

## CUTANEOUS RESPONSES TO LEPROMIN AND TO OTHER MYCOBACTERIAL ANTIGENS<sup>1</sup>

(HUMAN, AVIAN AND BATTEY TUBERCULINS,  
AND HUMAN AND AVIAN TUBERCLE BACILLI)

RICARDO S. GUINTO, M.D., MARIO C. MABALAY, M.D.

*Epidemiologist and Assistant Epidemiologist, respectively*

AND JAMES A. DOULL, M.D.

*Medical Director, Leonard Wood Memorial  
Cebu, Philippines, and Washington, D. C.*

Because the leprosy bacillus has not been cultivated, the only available source of a bacillary antigen is the bacillus-laden leproma tissue excised from lesions of patients with active lepromatous leprosy. The classical Mitsuda-Hayashi lepromin, such as that used in this study, is prepared as follows:

The lepromatous material is heated for several hours, or autoclaved. The fluid is discarded, and the lepromas are cleansed as thoroughly as possible of nonlepromatous tissue elements (fibrous tissue, fat, epidermis, etc.). The leproma tissue is weighed before grinding to a thick paste in a sterile mortar. The paste is slowly diluted and mixed with normal saline solution, to which 0.5 per cent phenol is added, until smears show approximately 500 to 1,000 bacilli per field or until the concentration of bacilli matches that of a previously used batch of lepromin, this requiring about 20 cc. of diluent per gram of tissue. The coarser tissue particles are removed by filtering through gauze or—less wastefully—nylon fabric (bolting cloth) or by sedimentation. Lepromin thus differs from other antigens or suspensions of killed bacilli in that it has, of necessity, a human tissue component.

The test is performed by injecting 0.1 cc. of the lepromin intradermally, usually in the flexor surface of the forearm or in the deltoid region. The commonly-accepted response is the "late," or Mitsuda, reaction. It is a much-delayed reaction in the form of a nodule which becomes palpable after 7 to 10 days, reaches its maximum after 3 to 4 weeks, then gradually subsides. Occasionally there may be necrosis and ulceration of the center of the nodule and this may take a long time to heal.

An "early" lepromin reaction of the 48-hour tuberculin type is also recognized<sup>(6)</sup>. This reaction is not seen in the lepromatous type. In our experience, following injection of the regular Mitsuda-Hayashi lepromin, it occurs in a variable and often small proportion in the tuberculoid type and in normal individuals.

The lepromin test almost invariably gives negative results in cases of the lepromatous type of leprosy. This negativity, which is associated with the presence of vast numbers of bacilli in the lesions, is a specific feature of the test. It is widely held that the Mitsuda reaction is of greater frequency and greater intensity in persons with tuberculoid leprosy than in normal persons of comparable ages. This would be additional evidence of the specificity of the test, but statistical evidence is inadequate.

In those borderline cases in which the lepromatous element predominates, the test is usually negative; whereas, if the lesions are predominantly tuberculoid, the reverse may be true. In reactional tuberculoid leprosy, if there are many bacilli in the lesions the test may be negative or only weakly positive. Thus the lepromin test is of value in prognosis and in the clinical classification of leprosy.

<sup>1</sup>Read at the Leonard Wood Memorial-Johns Hopkins University Symposium on Research in Leprosy, Baltimore, Md., May 8-10, 1961.

Reactivity to lepromin has been shown to be very common among healthy persons, including individuals without contact with leprosy. Many workers have reported positive findings in places where leprosy is absent or extremely rare. From 50 to more than 75 per cent of adults tested in New York, Cleveland, Paris, Cardiff (Wales) and other non-endemic areas<sup>(1, 2, 3, 10, 11)</sup> have been shown to be reactive to lepromin. Thus infection with *M. leprae* is not a prerequisite to reactivity to lepromin.

The following figures from our studies in Cebu<sup>(4, 5, 7, 8, 9)</sup>, based on a large number of examinations, show that the proportions of normal persons giving positive Mitsuda reactions increase rapidly with age. They range from almost complete negativity in early infancy to all but universal positivity in the adult ages: 4.9 per cent of infants 6-11 months of age, 17.9 per cent of children 1-2 years of age, 36.5 per cent 2-3 years of age, 65 to 74 per cent 6-10 years of age, and more than 90 per cent of all adults.

It is well known that lepromatous leprosy is more frequent in males than in females—more than twice as frequent in Cebu—but that there is no sex disparity with respect to the tuberculoid type. A comparison of the results of the lepromin test in 776 males and 1,075 females in the general *adult* population of Cordoba, Cebu, showed 67 per cent of reactors among males and 70 per cent among females with no substantial differences among the age groups.

