

RESULTS OF THE USE OF DILUTE MITSUDA ANTIGEN¹

ORESTES DINIZ, *Director*
AND HISSA ABRAHÃO NETO, *Laboratory Assistant*
Department of Leprosy
Belo Horizonte, Minas Gerais, Brazil

In his study of the immunological relations between leprosy and tuberculosis, Floch (1) performed von Pirquet and Mitsuda tests on 128 children of the Marchoux School and of the B. de Cayena Dispensary. Their ages varied from 6 to 14 years. Forty-three of them suffered from tuberculoid leprosy, while 85 had the indeterminate form. In carrying out the Mitsuda test Floch used as his antigen, not the classical 1:20 preparation of Hayashi, but a 1:750 dilution. He stated that in this way he economized antigen, for he had discovered by means of a series of experiments that the positive reaction to the antigen thus diluted was as prompt as when much less dilute preparations were used. The reactions, he stated, could be correlated with those to ordinary lepromin by adding one plus (1+) to the readings.

As this was an interesting observation, we undertook to repeat the experiment with diluted lepromin to compare the results obtained with it and those obtained with the classical 1:20 antigen. The tests were made on a group of 216 children of both sexes, varying in age from 2 to 16, interned in the São Tarcisio Preventorium in this state. Most of them had been treated with BCG. We also tested 40 young boys, varying in age from 12 to 16, at the Technical School for Apprentices. These boys had not been treated with BCG. The total number of individuals tested was 256.

TECHNIQUE

The antigen was prepared from lepromas by the classical method of Hayashi. The normal 1:20 preparation was used as a control; the tests were made with 1:750 and 1:1000 dilutions. Of each dilution we used 0.2 cc., given intradermally in the usual manner. The tests were made in the following regions: antero-external surface of the thigh, anterior surface of the right arm, and anterior surface of the left forearm. The patients were divided into four groups, as follows:

Group A.—This group consisted of 198 children who received three applications of antigen in the thigh, the control being given uppermost, the 1:750 dilution in the middle, and the 1:1000 dilution below that.

Group B.—This group consisted of 18 children who received two applications of antigen, also on the thighs, the 1:750 dilution above and the 1:1000 dilution below. This group did not receive the 1:20 antigen, as we wished to determine whether there was a possibility that injection of that dose would influence the results of the higher dilutions.

¹ Presented at the VI International Congress of Leprology, Madrid, October 1953, in the Portuguese language. English translation supplied by the authors.

Group C.—This group consisted of 20 young boys not treated with BCG who received a single injection of the 1:1000 dilution in the anterior part of the right forearm.

Group D.—This group consisted also of 20 young boys not treated with BCG. These received a single application of the 1:750 dilution in the anterior part of the left forearm.

RESULTS

Two observations were made. The first was the Fernandez reaction after 48 hours, and the second was the Mitsuda reaction at the end of 21 days. Analysis of the results shows that both of these reactions with the dilute antigens were closely comparable to those obtained with the 1:20 control. There was, however, a slight lessening of positivity with increase of the dilution. The findings with the Mitsuda reaction are summarized as follows:

<i>Antigen</i>	<i>Tests</i>	<i>Positive</i> ^a	<i>Negative</i> ^b
1:20	212	167 (78.7%)	45 (21.3%)
1:750	233	153 (65.6%)	80 (34.4%)
1:1000	235	116 (49.3%)	119 (50.7%)

^a Reaction lesions larger than 3 mm. in diameter.

^b Reaction lesions smaller than and including 3 mm.

To demonstrate the Mitsuda reaction lesions which resulted from these tests, we show in Figs. 1 to 12 photographs of three cases of each of the four groups. In those of Groups A and B (Figs. 1 to 3 and 4 to 6, respectively) are shown reactions to the 1:750 and 1:1000 dilutions which were suitable for histological examination.

