THE MULTIPUNCTURE DEPOT LEPROMIN TEST: INVESTIGATIONS WITH DIFFERENT ANTIGENS

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The work of de Faria[1] (1950), Kooij and Gerritsen[2] (1956), Floch[3] (1956) and Davey[4] (1958) has shown that a local response can be elicited in some individuals by the intradermal injection of a saline suspension of normal skin taken from a non-leprous person. An early reaction at 24-48 hours or a late infiltration at three weeks is possible. This phenomenon might be taken to mean that the Fernandez and Mitsuda reactions to lepromin are only incidental and have little direct relationship to prognosis; alternatively, that an antigen prepared from normal skin might for practical purposes be substituted for that prepared from bacteriologically positive tissue. The response to normal skin, however, was generally much smaller, comparable with that to a very weak lepromin. It is allowed for in the standard method of calibrating the results of the usual lepromin test, by not regarding as positive any reaction that is less than a certain diameter.

We have already reported the use of depot lepromin injection by the multipuncture route, the results of which are easily correlated with those obtained with the standard saline suspension by the usual intradermal method (Kinnear Brown and Stone[5,6,7], 1958-9). The modified test has the advantages that the reaction is less violent, the method is economical, and the lepromin is retained in the skin sufficiently long to indicate if there is conversion from negative to positive within a few weeks. A number of children whose depot tests were negative at three, four and five weeks showed a change to positive within four weeks of BCG vaccination, whilst others who were not BCG vaccinated showed no change whatever. Apart from the inference that BCG vaccination was the cause of the conversions, the investigations showed that this method eliminates the need for a second test and, therefore, the complicating effect of a second dose of antigen.

We now decided to investigate by the multipuncture route the results of using various skin preparations suspended in the depot medium, and the subsequent effect of BCG vaccination.

We first tested 25 patients who were known to be positive to lepromin, the majority strongly so, with a 1:20 depot 'lepromin' made from the skin of an active tuberculoid lesion which was bacteriologically negative on staining by Ziehl Neelsen. Eight were

positive at three weeks; of these seven were Grade I, and one was Grade II. The remaining 17 were negative. Three of the 17 negatives (Nos. 5, 7 and 13) gave a Grade I response which was just palpable at some point in the first or second week, but which rapidly subsided; seven (Nos. 1, 3, 6, 8, 9, 10 and 23) became temporarily visible during the same period but were never palpable, the remaining seven showed no change whatever. Of this group of 25 patients 19 were subsequently tested with a 1:20 depot preparation made from normal skin, but none reacted positively at any time. The results are set out below in tabular form to make comparison easy.

REACTIONS AT THREE WEEKS IN 25 PATIENTS

Serial Nos. of Patients	*Size of Reaction in mm. of Original Lepromin Test and whether Ulceration Present*	*Grade* of Reaction*	*With Lepromin made from Tuberculoid Skin*	*With Lepromin made from Normal Skin*
1	14.U		–	
2	15.U		I	–
3		II	–	
4	11.U		I	–
5	14.U		–	
6	9		–	–
7		III	–	–
8	19		–	
9		II	–	
10		II	–	–
11		I	–	–
12		I	–	–
13	11			–
14	10.U		I	–
15	13.U		I	
16	8		–	–
17	26.U		II	–
18		I	–	–
19		II	–	–
20		I	–	–
21		III	I	–
22	24.U		I	–
23		II	–	
24		III	I	–
25		II	–	–

NOTES

U Ulceration.

* Grade according to response to previous multipuncture test with 1:20 lepromin.

Nos. 5, 7 and 13 gave a transient Grade I response in the 1st or 2nd week.

Nos. 1, 3, 6, 8, 9, 10 and 23 gave a transient visible change which was never palpable.