Judging from observed secondary attack rates, persons living in household association with cases of lepromatous leprosy have a much higher risk of infection than those exposed to cases of the tuberculoid type, and the latter in turn are somewhat more exposed than the remainder of the population. Our experience in Cebu<sup>(9)</sup> again indicates that there is little difference between these three classes in respect to reactivity to lepromin. Comparable age-adjusted percentages of contacts of all ages who reacted to lepromin were: for contacts of lepromatous cases, 73.4 per cent; for contacts of tuberculoid cases, 68.3 per cent; and for persons not known to have lived in household association with leprosy cases of either type, 68.2 per cent. For children of 10 to 14 years the proportion of reactors was somewhat higher for those of lepromatous households than for those of nonlepromatous; the difference was not significant in the statistical sense, but warrants further study.

In searching for a nonspecific origin of reactivity to lepromin, foremost consideration has been given to infection with *M. tuberculosis*. There are numerous reports of positive correlation between reactivity to tuberculin and to lepromin when both tests were performed simultaneously in apparently healthy persons. This association, together with the definitely ascertained fact that BCG vaccination induces reactivity to lepromin, constitutes the principal support for the theory that prior infection with *M. tuberculosis* causes reactivity to lepromin.

In another of our studies on Mactan Island, Cebu<sup>(5)</sup>, related to the effectiveness of BCG in producing reactivity to lepromin, a pertinent observation was made. A total of 483 children from 6 to 35 months of age, living in their homes and considered to be healthy, were tested with lepromin and with 0.0001 mgm. (5 TU) of PPD-S. Because of their young ages, any tuberculous infection in these children would obviously be recent. There were no cases of leprosy in any of the families concerned, and the prevalence rate in the general population was well below 2.0 per cent for all forms of the disease. The prevalence of tuberculosis was probably considerably above that of leprosy. Nevertheless, only 2.3 per cent reacted to tuberculin while 23.2 per cent re-

acted to lepromin. Insofar as a small dose of tuberculin is an indicator, this group of children was relatively free from infection with *M. tuberculosis*. These findings seem definitely to prove that reactivity to lepromin may be gained naturally in the absence of infection either with *M. tuberculosis* or with *M. leprae*.

The present study is an attempt to throw further light on the relationship of the lepromin reaction to the tuberculin reaction and, in particular, to possible prior infection with some other species of *Mycobacterium*.

#### MATERIALS AND METHODS

A comparison is made of the cutaneous responses of healthy children to lepromin, to purified tuberculins made from a human strain of *M. tuberculosis*, from one of the avian tubercle bacillus, and one from the Battey strain of *Mycobacterium*; and also to killed suspensions of whole *M. tuberculosis* and whole *M. avium*—the suspensions being used only in children nonreactive to the homologous PPD.

The purified tuberculins were of equivalent dosage, 0.0001 mgm./0.1 cc., or 5 TU, and were prepared by the U. S. Public Health Service. The heat-killed suspensions of whole *M. tuberculosis* (T3505) and whole *M. avium* (M. C.), in phenolized saline, were specially prepared for the study by the State Serum Institute of Copenhagen. They were also of equal dosage, 0.01 mgm./0.1 cc., and contained about the same number of bacilli per field as the lepromin.

The test subjects were 746 healthy children, 7 to 10 years of age, from two public schools in the towns of Opon and Talisay, in the province of Cebu, Philippines. Scars indicating previous BCG vaccination were found on the arms of 194 of these children. It was found that the vaccination had been performed in all instances at least one and one-half years before testing and in the great majority more than 3 years before. The children were divided into six groups of about 120 each. The method of assignment to groups was as follows: The cards for the children of each sex were arranged in order of ascending age; the six youngest males were each assigned to one of the six groups using a list of numbers obtained at random, then the second lot of males, and so on through the rest of the males and the females.

The preliminary tuberculin tests were given to the six groups as follows:

- Group 1. Human type PPD.
- Group 2. Avian type PPD.
- Group 3. Battey strain PPD.
- Group 4. Human and avian types, simultaneously.
- Group 5. Human type and Battey strain, simultaneously.
- Group 6. Avian type and Battey strain, simultaneously.

In the first phase of the study the children were given tuberculin tests in the right forearm. Readings were made after 72 hours. The average of two cross diameters of induration was adopted as the final measurement. Reactions of 6 mm. or larger were regarded as positive.

In the second phase of the study all children were tested with lepromin. In addition, children negative to human PPD were given an intradermal test with 0.1 cc. of the killed *M. tuberculosis* suspension. Those negative to avian PPD were similarly retested with the whole *M. avium* suspension. The lepromin and bacillary suspensions were given simultaneously in the left forearm, 72 hours after the tuberculin tests were made. Non-reactors to Battey tuberculin were not tested with a corresponding whole bacillus suspension, as none was available.