HISTOPATHOLOGY

In order to make sure of the nature of the reaction lesions, and to exclude factors capable of provoking false positive reactions, we made biopsies of cases with positive reactions to the 1:750 and 1:1000 dilutions in order to study the structure. The examinations were made by Dr. Yvon Rodrigues Vieira, chief of the Service of Pathological Anatomy of our department, who supplied us with partial results and the following comments:

Comments on the results of 12 biopsies made in connection with the lepromin reaction at a dilution of 1:1000. In 7 of the 12 biopsies we met with inflammatory activity and signs of specific immunity which at times was scarcely noticeable but at other times quite substantial, while in some cases the tuberculoid structure was already being formed. In the other 5 cases there was a somewhat confused inflammatory aspect, and no solid conclusions could be arrived at. As the interval between the injection of the antigen and the taking of the biopsy specimen was from 21 to 30 days, we are inclined to believe that the period was insufficient for the formation of structures as complex as the tuberculoid one. We therefore think it well to await for a longer period the local developments before giving any conclusive verdict. In the meantime the tuberculoid findings specific or about to become so, met with in 7 of the 12 cases seems to us very suggestive.

One of the advantages deriving from the use of dilute lepromin is the elimination of the difficulty met with by research workers and labora-

tories in the preparation of Hayashi's original antigen due to the present scarcity of lepromas resulting from the success of sulfone therapy in clearing up lepromatous lesions.

CONCLUSIONS

From these findings we conclude that, due to the ever increasing scarcity of lepromatous material for preparing the 1:20 antigen of Mitsuda, and because the percentages of positive reactions obtained with the dilute antigens are very nearly as great as that obtained with the 1:20 antigen, it is possible to use a much more dilute antigen than hitherto in the routine practice of the Mitsuda reaction. It is also possible to do with the Mitsuda test the same as is done in the cutireaction of Mantoux, i.e., verify the immunity to Hansen's bacillus by a preliminary test with a dilute Mitsuda antigen. If the result is positive according to the established rules for the Mitsuda reading, we would be dealing with a lepromin-positive individual. If the result is negative when a dilute antigen is used, we should then make use of more concentrated antigens, reserving the 1:20 concentration for those cases which do not react to the more dilute preparations.

RESÚMEN

Debido a la disminución de buen material para hacer lepromina hay interés en el hallazgo de Floch que lepromina muy diluida puede ser usada. Los autores probaron diluciones de 1:750 y 1:1000 en 256 niños en un preventivo la mayoría de los cuales habían recibido BCG.

Los resultados de la reacción tanto precoz (Fernandez) como tardía (Mitsuda) fueron comparables con los obtenidos con el control de 1:20, excepto que como es de esperarse hubo alguna disminución en la positividad según el aumento en la dilución. Algunas reacciones fueron examinadas por medio de la biopsia, y aunque el examinador sugirió que las biopsias habían sido tomadas antes de que la lesión específica tuberculoide se desarrollara se vió suficiente cambio para ser considerado como sugestivo.

Los autores sugieren que se practiquen pruebas repetidas usando concentraciones de lepromina en aumento tal como se hace con la tuberculina. Aquellos individuos que no reaccionen a las diluciones mas bajas deben ser probados con concentración mas alta.

REFERENCE

1. FLOCH, H. Réaction de Mitsuda et intradermoréaction au BCG tué dans la lèpre. Conclusions théoriques et pratiques. *Ann. Inst. Pasteur* **82** (1952) 517-527.

DESCRIPTION OF PLATES

PLATE (12)

FIGS. 1-3. Examples of the reactions on the thighs in Group A individuals. In each instance the uppermost lesion resulted from the normal (1:20) antigen, the middle and lowermost ones from the 1:750 and 1:1000 dilutions, respectively.

FIGS. 4-6. Examples of the reactions on the thighs in Group B individuals. In each instance the upper lesion is from the 1:750 dilution, and the lower one from the 1:1000 dilution.

