INTERNATIONAL JOURNAL OF LEPROSY

VOL. 8 JULY-SEPTEMBER, 1940 NO. 3

SKIN REACTION TESTS WITH TUBERCULIN-TYPE EXTRACTS OF LEPROUS SPLEENS[1]

BY THE JOINT COMMITTEE ON LEPROSY SKIN TESTS,
PHILIPPINE BUREAU OF HEALTH AND
THE LEONARD WOOD MEMORIAL[2]

The investigation here recorded represents a further effort to arrive at a test, comparable to the tuberculin test in tuberculosis, which would be of diagnostic value in leprosy. Much of the success of the antituberculosis campaign is credited to case-finding programs in which tuberculin is a valuable aid, its use being particularly important as a means of detecting the presence of tuberculous infection in persons in contact with actual patients. As for leprosy, unfortunately, no culture of the various microorganisms labelled *M. leprae* can be considered suitable for the preparation of an analogous test material. In the first place, proof is lacking that these cultures are specific for the disease. Furthermore, it has been shown by previous work of this committee with Dr. E. B. McKinley (1, 3) that tuberculin-like extracts of several representatives of such cultures are of no value as indicators of leprosy infection.

It still remains possible, however, that an active, specific tuberculin-like antigen can be obtained from the acid-fast bacilli present in leprous tissue itself, rather than from the doubtful bacilli of cultures. Recently the Leonard Wood Memorial has fostered an attempt to secure such a product from the bacilli pre-

[1]Prepared with the cooperation of Dr. Esmond R. Long. Published with the approval of Dr. E. D. Aguilar, Director of Health.

[2]Drs. S. Chiyuto (*Chairman*), C. B. Lara, W. de Leon, C. Manalang, J. N. Rodriguez, F. Velasco and H. W. Wade.

sent in large numbers in the spleens of patients dying from leprosy. The technique of preparation of certain of these products is described in a separate article (2).*

It was originally planned that Dr. McKinley, with the other members of the committee which carried out the previous tests with the bacillary proteins from cultures, should conduct a survey with these new preparations, and in July, 1938, he left the United States for Manila with a sufficient supply of the material. This project was abruptly terminated by the tragic loss of the trans-Pacific airplane in which he was travelling. Subsequently, with approval of the director of health, the committee undertook the contemplated tests along the lines planned by Dr. McKinley, using a further supply of the antigens provided by Dr. Long. The committee met on February 28, 1938, and one of us (H.W.W.), who had been in communication with Dr. Long, reported on the nature of the antigens. It was agreed that the work should be carried out and that, in order to avoid any possible bias in reading and interpreting the results of the tests, no other member of the committee should be informed of the sources of the individual antigens, which had been received in containers marked "A," "B," and "C." Their descriptions were deposited with the chairman, to remain sealed pending completion of the work.

After consideration of the possible communities in which the work might be done, it was decided that a small series of "range-finding" tests should be made on patients ("negatives") at San Lazaro Hospital, and that the main work should be begun on children at Welfareville immediately after the results were seen. Dr. McKinley had previously indicated those whom he wanted to test in the Welfareville groups, which included both children of lepers and, as noncontact controls, other minors under restraint for misdemeanors in another department of the same institution. As many of these children as were still available were to be tested, the desired numbers to be made up with new individuals.

The materials for use as antigens were as follows:

Solution "A": Protein from normal human spleen; 5 mgm. per cc.

Solution "B": Protein from a human leprosy spleen prepared by trichloracetic acid precipitation of the concentrated buffered extract, as in the preparation of the tuberculin product TPT; 5 mgm. per cc.

Solution "C": Protein from a human leprosy spleen prepared through simple concentration by ultrafiltration without trichloracetic acid precipi-

*Article by Mr. H. J. Henderson, Fellow of the Leonard Wood Memorial, working under the direction of Dr. Esmond R. Long of the Medical Advisory Board of the Memorial, at the Henry Phipps Institute, Philadelphia (the following article in this issue).

tation, as in the preparation of the tuberculin product TPU (4); 5 mgm. per cc.

Solution "T": Tuberculin.

Since it was assumed that the processing of the leprous tissues was such as to break up the bacilli contained therein, thus to obtain a tuberculin-like substance from them, it was decided that tuberculin be used in parallel with the other antigens, to ascertain if there might be any significant relationships in the reactions obtained with the two kinds of substances.[3]

The "range-finding" tests were made at San Lazaro Hospital, on nine "negatives" awaiting parole. Dr. Long had suggested trying 1:10, 1:100 and 1:1000 dilutions of the three antigens. It was decided, however, because of the limited amount of the materials, to use only 1:10 and 1:100 dilutions of the two lots A and B. The 24-hour and 48-hour readings were so nearly negative throughout that it was decided to use only the 1:10 dilution in the further tests. The principal tests were then made at Welfareville, on adequate numbers of children of both groups, namely, those born at Culion of leprous parents ("Department B") and the non-contact delinquents ("Training School" department). Thereafter a group of bacteriologically positive cases at San Lazaro Hospital was tested.