Reactions to lepromin and to the *M. tuberculosis* and *M. avium* suspensions were measured at 24, 48, and 72 hours for tuberculin-type early reactions, and at 10, 14, 18,

21, 28, 32, and 43 days for the late nodular or Mitsuda phenomenon type of reaction.

The tissue-free suspensions of killed *M. tuberculosis* and *M. avium* produced both early and late reactions which were very similar in appearance to the early and late reactions to lepromin. In judging positivity, therefore, the same criteria as for lepromin reactivity were applied to the early and late reactions to these suspensions; that is, early reactions of 10 mm. or larger were regarded as positive, as were late reactions (nodules) of 5 mm. or more.

## RESULTS

### TUBERCULIN REACTIONS

The results of the individual tuberculin tests are summarized in Table 1. The figures there show that significantly more children reacted to the avian and Battey tuberculins (40.2% and 48.8%, respectively) than to human PPD (26.7%), although the reactions to human tuberculin tended to be larger. An appreciable proportion of the responses to all three were of low or intermediate grades, this being particularly the case with respect to the avian and Battey tuberculins.

The comparison between children with scars indicating previous BCG vaccination and those without such scars is of great interest. This subject will be discussed in a subsequent report, but it will be noted that the vaccinated children showed a frequency of reactivity about 20 per cent higher than the unvaccinated, that this was the case for each of the tuberculins studied and was caused principally by an excess of smaller reactions among the vaccinated.

Simultaneous tests with the human and avian tuberculins were made in 122 children (Group 4), of whom 31 (or 25.4%) reacted to human PPD and 44 (or 36.1%) to the avian. Of the 31 reactors to the human PPD, 30 reacted also to the avian, while of the 91 nonreactors

TABLE 1.—Reactivity of Cebu children, 7 to 10 years of age, to human, avian, and Battey tuberculins according to size of reaction and presence or absence of scars indicating previous BCG vaccination.

PPD 0.0001 mgm.	Number tested	Per cent positive			Total
		1+	2+	3+	
Human					
No BCG scars	282	8.5	7.1	9.6	25.2
BCG scars	93	15.1	6.4	9.7	31.2
Total	375	10.1	7.0	9.6	26.7
Avian					
No BCG scars	276	17.7	16.7	3.6	38.0
BCG scars	90	20.0	24.5	2.2	46.7
Total	366	18.3	18.6	3.3	40.2
Battey					
No BCG scars	271	25.8	20.7	—	46.5
BCG scars	110	32.7	19.1	2.7	54.5
Total	381	27.8	20.2	0.8	48.8



TABLE 2.—*Correlation between reactivity to human and avian tuberculins in 122 children tested with both simultaneously.*

Avian	Human		Total children
	No. Positive	No. Negative	
No. Positive	30	14	44
No. Negative	1	77	78
Total Children	31	91	122

to the human only 14 (or 15.4%) reacted to the avian. Thus the two reactions were positively and highly correlated. (Table 2)

In simultaneous tests with the human and Battey tuberculins given to 129 children (Group 5), 30 (or 23.3%) were found to react to the former and 62 (or 48.1%) to the latter. Of the 30 positive to human PPD, 26 (or 86.7%) reacted to the Battey PPD, while of the 99 non-reactors to the human PPD, only 36 (or 36.4%) reacted to Battey. The correlation is again positive and significant, but not as high as in the case of the human vs. avian tuberculins. (Table 3)

TABLE 3.—*Correlation between reactivity to human and "Battey" tuberculins in 129 children tested with both simultaneously.*

Battey	Human		Total children
	No. positive	No. negative	
No. positive	26	36	62
No. negative	4	63	67
Total children	30	99	129

In simultaneous tests with avian and Battey tuberculins in 125 children (Group 6), 52 (or 41.6%) reacted to the avian, and 64 (or 51.2%) to the Battey. Of the 52 positives to the avian, all but 1 reacted to the Battey, while of the 73 nonreactors to avian only 13 (or 17.8%) reacted. This is an even closer positive association than that between human and avian. (Table 4)

If only reactions of 11 mm. or more of induration be considered as positive (instead of the usual 6 mm. limit), the positive associations still remain but are not so marked. Of 17 children giving such reactions to human PPD, 11 reacted in like degree to avian. Of 105 who would be considered negative at this level, 8 showed these larger reac-

TABLE 4.—*Correlation between reactivity to avian and Battey tuberculins in 125 children tested with both simultaneously.*

Battey	Avian		Total children
	No. positive	No. negative	
No. positive	51	13	64
No. Negative	1	60	61
Total children	52	73	125

tions to avian. If we go to a still higher level and consider as positive only those reactions with 21 mm. or more of induration, only 8 would be positive to human PPD. Of these 8 only one had a reaction of this size to avian. Of the 114 negative at this high level to human PPD, none would be considered positive to avian.

