

THE LEPROMIN TEST—A REVIEW.

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ORIGIN AND HISTORY OF THE TEST :

The lepromin test is the only immunological test of value in leprosy. It is also called the Mitsuda Reaction, after its originator. It has sometimes been called the leprolin test, possibly on the analogy of the tuberculin test, but obviously this analogy is not admissible, and the word lepromin is to be preferred (tuberculin from the tuber-cle bacillus, and lepromin from leprom-a).

Mitsuda (1916) first reported that intracutaneous injections of an emulsion of boiled leprosy tissue rich in bacilli usually produced no reaction in nodular leprosy, but a marked local reaction in neuro-macular leprosy, which took the form of a nodule in the skin, usually appearing two or three weeks after the injection. At the International Leprosy Conference, Strasbourg, Mitsuda (1924) reported positive results in neuro-macular cases, in healthy contacts and in non-contacts, and negative results in nodular cases. He interpreted these results as indicating resistance of healthy persons and neural cases, and the lack of resistance in cases of the nodular type.

Mariani (1924) published his observations on a test similar to Mitsuda's with similar results. Between 1924 and 1930 the matter was further investigated chiefly in the Dutch East Indies. Bargehr (1926) studied the results of inoculation by the percutaneous method as used in the Von-Pirquet reaction, and obtained results similar to Mitsuda's, except that he found that non-contacts gave a negative response. He therefore thought that a positive test was indicative of leprosy infection. Bargehr's results were partly confirmed by de Langen (1929) and de Vogel (1929). Later, other workers found that Bargehr's percutaneous method of performing the test was much less reliable than the intradermal injection used by Mitsuda.

Hayashi (1933) published in the International Journal of Leprosy an article in which Mitsuda's findings were confirmed. From this time interest in the test became general and several workers reported on it. In some respects early opinions on the lepromin test were confirmed and in other respects they were modified.

LEPROMIN TEST IN A CASE OF THE NEURAL TYPE
Positive Reaction.



ORIGINAL LEPROMIN
4 Weeks after Injection.
Note:—Nodulation and Ulceration.



REFINED LEPROMIN
24 Hours after Injection.
Note:—Erythema and Thickening.

LEPROMIN TEST IN A CASE OF THE LEPRIMATOUS TYPE
Negative Reaction.



ORIGINAL LEPROMIN
4 Weeks after Injection.
A slight Induration at site of Injection.



REFINED LEPROMIN
24 Hours after Injection.
No Reaction.

About 1940 interest in the lepromin test was revived. The recent work, specially of Dharmendra and co-workers in Calcutta, of Fernandez and other workers in South America, has helped to modify and simplify the test, and to explain some of the anomalous features of the original Mitsuda's reaction.

PREPARATION OF LEPROMIN :

Till recently the methods used by various workers for the preparation of lepromin consisted essentially of grinding the boiled leprosy nodules, suspending the ground up material in .5% carbol-saline, and eliminating the large particles of tissue by filtration or sedimentation. Mitsuda (1924) took fresh leproma, boiled it for two hours in water, ground it and suspended 1 gramme of the ground material in 10 c.c. of .5% carbol-saline. Mariani (1924), Bargehr (1926), Hayashi (1933), and most later workers have used similar methods. De Langen (1929) boiled and then dried the nodule, ground it into a powder and stored it in vacuum tubes. When required for use, the powder was suspended in normal saline and the major lumps were filtered out. Muir (1933) used a method similar to that of de Langen.

Lepromin prepared by methods such as the above could not be standardised by counting the number of bacilli, since the bacilli occur in big globi. Muir attempted an elementary standardisation by a rough relative count made by preparing a smear with a standard platinum loop, and comparing with a similar smear made from the preparation which had already given satisfactory results in the test.

In recent years methods have been developed for the preparation of standard solutions of lepromin. Dharmendra (1941a) described a method of making from autoclaved leprosy material a saline suspension containing single bacilli, and practically free from globi and tissue. This preparation was then standardised by means of Breed's method for counting bacteria to contain 15,000,000 bacilli per c.c. Dharmendra (1942) later evolved a method of preparing a standard lepromin from dried and partly de-fatted leprosy bacilli obtained by extracting the nodules with chloroform, and standardised by weight of the bacillary powder.

Fernandez and Castro (1941) described another method of preparing lepromin from separate dried bacilli and standardised by weight of the dry bacilli. They separate the bacterial powder from a suspension of leprosy material in water, by taking advantage of the difference in density between leprosy bacilli and the tissues. A 1% suspension by weight of the bacillary powder is

prepared and further dilutions of 1 in 10, 1 in 100, and 1 in 1,000 are made from this suspension.

The chloroform method appears to be better than the other method, since with the chloroform method, the yield of bacilli is about three times as great, and weight for weight, the chloroform treated bacterial powder is more potent than the one obtained by the other method. The details of the chloroform method are as under:—

Pieces of lepromatous material, usually nodules cut from ears, are autoclaved and then ground in chloroform in a glass-mortar. The chloroform is pipetted off. The grinding in chloroform is repeated till a smear from the remaining tissue is almost free from bacilli. All the lots of chloroform used in grindings are pooled, and the remaining tissue is discarded. (A smear from the pooled chloroform shows bacilli in very large numbers and the absence of tissue cells or debris.) The pooled chloroform extract is stored in a refrigerator for 4 days. At the end of this period the chloroform is completely evaporated on a water-bath, the residual substance consists of lipoids and bacilli. The residue is then suspended in ether and the ethereal suspension is centrifugalized in a refrigerator. The deposit consists of bacilli. To remove the lipoids more completely, the bacillary deposit is again suspended in ether, the suspension is centrifugalized and the deposited bacilli are separated and dried in vacuum: smears made from the dried powder show only bacilli and no tissue. Standard lepromin is prepared by suspending 1 mg. of the dry bacterial powder in 10 c.c. of 0.5 per cent. carbol-saline. The suspension is made by putting the powder in a mortar, adding a few drops of N/10 NaOH, grinding with a pestle and adding the requisite amount of carbol-saline. 0.1 c.c. of the suspension is used for the test.

Dharmendra (1941b) also described a method of obtaining a solution of protein antigen of the leprosy bacilli standardised by weight of the antigen. Since the isolation of the protein antigen entails extra laboratory work, and the use of special technique, and since the extra labour does not result in any special advantages, lepromin prepared from dried leprosy bacilli as described above is preferable for routine purposes.

METHODS OF INOCULATION :

Mitsuda, the originator of this test, used the intra-cutaneous method for giving injections of lepromin; later Bargehr, de Langen and some others used the per-cutaneous method. Most of the workers, however, found that the per-cutaneous method was un-

reliable; and the intradermal method as used by Mitsuda has become the standard method.

THE REACTIONS :

The injection of lepromin results in (1) an early reaction seen after 1 or 2 days and tending to disappear after 3 or 4 days, and (2) a late reaction beginning after about 7 days (sometimes later) and reaching its maximum in 3 or 4 weeks. The late reaction is the classical Mitsuda reaction.

The comparative strength of these two reactions varies markedly with the preparation used for the test: with the original lepromin, the early reaction is slight, but the late reaction is marked giving rise to big nodules which often ulcerate; with the preparations made from isolated and broken bacilli, the early reaction is marked, and the late reaction is slight, the nodule being smaller and often not ulcerating; with the isolated protein of the leprosy bacillus or with the filtrate from the lepromin, there is only an early reaction, and no late reaction at all. In appearance the early and the late reactions are quite different and will be described separately:

(a) *The classical Mitsuda reaction :*

The late reaction is the classical Mitsuda reaction. It is characterised by marked local infiltration of the skin, at first somewhat diffuse but becoming more localised as the reaction develops, and producing a definite nodule easily visible and palpable. In cases in which the reaction is marked there is sometimes necrosis in the centre of the nodule, and the epidermis breaks down with the formation of an ulcer which discharges white cheesy matter, and may take a considerable time to heal. In many cases, however, a positive reaction is not associated with ulceration. The nodule attains its maximum size after about three or four weeks, and then gradually subsides, but it may be many weeks before it definitely disappears.

(b) *The early reaction :*

The early reaction is a reaction of the tuberculin type, and is characterised by the appearance of a definite area of erythema about half an inch or more in diameter, accompanied by an appreciable degree of oedema and thickening. Till recently the early reaction did not attract much attention, and was not considered to be of great significance. Fernandez (1940) however reported a special study of this early reaction. He found that it was always present in cases giving a marked late reaction, that the early reaction had the same significance as the late reaction,

and that the early reaction could be induced by injections of lepromin freed from bacilli by filtration. In non-cases, however, the early and late reactions often gave divergent results. Lowe and Dharmendra (1941) confirmed the report of Fernandez regarding the early reaction in a high percentage of the cases tested. They also reported that breaking down the bacilli by grinding accelerated and increased the early reactions and diminished the late reaction.

