TUBERCULIZATION AND REACTIVITY TO LEPROMIN

ASSOCIATION BETWEEN LEPROMIN AND TUBERCULIN REACTIONS IN SCHOOL CHILDREN IN CORDOVA AND OPON CEBU, PHILIPPINES

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The possibility of an association between leprosy and tuberculosis is currently attracting attention. Chaussinand (4) has gone so far as to suggest that the decline of leprosy in Europe in the Middle Ages was attributable to the rise of tuberculosis, the implication being that increasing tuberculization carried with it immunization to leprosy. If it be granted that reactivity to lepromin indicates some degree of resistance to multiplication and spread of *Mycobacterium leprae* within the body, two observations may be interpreted as supporting this hypothesis: (*a*) Fernandez (17) and others have found that vaccination of lepromin negative persons with the bovine type of *M. tuberculosis* (BCG) induces reactivity to lepromin; and (*b*) several workers have reported positive association between natural tuberculization and response to lepromin when tuberculin and lepromin tests are applied simultaneously to the same individual.

The fundamental assumption that a positive lepromin reaction indicates resistance to leprosy may be correct, but at present it lacks adequate support. It is based principally upon the observation that patients with lepromatous leprosy are almost universally negative, whereas a high proportion with the more benign tuberculoid type are positive. It is possible, however, that some of the persons who are naturally lepromin positive may, after infection with leprosy occurs, lose that reactivity and develop the lepromatous form of the disease, while others retain their natural reactivity and develop the tuberculoid type. To determine what actually occurs, field studies in endemic areas are required, designed to measure attack rates in groups of persons differing in response to lepromin but comparable in other respects. In the meantime, it is of importance to examine the validity of the conclusion that lepromin and tuberculin reactions are positively associated in healthy persons, and if so the extent of this association. The present report deals only with this aspect of the problem. It is based upon results obtained in apparently normal school children tested with the purified protein derivative of tuberculin (PPD) and the usual Mitsuda-Hayashi lepromin.

PRESENT STUDY

Area, population, and leprosy prevalence.—The study was made in 1950 in Cordova and Opon, the two municipalities of Mactan Island, which is separated from the mainland of Cebu by a narrow channel. The approximate population of Opon is 30,000 and that of Cordova, 7,000. Cordova has been the locus of epidemiological studies of leprosy by the Leonard Wood Memorial and the Department of Health of the Philippines since 1933 (12, 13, 21, 23, 24). In the last complete survey, in 1948, the prevalence of leprosy was about 20 cases per 1,000 population, one-third of the cases being lepromatous. The prevalence of leprosy in Opon is not as precisely known. Bacteriologically positive patients from both municipalities are sent to the Eversley Childs Sanitarium in Mandawe, near Cebu City. The numbers received from Cordova and Opon in recent years indicate that about the same prevalence rates prevail in the two municipalities.

Tuberculosis is widespread in both municipalities, but its prevalence cannot be stated. In most instances certificates of death are not made by a physician. X-ray studies have not been made, but cases of frank pulmonary tuberculosis have been seen frequently during the leprosy surveys. In the present study, as noted below, the proportion of school children of 7 to 9 years of age reacting to the first strength PPD was 14.0 per cent, and to either first or second strength, 71.9 per cent.

Selection of children for testing.—There were no criteria for selection other than age and school attendance, except that in Cordova efforts were made to exclude those who had been tested with lepromin during the previous year, although some of them were included. None of the Opon children had received lepromin previously. BCG had not been used on Mactan Island prior to 1954. All children tested were from 7 to 9 years of age, inclusive. The total number was 550—204 in Cordova and 346 in Opon. Of the total, 267 were boys and 283 girls. Readings for 6 children were incomplete, but their omission affects the tabulations very slightly; only the results on 544 are considered.

Methods and materials.—Both tests were made intradermally on the flexor surfaces of the forearms, tuberculin on the right and lepromin on the left, the standard dose being 0.1 cc. All tuberculin tests, some of the lepromin tests, and all readings of both tests were made by one of us (R.S.G.). To facilitate the work most of the lepromin tests were made, simultaneously with the tuberculin tests, by an experienced nurse, Mr. Roman L. Ponce, of the staff of the Leonard Wood Memorial.

