American Rev. Tuberc. **44** (1941) 9-25.
16. SOUZA CAMPOS, N. O B.C.G. na profilaxia da lepra (revisão bibliográfica). Rev. brasileira Leprol. **21** (1953) 292-314.
17. WADE, H. W. The immunology problem. Internat. J. Leprosy **6** (1938) 95-101 (editorial).

APPENDIX.

TABLE A.—*Geographic distribution of children completing the study, by barrio of residence and study group.*

Barrio of Opon	Subgroups[a]				Initial positives	Basic controls[b]	Total
	A	B	C	D			
1. Poblacion	26	21	21	20	27	24	139
2. Lo-oc	7	4	9	9	13	11	53
3. Gon-ob	9	8	8	8	8	13	54
4. Babag	16	17	14	15	11	16	89
5. Calawisan	9	18	11	14	21	15	88
6. Timpoloc	3	5	1	4	4	3	20
7. Canjulao	3	4	5	1	7	3	23
8. Basak	2	2	1	–	1	1	7
9. Pajo	1	1	1	2	4	1	10
TOTAL	76	80	71	73	96	87	483

a Negative to lepromin (Mitsuda) and to PPD-S (5 TU) on initial testing.
b Not tested initially.

TABLE B.—*Age distribution on entry of all children completing the study, according to subgroups to which they were assigned.*

Age on entry	Children initially tested						Basic controls	Grand total
	Subgroups				Positives	Total		
	A	B	C	D				
6-12 mos.	17	20	18	20	6	81	23	104
1-2 yrs.	33	33	27	26	26	145	34	179
2-3 yrs.	26	27	26	27	64	170	30	200
TOTAL	76	80	71	73	96	396	87	483

APPENDIX—Continued.

TABLE C.—*Estimated percentages of children gaining reactivity of the Mitsuda type from natural causes, lepromin test and BCG.*[a]

Lepromin status (Mitsuda)	Basic controls (87)		Reconstituted groups			
			Toxoid & saline subgroups C & D[b] (190)		BCG subgroups A & B[b] (206)	
	Per cent	S.E.	Per cent	S.E.	Per cent	S.E.
Originally positive	23.2[c]	2.1	23.2	2.1	23.2	2.1
Became reactive:						
Natural (unknown causes)	11.5	5.5	11.5	5.5	11.5	5.5
Lepromin induced	—		7.2	5.9	7.2	5.9
BCG induced	—		—		33.4	4.1
Positive–Total	34.7[d]	5.1	41.9	2.9	75.3	2.8
Negative–Total	65.3	—	58.1	—	24.7	—
TOTAL	100.0		100.0		100.0	

a The standard errors of Tables C, D, and E for percentages attributed to lepromin and BCG, and for the total percentages of positives of the reconstituted groups are computed from the formula: Variance = $\Sigma \frac{w^2pq}{n}$, where w is the weight or proportion in each class and p the percentage positive on final test, as given in the text, Tables 4, 5 and 6.

b These numbers were obtained by assuming that the negatives constituted 75.7 per cent (see text, Table 4). For toxoid and saline subgroups 75.7 per cent = 144, and total = 190. For the BCG subgroups 75.7 per cent = 156, and total = 206.

c Not originally tested; assumed to be same as for other children.

d Adjusted for age, using the age distribution of the four-fifths who were tested. The age distribution of Subgroups A, B, C, and D are very close to one another (Table B).

APPENDIX—Continued.