Turning to the human PPD and the Battey, and again considering as positive only those reactions of 11 mm. or larger, it is found that of 15 children strongly reactive to human PPD, 8 reacted in the same manner to Battey. Of 114 negatives to human PPD by this standard, 17 reacted to Battey. The association is still a positive one, again not so close as between human and avian and not so high as at the lower level. Moving to the level of 21 mm. and over, there would be 8 positives to human PPD, of whom none would be considered positive to Battey. Of the 121 negative to human, likewise none was positive to Battey.

Finally, in the comparison of the avian and Battey tuberculins on this higher standard of positivity, it is found that of 32 children positive to avian PPD at the 11 mm. level, 27 showed reactions of similar size to the Battey, while of 93 negative to avian only 6 were positive to Battey. This is a closer association than that seen between human and avian or between human and Battey, but again not so high as at the lower level. Taking 21 mm. as the standard of positivity there was only one positive to avian and none to Battey.

EARLY REACTIONS TO LEPRIMIN AND TO THE SUSPENSIONS OF  
KILLED *M. tuberculosis* AND *M. avium*

Tuberculin-like early reactions were observed with the suspensions of killed human and avian tubercle bacilli as well as with lepromin. All of the 746 children in the study were tested with lepromin, and 114 (or 15.3%) gave positive early reactions measuring 10 mm. or more. Of these positive reactors, 99 (or 86.8%) showed late (Mitsuda) reactions, while of 632 who were negative for the early (24-48 hours) reaction, 454 (or 71.8%) showed late reactions. Although the correlation is positive, the agreement between the early and late reactions to lepromin is not nearly as close as reported by other workers.

As stated previously, to avoid severe reactions and because the assumption seems justified that those reacting to PPD would also react to the whole bacilli, only the children negative to the 0.1 microgram dose of the human and avian tuberculins, respectively, were tested intradermally with 0.1 cc. of the suspensions of the corresponding killed tubercle bacilli. The results are shown in Table 5.

The apparent early reactions to the intradermal tests with the tubercle-bacillus suspensions were much more frequent and larger in the tuberculin-negative children than were the early (Fernandez) reactions to lepromin. Of 197 children, tested with the human tuberculosis antigen, 75 (or 38.1%) gave early reactions of 10 mm. or more; of

TABLE 5.—Early reactions to lepromin and to suspensions of killed *M. tuberculosis* and *M. avium* in Cebu children negative to human or avian PPD respectively, according to presence or absence of scars indicating previous BCG vaccination.

Antigen	Number Tested	Doubtful 5-9 mm.	(Per cent reactive)			Total
			1+ 10-14 mm.	2+ 15-19 mm.	3+ 20 mm. +	
Lepromin						
No BCG scar	276	30.8	6.2	0.4	-----	6.6
BCG scar	63	20.6	7.9	-----	-----	7.9
Total	339	28.9	6.5	0.3	0	6.8
<i>M. tuberculosis</i> (0.01 mgm)						
No BCG scar	158	42.4	26.8	10.6	1.2	38.6
BCG scar	39	41.0	17.9	12.8	2.6	33.3
Total	197	42.6	25.4	11.2	1.5	38.1
<i>M. avium</i> (0.01 mgm)						
No BCG scar	118	23.7	35.6	17.8	10.2	63.6
BCG scar	24	25.0	41.7	16.7	-----	58.4
Total	142	23.9	36.6	17.6	8.5	62.7

142 tested with the avian antigen, 89 (or 62.7%) gave similar early reactions. In contrast, only 6.8 per cent of the tuberculin-negative children gave positive early lepromin (Fernandez) reactions of the same size. The children with scars indicating BCG vaccination did not differ materially from nonvaccinated children in respect to early reactivity to any of these antigens.

There were indications, however, that the apparent early reactions to the tubercle-bacillus suspensions were of a nonspecific inflammatory nature not definitely related to the more significant late reactions. No association was observed between the early and the late reactions to the *M. avium* antigen; that is, the proportions of positive late reactions were almost equal for early reactors as for early nonreactors, and, at the most, only a doubtful positive association was found between the early and the late reactions to the *M. tuberculosis* suspension.

#### LATE REACTIONS TO LEPROMIN AND TO THE *M. tuberculosis* AND *M. avium* SUSPENSIONS

In spite of their young ages—7 to 10 years—the Cebu children in this experiment were highly reactive to lepromin as measured by the late (Mitsuda) reaction; 533 (or 74.1%) of them showed nodules of 5 mm. or larger size. The Opon children and those of Talisay did not differ in the frequency of these reactions. This percentage is higher than that observed in a study made in 1950 on Opon children of 7 to 9 years, in which the proportion reacting was 65.3 per cent.