PPD was purchased from Parke, Davis and Company, Detroit, and forwarded to Cebu by air. Fresh solutions were made each working day. The standard first and second dosages were used; that is, 0.00002 mgm. and 0.005 mgm. respectively. Readings were made after 48 hours. Results were recorded as follows: 1 +, erythema 5-10 mm. in diameter with definite edema; 2 +, erythema 11-20 mm. with edema; 3 +, erythema exceeding 20 mm. with edema; and 4 +, marked reactions with central necrosis. Erythema alone was recorded as negative.

Lepromin was prepared by one of us (E.B.M.) as follows: Lepromatous tissue (4 + earlobes) was placed in a small cotton-stoppered flask and heated in an Arnold sterilizer for at least 4 hours. Discarding the melted fat and fluids, it was cut into small pieces, weighed, and ground into a thick paste in a sterile mortar. The paste was slowly diluted and mixed with normal salt solution, with 0.5 per cent phenol, until

smears showed approximately 500 to 1,000 bacilli per field, this requiring 10 to 20 cc. per gram of tissue. The suspension was filtered through six layers of gauze, sterilized in the Arnold for 2 hours, and placed in rubber-stoppered vials.

Early lepromin readings (Fernandez reaction) were made at 48 hours ([18]). Late readings (Mitsuda reaction) were made on or about the 22nd day. The early reactions were recorded as measurements of the average diameter of the area of edema:–(negative) less than 5 mm.; ± (doubtful), 5-9 mm.; 1 +, 10-14 mm.; 2 +, 15-19 mm.; and 3 +, 20 mm. and over. The late reactions were recorded as follows:–(negative), less than 3 mm.; ± (doubtful), 3-4 mm.; 1 +, 5-7 mm.; 2 +, 8-9 mm.; 3 +, 10 mm. and over and all reactions with ulceration.

RESULTS

Of the 544 children tested, only 24, or 4.4 per cent, had an early lepromin response of sufficient size to be classed as positive, and all of these showed a typical late reaction, in 10 instances with ulceration. Of the total, 355, or 65.3 per cent, gave late reactions.

The number of reactors to the first strength PPD was 76, or 14.0 per cent. Of 468 children who were negative to the first strength, 315, or 67.3 per cent, reacted to the second. Thus the total number reacting to either strength was 391, or 71.9 per cent, of the 544 children tested.

Early lepromin vs *PPD.*—Of 76 children responding to the first strength PPD, 8, or 10.5 per cent, showed the early lepromin reaction. Among 468 negative to the first strength, there were 16, or 3.4 per cent, who gave an early response to lepromin. Of 391 children responding to either first or second strength PPD, 24, or 6.1 per cent, showed the early lepromin response. Among 153 who were negative to both strengths PPD, there was none who had an early lepromin reaction. From the point of view of reaction to lepromin, of 24 showing an early reaction, 8, or 33.3 per cent, were positive to the first strength PPD; while of 520 not showing an early reaction, 68, or 13.1 per cent, reacted to the first strength PPD. All of the 24 positives reacted to either first or second strength PPD. Thus, there is some evidence of positive association between the early lepromin reaction and the results of the tuberculin test, but the number of Fernandez reactors is too small to give much weight to the observation.

Late lepromin vs *first strength PPD.*—The results of a study of the degree of association between late lepromin and first strength PPD reactions are shown in the first part of Table 1. Of 76 children positive to first strength PPD, 84.2 per cent showed a late response to lepromin, as compared to 62.2 per cent of 468 negative to that tuberculin dose, and to 65.3 per cent for the entire group. From the viewpoint of response to PPD, 18.0 per cent of 355 lepromin positives reacted to the first strength PPD, as compared to 6.3 per cent of 189 lepromin negatives and to 14.0 per cent of all children. Some positive association between the reactions is indicated, but the relationship is not striking. In 241 children, or 44.3 per cent, there was agreement between the results. In

303, or 55.7 per cent, there was disagreement: 291 were positive to lepromin and negative to tuberculin, and 12 were positive to tuberculin and negative to lepromin.