THE CRITERION OF POSITIVITY:

Since the early and the late reactions differ markedly in appearance, the criteria of positivity have to be considered separately for the two reactions:

(a) *The late reaction:*

Hayashi (1933) graded the positive lepromin reaction arbitrarily into three degrees: infiltrations of 3 to 5 m.m. diameter were classed as one plus (+), those with 5 to 10 m.m. as two plus (++), and those over 10 m.m., or which had suppurred as three plus (+++). Muir (1933) adopted a similar method of grading the reactions.

Rotberg (1939) tried to get a rational criterion for a negative and a positive result by studying the reaction in the two immunologically distinct groups of cases—typically lepromatous, and definitely tuberculoid. Any reactions seen in the lepromatous cases were recorded as negative, since according to Rotberg, they could not have any immunological significance. The reaction in a definitely tuberculoid case was considered positive and Rotberg laid down the following criterion of positivity. “Nodular lesion, often suppurred, usually belated in appearing, of progressive evolution, generally reaching its maximum from the second to sixth week, seldom less than 5 m.m. in diameter in the fourth week.”

Dharmendra and Lowe (1942) studied the matter in a large number of cases of the lepromatous and the tuberculoid types and were in general agreement with Rotberg, but could not subscribe to his view that any reaction seen in a lepromatous case should be considered as negative, since in a small percentage of lepromatous cases there is seen a definite reaction, though slight in degree, and since this kind of slight reaction is found in about 20% of the tuberculoid cases. This slight reaction differs from the marked reaction only in producing a smaller size of nodule which never ulcerates; it resembles a marked reaction in producing a definite nodule which is progressive and persistent and which is of the same histology as the nodules of the definitely positive

reactions. These workers defined a positive result as follows: "A progressive infiltration leading to definite nodulation from the second or third week onwards, persisting till at least the fifth or sixth week, often longer, the nodule in most cases measuring 5 m.m. or more in diameter at the end of the fourth week, but occasionally being smaller." Thus the characteristic feature of a positive result is the nature of the reaction (nodular, progressive and persistent) and not its size. The size of the nodule may, however, be used to grade the degree of positive reaction. For example, a nodule of 3 to $4\frac{1}{2}$ m.m. may be called weak positive, of 5 to $7\frac{1}{2}$ m.m. moderately positive, and over $7\frac{1}{2}$ m.m. strongly positive.

(b) *The early reaction :*

The early reaction is a reaction of the tuberculin type and is characterised by the appearance of a definite area of erythema about half an inch or more in diameter, the erythema being accompanied by an appreciable degree of oedema and thickening of the skin of the whole area and not merely at the point of puncture. The combined effect of the erythema and oedema of the skin is to produce a definite erythematous "flare."

RESULTS OF THE TEST IN CASES OF LEPROSY :

The results of the test in cases of leprosy vary markedly according to the type and the sub-type of the disease. Practically all workers are agreed that in typical cases of neural type, a vast majority give a positive result, the percentage recorded usually being over 90%. Similarly in active lepromatous cases, practically all workers have recorded negative results in over 90% cases; there are some workers who believe that a positive result is never seen in cases of the lepromatous type. The results in the types and sub-types of leprosy may be summarised as under:

Of the active lepromatous cases over 90% give a negative result, while less than 10% give a weak positive but not a strong positive result. The positive results are common in cases which show clinical and/or histological abnormality, but are not confined to them.

Of the 'doubtful' cases, about half give positive results. Correlation of the results with histological findings in these cases has shown that positive results are seen chiefly in cases which are either definitely tuberculoid or else show a tuberculoid element in histology.

In cases classified as 'neuro-anaesthetic,' the incidence and degree of positive results is fairly high; some workers, however, report a low incidence.

In the neuro-macular cases there is a high incidence of positive results, the incidence and degree of positive reactions increasing from 'simple' through 'tuberculoid not major' to 'major tuberculoid,' with a rising incidence and degree of tuberculoid activity. Of the simple cases about 20% give negative results, about 35% weak positive, and about 45% moderate or strong positive; whereas of the major tuberculoid cases, almost all give positive results, more than 80% being moderate or strong positive; in cases classified as 'tuberculoid not major' the figures are intermediate. The incidence and degree of positive results in all the sub-types of the neuromacular cases is higher in the bacteriologically negative cases than in the bacteriologically positive ones.

RESULTS OF THE TEST IN CONTACTS :

Bargehr (1926) and Mitsuda (1924) reported positive results, but de Vogel (1929) reported some negative results. Later workers have mostly reported positive results in healthy contacts particularly in adult life. In an endemic area in Bengal, Dharmendra and Jaikaria (1941) reported positive results in 60% of the contacts tested, the incidence of positive reactions increasing with age—in the age group 0-5 years it was 21%, but by the age of 30 this incidence rose up to 98%.

RESULTS OF THE TEST IN NON-CONTACTS :

Positive results in the non-contacts have been reported in countries with little or no leprosy, though the incidence and strength of such reactions have been lower than in countries where leprosy is endemic. Positive results in non-contacts have been reported by Cummins and Williams (1934) in England, Dubois (1936) in Belgium, Boncinelli (1937) in Italy, Esseveld (1937) in Netherlands and Bechelli *et al* (1945) in New York. Rotberg (1937), however, has challenged these results, expressing the opinion that few nodular reactions of more than 5 m.m. were observed in these cases, and that lesser degrees of reactions should not usually be recorded as positive. However, it appears that the occurrence of positive reactions in healthy individuals in non-endemic countries cannot be denied. Dharmendra and Jaikaria (1941) obtained positive results in healthy persons in parts of India where leprosy is very rare and under circumstances that make the chances of exposure to infection very remote. With the original Mitsuda antigen, they found positive results in 36% of persons as against 60% in healthy persons in endemic areas; as in the endemic area, the incidence of positive results was low in the 0-5 years age-group, but rose steadily with increasing age till the age of

30. With the improved and refined antigen they (Dharmendra and Jaikaria, 1943) reported the percentage of positive results in non-contacts were considerably lower (3 to 5%), but with no preparation were uniformly negative results obtained.

RESULTS OF THE TEST IN CHILDREN :

The results of the lepromin test in children are of special significance and deserve special consideration. Besides the presence or absence of leprosy, the various factors which influence the results of the test are age, exposure to infection and heredity.

(a) Age—

Most workers have reported a low incidence of positive results in lower age-groups, which rises progressively with increasing age. Chiyuto (1932) was the first to point out that in very young children the results of the test were usually negative. He reported on the test in 266 children, some of leprosy parents, some of healthy parents. Of the 23 children below the age of one year, all gave negative results; between 1 and 3 years, the results were variable; and above 3 years, nearly all were positive. Numerous other workers including Muir (1933), Burnet (1938), Bhattacharya (1935), Chatterji (1937), Pereira (1935), Rotberg (1937) and de Souza-Campos (1937) have reported similar results. Lara (1939) studied the test in children of leper parents and found that children below one year often gave weak positive results, and below two years about 50% were positive. Cochrane *et al* (1941) have reported the rising incidence of positive results with increasing age in children, mostly cases of leprosy. Dharmendra and Jaikaria (1941) made similar studies in healthy populations in endemic and non-endemic areas. In both the areas, the incidence of positive results was lowest in the 0-5 years age-group, and it gradually increased with increasing age.

Most workers have interpreted these results as indicating the relative susceptibility of children and increasing immunity with increasing age, even apart from exposure to infection. This view is, however, not shared by some workers who believe that the rising incidence of positive reaction with increasing age is caused by increasing chances of exposure to infection.

(b) *Exposure to infection and heredity—*

The two factors are so intimately interrelated that they will be discussed together. Chatterjee (1936) found that children with family contact showed a lower percentage of positive results than non-contacts or slight contacts in the same age group. He suggested that while slight contact increased the reaction to lepromin,

close contact and massive infection diminished it. He did not mention the possible influence of heredity in producing the differences. de Souza-Campos (1937) studied the test in children of leprous parents. He reported that those isolated at birth gave negative results, while those remaining longer with their parents showed a higher percentage and degree of positive results; and that the children of cases of lepromatous type usually gave negative results, while the children of cases of neural type commonly gave positive results. It would appear that de Souza-Campos considers that both heredity and exposure to infection influence the results of the test. The report of Muneuchi (1936) on the results of the test in leprous and healthy children, points to a similar conclusion.