Even in the tuberculin-negative children, definite late reactions were observed with the tissue-free suspensions of killed *M. tuberculosis*



and *M. avium* which were macroscopically very similar to the nodules or late reactions produced by standard lepromin. The late reactions to lepromin and to the suspensions in the tuberculin-negative children are given in Table 6.

The figures of Table 6 show that if small reactions (less than 5 mm.) are excluded, then about the same proportions of children, negative to the homologous tuberculins, reacted to the whole-bacillus antigens of *M. tuberculosis* and *M. avium*, 37.0 per cent and 40.8 per cent, respectively. A much higher proportion, 67.6 per cent, reacted to lepromin.

TABLE 6.—Late reactions to lepromin and to suspensions of *M. tuberculosis* or *M. avium* respectively, according to presence or absence of scars indicating previous BCG vaccination.

Antigen	Number Tested	Doubtful 3-4 mm	1+ 5-7 mm	2+ 8-9 mm	3+ 10 mm +	Total 1+ to 3+
Lepromin						
No BCG scar	276	27.2	62.7	2.5	2.2	67.4
BCG scar	63	28.6	57.1	7.9	3.2	68.2
Total	339	27.4	61.7	3.5	2.4	67.6
<i>M. tuberculosis</i> (0.01 mgm)						
No BCG scar	158	53.6	31.1	1.3	1.3	33.7
BCG scar	39	41.0	41.0	—	7.7	48.7
Total	197	51.8	33.5	1.0	2.5	37.0
<i>M. avium</i> (0.01 mgm)						
No BCG scar	24	62.5	29.2	—	—	29.2
BCG scar	118	53.4	39.0	3.4	0.8	43.2
Total	142	54.9	37.3	2.8	0.7	40.8

It is evident from inspection of Table 6 that late reactions to lepromin were of no greater frequency in previously vaccinated children than in others. In the case of *M. tuberculosis* and *M. avium* there is some indication of greater frequency and of larger size of reactions among the vaccinated.

With regard to the nature of the reactions following injection of the whole bacillus suspensions, there seemed to be two distinct types: (a) the early and perhaps nonspecific inflammatory reaction which reached its maximum at about 48 hours and then rapidly subsided, and (b) the second or late reaction the nodule of which was observed about the 10th day and on the average reached its maximum earlier than did the lepromin reaction. It began to disappear about the 21st day when the lepromin nodules were still increasing in size. The lepromin reaction showed its greatest average size about the 28th day. The mean sizes of all measurable reactions, including those of doubtful significance, on successive readings are shown in Fig. 1.



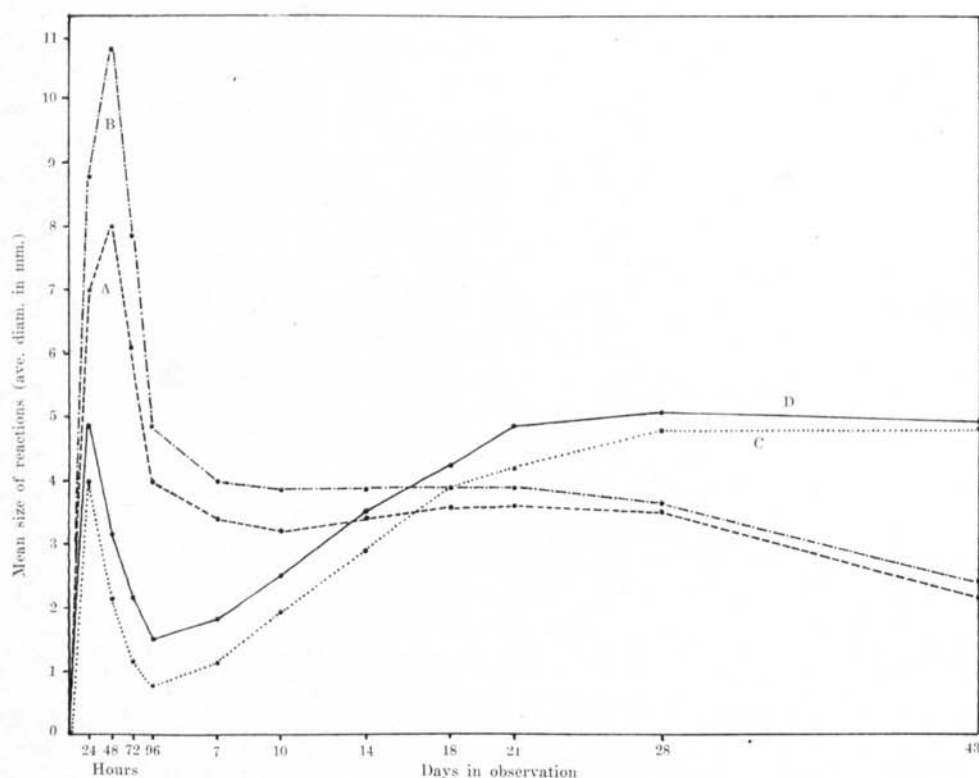


FIG. 1.—Mean size of reactions to lepromin and to heat-killed suspensions of whole *M. tuberculosis* and *M. avium*. Schoolchildren 7 to 10 years of age, Mactan and Talisay, Cebu. **Curve A:** *M. tuberculosis* in 197 children negative to human PPD. **Curve B:** *M. avium* in 142 children negative to avian PPD. **Curve C:** lepromin in 339 children negative to human or avian PPD. **Curve D:** lepromin, all (746) children in study.