TABLE 1.—*Association between the late (Mitsuda) reaction to lepromin and reactions to tuberculin,* (a) *first strength PPD,* (b) *second strength PPD, and* (c) *both combined.*

PPD	Total children	Lepromin		
		Positive	Negative	Per cent positive
(a) *First dose PPD*				
Positive	76	64	12	84.2
Negative	468	291	177	62.2
Total	544	355	189	65.3
Per cent positive to PPD	14.0	18.0	6.3	
(b) *Second dose PPD*				
Positive	315	239	76	75.9
Negative	153	52	101	34.0
Total	468	291	177	62.2
Per cent positive to PPD	67.3	82.1	42.9	
(c) *Total PPD, first and second doses*				
Positive	391	303	88	77.5
Negative	153	52	101	34.0
Total	544	355	189	65.3
Per cent positive to PPD	71.9	85.4	46.6	

Late lepromin vs *second strength PPD.*—The Mitsuda reactions are compared to those obtained with the second strength PPD, in children negative to the first strength, in the second part of Table 1. Among 315 such children who were positive to the second strength, 75.9 per cent showed a late lepromin reaction as compared to 34.0 per cent of 153 children negative to both strengths of tuberculin. From the viewpoint of response to PPD, 82.1 per cent of 291 children reacting to lepromin were positive to the second strength PPD as compared to 42.9 per cent of 177 negative to lepromin. Obviously, there is a positive association between the reactions, and it is much more marked than that indicated in the previous analysis. In 340 children, or 72.6 per cent, there was

agreement between the two reactions. There was still disagreement in both directions; 52 children, or 11.1 per cent, were positive to lepromin and negative to PPD, while 76, or 16.2 per cent, were negative to lepromin and positive to PPD.

Late lepromin vs *both strengths PPD.*—To obtain the complete picture, the data of the first two sections of Table 1 are combined in the third section. Of 391 children who were positive to PPD in either test (first or second strength), 77.5 per cent showed late reactions to lepromin as compared to 34.0 per cent of 153 children negative to both strengths of PPD. From the viewpoint of reaction to PPD, 85.4 per cent of 355 lepromin reactors were positive either to the first or the second strength, and only 46.6 per cent of 189 lepromin negatives. There was agreement between the results in 404 children, or 74.3 per cent of the total tested. Discordance was seen in 52 children or 9.5 per cent, who were positive to lepromin and negative to PPD, and in 88, or 16.2 per cent, who were negative to lepromin and positive to PPD.

TABLE 2.—*Association between intensity of reaction to lepromin (Mitsuda) and to both strengths PPD.*

PPD	Total children	Lepromin				
		Number			Per cent	
		Neg. or ±	1+	2+ or 3+	2+ or 3+	Neg. or ±
Neg. or doubtful (±)	153	101	51	1	0.6	66.0
Positive, 1+	93	32	58	3	3.2	34.4
Positive, 2+	156	40	104	12	7.7	25.6
Positive, 3+ or 4+[a]	142	16	101	25	17.6	11.3
Total	544	189	314	41	—	—
Per cent 3+ or 4+	—	8.5	32.2	61.0	—	—
Per cent neg. or ±	—	53.4	16.2	2.4	—	—

a This group includes all children positive to the first strength PPD test.

Intensity of reaction to lepromin and PPD.—Among the children so highly sensitive to tuberculin that they reacted to the first strength PPD, the proportion showing the late response to lepromin was 84.2 per cent. Of those negative to the first strength but reacting to the second, 75.9 per cent gave Mitsuda reactions. This difference is small, and not significant from the statistical point of view. The question of relationship between the intensity of reaction to PPD and that of reaction to lepromin was, therefore, subjected to further study, with the results given in Table

2. In this table all children reacting to the first strength PPD are included with those showing a 3 + or 4 + reaction to the second strength PPD.

Examining first the lepromin results, it is seen that the proportions of 2 + and 3 + Mitsuda reactions increased with the intensity of response to PPD, the percentages being: 0.6 for children negative to tuberculin, 3.2 for those with 1 + tuberculin reactions, 7.7 for those with 2 +, and 17.6 for those with 3 + or 4 + reactions. From the standpoint of tuberculin, the percentage of 3 + and 4 + PPD reactions increased from 8.5 for lepromin negatives to 61.0 for those showing 2 + or 3 + Mitsuda reactions.