What responses there were to the preparation made from tuberculoid skin were much less pronounced than those to normal lepromin and more transient, but they appeared to be related, e.g., those who were weakly positive at 21 days to a 'tuberculoid' lepromin were strongly positive with ulceration to ordinary lepromin or Grade III by the multipuncture method. There was no reaction to the normal skin preparation in any patient. This was not expected, as the responses reported to 0.1 ml. by intradermal injection were proportionally weak (Floch[3], Kooij *et al.*[2,8], and Davey[4]) and the quantity of antigen injection by the multipuncture route is very much less.

Of the 19 patients who had 'tuberculoid' lepromin and 'normal skin' antigen ten were tuberculin negative. They were vaccinated with BCG three weeks after their depot tests but none showed any subsequent change at the sites of the tests.

We now applied various suspensions by the multipuncture method to 56 non-leprous school children.

Of these Group A comprised 37 who were tested simultaneously in the interscapular area with four different 1:20 antigens made up with depot medium as follows: (*i*) normal skin from a healthy individual; (*ii*) active tuberculoid tissue, using the same preparation as in the above 25 patients; (*iii*) bacteriologically negative skin from a lepromatous patient; (*iv*) normal lepromin from bacteriologically positive tissue.

Group B included 19 children who were given antigens from the same sources but made up with saline.

Neither group gave any responses to the preparations made from normal or tuberculoid skin. There were 28 of the 37 in Group A positive to normal lepromin, and three of those who had a Grade III reaction gave a Grade I to the lepromin from bacteriologically negative tissue. In Group B all 19 were positive to normal lepromin and five gave also a feeble positive to that from the bacteriologically negative tissue from a lepromatous patient.

Of the 37 children in Group A 36 were now vaccinated with BCG; nine were weakly tuberculin positive (Grade I Heaf) and 27 negative. There was no untoward reaction in the nine who were weakly tuberculin positive. Of these, six were lepromin positive and three negative. After BCG vaccination the depots in the three negatives showed conversion and the other six showed an increase in positivity.

Of the 27 tuberculin negatives 22 had been lepromin positive and five lepromin negative. After BCG the depots in the five negatives showed conversion and 16 of the 22 positives registered an increase in positivity. There was no change in any of the other depots.

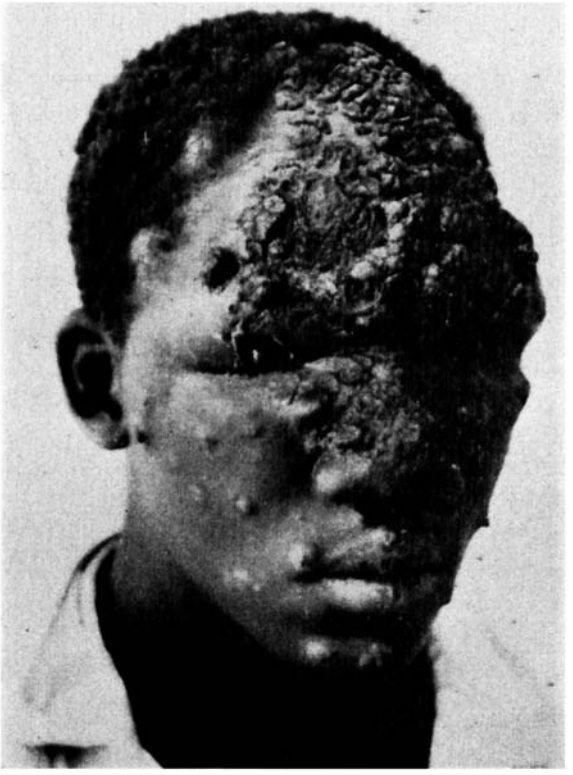
Summary and Discussion

1. The injection of a normal skin preparation by the multipuncture route did not produce any reaction in either lepromin positive patients or uninfected children. It would, therefore, appear that any response to lepromin *by this route* is independent of any normal tissue element it contains, which is another point in favour of this method of testing.