Rotberg (1937) attributed the rise in the proportion of positive results with increasing age in children to increasing exposure to infection, but considered that heredity played an important part.

Cochrane *et al* (1941) have analysed the results of lepromin test in 276 children, mostly cases of leprosy. They found that the incidence of positive results in children with a history of intra-familial contact was lower than in the children with a history of only extra-familial contact; and concluded that the lepromin reaction tended more often to be negative in those where a history of contact was maximum. They tried to separate the two factors of contact and familial susceptibility, and came to the conclusion that the lepromin reaction was not significantly influenced by family predisposition, and that the most important single factor in bringing down cellular resistance in leprosy is continued contact with an open case.

It will be seen that studies of lepromin test in children have given varying results with different interpretations, and that even similar results have been interpreted differently.

RESULTS OF REPEATED LEPROMIN TESTING:

In the comparatively early days of work on the lepromin test, Bargehr 1926, de Langen (1929), and later Pereira (1935) reported that a negative reaction in healthy persons could be changed into a positive reaction by repeatedly testing these individuals with lepromin. The change could be more easily brought about in adults than in children, and this change was considered to be accompanied by immunity. Recently Lara (1939, 1940) has reported that in children of leprous parents repeated testing has a tendency to change a negative reaction into a positive one and to intensify the previously positive reactions. Lara, however, did not find this increase in reaction to be associated with an increased resistance to disease since some of such children later developed the disease.

Lagrosa (1939), Ignacio (1939), and Nolasco (1940) have reported on the effects of repeated testing in cases of leprosy; Lagrosa tested bacteriologically-negative cases of all types, Ignacio tested bacteriologically-positive active cases of all types, and Nolasco tested lepromatous cases in the phase of reaction. All these workers found that with repeated testing the doubtful and negative reactions gradually became positive. The induced positive reactions were, however, found neither to be associated with enhanced resistance, nor to be of any prognostic value.

Dharmendra, Lowe and Mukherji (1942b), however, did not find any increase in the reaction on repeated testing of cases with a negative or a weak initial lepromin reaction. Monthly tests for a period of up to 18 months failed to enhance the reaction in the large majority of the neural and lepromatous cases tested; in only 10% of the cases there was a slight increase observed, mostly in the neural cases. In several cases the reaction to repeated test was even weaker than to the initial test.

VARIATIONS IN THE RESULTS OF THE TEST IN THE SAME CASES :

In cases of leprosy the results of the lepromin test vary according to the types and sub-types of the disease. In the same case the results of the test may vary according to the varying circumstances in which the test is done. The deterioration of lepromin on keeping, and the use of different lots of lepromin may perhaps be responsible for some of the differences, but variations are seen even when these two factors are eliminated.

The most important factors in influencing the result of the test in the same individual are variations in (a) clinical activity, (b) the time of the year when the test is done, and (c) site of injection. The repeated testing with lepromin and the occurrence of intercurrent diseases and debilitating conditions have also been reported to influence the results of the test.

(a) *The variations in clinical activity—*

(i) *The neural cases—*Dharmendra, Lowe and Mukherji (1942a) reported a study of the variations in the results of the lepromin test seen in cases of the neuro-macular type demonstrated by repeated testing of the same cases in different circumstances. They concluded that the two most important factors were the variations in the clinical activity and the time of the year when the test was done. In cases in which the factor of seasonal variation was eliminated they found that the subsidence of clinical activity was associated with a diminution in reaction to lepromin.

(ii) *The lepromatous cases—*Various workers have reported that

in the lepromatous cases the subsidence of activity of the disease is sometimes associated with a negative lepromin reaction becoming positive. Hayashi (1933), Lagrosa (1939), Rodriguez (1938), Radna (1938) and Igarashi and Hayashi (1941) have made such reports. Dharmendra and Mukherji (1947) have partly confirmed these findings; they found that the subsidence is often associated with an increase in reaction to lepromin, although no definitely positive reactions were seen.

(b) *Seasonal Variations*—

By eliminating the other factors, Dharmendra, Lowe and Mukherji (1942a) investigated the influence of the seasonal variations on the results of the lepromin test in the neural cases. They found that the same lot of lepromin used in the same patients showing no change in clinical conditions gave a stronger reaction in summer and a weaker reaction in winter.

(c) *The site of injection*—

Variations in the results of the test have been reported with injections given in different parts of the body, and with the injections given inside the leprous patch or in the normal skin outside. Tisseuil (1931) reported that injection into the lesions produced a reaction within 48 hours with infiltration and redness, while in healthy tissues the reaction commenced after a fortnight. Hayashi (1933) also reported that quicker and stronger reactions occurred inside the patches than outside, specially in cases of tuberculoid macules; macules in the nodular cases gave negative reactions. Cerqueira (1938) reported on an advanced case of leprosy with macules and nodules, in whom the reaction to lepromin was positive in the macules and negative in the nodules and healthy areas. Mendes and Cerqueira (1939) reported marked variations in the results of the test with injections given in different parts of the body; it was even possible that strong positive reaction might be seen in one region and negative in another. They found that in the neural cases the macules sometimes showed stronger reactions than in apparently healthy areas, but that in bacteriologically positive macules the reaction was generally negative. They concluded that the intensity of reactions was not entirely conditioned by the lepromin, but also by regional differences in immunity.

Recently Davey (1946) has reported a detailed study of the results of the test inside and outside the macules of leprosy of various types. He found that the injections inside the patches always produced stronger reactions and higher incidence of positive results than those in normal skin. This was specially marked in

cases of the lepromatous type; while his results in normal skin in cases of this type were in common agreement with most published results, he reported a high percentage of positive results in the lepromatous macules themselves. These findings of Davey regarding the lepromatous cases, however, have yet to be confirmed. The author of this 'Review' is investigating the matter, and his findings do not confirm those of Davey; in both the flat and the tuberculoid patches of the neural type the reaction is usually stronger inside the patch than in healthy skin outside, when the patches are bacteriologically negative; in bacteriologically positive similar patches, and in patches of the lepromatous type, there is usually no difference in the reaction at the two sites.

In conclusion it may be said that there is undoubtedly some evidence regarding the regional differences in reactions to injections of lepromin, evidently depending on variations in local tissue immunity. However, this may not interfere with the utility of the test if the same site (say the inside of the arm) is always selected for the test and if the injection is given outside the patch.

(d) *Debilitating conditions*—

Muir and Chatterji (1934) reported that in cases of leprosy the occurrence of intercurrent diseases and debilitating conditions could markedly reduce the reactions to lepromin, and may make some positive cases turn negative; the reactions increasing again when the debilitating factors disappeared. Other workers including Rotberg (1937) and Lara (1940) have failed to verify this.

(e) *Repeated testing*—

As already stated various workers have reported that with repeated testings doubtful and negative reactions become positive. However, Dharmendra, Lowe and Mukerjee (1942b) have failed to verify this.

THE ACTIVE PRINCIPLE OF LEPROMIN :

(a) *The original lepromin*—

The original lepromin consisted of a mixture of the leprous tissue and the lepra bacilli. Hayashi (1933) was of the opinion that the reaction was dependent on the presence of the bacilli. He prepared lepromin from a lymphatic gland of a subsided case of the lepromatous type, consisting entirely of leprous tissue but containing very few bacilli; the vaccine prepared from this material gave very much feebler reactions than those produced by vaccines prepared from the usual leprous tissue. He, therefore, concluded that the leprosy bacilli were responsible for the production of a positive reaction. This view has been held by all the late workers.

(b) *Fraction of the leprous tissue*

A few workers have attempted to isolate the antigenic fraction or fractions from emulsions made by grinding up lepromatous tissue. Villela (1938) and Rabello, Thiers-Pinto and Villela (1938), at the Cairo Congress reported the isolation from leprous tissue of an active non-lipoid fraction which on injection into the patients produced a reaction similar to that produced by ordinary lepromin. Rabello (1938), Rabello and Villela (1938), and Rabello, Villela and Tostes (1939), re-stated the same findings. Villela (1939) has described a method of the preparation of the active fraction "apparently of protein nature." From the nature of reaction produced by this fraction, it appears that these workers were working with unbroken and incompletely broken leprosy bacilli and not with protein fraction of the bacilli. This belief receives support from the account given of the method employed by these workers, which is not likely to break up the bacilli and liberate the bacillary protein.