DISCUSSION

Lepromin and tuberculin reactions were positively associated in the children tested. Only 4.4 per cent showed an early lepromin reaction, but all of these were positive to either first or second strength PPD. A relationship between the late lepromin response and that to PPD was suggested when first strength PPD was used, but was much more conspicuous on retesting negatives with second strength PPD. Positive association was also indicated by the relationship between the intensities of the two reactions; the proportion of children showing moderate or strong reactions to PPD was almost twice as high for those with strong reactions to lepromin as for those with weak (1 +) reactions.

These results are consistent with the view that infection with the tubercle bacillus may induce reactivity to lepromin, or, conversely, that infection with the leprosy bacillus may sensitize to tuberculin. Because of the occurrence of reactivity to lepromin in persons living in areas where leprosy is absent or extremely rare (2, 3, 5, 7, 9, 10, 14, 25, 38), and of the usual higher frequency of active tuberculosis than of leprosy in areas where both are endemic, it is logical to give preference to the hypothesis that all reactions to lepromin may be caused by infection with the tubercle bacillus. Before examining further the extent to which the results of the present series can be explained by this hypothesis, the principal facts concerning the nature of the two reactions may be reviewed briefly.

Tuberculin reaction.[1]—The tuberculin reaction is inflammatory in nature and attributable to hypersensitivity to proteins of the tubercle bacillus. For maintenance of the hypersensitive state, the presence in the body of living tubercle bacilli (or possibly other mycobacteria) is probably necessary. Animals can be sensitized by inoculation of either living or killed tubercle bacilli; of a combination of fractions, including a lipo-carbohydrate, from a paraffin oil extract of heat-killed tubercle bacilli, as shown by Choucroun (6); or of tuberculoprotein plus the

[1] For a discussion of the constituents of tuberculin, see Seibert, F.B. (39).

"purified wax" fraction of the tubercle bacillus, but not by either purified protein or purified wax alone, as demonstrated by Raffel (30, 31, 32), Raffel and Forney (34), and Raffel *et al* (33). Temporary sensitization to tuberculin is produced in man by inoculation with a living culture of BCG. Also Wells (42) sensitized to tuberculin very rapidly all of 121 tuberculin negative human subjects by injection of living cultures of *M. tuberculosis muris* as had been done previously in calves by Griffith and Dalling (20). The capacity to produce hypersensitivity is probably shared by all mycobacteria although there are wide differences in this respect not only between species but also between strains of the same species. There are also wide differences between susceptible animal species in the degree to which they exhibit hypersensitivity following infection with the tubercle bacillus, the guinea pig developing a much higher degree of hypersensitivity than other animals.

The proteins which elicit the tuberculin reaction are also apparently present in widely different quantities in "tuberculins" made from different species of mycobacteria. It was found by Green (19) that following inoculation of groups of guinea pigs with various mycobacteria, the tuberculin type of reaction was obtained by injection both of homologous and heterologous PPD preparations, but guinea pigs inoculated with living tubercle bacilli of the bovine type, for example, required 150 units of *M. phlei* PPD to elicit a reaction comparable in size to that produced by one unit of PPD prepared from the homologous bacillus—*M. tuberculosis* var. *bovis*.

While therefore reaction to small doses of tuberculin probably indicates prior infection with the tubercle bacillus, there is no assurance that this is necessarily true of reaction to large doses. In the present series, children reacting to the second strength PPD represented a wide range of hypersensitivity and there is no doubt that a major proportion would have responded to a much smaller dose. Evidence of this is seen in the fact that in 315 children, who reacted to the second strength PPD but not to the first, the reactions were 2 + or larger in 222, or 70.5 per cent. It cannot be denied however that some reactions to the second strength may have resulted from infection with mycobacteria other than the tubercle bacillus. The probability that a substantial proportion of the children had latent leprosy infections is very remote and no other mycobacterial disease is known to exist in the area. Until some better explanation is forthcoming, therefore, it may be assumed that the great majority of reactions to PPD were caused, and that all may have been caused, by infection with the tubercle bacillus.

Lepromin reaction.—The early lepromin reaction is inflammatory in appearance, and reaches its maximum between 24 and 72 hours after injection into the skin either of lepromin or of proteins derived from the leprosy bacillus. As originally described by Fernandez (18), the reaction is induced both by filtered and unfiltered lepromin and "resembles in its

appearance and evolution the one that is induced by intradermal injection of tuberculin (Mantoux)."