As far as could be judged by inspection and palpation, the reactions to the bacillus suspensions were similar to one another and to the Mitsuda reaction in every way, except that they were of smaller average size and reached their maximum at an earlier date than did the lepromin nodule. As with lepromin, a few instances of necrosis were seen with both suspensions. A third and less obvious difference was that the *M. tuberculosis* and *M. avium* nodules receded somewhat more rapidly than the lepromin nodules.

#### ASSOCIATION OF THE LATE LEPRIMIN REACTION WITH REACTIONS TO THE TUBERCULINS AND WHOLE BACILLUS ANTIGENS

Omitting all reactions considered to be doubtful, comparisons were made of the frequencies of the late lepromin response in (a) positives and negatives, respectively, to each of the three tuberculin; (b) positives and negatives to the *M. tuberculosis* and *M. avium* suspensions among nonreactors to the homologous tuberculin; and (c) positives and

negatives to each of the tuberculins and its homologous bacillary antigen. The data are summarized in Table 7. The difference between the proportion of lepromin reactors for the tuberculin or whole-bacillus positives on the one hand and for the tuberculin or whole-bacillus negatives on the other, constitutes the measure of the association between the types of reactivity being compared.

TABLE 7.—Association in Cebu children between the late lepromin reaction and the reactions to (a) human, avian and Battey tuberculins, (b) whole *M. tuberculosis* or *M. avium* suspension in tuberculin negatives, and (c) tuberculin and whole-bacillus antigens taken together.

Antigen	Number tested	Positive To (a), (b) or (c)		Negative To (a), (b) or (c)		Difference	Difference <sup>a</sup> SE of diff.
		No.	% Lep- romin positive	No.	% Lep- romin positive		
(a) Human, avian and Battey tuberculins (0.0001 mgm.) in all children:							
Human PPD	375	100	89.0	275	67.3	21.7	4.2
Avian PPD	366	147	85.0	219	63.5	21.5	4.5
Battey PPD	381	186	89.8	195	62.6	27.2	6.2
(b) Killed <i>M. tuberculosis</i> or <i>M. avium</i> (0.01 mgm.) in tuberculin negatives:							
<i>M. tuberculosis</i>	197	73	97.3	124	53.2	44.1	6.5
<i>M. avium</i>	142	58	86.2	84	50.0	36.2	4.5
(c) Tuberculin and whole-bacillus antigens taken together:							
PPD-H and <i>M. tuberculosis</i>	375	173	92.5	202	56.4	36.1	7.9
PPD-A and <i>M. avium</i>	366	205	85.4	161	55.3	30.1	6.4

<sup>a</sup>In random sampling a difference as great or greater than 2 times SE in either direction is expected about 5 times in 100; of 3 times SE about 3 times in 1,000.

Inspection of section (a) shows that of the three tuberculins, the Battey showed the highest degree of association, although all were positively and highly associated with the lepromin reaction. Of the two whole-bacillus suspensions, that prepared from the human bacillus showed a closer association to lepromin than did the avian bacillus suspension. The very close association with the lepromin reaction was even more evident with the bacillary suspensions in the tuberculin negatives than it was with the tuberculins among all the children.

There is another point not previously mentioned which indicates a positive relationship between the Mitsuda reaction and reactivity to the whole bacillus antigens of *M. tuberculosis* and *M. avium*. Inspection of Figure 1 shows that the average size of the Mitsuda reaction at each reading was greater for all children in the study (D) than for those who were negative to both of the whole bacillus antigens (C). That is to say, the average size of the Mitsuda reactions among children reacting to

the whole bacillus antigens exceeded that among the negatives sufficiently to raise substantially the average size of the Mitsuda reaction for all children above that for the negatives.

Clearly, therefore, infection with *M. tuberculosis*, *M. avium* or with the Battey organism might have caused some positivity to lepromin. If, however, negativity to tuberculin or to the whole bacillus indicates absence of such infection, then at least in the case of *M. tuberculosis* and *M. avium*, such infection cannot account fully for lepromin reactivity. This is evident from inspection of the frequency of lepromin reactors among the negatives (Table 7).