Paras (1938) isolated the major lipid components (phosphatides, acetone-soluble fat, and wax) of leprous nodules. (Isolation of the non-lipoid fraction has not yet been reported). Skin tests on a few cases of leprosy showed that of these lipid fractions only the wax produced definite reactions similar to, but not as intense as, those produced by ordinary lepromin. A consideration of the method employed by this worker makes one believe that it was quite likely that some leprosy bacilli were present in the wax and were responsible for the weak late reaction produced by it.

Henderson (1940) isolated proteins from leprous spleens rich in acid-fast bacilli, by grinding the dried spleen in a ball-mill, and by extracting the ground material with distilled water or with phosphate buffer. The Joint Committee of Leprosy Skin Tests (1940) used these preparations for making skin tests on cases of leprosy, contacts and non-contacts. No late reaction of Mitsuda type was seen, early (24-48 hours) reactions of the tuberculin type were seen in some persons in all the three groups. These reactions were, however, very weak.

It would thus appear that of all the above workers only Henderson was successful in obtaining the protein antigen from the
(c) *Fractions of the leprosy bacillus*—
leprous material.

Dharmendra (1941b) reported a detailed study on the active principle in the lepromin. He worked not with leprous tissue but with the leprosy bacilli separated from these tissues by his chloroform method. From these bacilli, he isolated various chemical

fractions by means of breaking down the bacilli, by grinding, and extracting them with different solvents. In this way he isolated the following fractions of the leprosy bacilli: protein, polysaccharide, glyceride, phosphatide and wax. Of the protein itself, three different fractions were obtained: the nucleo-protein, the acid-soluble protein, and the alcohol-soluble protein. The antigenic action of these various preparations, as also of the lipoids of the nodule and the residue left after the removal of the leprosy bacilli was tested by intradermal tests in cases of leprosy, and the following conclusions were drawn:

(1) In the lepromatous nodule only the bacillary matter is antigenic. The bacilli produce both the early reaction (24 to 48 hours) and the late reaction of Mitsuda type. The lipoids from the nodule showed slight activity, but this is believed to be caused by traces of bacillary matter retained in it. The nodular tissue freed from bacilli is not antigenically active.

(2) Of all the fractions isolated from the bacilli, only the protein is definitely antigenic, producing only an early reaction of the tuberculin type. None of the lipid fractions produced any significant reaction, but the polysaccharide produced slight early reactions in some cases. This weak antigenic activity of the polysaccharide fraction is most probably caused by traces of protein in it.

(3) All the three protein fractions were antigenic, the nucleo-protein being the strongest.

(d) *Conclusions—*

Dharmendra's work has, therefore, definitely proved that the active principle of lepromin is the protein antigen of the leprosy bacillus, which when injected in solution produces only an early reaction.

IS THERE MORE THAN ONE ANTIGEN?

(a) *The different reactions—*

The intradermal injections of the preparations derived from human leprosy tissue are capable of causing reactions of three different types: (i) the classical Mitsuda reaction characterised by the formation of a nodule 3 to 4 weeks after the injection, (ii) the early erythematous reaction of the tuberculin type preceding the classical reaction, and (iii) the early erythematous reaction of the tuberculin type followed by no late reaction. All the three different types of reactions can be explained on the basis of the protein antigen isolated by Dharmendra and it is, therefore, believed that all these are actually produced by the bacillary

protein. The differences in the type of reaction appear to be determined by the nature of the material to elicit the reaction.

(b) *Separate antigen for the early and the late reactions?*

Some workers, however, have postulated the existence of two separate antigens; one for the early, and the other for the late reaction. Fernandez (1940) made a comparative study of the early and late reactions to lepromin, and to a filtrate from that preparation. He found that the ordinary lepromin produced late reactions in all the cases in which it had produced early reactions. The filtrate, however, produced the early reactions only. From these results Fernandez concluded that "early and late reactions are probably brought about by different substances or toxins of the Hansen's bacillus." Harrell and Horne (1945) have also suggested the possibility of the existence of two antigens, a soluble protein or polysaccharide antigen responsible for the early lepromin reaction, and an insoluble firmly bound lipoid fraction responsible for the late reaction.

(c) *One antigen for both the reactions—*

Dharmendra's work, however, does not confirm the existence of the two antigens. His findings show two things; firstly, that to explain the early and late reactions, it is not necessary to postulate the presence of two antigens and, secondly, that actually none of the chemical fractions obtained from the leprosy bacillus produce any late reaction.

Early and late reaction explained by the same antigen—

The early and the late reactions can very well be explained on the basis of only one antigen, the early reaction being caused by the free antigen in the injected material, and the late reaction by the same antigen which is slowly liberated from the breaking down by the tissues of the bacilli contained in the material. This view is supported by the findings that the grinding of the bacilli enhances the early and reduces the late reaction, the preliminary breaking down of the bacilli being accompanied by the liberation of a large amount of antigen and leaving less antigen to be liberated later. The work of Kitano and Inoue (1941) lends support to this view. Kitano and Inoue treated ordinary lepromin with ultra-supersonic rays to break the bacilli contained in it. The lepromin thus treated was found to produce stronger early, but weaker late reactions than ordinary lepromin. The filtrate from this treated material was found to give early reactions stronger than those produced by filtrate or ordinary lepromin, and no late reactions at all. These workers attribute the early reaction to the dissolved components of the bacilli. They conclude that the soluble elements

are unable to produce Mitsuda reaction, which depends on the presence of solid bacillary elements in the injected material. They do not appear to have realised the significance of the enhanced early reaction produced by breaking down of the bacilli.

The chemical fractions of the bacillus—The work on the fractionation of the bacillus supports this view. A thorough fractionation of the bacillary powder has shown that none of the isolated fractions, nor the final bacterial residue gives rise to a late reaction.

(d) '*Species*' and '*Type*' specific antigens—

Another worker has brought up the question of the plurality of antigens in quite a different sense. de Souza Lima (1938) suggested that the Mitsuda antigen is a three part complex; one part coming from the tissue cells and being non-specific, the second part being common to acid-fast bacilli in general, and the third part being specific for the leprosy bacillus. Dharmendra has shown that the tissue cells completely freed from the bacilli are not antigenic. The statement of de Souza Lima regarding the part played by the tissue cells in bringing about the lepromin reaction has therefore not been confirmed since it has been shown that the whole lepromin reaction depends on the bacilli. Dharmendra's work, however, shows that of the protein antigen there are at least three fractions and that it is not impossible that one or more of these fractions is "species specific" and one "type specific." This conception is quite in keeping with the knowledge of the antigenic make up of other acid-fast bacilli.

(e) *Protein fractions of other acid-fast bacilli*—

Various workers have studied in cases of leprosy and in contacts the results of intradermal injections of acid-fast bacilli other than those of human leprosy. Such bacilli have included the tubercle bacillus, the rat leprosy bacillus, the bacilli isolated from leprous lesions, and the saprophytic acid-fasts. All these bacilli produce reactions in all cases of leprosy, including the lepromatous cases which are practically always lepromin-negative. These findings indicate that in lepromin-negative cases at any rate, the tissues react to other acid-fast bacilli differently from the way they react to the leprosy bacillus, and that there is at least this element of specificity in the lepromin test.

As stated earlier, Dharmendra isolated three protein fractions from the leprosy bacilli—the acid-soluble protein, the nucleoprotein, and alcohol-soluble protein. All these protein fractions produced early reactions in neural cases of leprosy, and negative reactions in the lepromatous cases. In the hope that some of

these fractions might be shared by other members of the acid-fast group of bacilli, and that at least one fraction might be "type-specific" for the leprosy bacillus, similar fractions were prepared from 6 other acid-fast bacilli, including the tubercle bacillus and the rat leprosy bacillus. All these fractions were tested by intradermal injections in cases of leprosy of both the neural and the lepromatous type with the following results:

Nucleo-protein—The nucleo-protein obtained from the leprosy bacillus produced positive results in 86% of the neural cases, and negative results in all the lepromatous cases tested. With the nucleo-proteins isolated from the other acid-fast bacilli, positive results were obtained in cases of both the neural and the lepromatous type, although the incidence of positive results was lower in most cases in the lepromatous type. The nucleo-protein from the tubercle bacillus was, however, equally active in both the neural and the lepromatous types of cases, producing positive results in 100% of the cases tested.