The late reaction, on the other hand, is marked by a papule which appears about a week after injection of lepromin and increases in size to about the third or fourth week. Ulceration often occurs. The intensity and frequency of the reactions vary with the content of intact bacilli, as noted originally by Mitsuda (29). Negative results were reported by F. Hayashi (26) with a filtrate obtained by passing lepromin through a bacterial filter. Dharmendra (11) also has shown that nodular tissue, freed from bacilli, is inactive. The histological structure of the lepromin-induced nodule is tuberculoid, showing epithelioid and giant cells bounded by a lymphocytic area.

There is little experimental or epidemiological evidence that reactivity to lepromin is attributable to prior infection with *M. leprae* and there is good evidence, such as the reactivity of persons resident in nonendemic areas, already mentioned, that this reactivity may occur without infection with *M. leprae*. The fact that patients suffering from the lepromatous type are usually nonreactive to lepromin, although they remain hypersensitive to tuberculin, is suggestive of a specific relationship. Patients suffering from the tuberculoid type usually react to lepromin, but a careful search of the literature has not disclosed a valid comparison of lepromin reactions in such patients with those in normal persons of equivalent ages living in the same areas. The number of Cordova residents with tuberculoid leprosy who have been tested with lepromin is relatively small and it is planned to discuss this question further when additional data are obtained. It may be noted that household associates of Cordova patients suffering from the lepromatous type did not show a definitely higher proportion of reactors to lepromin than did unexposed persons (22).

Lara (27) stated that young children became Mitsuda positive following injections of lepromin. This is an important finding and confirmation should be sought in a larger experiment designed specifically for the purpose. Wade (41) has made the suggestion that the late lepromin reaction is the result of sensitization caused by the test dose itself. As has been mentioned, Fernandez (17) and others have shown that conversion from lepromin negative to positive can be achieved by vaccination with BCG. The duration of the reactive state, induced by either of these methods, has not been established.

Rodriguez (36) first reported natural reactivity to lepromin in dogs. All older animals were positive but he found two pups to be negative. Wade (personal communication) states 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* (15, 16) have reported that lepromin negative dogs became reactive after repeated injections of lepromin. The animals also became reactive to rat lepromin and to cultures of a so-

called leprosy bacillus and of a mycobacterium from soil, but not to Old Tuberculin (1:100).

If there were some means of obtaining approximately equivalent dosages, perhaps in terms of nitrogen content, a comparison of response to tuberculin with the early reaction to lepromin would throw light on the respective "tuberculin" activities of the proteins of *M. tuberculosis* and *M. leprae*. Such a comparison cannot be made with available materials and may not be possible until *M. leprae* has been cultivated.

Fernandez (18) found disagreement between early reaction to lepromin and reaction to tuberculin in 39.7 per cent of leprous children and in 50.8 per cent of contacts. In the present series only 76 of the children, or 14.0 per cent, reacted to the first strength PPD, and only 24, or 4.4 per cent, showed an early response to lepromin. The report therefore deals principally with a comparison of reactions to first or second doses of PPD with the late lepromin reaction. The former demonstrate hypersentitivity to proteins contained in the tubercle bacillus; the late lepromin reaction indicates localized tissue response to some component of the leprosy bacillus, of which, by analogy with the tubercle bacillus, the phosphatides are probably an essential part. Positive association of this tissue response with hypersensitivity to tuberculin suggests that this component may be common to leprosy and tubercle bacilli, and that reactivity to lepromin may be explained by prior infection with the tubercle bacillus. It is of interest to examine the disagreement between the reactions from this point of view.

Tuberculization theory in relation to disagreement between results.— A. Tuberculin negative, lepromin positive: If reaction to lepromin is attributable to prior infection with the tubercle bacillus, failure to react to tuberculin in the presence of lepromin reaction may be explained by: (1) an insufficient dose of tuberculin; (2) false lepromin reaction; (3) dissociation between hypersensitivity to tuberculin and the capacity of the infected body to localize intact bacilli in the skin by formation of what is essentially an "accelerated tubercle."