TABLE D.—*Estimated percentages of children gaining reactivity of the Fernandez type from natural causes, lepromin testing, and BCG.*

Lepromin status (Fernandez)	Basic controls (87)		Reconstituted groups			
			Toxoid & saline subgroups C & D (190)		BCG subgroups A & B (206)	
	Per cent	S. E.	Per cent	S. E.	Per cent	S.E.
Originally positive	3.3	0.9	3.3	0.9	3.3	0.9
Became reactive:						
Natural (unknown causes)	8.2	3.5	8.2	3.5	8.2	3.5
Lepromin induced	—		7.8	4.1	7.8	4.1
BCG induced	—		—		17.9	3.9
Positive—Total	11.5	3.4	19.3	2.3	37.2	3.1
Negative—Total	88.5	—	80.7	—	62.8	—
TOTAL	100.0		100.0		100.0	

TABLE E.—*Estimated percentages of children gaining hypersensitivity to PPD-S from natural causes, lepromin testing, and BCG.*

Tuberculin status (PPD-S, 0.0001 mgm.)	Basic controls (87)		Reconstituted groups			
			Toxoid & saline subgroups C & D (190)		BCG subgroups A & B (206)	
	Per cent	S.E.	Per cent	S.E.	Per cent	S.E.
Originally positive	2.3	0.8	2.3	0.8	2.3	0.8
Became reactive:						
Natural (unknown causes)	3.4	2.6	3.4	2.6	3.4	2.6
Lepromin induced	—		6.1	3.1	6.1	3.1
BCG induced	—		—		39.5	3.4
Positive—Total	5.7	2.5	11.8	1.9	51.3	3.2
Negative—Total	94.3	—	88.2	—	48.7	—
TOTAL	100.0		100.0		100.0	

APPENDIX—Continued.

TABLE F.—*Children of BCG, toxoid or saline, and originally positive groups classified according to diameter of Mitsuda nodule on initial (I) and final (F) tests.*

Diam. in mm.	BCG subgroups						Toxoid and saline subgroups						Orig. positive		GRAND TOTAL	
	A		B		AB		C		D		CD		E			
	(I)	(F)	(I)	(F)	(I)	(F)	(I)	(F)	(I)	(F)	(I)	(F)	(I)	(F)	(I)	(F)
0–2	35	—	29	—	64	—	30	14	28	7	58	21	1*	—	123	21
2	3	—	8	1	11	1	3	3	6	8	9	11	1*	—	21	12
3	22	7	21	7	43	14	24	22	25	20	49	42	—	—	92	56
4	16	16	22	14	38	30	14	15	14	16	28	31	2*	11	68	72
5	—	46	—	46	—	92	—	14	—	19	—	33	74	52	74	177
6–7	—	2	—	10	—	12	—	2	—	3	—	5	12	18	12	35
8–9	—	1	—	1	—	2	—	—	—	—	—	—	2	3	2	5
10	—	1	—	—	—	1	—	—	—	—	—	—	2	3	2	4
11+	—	—	—	—	—	—	—	—	—	—	—	—	1	4	1	4
Ulcer	—	3	—	1	—	4	—	1	—	—	—	1	1	5	1	10
TOTAL	76	76	80	80	156	156	71	71	73	73	144	144	96	96	396	396

* Originally positive to PPD-S; negative to lepromin.

TABLE G.—*Children of BCG, toxoid or saline, originally positive, and basic control groups, classified according to diameter of edema on initial (I) and final (F) tuberculin tests.*

Diam. in mm.	BCG subgroups						Toxoid and saline subgroups						Orig. positive		Basic controls		GRAND TOTAL	
	A		B		AB		C		D		CD		E					
	(I)	(F)	(I)	(F)	(I)	(F)	(I)	(F)	(I)	(F)	(I)	(F)	(I)	(F)	(I)	(F)	(I)	(F)
0–4	76	33	80	31	156	64	71	66	73	68	144	134	87*	70	86	82	473	350
5	—	5	—	7	—	12	—	—	—	—	—	—	1	5	—	1	1	18
6	—	6	—	8	—	14	—	3	—	2	—	5	—	1	—	—	—	20
7	—	6	—	7	—	13	—	2	—	—	—	2	1	2	—	3	1	20
8–10	—	10	—	13	—	23	—	—	—	1	—	1	2	3	—	—	2	27
11–20	—	16	—	14	—	30	—	—	—	2	—	2	5	13	1	1	6	46
21+	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—	2
TOTAL	76	76	80	80	156	156	71	71	73	73	144	144	96	96	87	87	483	483

* Positive to lepromin only.