## IMMUNOLOGY

For the first time this subject is included among the themes of an international congress of leprology. The decision to do this results from the importance now ascribed to the lepromin reaction, after many years of experience, and also from the results obtained in certain countries with B.C.G. which may open up new horizons in the prophylaxis of this disease.

## THE LEPROMIN REACTION

The use of the lepromin reaction as an index of the degree of resistance to *Mycobacterium leprae* is constantly increasing. It offers a useful element in respect to prognosis and classification of cases of leprosy, and consequently its use in practice is recommended.

*Antigens.*—For the preparation of the antigen the Committee recommends the method which fulfills most closely the following requirements: (*a*) susceptibility of standardization; (*b*) maximal utilization of the bacillary element of the material used; and (*c*) the greatest simplicity of preparation.

The method of Dharmendra gives an antigen which can be standardized with minimal loss of bacilli. On the other hand, the late reaction is weaker than with other lepromins, perhaps because the chloroform and ether employed in its preparation modify the composition of the bacilli.

The method of Fernández and Olmos Castro gives a standardized antigen with bacilli very little changed in their composition, but it has the disadvantage that a great many bacilli are wasted in its preparation.

The Mitsuda-Hayashi method, in spite of the fact that it gives a cruder antigen which cannot be standardized, is most widely used because of the simplicity of its preparation and its practical efficacy.

With these considerations in mind, the Committee recommends as preferable: (*a*) for routine work, the Mitsuda-Hayashi antigen as modified by Wade; (*b*) for investigations, the more purified and standardizable antigens, the method of preparation of which should always be specified.

Because of the increasing scarcity of material for the preparation of lepromin, the Committee recommends increased studies of new methods and refined techniques of preparation (see appendix), and also the use of higher dilutions of the antigen. The use of visceral lepromin as suggested by Campos should also be investigated further.

Finally, the Committee suggests that it would be desirable to ask central laboratories, with facilities for the purpose, to undertake the preparation of the antigen for distribution to those who may need it. An antigen of the Mitsuda type will be the more uniform, the more numerous the lesions from which it is made.

## READING OF THE LEPROMIN REACTION

The intradermal injection of lepromin provokes, in those who react positively, a double response: (*a*) an early reaction in 24 to

48 hours—the reaction of Fernández; (*b*) a delayed reaction read at about the fourth week—the reaction of Mitsuda.

*The Early Reaction.*—This consists of an erythematous infiltrated lesion, sometimes evident twelve hours after the injection, the aspect and evolution of which resembles the reactions of the tuberculin type. It reaches its maximum after 24 to 48 hours, and begins to diminish after 72 hours. In strongly positive cases it persists for a longer time in the form of a dark halo surrounding the late nodule.

In the reading of the reaction the only element of importance is the infiltration. Reactions which present only erythema should be considered doubtful or negative, and also reactions which appear very early and regress or disappear before 48 hours. A sharp margin of ameboid configuration is peculiar to very strong positive reactions.

It is recommended that the results should be read after 48 hours, conforming to the following criteria:

Negative (—): Absence of reaction, or erythema without infiltration, or erythema with infiltration less than 5 mm. in diameter.

Doubtful (±): An erythematous-infiltrated reaction with infiltration more than 5 mm. and less than 10 mm. in diameter.

Weak positive (+): An erythematous-infiltrated reaction with infiltration more than 10 mm. and less than 15 mm. in diameter.

Moderate positive (++): An erythematous-infiltrated reaction with infiltration more than 15 mm. and less than 20 mm. in diameter.

Strong positive (+++): An erythematous infiltrated reaction with infiltration more than 20 mm. in diameter.

*The Delayed Reaction.*—This consists of a nodular infiltration which begins after the first week after the injection, reaches its maximum about the fourth week, and later regresses, frequently leaving atrophy or a scar. Intensely strong reactions may result in ulceration. Sometimes the evolution is accelerated and reaches its peak before the third week, while at other times it is delayed, reaching its peak after the fourth week. In negative or doubtful cases it may be well to make later readings up to 60 days.

The criterion of reading should be based not only on the size of the infiltration, but also on its appearance and evolution.

Negative (=): Absence of all local reaction between the first and fourth weeks.

Doubtful (±): Slight infiltration, difficult to detect and less than 3 mm. at the point of inoculation.

Weak positive (+): Frank infiltration between 3 and 5 mm. in diameter.

Moderate positive (++): Nodular infiltration larger than 5 mm. in diameter.

