IMMUNOLOGY

18 NOVEMBER, 1958
9.00 A.M.

Chairman: DR. J. M. M. FERNANDEZ
Rapporteur: DR. K. YANAGISAWA

DR. FERNANDEZ gave his report of the committee on this subject.

1. *The lepromin reaction* is useful in prognosis and classification and its use is recommended. Lepromin should be made simple: there are various methods and modifications. The Mitsuda-Hayashi antigen is recommended as modified by Wade. As for bacillary extracts, further studies are needed (the name of 'leprolin' is recommended). Standardisation of lepromin is advised.

   Readings: Fernandez at 24 hrs, Mitsuda at 3 to 4 weeks: either can be read separately. The Fernandez means a hypersensitivity to the leprosy bacilli: a doubtful result is less than 5 mm. in diameter; a strong positive is 20 mm. or more in diameter.

   The Mitsuda may reach its peak in 3 weeks or after 4 weeks and should be observed up to 60 days. The doubtful reading is less than 3 mm. In strong positive there is ulceration. In all cases, record diameter in mm. The significance of the lepromin reaction is one of sensitivity, with or without previous contact with leprosy.
2. *BCG and lepromin reaction.* Conversion does occur but the evidence for change in resistance to leprosy is still inconclusive. A standard experiment applied in many countries is needed. Preliminary tuberculin testing may be given to one group and not to another. A reliable dried BCG is advisable (as Dr. Yanagisawa offers to supply.)

Paper

DR. K. YANAGISAWA (Japan):

Criteria of Reading the Lepromin Reaction

There is a great correspondence between the early and late reactions. No standard lepromin antigen is available at present. In Japan we make our lepromin in a central institute and try to standardise it before issue. We suggest the following standards for reading:

Using the Mitsuda antigen we suggest

0 to 4 mm.	negative
5 to 6 mm.	doubtful
larger than 7 mm.	positive

Using the Dharmendra antigen for the late reaction read at 15 days.

0 to 3 mm.	negative
4 to 5 mm.	doubtful
larger than 6 mm.	positive

For the early reaction at 48 hours with the *Mitsuda antigen*, 0 to 6 mm. is negative, 7 to 10 mm. doubtful, and larger than 11 mm. is

positive. With the *Dharmendra antigen*, 0 to 9 mm. is negative, 10 to 12 mm. is doubtful, larger than 13 mm. is negative.

There is a great deal of experimental and statistical analysis behind these figures.

DR. L. M. BECHELLI (Brazil):

The Influence of Repeated Lepromin Tests

We tried repeated injection of lepromin to convert negative lepromin reaction in children. The results were excellent, more than 70% of conversions or intensifications. Results were similar to that obtained with BCG. There were some variations in age groups. The lepromin acts as a sensitizing agent and may perhaps increase the resistance, though this is more uncertain. Most positivizations were in 5 to 14 years age groups.

DR. H. W. WADE (Philippines):

Nomenclature and Classification of Skin Test Antigens

We need to avoid confusion in the literature. Several lepromin antigens are now used and the name 'lepromin' can be applied to all of them. The term is taken to mean the Mitsuda-Hayashi type of antigen. 'Stefansky antigen' will be suitable for the *M. lepraemurium antigen*. The tissue elements lead to 'crude lepromin' and the removal of these leads to 'integral lepromin'. The extracted bacilli type could be 'purified bacillus suspension' or 'bacillary lepromin'.

The Dharmendra antigen is non-acidfast or defatted and the bacilli will not stain: it is not a lepromin: call it the 'Dharmendra antigen'. Special preparations to elicit the early reaction only may best be called 'leprolins'. The skin test antigen produces an early reaction (the Fernandez): the state produced is a hypersensitivity (in analogy with tuberculin sensitivity).

Discussion

DR. DHARMENDRA (India): "I have listened with great interest to the Report of the Committee on Immunology and to the papers of Dr. Yanagisawa and Dr. Wade.

"With reference to the antigen for the lepromin test prepared by the chloroform method and which has been associated with my name, I agree that the chloroform treatment will modify the bacilli in some respects. But the question is to what extent the antigenic activity of the bacilli is changed. When this antigen was first prepared, the results with it were compared in a large number of patients of various types and a very high degree of correlation was found between the results with this antigen and with the original Mitsuda-Hayashi antigen. I am glad to note that Dr. Yanagisawa in Japan has found the same results in a very large number of patients.

"A few years ago we compared the results of this antigen with that of Hayashi-Mitsuda lepromin prepared by Dr. Wade's modification. The same degree of high correlation in the results was seen.

