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THE IMMUNOLOGICAL SKIN TEST IN LEPROSY.

Part III.

THE ISOLATED PROTEIN ANTIGEN IN RELATION TO THE ANTIGENS USED BY OTHER WORKERS.

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In the two preceding articles [Parts I and II of this series (Dharmendra, 1942; Dharmendra and Lowe, 1942)] the reactions to lepromin (suspension of ground leprous nodules) to filtrate from it, and to the protein antigen isolated from the leprosy bacilli have been discussed. A mention has been made of some other preparations which have been used by different workers mainly with a view to elucidating the mechanism of the Mitsuda reaction. In the present article it is proposed to discuss the bearing of some of these findings on the present work.

Reactions to disintegrated bacilli.

Nagai (1938) found that keeping leprous nodules over a prolonged period in 5 to 10 per cent lecithin or boiling the nodules in the same solution for half an hour resulted in the loss of acid-fastness of the leprosy bacilli and in their degeneration and granulation. Intradermal injections of a suspension of the nodule boiled in lecithin gave rise to reactions similar to those produced by ordinary lepromin.

Thus, this work gave no additional information regarding the mechanism of the test.

Kitano and Inoue (1941) broke down the bacilli by physical, instead of chemical, methods. These workers treated ordinary lepromin with ultra-supersonic waves 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 of ordinary lepromin and no late reactions at all. These workers attribute the early reactions to the dissolved components of the bacilli. They concluded that the soluble elements are unable to produce the Mitsuda reaction which depends on the presence of solid bacillary elements in the injected material. These findings agree with the present findings that breaking down of the bacilli is accompanied by the liberation of larger amounts of soluble antigen, and that this soluble antigen produces an early reaction only. These workers do not appear, however, to have realized the significance of the enhanced early reaction produced by the breaking of bacilli.

Reaction to the different fractions of the leprous nodule.—No other workers have reported on the isolation of chemical fractions of the bacilli themselves. A few workers have, however, 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 tissues of an active non-lipoid fraction. On injection into patients this fraction produced a reaction similar to that produced by ordinary lepromin. Rabello (1938), Rabello and Villela (1938) and Rabello, Villela and Tostes (1939) re-state the same findings. Villela (1939) has described the method of the preparation of the active fraction, 'apparently of protein nature'. The present findings agree with the findings of these workers to the extent that the lipoid fractions of the nodule are inactive, and that activity is confined to the non-lipoid fraction. The reaction to the active substance of protein nature separated by these workers is, however, of the 'delayed' type, like the classical Mitsuda reaction. This protein fraction isolated from leproma emulsion, therefore, differs markedly from the fraction isolated as here reported from the bacilli themselves, which gives only an early reaction of the 'tuberculin' type and no late reaction at all.

It is believed that these workers could not have actually separated the protein fraction of the bacilli, and were really working with unbroken and incompletely broken bacilli together with the proteins from the tissues. This belief receives support from the account given of the method employed by these workers to obtain their non-lipoid fraction. 'Ground leproma, boiled in distilled water, is extracted with petroleum ether and an ether-alcohol mixture is added to the aqueous phase. The precipitate forms the non-lipoid fraction.' Our experience indicates that such treatment is not likely to break up the bacilli and liberate bacillary protein, for the leprosy bacilli are very resistant to chemical agents.

Paras (1938) isolated the major lipoid components (phosphatide, acetone-soluble fat and wax) of leprous nodules (the isolation of the non-lipoid fractions

has not yet been reported on). Skin tests on a few cases of leprosy showed that, of these lipoid fractions, only the wax produced definite reactions similar to, but not as intense as, those produced by ordinary lepromin. The biological activity of the wax separated by Paras can, it is believed, be explained by assuming that the wax contained some lepra bacilli, and this idea also is supported by his account of his methods. The ground leprous tissue, after having been treated with alcohol-ether mixture, was macerated with chloroform, and the chloroform with the dissolved wax was separated from the tissue by filtration through a Buchner funnel. The great affinity possessed by chloroform for the bacilli has been mentioned in the first article of this series, and it is quite conceivable that some bacilli were carried with the chloroform and were present in the wax obtained by evaporating the filtrate.

Reaction to a protein isolated from leprous spleen.—Henderson (1940) isolated proteins from leprous spleens rich in acid-fast bacilli, by grinding the dried spleen in a ball mill at — 70°C. and by extracting the ground material with distilled water or with phosphate buffer. The work was undertaken in the hope of obtaining specific proteins of the leprosy bacillus, which could be used in serological or skin tests for the diagnosis of leprosy. The Joint Committee on Leprosy Skin Tests (1940) used these preparations for making skin tests on (*i*) bacteriologically positive cases of leprosy, (*ii*) children of leprous parents (contact group) and (*iii*) children of healthy parents, with no history of exposure to leprous infection (control group). 0·05 mg. of the isolated protein in 0·1 c.c. was used for the injection. No late reactions of 'Mitsuda' type were seen. Early (24 to 48 hours) reactions of 'tuberculin' type were seen in some persons in all the three groups. These reactions were, however, very weak, usually consisting of an œdematous area of less than 10 mm. The incidence of positive early reactions in the cases, contacts and non-contacts was 4, 19 and 11 per cent respectively. The Committee concluded that the protein extracts of leprous spleens 'do not contain any substance to which persons suffering from active leprosy (bacteriologically positive cases), or previously exposed to infection by leprosy, react specifically'. The Committee has explained this lack of reaction as follows :—

'Either the material, although derived from spleens rich in acid-fast bacilli, did not contain enough specific protein to elicit a positive reaction in the doses used, or sensitiveness comparable to the tuberculin-sensitiveness of tuberculosis does not exist in leprous patients of the kind used or in leprosy contacts.'