All injections were made intradermally (Mantoux technique), exactly 0.1 cc. of each solution being employed. All four test materials were injected in each subject, with care to observe proper spacing. Readings were made at the end of 24 hours and 48 hours, and again after 2 weeks in order to observe any delayed, lepromin-like reactions. The readings were made exactly as in the case of tuberculin reactions. The committee operated as it did in 1937. All injections were made by two of us (W.deL. and C.B.L.); two (S.C. and F.V.) checked the lists and, while the injections were being made, prepared the record sheets; and the readings were made for the most part by two others (J.N.R. and C.M.). The recording was carried out in a manner essentially similar to that followed in the 1937 work. The results are given briefly below. Summarized data appear in Table 1.

SPECIAL ANTIGEN "A"

(1) *Contact group.*—In this group of children (Department B) three positive reactions occurred among the 50 males and eight

[3] A sufficient quantity of "purified protein derivative tuberculin, PPD" was kindly provided by Parke, Davis and Co. through its local representative.

among the 50 girls, giving an average of 11 percent positive reactions for this group.

(2) *Control group.*—In this group (Training School) nine positive reactions occurred among 61 males and three among 30 females, or an average of 13 percent for the whole group.

(3) *Leprous group.*—In the bacteriologically positive San Lazaro Hospital cases no positive reaction occurred in any of the 100 male and female lepers in 24 hours.

SPECIAL ANTIGEN "B"

(1) *Contact group.*—Eight positive reactions occurred among the males and eleven among the females, after 24 hours, or 19 percent.

(2) *Control group.*—Four positive reactions occurred among the males and five among the females, after 24 hours, or about 10 percent.

(3) *Leprous group.*—One positive reaction occurred among the males and none among the females after 24 hours; the incidence of reaction was thus 1 percent.

SPECIAL ANTIGEN "C"

(1) *Contact group.*—Two positive reactions occurred among the males and nine among the females, or 11 percent for the whole group.

(2) *Control group.*—Two positive reactions occurred among the males and four among the females, or 6.6 percent for the whole group.

(3) *Leprous group.*—Two positive reactions occurred among the males and two among the females, or 4 percent for the whole group.

TUBERCULIN TEST

As only the first-strength solution was employed, no conclusions concerning the total incidence of reactivity to this substance in the three groups of persons tested can be made. It was decided to employ the first strength in the present experiment simply for comparison with the test materials at approximately the same concentrations, the purpose being to determine if tuberculin-allergy might be responsible for positive reactions with solutions A, B and C.

(1) *Contact group.*—This test was positive in 34 percent

among the 50 boys, and 46 percent among the 50 girls, or an average of 40 percent for the 100 individuals of both sexes.

(2) *Control group.*—In this group the test was positive in 44 percent of the 61 males and 50 percent of the 30 females, or 46 percent of the total of 91 individuals tested.

TABLE 1. *Results of tests of* (A) *contact children,* (B) *noncontact children, and* (C) *San Lazaro Hospital patients, bacteriologically positive cases and negatives awaiting parole.*

Antigen	No. of cases	Readings after 24 hrs.				Positive		Negative		Readings after 48 hrs.				Positive		Negative	
		1+[a]	2+	3+	4+	No.	Pct.	No.	Pct.	1+[a]	2+	3+	4+	No.	Pct.	No.	Pct.
		A1. Contact children, boys															
A	50	2	1	0	0	3	6.0	47	94.0	0	1	0	0	1	2.0	49	98.0
B	50	6	1	1	0	8	16.0	42	84.0	5	1	0	0	6	12.0	44	88.0
C	50	1	0	1	0	2	4.0	48	96.0	0	0	0	0	0	0	50	100.0
T	50	9	3	5	0	17	34.0	33	66.0	7	3	3	4	17	34.0	33	66.0
		A2. Contact children, girls															
A	50	7	1	0	0	8	16.0	42	84.0	3	0	0	0	3	6.0	47	94.0
B	50	9	2	0	0	11	22.0	39	78.0	6	2	0	0	8	16.0	42	84.0
C	50	6	2	1	0	9	18.0	42	92.0	5	0	1	0	6	12.0	44	88.0
T	50	13	3	7	0	23	46.0	27	54.0	7	7	5	2	21	42.0	29	58.0
		B1. Noncontact children, boys															
A	61	9	0	0	0	9	14.7	52	85.2	7	0	0	0	7	11.5	54	88.5
B	61	4	0	0	0	4	6.6	57	93.4	3	0	0	0	3	4.9	58	95.1
C	61	2	0	0	0	2	3.3	59	96.7	1	0	0	0	1	1.6	60	98.4
T	61	16	7	4	0	27	44.3	34	55.7	13	5	4	4	26	42.6	35	57.4
		B2. Noncontact children, girls															
A	30	3	0	0	0	3	10.0	27	90.0	1	0	0	0	1	3.3	29	96.6
B	30	5	0	0	0	5	16.6	25	83.3	0	1	0	0	1	3.3	29	96.6
C	30	4	0	0	0	4	13.3	26	86.6	1	0	0	0	1	3.3	29	96.6
T	30	12	0	3	0	15	50.0	15	50.0	7	1	1	1	10	33.3	20	66.6
		C1. San Lazaro patients, males															
A	50	0	0	0	0	0	0	50	100.0	0	0	0	0	0	0	50	100.0
B	50	1	0	0	0	1	2.0	49	98.0	1	0	0	0	1	2.0	49	98.0
C	50	2	0	0	0	2	4.0	48	96.0	0	0	0	0	0	0	50	100.0
T	50	9	4	1	0	14	28.0	36	72.0	7	3	2	1	13	26.0	37	74.0
		C2. San Lazaro patients, females															
A	50	0	0	0	0	0	0	50	100.0	0	0	0	0	0	0	50	100.0
B	50	0	0	0	0	0	0	50	100.0	0	1	0	0	1	2.0	49	98.0
C	50	2	0	0	0	2	4.0	48	96.0	0	0	0	0	0	0	50	100.0
T	50	7	2	2	0	11	22.0	39	78.0	1	3	1	3	8	16.0	42	84.0
		C3. Negatives (San Lazaro)															
A	10	1	0	0	0	1	10.0	9	90.0	0	0	0	0	0	0	10	100.0
B	10	3	0	0	0	3	30.0	7	70.0	0	0	0	0	0	0	10	100.0