Alcohol-soluble protein—The alcohol-soluble protein from the leprosy bacillus produced positive results in 61% of the neural cases, and negative results in the lepromatous cases. The alcohol soluble protein from the other acid-fast bacilli produced positive results in both neural and lepromatous cases, the incidence of positivity being less in the lepromatous cases except in the case of the fraction isolated from the tubercle bacillus which produced positive results in 100% of all neural and the lepromatous cases. The alcohol-soluble protein from the rat leprosy bacillus gave results which were closest to the results obtained by the similar fraction of the leprosy bacillus, since it produced positive results in 63% of the neural and in only 13% of the lepromatous cases.

Acid-soluble protein—Acid-soluble protein from the leprosy bacillus produced positive results in 54% of the neural cases, and negative results in the lepromatous cases. Similar preparations from the other acid-fast bacilli produced positive results in both the neural and the lepromatous cases, although in the lepromatous cases the incidence of positive results was lower, specially in cases of the acid-soluble fractions of the rat leprosy bacillus and the Kedrowsky's bacillus. To begin with the acid-soluble fraction from the rat leprosy bacillus behaved exactly like the similar fraction of the leprosy bacillus, giving positive results in the 'neural' and negative results in the 'lepromatous' cases. Consequently it was hoped that these fractions from the two bacilli were antigenically similar and that it might be possible to use for the lepromin test the acid-soluble fraction of the rat leprosy bacillus in place of the lepromin prepared from human leprosy

tissue. As work progressed, however, this hope did not materialise as it produced positive results in 10% of the lepromatous cases.

The above work gives some indication of the existence of an antigenic relationship between the acid-fast bacilli; of all the bacilli tested, the bacillus of rat leprosy appears to be closest to the leprosy bacillus. The nucleo-protein and the alcohol-soluble protein fractions of other acid-fast bacilli appear to be different from similar fractions of the leprosy bacillus; the acid-soluble protein fraction appears to serve as a link between the leprosy bacilli and some other acid fasts.

(f) *Conclusions*—

Dharmendra's work does not confirm the existence of two antigens of the leprosy bacillus, one for the early and the other for the late reaction. It, however, lends support to the view that there may be more than one antigen in the leprosy bacillus, some of which may be shared by the other members of the acid-fast group of bacilli, and at least one may be specific for the leprosy bacillus.

GROUP SENSITIVITY OF ACID-FAST BACILLI:

As stated earlier, positive results with lepromin are seen in non-contacts, i.e., persons who have not been exposed to leprous infection although the incidence is lower than in contacts. While some workers, who consider the test as one of specific allergy for leprosy, have questioned the occurrence of positive results in non-contacts, other workers, who consider the test as one of allergy but not necessarily specific, have attempted to explain the positive results in non-contacts on the hypothesis that infection with acid-fast bacilli, specially the tubercle bacillus, may make persons allergic to the leprosy bacillus.

The question regarding the group sensitivity of the tubercle and the leprosy bacillus can be viewed from four aspects; firstly, whether infection with the tubercle bacillus can make a person lepromin-positive, secondly, whether leprosy can cause a positive tuberculin test; thirdly, whether injection of the tubercle bacillus can make a lepromin-negative person lepromin-positive; and fourthly, a consideration of the antigenic make up of the acid-fast bacilli.

(a) *Tuberculous infection and the lepromin test*—

Rotberg and Oliveira (1937) lepromin tested patients with tuberculosis in a hospital in Brazil, and reported that the percentage of positive results was similar to that in healthy adults in areas endemic for leprosy. They concluded that they did not find any

evidence for a correlation of immunities in leprosy and tuberculosis.

Other workers, however, have reported results which support the opposite view. Such reports have been made by Fernandez (1943a), Convit *et al* (1944), Harrell and Horne (1945), and Weeks and Smith (1945). These workers studied the reaction to lepromin of persons suffering from various forms of tuberculosis and came to the conclusion that the positive lepromin reactions seen in such persons were caused by infection with the tubercle bacillus. Bechelli *et al* (1945) tested tuberculin and lepromin reactions in persons not exposed to leprosy infection and came to a similar conclusion.

(b) *Tuberculin test in cases of leprosy—*

Adant (1932), Dubois (1932) and Rotberg (1938) tuberculin tested cases of leprosy and non-cases in the same area, and reported identical results in both the groups. These workers were of the opinion that a positive tuberculin test in cases of leprosy indicated infection with the tubercle bacillus.

Other workers, however, have reported results which indicate that infection with the leprosy bacillus may itself produce a positive tuberculin test. Such findings have been reported by Nitto (1937), Fernandez (1939c), and Schujman (1945). This view is supported by the experimental work of Melsom (1938) in guinea-pigs. Melsom reported that normal guinea-pigs inoculated intradermally with macerated leprosy nodules very often developed tuberculin hypersensitiveness persisting over one year.

In cases of the lepromatous type divergent results are often seen with the tuberculin and the lepromin tests. The lepromatous cases are almost always lepromin-negative, but a large proportion of them are tuberculin-positive. Dharmendra found that the incidence and the strength of tuberculin reactions was higher in the lepromatous cases than in the neural cases. Some other workers have also reported similar findings, and have attributed this to a higher incidence of tuberculosis in the lepromatous cases.

(c) *Sensitisation experiments—*

Fernandez (1939c) reported that many lepromin—and tuberculin-negative healthy persons could be made lepromin—and tuberculin-positive by BCG vaccination. Fernandez and Castro (1942) reported that it was possible to sensitise lepromin-negative persons by an intradermal injection of lepromin. Fernandez (1943b) reported that sensitisation to lepromin in lepromin-negative persons could be produced not only by the leprosy bacillus but also by an injection of a suspension of the

tubercle bacillus. Dharmendra and Jaikaria (1947), however, failed to sensitise lepromin-negative persons by a single injection of suspension of the leprosy bacillus.

(d) *Antigenic make-up of the acid-fast bacilli—*

As stated earlier, Dharmendra's work on the protein fractions of the leprosy bacillus and some other acid-fast bacilli indicates that one of the protein fractions, i.e. the acid-soluble protein fraction may be common to the various acid-fast bacilli included in the study. This work, therefore, lends support to the possibility of co-sensitivity by the different members of the acid-fast bacilli group.

(e) *Conclusions—*

It will be seen that divergent views have been expressed regarding the co-sensitivity of the tubercle and the leprosy bacilli. The consensus of opinion, however, is that group sensitivity is possible. It appears that in a large number, but not in all, of the cases of positive lepromin results seen in persons not exposed to leprosy, the positive reaction is caused by infection with the tubercle bacillus. Dharmendra and Jaikaria (1941) tested with lepromin and tuberculin 260 persons in various age-groups in a part of India where the chances of leprosy infection were remote. They found that the incidence of positive reaction to both lepromin and tuberculin rises with increasing age. The lepromin sensitivity was higher (55%) in the tuberculin-positive than in the tuberculin-negative persons (15%). The facts that 45% of the tuberculin positive persons were lepromin-negative, and that 15% of the tuberculin-negative persons were lepromin-positive indicate that infection to the tubercle bacillus is not always accompanied by sensitivity to lepromin, and that sensitivity to lepromin may exist in the absence of tuberculous infection. Radna (1938) also reported positive lepromin reaction in a large proportion of tuberculin-negative healthy persons belonging to an area where leprosy is very rare.

WORK ON ANIMALS :

The work on animals has produced variable results and has not contributed much towards clearing up the nature of the lepromin reaction. Sugai *et al.* (1918) reported that in guinea-pigs a slight degree of immunity to tuberculosis was conferred by previous injection of leprosy organisms. Rodriguez (1937) found that the natural immunity in animals is not necessarily accompanied by the power to react allergically to the leprosy bacillus. He reported that old dogs reacted to the intracutaneous injections of lepromin while young dogs did not.

Watanabe (1937) produced in rats and apes "certain allergy" to the leprosy bacillus by repeated injections. Melsom (1938) found that by injections of leprosy material some guinea-pigs could be made allergic to tuberculin. Harris and Schatenburg (1938) on injecting various acid-fast bacilli into rabbits obtained some evidence of a relatively specific allergy. Dharmendra attempted to confirm the report but found that the results were so variable that no definite conclusions could be drawn. Wade (1941) reported on lepromin reaction in normal dogs, and confirmed the findings of Rodriguez that dogs react positively to intracutaneous injections of lepromin. He concluded that the reaction while undoubtedly of allergic nature is not a test of the existence of hypersensitiveness but rather one of capability of developing the allergic state after the introduction of antigen. Fielding (1944) reported on lepromin tests in rabbits, guinea-pigs, dogs, cats, and rats. He found that the test in animals gave variable results, except in the case of rats which consistently failed to react.