"The one constant difference that has been observed is that the earlier reactions with this antigen are stronger and the late reactions weaker, giving rise to a nodule of smaller size and less frequent ulcerations than with the original

lepromin. This was considered a point in favour of this particular antigen. It is an antigen which can easily be prepared and gives conformable results with the original antigen."

DR. MONTESTRUC (France): "Concerning repeated lepromin injections to positivize the lepromin test, I point out that repeated tuberculin will positivize to that reaction. A certain number *of living* germs were needed."

DR. K. R. CHATTERJEE (India): "We have been trying to standardize lepromin. We found a saprophyte (Kedrowsky) which gave a good lepromin, the same as the Dharmendra. We tested it in India, and in Japan they found a very high correspondence of Kedrowsky to Dharmendra antigen. In Japan they made it themselves and got the same results. Repeated inoculations with Mitsuda antigen every 3 months we found to give a high rate of conversion and a high degree of protection from leprosy. Concerning nomenclature, how would Dr. Wade name this Kedrowsky one?"

DR. ROLLIER (Morocco): "I do not believe a figure of reading below 20 mm. is of any importance. In tuberculosis patients I did not find any relation between Fernández and early tuberculin reaction. This is against any inter-sensitivity. I saw 8 lepromatous cases where early pulmonary tuberculosis occurred."

DR. FROEHLICH (Taiwan): "Proteins become changed by physical measures (boiling, etc.). We should remember this in lepromin tests. Why not eliminate the human proteins in lepromin?"

DR. ESTRADA (Mexico): "Re Yanagisawa's paper, I was surprised he established Mitsuda positivity in lepromatous patients. We in Mexico believe in the complete lack of resistance in lepromatous patients. The measurements in mm are confusing and one can make mistakes. The reactions are useful however."

DR. AZULAY (Brazil) Re Dr. Wade's paper: "I have made Dharmendra antigen. Wade says the bacilli lose their acid-fastness. I find they become weaker in acid-fastness but do not necessarily lose it. We have tried Dharmendra against integral; we find it shows weaker late but stronger early reaction; this is sometimes reversed, perhaps by small variations in preparation. Re BCG: I agree much of the work lacks control, but my works had controls. Re Bechelli's remarks: it is difficult to control the variations. The youngest children are better controlled."

DR. G. L. FITE (U.S.A.): "Wade is right when he says we are still dealing with impure antigens. His idea of the names is sound. His purified antigen should be called bacillary antigen. I advise caution in borrowing from terms in tuberculosis. The tuberculin test is a late reaction (Fernandez is the early). Tuberculin and leprolin are two very different substances, and I do not like 'leprolin'."

DR. T. OGATA (Japan) showed a slide showing cross-reaction between sera from syphilis and leprosy, also from tuberculosis. He stated there was some kind of similarity in the antigens between leprosy and syphilis.

DR. H. W. WADE: "One point ought to be stressed. There is a big difference between young leprosy patients and normal individuals. In Japan tests on the young are difficult.

"Chloroform in antigen of Dharmendra also includes ether: the ether extracts the lipids. Acid-fastness does disappear. It is hard to find any acidfast material in the samples from Dharmendra or Japan.

"Tubercle bacilli can be made non-acidfast by growing with INH, and this removes their power to produce reactions. Even young children can be converted in lepromin reaction by repeated lepromin. Dr. Fite's remarks overwhelm me (re terminology) that the antigens are not pure. My purified antigen was tested in many countries and all reported its positivity, though the readings were lower."

(Intermission of 10 minutes at 10.45 a.m.)

Chairman: DR. R. G. COCHRANE

Rapporteur: DR. BUU-HOI

DRS. J. ALEIXO, J. STANCIOLI, J. MARIANO and A. SALOMAO, of Brazil.
(Read by DR. ALEIXO)

Mitsuda Test after Mass BCG

This was done in areas of severe leprosy prevalence where no previous immunological tests had been given. The Mitsuda not having been given, any possible converting by it was ruled out. That BCG was effective was known in Brazil from 1 and ¼ million previous BCG vaccinations. This paper reports the study of 1,889 inhabitants. The conversion was very satisfactory.

DRS. L. M. BECHELLI, RATH DE SOUZA, and R. QUAGLIATO, of Brazil.
(Read by DR. BECHELLI)

Correlation of Clinical and Histological Mitsuda Reaction

We reported this in 1953 on 159 biopsies. Now we report on 253 biopsies. In histology a clearly tuberculoid picture was necessary for us to read a positive. A negative showed only a general cell reaction of inflammatory nature.

There was an intermediate picture of 'tending to a positive', and this is found even in some doubtful reactions clinically, and even in some clinical negatives. The histology is almost similar in the 1 plus and 2 plus. With more work we propose the readings should be re-arranged.