(1) Dosage of tuberculin: Judging from the reported experience of the majority of other workers and the results of the present series of tests, this type of disagreement between the two reactions usually occurs only in a small proportion of persons when the test dose of tuberculin is high. In the present series, as shown in Table 1, the proportion of children tested who were negative to the first strength PPD and positive to lepromin was 53.5 per cent, while the proportion negative to both the first and second strengths but positive to lepromin was only 9.5 per cent.

Convit *et al* (8) tested 313 healthy persons of all ages in Venezuela, all being contacts of leprosy patients. They found 36.4 per cent to be negative to ten times the first strength PPD but positive to Mitsuda-Hayashi lepromin. Lowe and McNulty (28) tested children and adults in Nigeria. They applied Old Tuberculin by the von Pirquet method, followed

in doubtful and negative cases by an intradermal injection of 50 international units (T.U.) of purified tuberculin. They used lepromin extracted with chloroform, dried, and diluted with saline until it corresponded with a "standard" prepared by Dharmendra's method. Of 81 children, 1 to 15 years of age, none was tuberculin negative and lepromin positive; but of 278 adults, 8.3 per cent showed this type of disagreement. Dharmendra and Jaikaria (10) tested 260 persons of all ages resident in villages of the Punjab with Old Tuberculin (1:100) and with Mitsuda-Hayashi lepromin. Of these 7.7 per cent were negative to tuberculin and positive to lepromin. Chaussinand (5) tested 231 children, 1 to 8 years of age, resident in Indo-China, with Old Tuberculin (1:10) and with Mitsuda-Hayashi lepromin; 7.4 per cent were negative to tuberculin and positive to lepromin. In similar testing in Paris, of 38 children, 10 to 17 years of age, only 2.6 per cent (one child) showed this type of disagreement. Quite different results, however, were obtained by de Souza Campos *et al* (40), who tested 185 healthy children, 2 to 16 years of age, of leprous parentage, resident in an orphanage in São Paulo, with Mitsuda-Hayashi lepromin in October 1949 and with Old Tuberculin (1:10) in December of the same year. Of the total tested, 33.0 per cent were negative to tuberculin but showed definite (1+ or 2+) late lepromin reactions.

TABLE 3.—*Results obtained with tuberculin and lepromin tests by various workers, arranged according to approximate strength of tuberculin used.*

Author	No. tested	Ages (years)	Strength of tuberculin	Probable T. U.[a]	Agreement %	Disagreement per cent[b]	
						M+, T—	M—, T+
(c)	544	7-9	1st PPD	1	44.3	53.5	2.2
(8)	313	all ages	PPD (10 units)	10	61.0	36.4	2.6
(28)	81	1-15	50 TU	50	81.3	0.0	19.7
(28)	278	adults	50 TU	50	83.8	8.3	7.9
(10)	260	all ages	OT (1:100)	100	68.1	7.7	24.2
(c)	544	7-9	1st & 2nd PPD	250	74.3	9.5	16.2
(5)	231	1-8	OT (1:10)	1000	89.6	7.4	3.0
(5)	38	10-17	OT (1:10)	1000	97.4	[2.6	0.0
(40)	185	2-16	OT (1:10)	1000	65.9	33.0	1.1

a 1 T.U. is taken as equivalent to 0.00002 PPD or 0.1 cc. of 1:10,000 O.T.
b M = Mitsuda, T = tuberculin.
c Refers to present paper.

The approximate dosage of tuberculin injected, in each of these studies, has been estimated and expressed in international units (T.U.). The

percentages of tuberculin negative, lepromin positive individuals are arranged in ascending order of dosage in Table 3, which shows also the percentages of disagreement in the other direction to be discussed later.

Of the several studies referred to in Table 3, the highest percentage of tuberculin negative, lepromin positive persons (53.5%) occurred in the present series with the weaker dose of tuberculin, the first strength PPD. A fall to less than 10 per cent is seen with all doses of 50 T.U. or higher except with that used by de Souza Campos *et al* (40). These workers gave a first injection of 0.1 cc. of Old Tuberculin 1:1000 and retested those negative with 0.1 cc. of a 1:10 dilution. Nevertheless, the proportion of their subjects failing to react to tuberculin but reacting to lepromin was very much higher (33.0%) than the proportions observed by other workers who used 50 or more T.U.