HISTOLOGY OF THE LEPROMIN TEST :

The histology of the test has been described by Hayashi (1933), Manalang (1932), Schujman (1936), Rabello and Rotberg (1937), Nagai (1938), Tachikawa (1939), and Fernandez (1939b). Hayashi, Manalang and Schujman have described the early changes at the site of injection as those of acute inflammation, and the later changes of the tuberculoid nature characteristic of allergic skin reaction, whereas Nagai, Tachikawa, and Fernandez described the early changes as also being characteristic of an allergic reaction.

THE NATURE OF THE REACTION :

The exact nature of the lepromin reaction has been a controversial matter although the consensus of opinion is that a positive reaction is an allergic phenomenon. The various views are discussed below:

(a) *A test of tissue resistance?*

Mitsuda's original view was that a positive reaction was not the result of exposure to infection, but merely represented the resisting powers of healthy tissues to the bacilli. The only specific feature about the test was the negative response in lepromatous cases showing absence of resistance.

Various workers, while adopting the main position of Mitsuda regarding the non-specific nature of the test, have pointed out how factors such as age and intercurrent disease may

influence the results of the test. Such workers explain the absence of reaction in young people, and the increasing incidence of positive reactions with increasing age, on the basis of "serological ripening," or the development with age of higher powers of resistance, the reaction being non-specific.

A few workers have considered that while the basic reaction is non-specific, it may be enhanced by the specific factor of exposure to infection since in lepromin-positive contacts the reaction has been found stronger than in lepromin positive non-contacts.

(b) *A test of specific allergy?*

Bargehr expressed the view that a positive reaction was the result of specific allergy and immunity, and was produced by infection with the leprosy bacillus. Several other workers have supported this idea of Bargehr. Such workers include de Langen, Pereira, Rotberg, Stein and Steperin. These workers report that non-contacts give negative results while contacts usually give positive results. They also tend to emphasise the importance of the duration and closeness of the contact in the production of this difference; Stein and Steperin (1934) reported that the percentage of the positives and the intensity of reaction was proportionate to the duration and closeness of contact.

It is difficult to reconcile this view with the positive lepromin reactions in non-contacts reported by several workers.

(c) *A test of non-specific allergy?*

In view of the facts that positive results are seen in persons who have never been exposed to leprosy infection, but that a positive test is otherwise strongly suggestive of an allergic phenomenon, some workers have considered a positive test as allergic but not necessarily specific. This view is supported by the possibility of the existence of group sensitivity amongst the various members of the acid-fast group of bacilli.

(d) *Reaction to injection of an irritant substance?*

Some workers are of the view that a positive reaction of the nature of a tissue response to the injection of an irritating substance. However, this does not appear to be a likely explanation since the lack of power to react to the antigen of leprosy bacillus found in the tissues of patients suffering from the lepromatous type is a specific one; the tissues of such patients retain the power to react to other acid-fast bacilli and to some irritating substances that have nothing in common with the acid-fast bacilli.

A few workers from Argentina, however, have reported certain

observations to the effect that reactions of the same significance as the lepromin reaction can be obtained with a non-specific non-allergic irritant substance. Basombrio *et al.* (1943), Mom and Basombrio (1943), Basombrio (1945) reported that the injection of a solution in acetone of 2.4 dinitrochlorobenzene 1 in 1,000, produced in neural cases, lepromatous cases, contacts, and non-contacts reactions similar to those produced by lepromin. This work has not yet been confirmed by other workers. In an attempt to confirm the above reports Dharmendra found that the solution of dinitrochlorobenzene in acetone, acted as an irritant and produced positive reactions in both neural and lepromatous cases, and that in appearance these reactions were different from those produced by injections of lepromin.

(e) *The anomalous feature of the reaction—*

The consensus of opinion is that positive reaction strongly suggests an allergic phenomenon, though not necessarily specific. There are, however, certain anomalous features of the reaction which distinguish it from other allergic skin reactions. These features are (i) the lateness and the nodular nature of reaction. (ii) positive results seen in non-contacts, and (iii) negative results in the lepromatous type of case, and in some persons after repeated exposure to leprous infection. These features are briefly discussed below:—

(i) *Lateness of the reaction.*—The lateness of the classical Mitsuda reaction and its nodular character distinguish it from the allergic skin reactions. Dharmendra's work has explained away these anomalous features of the test. It has been shown that differences observed in the lepromin reaction, and other allergic skin reactions are caused by differences in the nature of the material injected to elicit the reaction. Whereas in the other allergic skin tests a soluble antigen which is free to act at once is injected, in the lepromin test whole bacilli (together with the nodular tissue) are injected, the antigen being liberated slowly in minute amounts over a prolonged period. It is this constant liberation of minute amounts of antigen, reaching its height three weeks or more after the injection, that causes the characteristic late nodular reaction. This view receives support from the following findings:

(1) After injection of ordinary lepromin into tissues, intact leprosy bacilli can be found for several weeks at the site of injection,

(2) Breaking down of the bacilli enhances the early and reduces the late reaction,

(3) None of the chemical fractions separated from the leprosy bacilli produces any late reaction.

By providing an explanation for the lateness of the reaction, and by isolating the antigen which produces only an early reaction, the anomaly regarding the lateness of the reaction has been explained away. As stated earlier, some workers have postulated the existence of two antigens, one for the early and the other for the late reactions; since both can be explained on the basis of the same antigen, it is not necessary to postulate the existence of two antigens.

At one time in the initial stages of their work, Lowe and Dharmendra (1941) formulated a hypothesis which was capable of explaining both the lateness of the Mitsuda reaction and the positive results in non-contacts, but it was later abandoned as untenable. It was believed that non-contacts were possibly not allergic at the time of injection of lepromin, but being potentially allergic, might be sensitised and rendered allergic by the antigen liberated in the first few days after the injection, and that later their tissues might react allergically to the antigen still being liberated at the site of injection. This idea would mean that the same dose of lepromin might both induce allergy and demonstrate allergy by the nodular reaction three weeks later. This hypothesis was found untenable because soluble antigen of the leprosy bacillus was found to produce early positive reaction in a large number of the non-contacts. Wade (1941) formulated a similar hypothesis.

(ii) *Positive results in non-contacts.*—A few workers, who believe the test to be one of specific allergy, have denied the existence of such reactions, but there is no doubt that positive reactions are seen in non-contacts, although the incidence is less than in contacts. The most probable explanation for these reactions appears to be one based on group sensitivity of the acid-fast bacilli.

(iii) *Negative reactions in lepromatous cases.*—It is common knowledge that injections of lepromin produce negative results in the vast majority of lepromatous cases. This lack of reaction on the part of lepromatous cases is specific for leprosy bacilli and their products, since their tissues still retain the power to react to injections of other acid-fast bacilli and some irritant substances.

The cause of this specific lack of reaction is not clear. There are two main lines of thought bearing on this matter: firstly that the lack of response of the tissues in these cases is associated with

a heavy bacillary infection and may be similar to the negative tuberculin test seen in very advanced cases of tuberculosis; secondly, that this lack of activity may be inherent in the tissues and not related to the presence of leprosy bacilli in the body.

According to the first view, heavy or repeated infection would tend to break down or undermine the resistance of the body, causing lepromin reaction to be negative, and leading to the development of the lepromatous type of leprosy. Cochrane *et al.* (1941) have found that in children the proportion of positive lepromin reactions appeared to be lower in those who had had closer contact. In the opinion of these authors, "the most important single factor in breaking down cellular resistance in leprosy is continued contact with an open case." Dharmendra and Lowe (1942) reported a relation between the presence of leprosy bacilli in the lesions and the results of the lepromin test. Even in neural cases, the finding of bacilli in the lesions is very often associated with a weaker reaction than would be seen in similar but bacteriologically negative cases. Similar correlation with the bacteriological findings and the results of the lepromin test have been reported by some other workers. Mendes and Cerqueira (1939) reported that stronger reactions are sometimes seen in patches of leprosy than in apparently healthy areas, but that in bacteriologically positive patches, the reactions are always weaker. A study by Dharmendra of the reactions to lepromin injected in the patches and in the healthy skin outside has given similar results. The observations of Mom and Basombrio (1944) on the diffusion factor (R-factor) in leprosy skin have a bearing on the matter. These workers found that the presence of the leprosy bacilli modifies the diffusion factor found in normal skin, and that the intensity of the modification varies directly with the number of bacilli in the skin. While in tuberculoid cases the normal diffusion rate is present, in lepromatous cases the diffusion activity disappears completely from the skin.