[a] Including doubtful (±?) reactions.

(3) *Leprous group.*—In this group the test was positive (after 24 hours) in 28 percent of the 50 males and 22 percent of the 50 females, an average of 25 percent in the 100 patients of both sexes.

Thus the test was distinctly more frequently positive in the contact and the control groups than among the lepers.

REMARKS

1. Most of the positive reactions following injections of solutions A, B and C (spleen antigens) subsided within 24 hours. There were a number of delayed reactions occurring between 24 and 48 hours, but the majority of these also subsided within a further 24 hours.

2. No constant relation was observed between the action of the tuberculin and that of any of the three spleen antigens; nor, it may be added (though the matter has not been mentioned), was a relationship observed between the results of the tests with any of these four antigens on one hand and the occurrence of lepra reaction in the lepers on the other hand.

3. There were few reactions to any of the spleen antigens stronger than one-plus (1+) in any of the three groups tested. Most of the stronger reactions occurred in the contact group; these included two 2+ reactions to solution A (normal spleen extract) and three 2+ reactions and one 3+ reaction to solution C (the TPU-type spleen extract). Solutions B (the TPT-type spleen extract) and C seemed to cause somewhat stronger reactions than solution A in this group. In the control group only one 2+ reaction was observed; this occurred 48 hours after an injection of solution B. In the group of 100 leprous patients, also, there was only one 2+ reaction, that again elicited by solution B.

4. The fact that most of the comparatively few positive reactions produced by the spleen antigens occurred in the contact group may be considered as suggestive, but the percentage of positives was not high enough to appear significant.

5. Solution B gave slightly more reactions in the contact group than either solution C or the control extract (solution A), but the differences noted were not significant.

6. A number of cases were of some interest in that they proved to be "general reactors," showing rather strong reactions to all three spleen antigens after 24 hours, usually beginning to diminish at the 48-hour reading. Since, in these cases, the

reactions to the normal spleen extract were usually as marked, or nearly so, as those to the preparations from leprous spleens, these reactions can hardly be of any significance as regards a specific diagnostic test.

7. No reactions were present at the end of two weeks in any member of the three groups; i.e., no evidence of a late, lepromin-like reaction was seen.

8. Most of the individuals tested with the spleen antigens were patients previously tested in cooperation with Dr. McKinley. No interrelationship between the results of the previous tests and the present ones in these individuals could be observed.

CONCLUSION

It is concluded that the protein extracts of leprous spleens that were used in these tests do not contain any substance to which persons suffering from active leprosy (bacteriologically positive cases), or previously exposed to infection by leprosy, react specifically. The failure of reaction might be explained on either of two grounds. Either the material, although derived from spleens rich in acid-fast bacilli, did not contain enough specific protein to elicit a positive reaction in the doses used, or sensitiveness comparable to the tuberculin-sensitiveness of tuberculosis does not exist in leprous patients of the kind used or in leprosy contacts.

REFERENCES

(1) [Committee on Leprosy Skin Tests] Summary report on antigenic skin tests in leprosy. Month. Bull. Bu. Health (Manila) **17** (1937) 265-275.

(2) Henderson, H. J. An attempt to obtain specific protein antigens from leprous spleens. Internat. Jour. Lep. **8** (1940) 271-283.

(3) McKinley, E. B. The present status of diagnostic skin tests in leprosy. Internat. Jour. Lep. **6** (1938) 33-46.

(4) Seibert, F. B. Chemical composition of active principle of tuberculin; improved and simplified method for making standard undenatured tuberculin of any desired strength and method of chemical assay. Jour. Biol. Chem. **78** (1928) 345-362.