Strong positive (+++): When the infiltration undergoes ulceration.

## INTERPRETATION OF THE RESULTS

A positive lepromin reaction is regarded as an expression of

a certain amount of resistance to *Mycobacterium leprae*, directly proportionate to the degree of positivity.[1]

A negative lepromin reaction is interpreted as follows:

(*a*) In patients with leprosy, and contacts living with open cases, it is generally regarded as a sign of deficient resistance.

(*b*) In healthy individuals not contaminated with leprosy, it is without significance.

### B.C.G. AND LEPROMIN REACTION

Studies of conversion of lepromin negative individuals to positive by means of B.C.G. have been widely undertaken in recent times. There is no doubt that if experience shows that this artificially induced change is of value in immunity, this will have a decisive influence on the future orientation of the prophylaxis of leprosy. The Committee is in agreement in accepting:

(1) Healthy people with positive lepromin reaction, not artificially produced, frequently present a state of biological resistance to *Mycobacterium leprae*.

(2) In leprosy patients, a positive lepromin reaction, not artificially produced, gives, from the biological point of view, a favourable prognosis.

(3) Spontaneous or natural conversion of the reaction takes place in a large proportion of cases.

(4) The administration of B.C.G. to healthy individuals who are negative to lepromin, causes a change of the reaction in a large proportion of cases.

(5 The administration of B.C.G. in the usual doses by mouth is free from risk, even in allergic individuals.

The question of whether or not a positive lepromin reaction artificially induced by B.C.G. indicates immunity is being studied, and as yet no conclusive statement can be made regarding the matter.

The Committee recommends that experimentation be intensified to determine the value which this vaccine may have, and also that wider investigation be made with a view to finding other procedures equally capable of converting the lepromin reaction.

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1 It is certain that intense reactivity to lepromin reflects biologically a favourable prognosis. This state of hypersensitivity may, in certain cases, result in clinical changes prejudicial to the patient (atrophies, mutilations, reactional occurrences, etc.).

## APPENDIX

The following description of the improved (Wade) technique of preparation of the Mitsuda-Hayashi antigen, referred to in the text of this report, is taken verbatim from the report of the W.H.O. Expert Committee on Leprosy (W.H.O. Technical Reports Series No. 71, September 1953).

(1) For each batch of lepromin, lesion-tissue from several cases should be used, and reliance should not be placed alone on a tissue such as the ear lobe. The purpose of this "pooling" of material is to compensate for possible antigenic deficiencies of material from one or more cases by inclusion of material from others which may be more favourable.

(2) Each specimen used should be incised and a bacteriological smear examined, to ensure that only those which contain abundant bacilli will be used. Those poor in bacilli should be discarded.

(3) All tissue extraneous to the actual lesion mass should be trimmed off and discarded. This includes subcutaneous fat and loose connective tissue, as well as the epidermis if the lesion is a cutaneous nodule or infiltration, and the skin itself if it is removed with a subcutaneous nodule and is not involved in the lesion.

(4) It is probably preferable to weigh the tissues to be used before they are heated. (A material loss of weight occurs in the heating, whether that be done by boiling or by autoclaving, and whether it be done in saline solution or without it.)

(5) The trimmed tissue is heated either at boiling temperature or by autoclaving. The latter form of sterilization is to be used if the tissue is to be shipped to a distant laboratory for processing.

(6) The heated material is ground fine in a mortar with gradual addition of saline up to 20 ml per gram of tissue.

(7) The material is then filtered. Filtration is best done through a single layer of the finest mesh bolting cloth of silk, or preferably of nylon, the latter having no capillary attraction for water. (This process avoids the loss of a great deal of tissue suspension which occurs when highly absorbent multiple layer cotton gauze filters are used.) The nylon fabric is applied, provision being made for a pouch, to a wire ring made to fit the funnel to be used. The suspension is worked through by gentle scraping with a spatula. The nylon filter, properly cleaned, can be sterilized and used repeatedly.

(8) The residue left on the filter may be returned to the mortar, reground for some minutes, suspended in fresh saline, and put back into the same filter. (In this way 20 ml of saline per gram of tissue can be used in the first instance and 10 ml per gram in the second instance, thus obtaining 50% more of the final preparation than when the tissue pulp is not reground.)

(9) 0·5% of phenol is added to the filtered suspension which is then distributed in the desired containers, which are sealed and reheated to ensure sterility, although asepsis is practiced throughout.