According to the second view the lack of resistance is independent of infection, and is caused by the inherent incapacity of the tissues to react allergically to the presence of the leprosy bacilli; it is because of this inherent lack of resistance that the lepromin reaction remains negative even after exposure, and that infection results in development of the lepromatous type of the disease. Rotberg (1937) who regards a positive reaction as the result of specific allergy and immunity, believes that this inherent lack of reactivity is caused by hereditary factors. He has postulated the existence of a hereditary factor, on the presence of which depends the capability of the body to react allergically

to the leprosy bacillus. He believes that it is the absence of this hereditary factor, which he calls the "N. factor," which prevents certain individuals from becoming allergic when exposed to infection. This hypothesis is an interesting one. However, the reports of some workers to the effect that lepromin-negative cases can be made lepromin-positive by repeated testing, and that in the lepromatous cases the subsidence of the disease is sometimes associated with the negative reaction becoming positive, are evidence against Rotberg's hypothesis.

(f) *Conclusions*—

In conclusion it may be stated that a positive lepromin reaction is an allergic phenomenon though the allergy is not always specific, but may be dependent in some persons on sensitisation with other acid-fast bacilli, the most important being the tubercle bacillus. The evidence for the test being of an allergic nature may be summarised as under:—

(i) In appearance the local changes in the skin produced by intradermal injections of isolated antigens are similar to those seen in other allergic reactions, e.g. the tuberculin test. It is believed that the same antigen is responsible for the late nodular reaction seen in the classical Mitsuda test, the lateness and the nodular character of the reaction in this test being caused by the nature of the material injected.

(ii) In healthy persons living in areas heavily infected with leprosy, the incidence of positive results is much higher and the degree of reaction is much greater than in healthy persons living in areas where there is little or no leprosy.

(iii) The response to the injection of active fractions of *Mycobacterium leprae* is seen not only at the site of injection, but not infrequently in leprosy lesions away from the site of the injection, and also at the site of previous injections of lepromin. This means that the response is not only local, but not infrequently focal also. This is a strong indication of the reaction being allergic.

(iv) The reported sensitisation to lepromin of lepromin-negative persons by means of injections with leprosy or tubercle bacillus is strongly indicative of allergy.

(v) Definitely tubercloid and definitely lepromatous cases of leprosy are generally believed to be two immunologically distinct groups. The lepromin test gives almost uniformly positive results in one of these groups (tubercloid) and almost uniformly negative results in the other group (lepromatous).

(vi) The lack of power to react to the antigen of *Mycobacterium leprae*

found in the tissues of patients suffering from the lepromatous type is a specific one. They retain the power to react to other acid-fast bacilli and irritant substances.

THE VALUE OF THE TEST :

(a) *Value in diagnosis*—

The test is of no practical value in the diagnosis of leprosy since positive results are seen in non-cases. Mitsuda did not advocate the test as of value in diagnosis, but a few workers including Bargehr (1926), de Langen (1929), and recently Rotberg (1937) have regarded a positive result as indicating infection, but not necessarily clinical disease. Rogers (1930) thought that a negative reaction to lepromin amongst contacts indicated early and latent leprosy. Most workers do not support these views.

However, a negative test under certain conditions may be of value in excluding a diagnosis of leprosy, as in the case of a person having a lesion with the typical appearance of tuberculoid leprosy.

Fernandez (1939a) has stated that subcutaneous injections of 1 to 1.5 c.c. of lepromin may be helpful in differentiating between tuberculoid leprosy and certain other conditions, such as Boeck's sarcoid. In cases of leprosy this injection produces within 24 hours (a) general reaction consisting of rigor, fever, and joint pains, (b) local reaction at the site of injection, and (c) a focal reaction with erythema and generalisation of pre-existing lesions. In cases of conditions like Boeck's sarcoid, lupus vulgaris, and lupus erythematosus, the injection produces moderate general and local reaction, but no change in the lesions.

(b) *Value in classification*—

Practically all workers are agreed that the test is of considerable value in confirming the results of the classification of cases of leprosy based on clinical and bacteriological grounds, cases of the lepromatous type being almost always negative and cases of the tuberculoid type being positive. Some workers have proposed to give the lepromin test a more prominent place in this connection, and have suggested that it should be made a primary criterion for the classification of cases.

The present position of the test with reference to classification may be summarised as below:—

(i) *Typical tuberculoid and lepromatous cases*.—The test is positive in almost all typical tuberculoid cases, and negative in almost all typical lepromatous cases, but the clinical features in such cases are clear-cut and they can be classified on clinical

grounds alone. However, a negative reaction in a clinically tuberculoid case should be sufficient to throw doubt on the true nature of the lesion.

(ii) *Cases of doubtful classification.*—The test is of value in sorting out cases with the clinical findings not typical of either type, i.e. cases of “doubtful” classification. A definitely positive result in such cases would provide an indication for the case being tuberculoid, while a negative result would point towards its being lepromatous.

(iii) *Flat patches of the neural type.*—In cases with flat patches of the neural type and cases with nerve involvement without any patches, the results of the test are positive in about 50%. Apart from the difference in the results of the lepromin test, there are no clinical or histological differences in the two groups—the lepromin positive and the lepromin-negative. If the lepromin test were to be used as a criterion for classification, cases with similar clinical and histological findings will have to be put in two separate groups without any justification. No doubt, the result of the lepromin test in such cases does have a prognostic value, and the change of type that is sometimes seen from the neural to the lepromatous, is practically confined to the lepromin-negative group, but the change is not a constant feature of this group, many of the cases showing improvement similar to those in the lepromin-positive group.

(iv) *Variation in the results of the test.*—The variations observed in the strength of the lepromin reaction under different conditions may sometimes be sufficiently marked to make the once-positive reaction look negative and vice versa.

(v) *Conclusion.*—The above facts would lead one to conclude that while the lepromin test is of value in classification of certain cases, it cannot be used as a primary criterion for the general classification of cases of leprosy, which should be essentially clinical.

(c) *Value in prognosis*—

The lepromin test is of great prognostic value in cases of leprosy of all types, a positive reaction indicating a good prognosis. The prognostic value of the test is, however, best illustrated in cases of the neural type since in the lepromatous type a great majority of the cases have a negative reaction. The test is also of great prognostic value in cases of “doubtful” classification since it clears up the classification and thereby the prognosis. The question of the prognostic value of the test may be considered in a little detail in the various types of cases.

Cases of the lepromatous type.—A study of the results of the test in lepromatous cases brings out the following points. (1) of the active lepromatous cases, those with a positive lepromin test are more likely to improve; (2) when lepromin-negative lepromatous cases show subsidence of the disease, the test sometimes becomes positive with the subsidence; and (3) the subsided lepromatous cases that are lepromin-positive are less likely to relapse than similar lepromin-negative cases.

(1) Regarding the prognostic value of the test in active lepromatous cases, Hayashi (1933) expressed the opinion that the test has a prognostic value and Ignacio (1939) produced data indicating that in lepromin-positive lepromatous cases, clinical improvement was much more common than in the lepromin-negative ones. Dharmendra and Santra (1946) have reported similar results.

(2) Regarding the occurrence of a positive result in subsided lepromatous cases, Hayashi (1933), Lagrosa (1939) and Rodriguez (1938) have each made reports. Hayashi reported on the results of the test in 68 cases before and after subsidence; all the 68 cases had been clinically active and lepromin-negative in 1917, but 13 years later all were clinically inactive and 15 (i.e. 22%) had become lepromin-positive. Rodriguez tested lepromatous cases which had become bacteriologically negative and clinically inactive and concluded that lepromatous cases which showed marked improvement under treatment tended to become lepromin-positive. Lagrosa tested 41 subsided lepromatous cases and reported positive results in 25 (weak positive in 11, and strong positive in 14). Dharmendra and Mukherji (1947) reported on the lepromin test during the stage of activity and later when the disease had become clinically inactive and bacteriologically negative in 17 cases of the lepromatous type. In most of the cases the classification had been confirmed by histological examination of the biopsy material from the lesions. An initial lepromin test in the stage of activity gave a negative result in 15 and a weak positive in 2 cases, with subsidence of the disease; there was no change in lepromin reaction in 8 cases; in the remaining 9 the subsidence was associated with an increase in reaction, although a definite positive reaction was not seen.

