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THE IMMUNOLOGICAL SKIN TESTS IN LEPROSY.

Part II.

THE ISOLATED PROTEIN ANTIGEN IN RELATION TO THE CLASSICAL MITSUDA REACTION AND THE EARLY REACTION TO LEPROMIN.

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Three types of reaction.

In the present series of articles and in the literature of leprosy, intradermal injections of various preparations from leprous material have been reported as causing three different types of reaction.

(*a*) *The classical Mitsuda reaction.*—The main features of the classical Mitsuda reaction have been briefly outlined in Part I of this series (Dharmendra, 1942). Clinically, it is characterized by the formation of a definite nodule, sometimes accompanied by ulceration, at the site of the intradermal injection of lepromin. This reaction is best seen three or four weeks after the injection.

(*b*) *The early reaction preceding the classical reaction.*—An early reaction is sometimes seen one or two days after the injection of lepromin and preceding the classical late reaction. Clinically this early reaction is quite different from the

classical Mitsuda reaction, for it is of the ' tuberculin ' type and consists of an area of erythema accompanied by some œdema and thickening of the skin.

Most workers have ignored these reactions as being of no significance but Fernandez (1940) made a special study of the early reaction. He found that it was always present in cases giving a marked late reaction and concluded that results of the early reaction are of the same significance as the late reaction. The findings of Fernandez regarding the significance of the early reaction have been confirmed by Lowe and Dharmendra (1941).

(c) *Early reaction followed by little or no late reaction.*—Fernandez (*loc. cit.*) reported that the injection of a filtrate from lepromin induced an early reaction followed by no late reaction. This finding of Fernandez has also been confirmed by Lowe and Dharmendra (*loc. cit.*), although filtration was found to reduce the number and degree of definite reactions. The Joint Committee on Leprosy Skin Tests (1940) reported some early reactions followed by no late reaction to a protein isolated from leprous spleen by Henderson (1940). Kitano and Inoue (1941) have reported early reactions unaccompanied by any late reactions to filtrate of lepromin treated by ultra-supersonic waves to break the bacilli contained in it. Early reactions, unaccompanied by any late reaction to a protein isolated from the leprosy bacillus, have been reported by one of us (D.) in the preceding article (*loc. cit.*).

Thus, intradermal injections of the preparations derived from human material are capable of causing reactions of three different clinical types; the classical Mitsuda reaction, the early reaction preceding the classical reaction and the early erythematous reaction followed by no late reaction.

The different reactions are caused by the same antigen.

In Part I it has been shown (*a*) that the lepra bacilli are the only antigenic element contained in lepromin and (*b*) that the protein is the only definitely antigenic fraction of the bacillus. As shown later all the three different types of reaction can be explained on the basis of this one antigen. It is therefore believed that all the three different types of reaction described are actually produced by the bacillary protein.

Is there more than one antigen?

Fernandez (*loc. cit.*) made a comparative study of the early and late reaction to lepromin and to 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, usually 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 bacillus '.

The reported work in the preceding article does not confirm the existence of the two antigens, one responsible for the early reaction and the other for the late. This work shows two things: Firstly, that to explain the early and late reactions

it is not necessary to postulate the presence of two antigens; secondly, that none of the chemical fractions obtained from the leprosy bacillus produces a late reaction.

The findings of Fernandez 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 breaking down of the bacilli contained in that material. This view is suggested by the finding 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 the antigen leaving less antigen to be liberated later. The work on the fractionation of the bacillus supports this view.

Our first attempts at fractionation of the bacillus into soluble and insoluble portions gave results which were not clear-cut, for while the saline extract of the bacilli gave rise to early reactions, the bacterial residue left after thorough grinding and extraction with saline was still antigenically active, producing both the early and late reactions. (The degree of late reaction was, however, much reduced.) This observation could have been interpreted as showing the existence of an antigen other than the one removed by saline extraction. More thorough fractionation of the bacillary powder has, however, shown that most, if not all, of the activity of the insoluble residue had been caused by incomplete fractionation. 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.