The antigen used by the Committee and the results obtained appear to show certain similarities to and certain marked differences from the antigen used by us, and from the results obtained by us. We will discuss these similarities and differences.

The reaction to the protein isolated by Henderson from leprous spleens was similar in type, although seen so rarely, to the reaction to the protein isolated by us from leprosy bacilli, since both the preparations produce an early reaction only.

All the cases tested by the Commission were bacteriologically positive and most appear to have been of the 'lepromatous' type. The fact that slight reactions were produced in only 4 per cent of the cases seems to point to another feature common to the two preparations—the inability to elicit reaction in the 'lepromatous' cases.

On the data available it is impossible to make a comparison of the results in the neural cases. In contacts the incidence and the degree of reaction to Henderson's protein is much less than to the protein isolated by us.

The two preparations appear to differ very markedly in potency. 0·05 mg. of the protein prepared by Henderson produced weak and evanescent reactions only (usually less than 10 mm.), whereas 0·02 mg. of the protein prepared from the bacilli produced strong reactions, the area of erythematous swelling being usually more than 20 mm., often reaching 40 mm., and the reaction persisting for a week or more. This difference in the potency appears to be caused by the difference in the source of the two preparations. Henderson isolated protein from the whole spleen, the product being a mixture of protein from the splenic tissue and the leprosy bacilli. The protein isolated by us was prepared from the bacilli freed from tissue and thus consists of bacillary protein only.

As already stated the Joint Committee has put forward two alternative explanations of the absence of reactions to Henderson's protein both in the cases of leprosy and in contacts. According to the Committee either the material injected did not contain sufficient specific protein or 'sensitiveness comparable to the tuberculin sensitiveness of tuberculosis' does not exist in the type of cases tested and in contacts. We have shown that such sensitiveness does exist in contacts and in cases of the neural type but not in cases of the lepromatous type. In the contacts and in the neural cases (if any) the absence of reaction was, therefore, probably caused by the insufficient amounts of antigen. In the lepromatous cases, which constitute most, if not all, of the cases tested by them, the other factor, lack of sensitiveness, also operates, and is the chief factor since even large doses of antigen will not induce reaction in them. The two explanations given by the Committee are thus not truly alternative explanations; neither will explain *all* the observations, a part of them being explained by one and a part by the other.

Reaction to a substance isolated from the urine of cases of leprosy.—Berny and Mauze (1940) have isolated a substance from the urine of bacteriologically positive 'lepromatous' cases. They consider skin reactions to this substance of diagnostic value. A papule exceeding 1 cm. in diameter, and accompanied by erythema and pain, is reported to appear 24 hours after the injection. Positive results have been reported in all the 199 cases of leprosy tested, none of the 91 healthy persons tested showing a positive reaction. We have attempted to confirm the findings of Berny and Mauze. A substance of proteose nature has been isolated from urine of bacteriologically positive lepromatous cases. The reaction produced by intradermal injections of this substance appears to be similar to that produced by other proteoses having nothing to do with leprosy and to be different from the reaction produced by the antigen isolated from the leprosy bacillus. This matter is being studied further.

Reaction to antigens prepared from cultures of acid-fast bacilli.—Apart from preparations obtained from human leprosy material, several preparations from other sources have been used for doing skin tests in leprosy. These preparations include proteins isolated from cultures of various acid-fast bacteria including some of the supposed cultures of *Myco. leprœ* and *Myco. leprœ muris*, and various chemical fractions of a supposed culture of *Myco. leprœ*.

In the present state of our knowledge it seems to us that no useful purpose will be served by analysing and discussing the findings. In order to assess the significance of the findings made with these preparations it is essential to make a comparative study of the antigenic properties of the different protein fractions, prepared by identical methods, from the Hansen's bacillus and the other acid-fast bacilli. This has not so far been done. A study of this matter is now being undertaken.

Summary.

1. The findings of other workers regarding the antigenic activity of lepromin treated by various methods and the bearing of these findings on the present work have been discussed.

2. The finding of some workers that the breaking down of the bacilli by physical means is accompanied by an increase in the amount of free antigen in lepromin is in accordance with the present findings.

3. The reports of other workers on fractionation of the leprous nodule (not the bacillus itself) are discussed. It is considered that the reaction produced indicated that the active non-lipoid fraction obtained by Villela and co-workers could not possibly have been a protein antigen. Their results are such as would be expected, and have been obtained by us, with unbroken or incompletely broken bacilli. The methods used by them would, it is believed, neither break down the bacilli nor liberate the antigen.

4. Only one worker has isolated protein by grinding leprous tissue (spleen) rich in acid-fast bacilli. The isolated protein produced only very slight early reactions of the 'tuberculin' type in a few cases and in some contacts. In producing an early reaction only, it resembles the protein isolated from the Hansen's bacillus by us, but it is of very much weaker potency, being a mixture of proteins from splenic tissue and from the bacilli.

5. A proteose isolated from the urine of leprous patients by the methods of Berny and Mauze has produced reaction different in nature from that produced by bacillary antigen.

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