Somewhat more than one-half of the negatives to the human-bacillus preparations, and also of the negatives to the avian-bacillus preparations, were positive to lepromin. The differences in each case indicate the approximate amount of lepromin reactivity which may be attributable to infection with *M. tuberculosis*, with *M. avium*, or with some other mycobacterium antigenically related to these organisms.

In a previous study of this question (<sup>1</sup>) a comparison was made between reactivity to lepromin and that to graded or increasing doses of tuberculin. The tentative conclusion was reached that, although there was a positive correlation, lepromin reactivity could not be fully explained by prior infection with *M. tuberculosis*.

The present study confirms this finding and goes farther by including, as a not fully satisfactory explanation, infection with *M. avium*.

There are other antigens which should be studied in comparison with lepromin, but it is now questionable whether any mycobacterial infection can account fully for lepromin reactivity. Possibly some of the fungi may have a relationship. More extensive studies are needed. These should include comparisons with all available mycobacterial antigens and with those prepared from various fungi known to infect man. Further evidence should be sought regarding the relative frequency of reactors among otherwise comparable children living under different environmental conditions.

#### SUMMARY

A comparative study has been made of the reactions in healthy Cebu children to lepromin, to human, avian and Battey PPD's, and to heat-killed suspensions of whole *M. tuberculosis* and *M. avium*. The bacillary suspensions were given only to nonreactors to the homologous tuberculins to avoid violent reactions and on the assumption that all tuberculin reactors would have responded to the homologous whole bacillus antigen.

Of the 746 children in the study, 15.3 per cent gave positive early lepromin (Fernandez) reactions, while 74.1 per cent showed positive late lepromin (Mitsuda) reactions. Of these children 194 had been vaccinated with BCG at some time in the past; there was no difference



between these children and others with respect to the frequency of either early or late reactions.

Significantly more children reacted to avian PPD (40.2%) and Battey PPD (48.8%) than to human PPD (26.7%). Most of the reactions to the three purified tuberculins were of low or intermediate grade. The previously vaccinated children showed a frequency of reaction about 20 per cent greater to each of the tuberculins than unvaccinated children.

Simultaneous tests showed the human, avian and Battey tuberculins to be positively and highly associated with one another.

An appreciable proportion of children negative to the human and avian tuberculins showed early and late reactions to the suspensions of killed *M. tuberculosis* and *M. avium*. The tuberculin-type early reactions appeared to be of a nonspecific inflammatory nature. The late reactions were in the form of definite nodules very similar macroscopically to late lepromin (Mitsuda) reactions, but they reached their maximum on the average somewhat earlier than did the late lepromin reactions.

The reactions to the human, avian and Battey tuberculins were positively and highly associated with the late lepromin reactions. The late reactions to the *M. tuberculosis* and *M. avium* suspensions were even more positively and highly associated with the Mitsuda reaction in the tuberculin-negative children. The average size of the Mitsuda reaction also was greater at each reading for all children in the study than for those negative to both bacillary suspensions. These findings clearly show that infection with the human or avian tubercle bacilli, or the Battey organism, or possibly with other antigenically related mycobacteria, might have caused some of the positivity to lepromin.

Nevertheless, more than 50 per cent of the children negative to the *M. tuberculosis* preparations (PPD and whole bacillus), and to the *M. avium* preparations, were still positive to lepromin. The tentative conclusion is reached that mycobacterial infection cannot account fully for the high frequency of reactivity to lepromin, and further studies are suggested.

#### RESUMEN

Este estudio versa sobre las reacciones observadas en niños sanos de Cebú a la lepromina, los DPP humano, aviario y de Battey y las suspensiones matadas al calor de los *M. tuberculosis* y *M. avium* íntegros. No se administraron las suspensiones bacilares más que a los que no reaccionaron a las tuberculinas homólogas, a fin de evitar reacciones violentas y partiendo de la suposición de que todos los reactores a las tuberculinas hubieran también respondido al antígeno del bacilo íntegro homólogo.

De los 746 niños del estudio, 15.3 por ciento acusaron reacciones positivas tempranas (de Fernández) mientras que 74.1 por ciento revelaron reacciones positivas tardías a la lepromina (de Mitsuda).

En forma significativa, más niños (40.2%) reaccionaron al DPP aviario y al DPP de Battey (48.8%) que al DPP humano (26.7%). La mayoría de las reacciones a las tres tuberculinas purificadas fueron de tenor bajo o intermedio.

Pruebas simultáneas demostraron que las tuberculinas humana, aviaria y de Battey esta positiva e intensamente asociadas entre sí.