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, a second part being common to the acid-fast bacilli in general, and a third part being specific for the leprosy bacillus. We have 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 Mitsuda reaction has, therefore, not been confirmed. It has been shown that the whole Mitsuda reaction depends on the bacilli. The present work has shown that the protein antigen can be divided into at least three fractions; it is not impossible that one or more of these fractions is ' species specific ' and one ' type specific '. This conception is, moreover, in keeping with knowledge of the antigenic make up of other bacteria.

Thus, our work disproves the presence of two antigens in the sense indicated by Fernandez (one antigen for the early reaction, and the other for the late) but shows that there is more than one antigen of protein nature.

The test with the isolated antigen and a comparison with the Mitsuda test.

The early reactions to the soluble antigen have been observed in 125 cases of leprosy, some ' neural ' and some ' lepromatous '.

In this work the antigen was used in doses of 0·02 mg., and positive results were obtained in most of the cases in which they could be expected. The not

infrequent occurrence of focal reactions however and the persistence of the early reaction for a week or more, have suggested that the dose used was too large, and that in cases of leprosy (but perhaps not in contacts), a considerably smaller dose could be used. In reading the results of this test a definite area of erythema of 10 mm. or more in diameter accompanied by infiltration and œdema has been recorded as a positive result. The average diameter of the erythema in positive cases was over 15 mm., the maximum being over 40 mm. The reading is best made at 24 hours.

Positive results were seen in cases of the ' neural ' type and negative results in cases of the ' lepromatous ' type. The significance of the test with this antigen, therefore, appears to be the same as that of the classical Mitsuda reaction.

A comparative study of the early reaction to the soluble antigen and of the late reaction to ordinary lepromin (the Mitsuda reaction) in the same patients has shown that the isolated antigen is at least as sensitive as, if not more sensitive than, ordinary lepromin.

The test with the isolated antigen has, moreover, great advantages over the classical Mitsuda reaction :—

(1) A pure antigen is used, which is of a known chemical nature and which can be accurately standardized by weight. This antigen is thus a great improvement on the crude antigen which consists of ground leprosy nodule and which is difficult to standardize.

(2) The results are obtained in 24 hours instead of three weeks or more—a great advantage to both the patient and the investigator.

(3) Undesirable late reactions, ulceration, etc., not uncommonly seen with the classical Mitsuda test are avoided—a very great advantage to the patient.

It is therefore suggested that for doing skin tests in leprosy the isolated antigen can, with great advantage, replace the ordinary lepromin of Mitsuda.

The anomalies of the Mitsuda reaction.

As already mentioned in the previous paper, the Mitsuda test has three marked anomalies, namely (*a*) its lateness, (*b*) the positive results in non-contacts and (*c*) the negative results in cases of lepromatous type. We will here discuss these three anomalies in the light of the present work.

(*a*) *The lateness of the Mitsuda reaction.*—In the preceding article it was suggested that the lateness of the reaction was caused by the nature of the material injected, most of the antigen not being free at first, but being liberated gradually in minute amounts over a prolonged period. Histological examination of the nodules produced by injection of ordinary lepromin in patients supported this theory ; intact and acid-fast bacilli could still be found several weeks after the injection. This observation is moreover in accordance with the previous

experience that Hansen's bacillus is extraordinarily resistant, for after injection into laboratory animals it can retain its form and acid-fastness for a year or more.

Further support was given to the theory by the finding that breaking down of the bacilli enhances the early reaction and reduces the late reaction. The final proof of the truth of the theory is provided by the work on the fractionation of the bacillus reported in the preceding article, the definitely antigenic fraction producing only an early reaction and no late reaction.

It appears, therefore, that it is the constant liberation of minute amounts of antigen, reaching its height three weeks or more after the injection, that causes the characteristic late nodular reaction of the Mitsuda test.

(*b*) *Positive results in non-contacts.*—As already stated positive results to the Mitsuda antigen in non-contacts have been reported by several workers and confirmed by Dharmendra and Jaikaria (1941). No satisfactory explanation has yet been given and the present work has not so far explained this phenomenon. Our work does, however, give some ground for the hope that an explanation may be forthcoming. The bacillary protein has been divided into three fractions, all antigenic, and it may be that one of the fractions is specific. If this is so, a specific allergic skin reaction will be made available.