(2) False lepromin reactions: Reference to Table 2 shows that among the 52 children negative to PPD but reacting to lepromin there was only one in whom the nodule was graded as more than 1+, whereas the proportion with these larger reactions among all lepromin reactors was 11.5 per cent. The matter is one requiring further exploration but it appears that the correlation between the two reactions would be improved by accepting as positive to lepromin only instances in which the nodule is graded as larger then 1+. That is, a question may be raised as to the nature of these smaller nodules; are they caused by the bacillary component, or are they merely evidence of reaction to a foreign body?

(3) Dissociation between hypersensitivity to tuberculin and tubercle formation: Rich (35) considers it to be established that tubercle formation (in internal organs) may occur in the absence of hypersensitivity to tuberculin, although the process is accelerated in the sensitized body. It seems highly probable that there are persons who either do not acquire or do not retain hypersensitivity to tuberculin and yet exhibit a local tuberculoid reaction when whole bacilli, either *M. tuberculosis* or *M. leprae,* are injected intradermally. As regards *M. tuberculosis,* evidence has been presented by many workers, including Fernandez (18), Rosemberg *et al* (37), and Aronson and McGettigan (1). The last mentioned found that of 2,694 medical students and nurses, not previously vaccinated with BCG, who gave negative results when tested with 0.1 mgm. O.T., 201 or 7.5 per cent, showed either a definite Koch phenomenon, or a slightly positive Koch with palpable edema, when inoculated intradermally with living BCG.[2]

B. Tuberculin positive, lepromin negative: In the present series only 12 children, 2.2 per cent of the total, were positive to first PPD and

[2] Floch [*Ann. Inst. Pasteur,* **82** (1952) 517-527] has compared results obtained with lepromin diluted 1:750 to those with a suspension of heat-killed BCG. In tuberculoid leprosy, 89% of 46 patients reacted to lepromin and 91% to BCG; in 50 indeterminate, 59% to lepromin and 79% to BCG, and in 40 lepromatous 5% to lepromin and 70% to BCG.

negative to lepromin (Mitsuda). When the PPD dosage was increased, the total was brought to 88, or 16.2 per cent, who reacted to PPD but not to lepromin. In Table 3 there are given the frequencies of this type of disagreement as observed in this and other studies. The proportions are substantial only in two other reports: Lowe and McNulty (28), children, 19.7 per cent, and Dharmendra and Jaikaria (10), all ages, 24.2 per cent.

There is no accepted method of standardization of the classical lepromin, and doubtless there is considerable variation in numbers of *M. leprae* which are contained in various lots. Variation in dosage therefore may account in part for the differences in frequency of this type of disagreement as given in the various reports.

In the case of lepromin reactions in tuberculin negative individuals, it was pointed out that in the present series the reactive nodules were of relatively small size in all of 52 children, with one exception. Tuberculin reactions in lepromin negatives, on the other hand, were frequently large (table 2). It is evident that in healthy persons, as well as in patients with lepromatous leprosy, hypersensitivity to tuberculin may exist independently of reactivity to lepromin. It may be noted here also that in the series of Aronson and McGettigan (1), already mentioned, there were 252 previously unvaccinated medical students or nurses who gave negative or doubtful reactions to 0.01 mgm. O.T. but were positive when tested with 0.1 mgm.; of these, 44 or 17.5 per cent, did not show even a slightly positive Koch phenomenon.

SUMMARY

1. A study has been made of the association between the reactions to tuberculin (first and second strength PPD) and early and late reactions to lepromin in 544 children of ages 7 to 9, inclusive, attending public schools in Cordova and Opon, Mactan Island, Cebu. In these municipalities the prevalence of leprosy is about 20 per 1,000 of which one-third is of the lepromatous type. Tuberculosis is known to be frequent.

2. The following percentages were positive: First PPD, 14.0; first and second PPD, 71.9; early lepromin (Fernandez), 4.4; and late lepromin (Mitsuda), 65.3.

3. Only 24 children showed an early lepromin reaction of 10 mm. or larger with infiltration. All of these had typical late reactions. Eight of the 24 were positive to first strength PPD; all of the remaining 16 reacted to the second strength.

4. The occurrence and intensity of the Mitsuda reaction were positively associated with occurrence and intensity of reaction to PPD. If it be granted that a high proportion of reactions to the second strength PPD were attributable to infection with the tubercle bacillus, then tuberculization of the population may have been responsible for concurrent acquirement of reactivity to lepromin.