Una proporción apreciable de los niños negativos a las tuberculi-humana y aviaria reveló reacciones tempranas y tardías a las suspensiones del *M. tuberculosis* y del *M. avium* matadas. Las reacciones tempranas de estilo tuberculínico parecieron ser de naturaleza inflamatoria inespecífica. Las tardías tomaron la forma de nódulos bien definidos, muy semejantes macroscópicamente a las reacciones tardías a la lepromina (Mitsuda), pero alcanzaron su máximo el 14.º día en tanto que las reacciones tardías a la lepromina alcanzaron por término medio su tamaño mayor al 28.º día.

Las reacciones a las tuberculinas humana, aviaria y de Battey se asociaron positiva e intensamente con las reacciones tardías a la lepromina. Las tardías a las suspensiones del *M. tuberculosis* y del *M. avium* estuvieron aun más positiva e intensamente asociadas con la reacción de Mitsuda en los niños negativos a las tuberculinas. Estos hallazgos demuestran claramente que la infección con los bacilos tuberculosos humanos o aviarios, o con el microbio de Battey o posiblemente con otras micobacterias antigénicamente afines, podría haber motivado parte de la positividad a la lepromina.

No obstante, más de 50 por ciento de los niños negativos a las preparaciones del *M. tuberculosis* (DPP y bacilo íntegro) y a las del *M. avium* eran todavía positivos a la lepromina. La conclusión tentativa es que la infección micobacteriana no puede explicar del todo la elevada frecuencia de la reactividad a la lepromina y se proponen estudios ulteriores.

#### RESUMÉ

Chez des enfants sains de Cebu, on a étudié et comparé les réactions à la lépromine, aux P.P.D. humain, aviaire, et de Battey, ainsi qu'à des suspensions bacillaires intégrales de *M. tuberculosis* et de *M. avium* tués par la chaleur. Les suspensions bacillaires n'ont été administrées qu'à des sujets qui ne réagissaient pas à la tuberculine correspondante, d'abord afin d'éviter des réactions violentes, et aussi parce qu'on a supposé que tous les sujets réagissant à la tuberculine auraient répondu à la suspension bacillaire intégrale correspondante.

Des 746 enfants inclus dans cette étude, 15,3% ont fourni des réactions précoces positives (Fernandez) à la lépromine, tandis que 74,1% ont témoigné de réponses tardives positives (Mitsuda) à ce même antigène.

Un nombre significativement plus élevé d'enfants réagissent au P.P.D. aviaire (40,2%) et au P.P.D. de Battey (48,8%) qu'au P.P.D. humain (26,7%). La plupart des réactions aux trois tuberculines purifiées ont été classées comme faibles ou intermédiaires.

Les tuberculines humaine, aviaire et de Battey, lorsqu'elles ont été testées simultanément, se sont révélées hautement associées les unes aux autres.

Une proportion non négligeable d'enfants négatifs aux tuberculines humaine et aviaire ont accusé des réactions précoces et tardives aux suspensions de *M. tuberculosis*, et à celles de *M. avium*, tués. Les réactions de type tuberculínique se sont révélées de nature inflammatoire non spécifique. Les réactions tardives ont revêtu la forme de nodules bien définis très semblables macroscopiquement aux nodules des réactions tardives (Mitsuda) à la lépromine; cependant, ils sont parvenus à leur maximum au 14.º jour, alors que les réactions tardives à la lépromine atteignent en moyenne leurs plus grandes dimensions au 28.º jour.

Les réactions aux tuberculines humaine, aviaire et de Battey, ont été fortement associées avec les réactions tardives à la lépromine. Les réactions tardives aux suspensions de *M. tuberculosis* et de *M. avium* ont encore été plus étroitement associées avec la réaction de Mitsuda chez les enfants négatifs à la tuberculine. Ces observations montrent clairement que l'infection par le bacille tuberculeux humain ou aviaire, ou par l'organisme de Battey, ou encore éventuellement par d'autres mycobactéries antigéniquement apparentées, ont pu intervenir pour entraîner une réaction positive à la lépromine.

Et cependant, plus de 50% des enfants réagissant négativement aux préparations de *M. tuberculosis* (P.P. D. et bacille intégral), ou aux préparations de *M. avium*, étaient

positifs à la lépromine. Les auteurs avancent la conclusion que l'infection par des mycobactéries ne peut pas rendre compte, entièrement, de la fréquence élevée des réactions positives à la lépromine; des études complémentaires devront être poursuivies.

*Acknowledgments*—We are indebted to Dr. Lydia B. Edwards of the Public Health Service, U. S. Department of Health, Education and Welfare, for the human, avian and Battey tuberculins used in this study, and to Mr. Mogens Magnusson of the Tuberculin Laboratory, State Serum Institute, Copenhagen, for the heat-killed suspensions of *M. tuberculosis* and *M. avium*. The lepromin used was prepared by Dr. Epifanio Mabalay, Eversley Childs Sanitarium, Cebu, Philippines.

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