At one time before the work had reached its present stage we formulated a hypothesis which was capable of explaining both the lateness of the Mitsuda reaction and the positive results seen in non-contacts. This hypothesis we have now abandoned as untenable and it is mentioned here largely because another leprosy worker (Wade, 1941) has recently advanced a very similar hypothesis. We thought that non-contacts were possibly not allergic at the time of the injection of lepromin, but being potentially allergic, might be sensitized 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 therefore means that the same dose of lepromin might both induce allergy and demonstrate allergy by the nodular reaction three weeks later.

If this theory had been true the soluble antigen should have given negative results in non-contacts. As already reported (Lowe and Dharmendra, *loc. cit.*) such results were not obtained. In 24 of the 39 healthy adults living in circumstances which made it highly improbable that they have ever had contact with cases of leprosy, positive results were seen within 24 hours on testing with the soluble antigen. This evidence showed that the hypothesis mentioned is untenable.

(*c*) *Negative results in cases of lepromatous type.*—The present work has no direct bearing on, and suggests no explanation of, this last anomaly, since there is little indication that the 'lepromatous' cases, while not reacting to ordinary lepromin, will react to isolated protein.

We will briefly outline two main lines of thought bearing on this matter. Firstly, the lack of the 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, this lack of activity may be inherent in the tissues, and not causally related to the presence of leprosy bacillus in the body.

According to the first view, heavy or repeated infections would tend to break down or undermine the resistance of the body, causing the lepromin reaction to be negative and leading to the development of the lepromatous type of the disease. We have found some relation between the presence of the 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. Cochrane *et al.* (1941) have found that in children the proportion of the positive lepromin reactions appeared to be lower in those children who had had closer contact. In the opinion of these authors 'the most important single factor in breaking down cellular resistance in leprosy is continuous contact with an open case'.

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 bacillus ; 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 this inherent lack of reactivity to be hereditary. He has postulated the existence of an 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 prevents certain individuals from becoming allergic when exposed to infection.

While either of these views will explain some of the observed facts, neither will explain all of them.

The bearing of the present work on the three main anomalies of the classical Mitsuda reaction may be summarized as follows : The lateness of the Mitsuda reaction has been explained. While no attempt has yet been made to apply the present findings to a study of the second anomaly, namely, the positive results in non-contacts, there is a hope that work with the different fractions of the protein antigen will give results of value. The present work has no direct bearing on the third anomaly, namely, the negative results in cases of lepromatous type, but work with the isolated antigen will facilitate a study of this matter.

Summary.

1. The intradermal injections of preparations from leprous material are capable of causing reactions of three different clinical types : The classical Mitsuda reaction (nodular), the early erythematous reaction preceding the classical reaction and the erythematous reaction followed by no late reaction.

2. Since protein is the only definitely antigenic fraction of the *Myco. lepræ* and since all the different types of reaction can be explained on the basis of this one antigen, it is believed they are actually caused by it. If at the time of injection all the antigen is free to act at once, only an early reaction is produced. If only part of the antigen is free, both early and late reactions are produced, the early reaction by the free antigen and the late by the same antigen which is liberated slowly from breaking down of the injected bacilli. If none of the antigen is free a late reaction only will be produced.

3. Since late reaction is not produced either by any of the isolated fractions or by the final residue of the bacilli, the idea that the early and late reactions are caused by different antigens is disproved.

Our work, however, shows the presence of more than one antigen of protein nature.

4. When compared to the classical Mitsuda test, the test with the isolated antigen is found to be at least as sensitive, to give results of the same significance and to have great advantages, particularly rapid results and the absence of unpleasant nodules and ulcers. It is, therefore, suggested that, for performing skin tests in leprosy, the isolated antigen may be used in place of ordinary lepromin.

5. By providing an explanation for the lateness of the reaction, the observations reported herein have brought the Mitsuda reaction more in line with the

allergic skin tests. The lack of specificity and the negative results in cases of the 'lepromatous' type have still to be explained before the reaction can be admitted as one of specific allergy. Work with the isolated antigen will facilitate a study of these phenomena.

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