2. The response to antigen made from tuberculoid skin in the patients tested was less marked and more transient than that to normal lepromin. That one occurred at all may have been due to undetected bacilli or tuberculoid tissue elements in the antigen. As no reaction with this same antigen was obtained in the lepromin positive healthy children, it may well be that lepromin positivity in patients includes an individual element that may be stimulated or exaggerated by the disease. In this connection one patient who had a large indolent ulcer in the site of the ordinary Mitsuda Test, a similar ulcer with weak lepromin, a Grade III response to the multipuncture method, and a Grade I to tuberculoid antigen, had also a violent contact dermatitis after applying an irritant herb to a large asymmetrical and clinically tuberculoid patch on his forehead which persisted for almost a year (see photograph). In this case biopsies of symmetrically distributed hypopigmented nodules elsewhere gave the picture of dimorphous leprosy. The response of a few individuals may, therefore, include two elements, one an allergic or foreign body reaction which is generally weak, and one which is stronger and more specific, owing to the presence of numbers of bacilli.

3. Of the 47 uninfected children in both groups who were lepromin positive, eight gave comparatively a much weaker response to antigen made from what was thought to be bacteriologically negative skin taken from a lepromatous patient. This patient had been consistently negative to Ziehl Neelsen for some time. The skin from which the lepromin was prepared had been infiltrated originally, but at the time the tissue was taken for the antigen it had the wrinkled appearance of resolution and a smear from it was negative. The weak response may therefore have been due to the presence of a few unidentified bacilli or to lepromatous tissue elements. This antigen has yet to be tried on lepromin positive patients.

4. BCG vaccination had no effect on any of the depots except those containing lepromin from bacteriologically positive lepromatous tissue. All the lepromin negatives became positive, and the majority of those who were positive showed an increase. The obvious and outstanding feature of these depot tests was the presence of large numbers of bacilli. The bacilli appear to be the important factor and BCG vaccination quickly alters the attitude of the host towards them in the majority of healthy individuals, a change which of course might take place later and more slowly by natural processes.



Violent contact dermatitis after the patient had applied an irritant herb to a tuberculoid lesion on the forehead.

5. Continued experience with the multipuncture depot test has convinced us of its simplicity, economy, and effectiveness. The response is less likely to include any factor due to normal tissue; conversely, and from a practical point of view, it does not appear possible to use any other skin preparation as a substitute, at least by this method.

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References

1. DE FARIA, J. L. The Mitsuda Test as an Indicator of Normal Resistance; Late Reaction with Normal Skin. Reviewed Internat. J. of Lep., **18,** 3; Oct. 1950, p. 443.
2. KOOIJ, R., and GERRITSEN, Th. Positive 'Lepromin' Reactions with Suspensions of Normal Tissue Particles. Internat. J. of Lep., **24,** 2: April, 1956, p. 171.
3. FLOCH, H. Sur la Reaction de Mitsuda: Intradermal Reaction a l'aide d'extrait phenique de Peau Normale. Internat. J. of Lep., **24,** 3; July, 1956, p. 292.
4. DAVEY, T. F. and DREWETT, S. E. Lepromin-like Activity of Normal Skin Tissue. *Leprosy Review*, **29,** 4: October, 1958, p. 197.
5. BROWN, J. A. KINNEAR. A Modification of the Lepromin Test, *Leprosy Review*, **29,** 4: October, 1958, p. 184.
6. BROWN, J. A. KINNEAR and STONE, M. M. The Depot Lepromin Test and BCG Vaccination, *Leprosy Review*, **30,** 2: April, 1959, p. 110.
7. BROWN, J. A. KINNEAR; SHORT, G. M.; BLENSKA, W. Lepromin Reactions with Normal Tissue Preparations. Correspondence, Internat. J. of Lep., **25,** 3: July, 1957, p. 271.
8. KOOIJ, R., and GERRITSEN, Th. Lepromin Reactions with Normal Tissue Preparations. Correspondence, Internat. J. of Lep., **25,** 3: July, 1957, p. 273.