(3) Regarding the recurrence of the disease in subsided lepromatous cases, Rodriguez (1938), Lima (1938), and Igarashi and Hayashi (1940) have found that of the subsided lepromatous cases, those that are lepromin-positive are much less likely to relapse than those that are lepromin-negative. Rodriguez studied

a large number of cases over one year, and found that none with a strong Mitsuda reaction had relapsed, and that the incidence of relapse in weak positive and negative reactors was 13% and 50% respectively. Igarashi and Hayashi studied a large number of subsided lepromatous cases over a ten year period, and concluded that the frequency of relapse varied inversely with the strength of the Mitsuda reaction; the incidence of relapse in strongly positive, weak positive and negative cases was 20%, 50% and "most" respectively. With the relapse, however, the lepromin reaction became negative. Dharmendra and Mukherji (1947) found that in subsided lepromatous cases associated with an increase in reaction to lepromin the relapses were less frequent than in similar cases showing little or no increase in lepromin reaction.

Cases of doubtful classification.—As stated earlier, the lepromin test is of value in clearing up the classification in several cases where the clinical findings are not clear-cut. Rotberg (1944) studied 182 cases which he describes as "non-characteristic," in half of the lepromin-negative cases, and in a quarter of the weak-positive lepromin cases, the disease had turned frankly lepromatous, while this change was seen in none of the cases with a moderate or strong lepromin reaction. This would indicate that the test is of definite prognostic value in cases of doubtful classification.

Cases of the neural type.—Hayashi (1933), Muir (1933) and Rodriguez (1938) on general grounds adopted the view that in the neural cases, a positive result indicated a favourable prognosis, and that a negative result indicated a marked tendency for the case to become lepromatous. Igarashi and Hayashi (1940) produced some data on this point. Of the neural cases tested in 1928, three were lepromin-negative and had become lepromatous in 1935, whereas of the large number of lepromin-positive neural cases, only one had become lepromatous, with a change in the lepromin reaction from positive to negative.

Rotberg (1944) had reported on the subsequent progress of 455 benign bacteriologically negative cases of leprosy under treatment from 1936 to 1942. In 91 of these cases the lepromin reaction was negative, in 101 it was weak positive, in 166 moderate positive, and in 147 strong positive. A change to the lepromatous type was seen among 59.3% of the lepromin-negative cases, and 32% of the weak positive cases; no such change was seen in cases with a moderate or a strong-lepromin reaction.

Dharmendra and Santra (1946) have reported similar results

in a follow-up of 229 neural cases in Bankura for 10 years. Improvement and subsidence was more frequent in the large lepromin-positive group than in the smaller lepromin-negative group; moreover, the change from the neural to the lepromatous type was seen in only the lepromin-negative group. In the larger lepromin-positive group of 171 neural cases, the disease became worse in only 11% while definite improvement, or total subsidence, was seen in about 54% of cases. In the small lepromin negative group of 58 cases, the disease became worse in 74% including the seven cases in which it changed to the lepromatous type; definite improvement or total subsidence was seen in only 10%.

Dharmendra and Mukherji (1946) have reported on a study of the progress of the disease over 6 years in relation to the results of the lepromin test in 109 cases of the neural type. Of these cases, the lepromin test was positive in 93, negative in 14 and doubtful in 3 cases. In the lepromin positive group, improvement was seen in 84% of the cases, the disease becoming worse in only 14%; in the lepromin-negative and doubtful group, improvement was seen in 53% of the cases, the disease becoming worse in 47%. In only one case did the disease change from the neural to the lepromatous type, and this occurred in a lepromin-negative case. The degree of positivity of the test was also found to influence the prognosis; the stronger the reaction, the better the prognosis.

(d) *Value in treatment*—

A few workers have reported favourably on the use of lepromin in the treatment of cases of leprosy. The consensus of opinion, however, is that it is of no value for this purpose. Other antigenic substances such as tuberculin, B.C.G., autolysed cultures of tubercle bacilli and other acid-fast organisms have been used by a few workers who reported encouraging results. The most widely used preparation was the "Nastin" of Deycke consisting of a killed suspension of acid-fast organism cultivated from a leprosy nodule. However, most of the workers who tried this preparation found either no improvement or only transitory results.

Recently Grasset and Davison (1942) have reported on the treatment of leprosy by means of a non-acid-fast variety of the tubercle bacillus referred to as NAC which had previously been recorded to be of value in the treatment of certain tuberculous infections by Grasset. These authors reported that no benefit was seen in lepromatous cases, but that in neural cases there was a definite improvement since a majority of cases who had positive nasal smears became negative.

(e) *Conclusions*—

Regarding the value of the test in cases of leprosy, it can therefore be concluded that the test is of definite prognostic value in all cases of leprosy, and is of value in the classification of certain cases. It is of practically no value in diagnosis or treatment.

THE VALUE OF THE TEST IN HEALTHY CONTACTS :

(a) *Likelihood of developing the disease*—

Apart from the prognostic value of the test in cases of leprosy, the test appears to be of prognostic value in contacts. Studies of the lepromin test in healthy contacts, children of leprosy parents, etc., made by de Souza Campos and Fernandez (1939) have indicated that the contacts most likely to develop the disease are those who are lepromin-negative; all the 9 children in whom the disease developed between 1936 and 1938 had been lepromin-negative. They confirm their opinion previously expressed that lepromin-negative children require greater care than those that are lepromin-positive. Various workers have expressed similar views. Lara (1939 and 1940), however, in his studies of children of leprosy parents has concluded that a positive lepromin test, either occurring naturally or induced by vaccination, is not accompanied by the decreased liability of developing leprosy.

(b) *Value in immunisation*—

Mitsuda, the originator of the test, as long ago as 1924, suggested the inoculation of healthy attendants of lepers with lepromin as a prophylactic measure. Manalang (1932) suggested the immunisation of children of lepers by injections of lepromin. As already indicated, various workers have found it possible by repeated testing to make lepromin-negative persons lepromin-positive. Lara (1940), however, found that this was of no prophylactic value. Rotberg (1937) suggested that only persons naturally possessing the "N factor" could be made allergic to lepra bacilli, and if this is so, the procedure of "immunisation" is likely to be of limited value since such persons are already potentially allergic and partly immune.

OTHER USES OF THE TEST :

Acid-fast bacilli other than the leprosy bacillus when injected into the skin of patients with leprosy, produce positive results in both "neural" and the "lepromatous" type of cases; the leprosy bacillus on the other hand produces positive results in neural cases only and negative results in lepromatous cases. An intracutaneous test with a supposed culture of the leprosy bacillus can therefore be used to test the genuineness of the culture,

Judged by this method, none of the several cultures so far obtained from the lesions of leprosy has been proved to be a genuine culture of leprosy bacillus.

SUMMARY.

(1) The origin and history of the lepromin test has been traced.

(2) A method has been described for the preparation of lepromin standardised by the weight of dried leprosy bacilli.

(3) The classical Mitsuda Reaction and the early (24-48 hours) reactions to lepromin are described.

(4) The results of the test in cases of leprosy, contacts and non-contacts, are discussed.

(5) The results of the test in children are discussed in relation to influence of age, exposure to infection, and heredity.

(6) The factors giving rise to variations in the results of the test in the same case are discussed, and it is concluded that the most important factors are the variations in clinical activity of the lesions, the presence or absence of leprosy bacilli in the lesions, and the time of the year when the test is done.

(7) The active principle of lepromin is considered to be the protein antigen of the leprosy bacillus, which when injected in solution, produces an early reaction. The late reaction (the classical Mitsuda reaction) is considered to be produced by the same antigen which is slowly liberated from the breaking down of the tissues contained in the injected material. The view of some workers regarding the plurality of antigens of the leprosy bacillus, one for the early and the other for the late reaction is not supported. However, the idea of plurality of antigen may be true in another sense; the leprosy bacillus may have more than one antigen, some of which may be "species" specific, and at least one "type" specific.

(8) The nature of the lepromin test is discussed, and it is concluded that a positive lepromin reaction is an allergic phenomenon, though the allergy is not always specific, but may be dependent in some persons on sensitisation with other acid-fast bacilli, the most important being the tubercle bacillus.

(9) Regarding the value of the test in cases of leprosy, it is concluded that the test is of definite prognostic value in all cases of leprosy, and is of value in the classification of certain cases. It is considered to be of little value in diagnosis or treatment.

(10) The other uses of the test include the possibility of its being used to test the genuineness of a supposed culture of the leprosy bacillus.

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