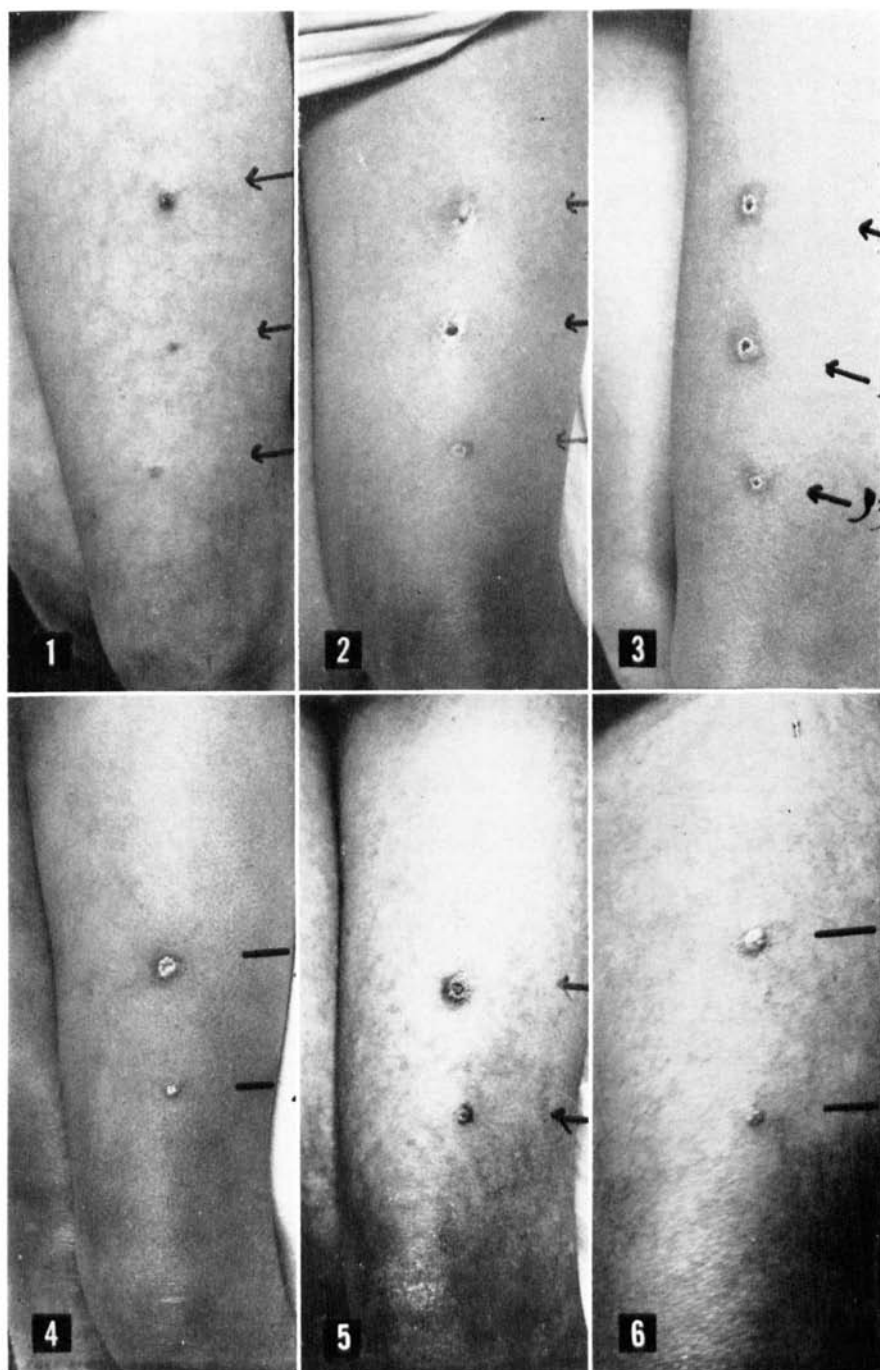


PLATE 12.

PLATE (13)

FIGS. 7-9. Examples of the reactions on the arms in Group C individuals, given—on this occasion—only the 1:1000 dilution. The lower leaders in Nos. 7 and 8 indicate remnants of previous lepromin reactions; that in No. 9 indicates the scar of an old Mitsuda reaction.

FIGS. 10-12. Examples of reactions on the arms in Group D individuals, given only the 1:750 dilution. The leader to the left in No. 11 points to the remnant of a previous lepromin reaction; that in No. 12 indicates an inflammatory reaction at the point of an intravenous injection.



PLATE 13.