5. There were, however, disagreements in both directions. There were 9.5 per cent of the children who were negative to the second PPD but positive to lepromin (Mitsuda). In all but one of these the lepromin reactions were small. On the other hand, there were 16.2 per cent who were positive to the second strength PPD but negative to lepromin. In 63.6 per cent of 88 children showing this type of disagreement, the reaction to tuberculin was 2 + or larger. The only explanation of these disagreements which is in conformity with the tuberculization hypothesis is that in some persons the two types of response are dissociated. Certain individuals are apparently capable of developing tuberculoid lesions when acid-fast bacilli are injected into the skin but have either lost or never possessed hypersensitivity to tuberculin. In other cases, hypersensitivity is present but capacity to localize the bacilli in the skin by formation of a tubercle is absent. Evidence is cited which suggests that the observed disagreements between the tuberculin and lepromin reactions were not more frequent than are disagreements between tuberculin and Koch reactions when tuberculin and BCG are injected simultaneously in the same individuals.

6. This report illustrates anew the need for a method of cultivation of *M. leprae.* Until cultures are available little progress can be made in the fundamental chemical studies which are essential for the elucidation of the nature of the lepromin reaction.

SUMARIO

1. Este estudio versó sobre la relación entre las reacciones a la tuberculina (primera y segunda diluciones del PPD) y las reacciones tempranas y tardías a la lepromina en 544 miños de 7 a 9 años de edad, inclusive, que asistían a las escuelas pública de Córdova y Opón, Isla de Mactán, Cebú, I. F. En esas municipalidades, la incidencia de la lepra representa unos 20 por 1,000, siendo la tercera parte de los casos de la forma lepromatosa. Es sabido que la tuberculosis es allí frecuente.

2. Resultaron positivos los siguientes porcentajes: primera dilución del PPD, 14.0; primera y segunda diluciones del PPD, 71.9; lepromina temprana o precoz (Fernández), 4.4, y lepromina tardía (Mitsuda), 65.3.

3. Solamente 24 niños acusaron una reacción temprana a la lepromina de 10 mm. o más con infiltración. Todas éstas fueron típicas reacciones tardías. Ocho de los 24 fueron positivos a la primera dilución de PPD; todos los 16 restantes reaccionaron a la segunda dilución.

4. La aparición y la intensidad de la reacción de Mitsuda se asociaron positivamente con la aparición e intensidad de la reación al PPD. Si se acepta que una alta proporción de las reacciones a la segunda dilución del PPD era imputable a infección por el bacilo tuberculoso, entonces la tuberculización de la población puede haber sido la causa de la adquisición concurrente de reactividad a la lepromina.

5. Hubo, sin embargo, desacuerdos en ambos sentidos. Hubo 9.5 por ciento de los niños que fueron negativos a la segunda dilución del PPD, pero positivos a la lepromina (Mitsuda). En todos éstos, menos uno, las reacciones a la lepromina fueron pequeñas. En cambio, hubo 16.2 por ciento que fueron positivos a la segunda dilución del PPD, pero negativos a la lepromina. En 63.6 por ciento de los niños que revelaron esta clase de desacuerdo, la reacción a la tuberculina fué de 2 + o mayor. La única explicación de estos desacuerdos que se conforma a la hipótesis de la tuberculización

es que, en algunas personas, están disociadas las dos formas de respuesta. Ciertos individuos poseen aparentemente la capacidad de manifestar reacciones tuberculoideas cuando se les inyectan bacilos ácidorresistentes en la piel, pero bien han perdido o jamás poseído hipersensibilidad a la tuberculina. En otros casos, existe hipersensibilidad, pero falta la capacidad para localizar los bacilos en la piel por la formación de un tubérculo. Cítanse datos que sugieren que los desacuerdos observados entre las reacciones a la tuberculina y la lepromina no fueron más frecuentes que los observados entre la reacción a la tuberculina y la de Koch al inyectarse simultáneamente tuberculina y BCG en los mismos individuos.

6. Esta comunicación demuestra de nuevo la necesidad de un método de cultivo del *M. leprae*. Hasta que se cuente con cultivos, poco puede acelantarse en los fundamentales estudios químicos que son indispensables para la dilucidación de la naturaleza de la reacción lepromínica.

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