DR. R. D. AZULAY (Brazil):

Lepromin Test in Guinea-pigs after Previous BCG and Dead M. Tuberculosis Vaccinations

We show the undoubted protection of guinea-pigs against rat leprosy. Effects vary with the dose of BCG (30 mg./kg. of guinea-pig body weight was the best). We tried larger doses of BCG for lepromin conversion and doses of killed *M. tuberculosis* (killed by irradiation). The positive results decreased with increased BCG, and no positive results with *M. tuberculosis*, but some doubtfuls. With ordinary doses of BCG the results are good. All doses were given by mouth. The optimum dose is similar to that for children.

DR. H. W. WADE and DR. R. E. GUINTO (Philippines):

Serial Dilutions of Lepromin in Normal Young Children

The tests were on healthy young children, 6–9 years. There were 5 groups of 100 children. Varying antigens were used and varying dilutions. In town children Dharmendra results were less than from the Mitsuda-Hayashi antigen. In country children, a full dose lepromin gave 91% of Mitsuda positives and lower dilutions were unsuitable for such children, though the reaction rate did not decrease in parallel with the dilutions. Early reactions were relatively few. There seemed in country children to be some conditioning factor, presumably environmental contact with non-pathogenic mycobacteria (this may operate also in tuberculin reactivity in rural areas in the tropics).

DRS. J. A. DOULL, R. S. GUINTO and M. MABALAY (Philippines):

Natural Reactivity to Lepromin: Association between Mitsuda and Tuberculin Reaction for Graded Doses of Tuberculin

The cause of natural reactivity to lepromin may be due to the effect of an antigen derived from another mycobacterium, e.g. *M. tuberculosis*, and this antigen may perhaps be present in some degree in other members of the group. As you increase the dose of tuberculin in our experiments you do not get increased correlation with lepromin. There is not any true explanation as yet for the greater part of Mitsuda reactivity.

DRS. T. OGATA and M. ABE (Japan):

Serological Agglutinations with Cardiolopin Lecithin Antigen in Leprosy Sera

I used Cardiolipin-lecithin antigen by a technique derived from my syphilis agglutination test. If there are equal parts of cardiolipin and lecithin, there will be a curve of highest end-titre which is in marked contrast with the syphilis one. This is characteristic enough to provide a lepro-agglutination test. The leprous antibodies are mostly in the beta-globulin of sera, and only to a less degree in the alpha-globulin. Lepromatous types give higher titres than in tuberculoid (and normal patients) so only high titres are useful. They are greater in L_3 than L_1, and in regressive leprosy.

DRS. T. FUJINAMI and H. HONDA (Japan):

Antigenicity of BCG Wax to Sera from Leprosy Tuberculosis

We studied the D-IV fraction of lipid and wax-P, and compared with brain lipid. Wax-IV obtained from BCG had the greatest antigenicity in the presence of mannose. The reaction against tuberculosis serum depends on the presence of mannose. This finding is of significance in explaining the Middlebrook reaction using tuberculin, and the specific and non-specific factors in leprosy. More experiments are planned.

DR. O. K. SKINSNES (Hong Kong):

The Defence Mechanism in Leprosy as Related to the Internal Lesion and Malnutrition

He showed diagrams of present concepts of immunity in leprosy. The inadequate defence mechanism is conspicuous in lepromatous leprosy. Leprosy is a systemic disease and one should not ignore the systemic aspects. There is a bacteriaemia and visceral lesions, and the endothelial and lymph systems are involved. There is propagation of the bacilli in visceral lesions.

The effect of severe protein deficiency on the progress of the infection in rats has been shown, and they lose ability to produce antibodies and even leucocytes. Depleted animals could not get rid of an infection, even when given penicillin. In leprosy patients we found a similar effect from protein malnutrition: the patients had high

morbidity and took long to respond to therapy. Hypoproteinaemia causes a severe type of leprosy which goes downhill and loses positive reactive power (Lazarine type of leprosy). It is not so much vitamin deficiency but a basic protein deficiency.

DR. J. M. M. FERNANDEZ (Argentina):

The Influence of the Factor of Tuberculosis on the Lepromin Reaction

He made an exhaustive study of all the work bearing on this subject by other authors and himself. Though some of the experiments have been technically imperfect and their conclusions debatable, he thinks there is a genuine para-specific influence on the lepromin reaction. Besides the specific factor of *M. leprae*, whether spontaneous in the leprosy infection, or provoked by the inoculation of lepromin, there is the para-specific factor of *M. tuberculosis* which also can be spontaneous in the shape of a tuberculous infection, or provoked by inoculation of BCG and suspensions of dead bacilli. Finally there is the non-specific factor represented by *M. lepraemurium* and other acidfast bacilli.

Session adjourned at 12.15 p.m.