

## THE USE OF DILUTE ANTIGENS IN LEPROMIN TESTS

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With the great advances in leprosy therapy during the last ten years, due to the new medicaments and the use of chaulmoogra in sufficient dosage, there has arisen a problem because of scarcity of bacillus-rich lepromas for the preparation of antigen for the lepromin test. This problem is becoming accentuated because of increasing demands for lepromin with intensification of immunological studies in leprosy. Consequently, attempts to utilize dilute suspensions which would entail significant economies of the antigenic material, are worthy of consideration. The matter should be studied until it is settled what can be done satisfactorily in that direction.

The first attempts to employ diluted antigens we owe to Floch (2), whose work was probably based on a statement by Pardo-Castello and Tiant (3) that, "A reacting patient will [react] to dilutions as high as 1:3,000 of lepromin, although with less intensity." Floch made his tests on one group of children with tuberculoid leprosy and another group with indeterminate leprosy, using as control the Mitsuda-Hayashi lepromin made in a 1:30 dilution, and making comparative tests with increasing dilutions down to 1:750. He concluded that the latter dilution, which represents a 25-times saving in the amount of material, is useful because it still gives positive responses, although they are somewhat less intense (one "plus" less).

Diniz and Neto (1) studied the value of dilute lepromin, not in leprosy patients but in apparently healthy contact children, using dilution of 1:750 and 1:1000 and, as the control, ordinary 1:20 Mitsuda-Hayashi antigen. In discussing the results they pointed out that, whereas the control suspension gave 78 per cent positives, the 1:750 dilution gave only 65 per cent, and the 1:1000 dilution only 49 per cent. These differences, as Wade (4) well pointed out, are material ones and indicate that the problem is not simple.

### PRESENT STUDY

For the same reasons that motivated the workers who have been mentioned, I undertook a study of the value of dilute antigens in positively-reacting leprosy patients, they having so responded—although with various degrees of positivity—in previous tests with normal lepromin. Forty cases of tuberculoid leprosy whose reactions had ranged from strong (3+) to weak (1+) were selected for this study.

*Antigen and technique.*—With 23 of these 40 patients the classical Mitsuda-Hayashi "integral" antigen was used, normal (1:20) and diluted to 1:100 and 1:750.

With the other 17 patients a "bacillary" antigen prepared according to the Dharmendra method was used, the normal control being at 1:1000 suspension and the dilution being 1:5000.

In all instances the dose injected was 0.1 cc., and the readings were made after 2 days for the early (Fernandez) reaction and between the third and fourth weeks for the late (Mitsuda) reaction.

#### RESULTS

Although records were kept of the early reactions, in analyzing the results I refer especially to the late reactions (Mitsuda phenomenon) because the previous investigators reported only on that one. I wish to say, however, that in general the early reactions are comparable to the late ones, although with the integral antigen the percentages are a little smaller. In the following, therefore, are given only the percentages of positivity of the Mitsuda reaction induced by each antigen in the strong and weak reactors.

*Integral antigen.*—Twenty-three cases were tested with the Mitsuda-Hayashi lepromin and dilutions. (a) Of the 15 cases considered strong reactors (8 of them 3+ and 7 of them 2+ to normal antigen), all were positive to the 1:100 dilution and 14 were positive to the 1:750 dilution, although with the latter the intensity was usually weak (Figs. 1-3).

(b) Of the 8 cases considered weak reactors to the normal antigen (1+ reactions, but with tuberculoid histology), only 1 was positive with the 1:100 dilution, and none reacted positively to the 1:750 dilution (Fig. 4).

*Bacillary antigen.*—Seventeen cases were tested with the Dharmendra antigen. (a) Of the 13 cases considered strong reactors (8 of them 3+ and 5 of them 2+ to the parent, or 1:1000 suspension), all responded also to the 1:5000 dilution although in most of them the intensity was weaker (Fig. 5).

(b) Of the 4 cases considered weak reactors (1+ to the 1:1000 suspension), none was positive to 1:5000 dilution (Fig. 6).

From this analysis of the results it appears that the strong reactors to normal antigens also respond, and in the same percentages although usually with weaker intensity, to a 1:750 dilution of the integral antigen and to a 1:5000 suspension of the Dharmendra bacillary antigen. On the other hand, the weak reactors show no response to these dilute antigens.

#### DISCUSSION

According to their response to the intradermal test with normal lepromin, both healthy individuals and leprous patients can be classified into three groups: nonreactors, weak reactors, and strong reactors.

I believe, as Wade does, that only the strong reactors have the capability of responding to very dilute antigens. The results here reported confirm this belief.

The fact that persons giving weak reactions to the normal antigen do not respond to the dilute ones should be taken clearly into account, since in prognosis and significance these weak reactors—which are seen with relative frequency in tuberculoid and indeterminate leprosy—are very different from the nonreactors. The use of a dilute antigen in these cases might lead us astray with respect to prognosis and classification.

The following question may, therefore, be asked: Do dilute lepromins have any practical use? I have no hesitancy in replying in the affirmative, provided they are properly used.

Whenever the lepromin test is made on a person for the first time, or when we do not know to what reaction-degree group he belongs, we should always use the regular concentration—either the Mitsuda-Hayashi lepromin 1:20, or the Dharmendra antigen 1:1000.

Dilute antigens should be reserved for the strong reactors only, for use in the successive tests that are made periodically to determine a patient's immunological curve. In this way dilute antigens offer a great advantage in economy of material. They also would avoid most of the residual scars caused by strong reactions.

In the weak reactors, on the other hand, we should always continue to use the normal, known antigens, because dilute antigens would cause loss of time and lead us into error.

#### SUMMARY AND CONCLUSIONS

In a study of the value of dilute antigens for the lepromin reactions, a group of 40 tuberculoid leprosy cases has been used. According to earlier lepromin tests with normal antigens (Mitsuda-Hayashi integral, 1:20, or the Dharmendra bacillary antigen, 1:1000), 28 of them were classed as strong reactors, and 12 as weak reactors.

The dilute antigens used for comparison were: integral, 1:100 and 1:750; bacillary, 1:5000. Both the early and the late reactions were observed, and on the basis of the results seen the following conclusions are arrived at:

1. Dilute antigens have value or practical use only in strong (2+ and 3+) reactors, in which they give the same percentages of positivity with the dilute antigens as with the normal ones although in lesser intensity.
2. Dilute antigens should not be used with weak (1+) reactors, because the results are virtually always negative and may lead to error.
3. With every individual who is lepromin tested for the first time, or whose capability to respond is not known, a normal antigen should always be used.
4. Dilute antigens should be used only with strong reactors, for the periodical examinations which are usually made to determine the immunological curve. In this way we would save antigen material, besides avoiding the residual scars which often occur after strong lepromin reactions.

## RESÚMEN Y CONCLUSIONES

Para estudiar el valor de los antígenos de Mitsuda diluidos, el autor los aplicó en un grupo de 40 casos de lepra tuberculoide de los cuales 28 fueron clasificados (por lepromino reacciones efectuadas anteriormente) como fuertes reactivos y 12 como reactivos débiles a los antígenos clásicos o normales (antígeno integral Mitsuda—Hayashi al 1:20 y antígeno bacilar de Dharmendra al 1:1000).

Con antígenos diluidos con fines comparativos utilizó: integral al 1:100 y al 1:750; y bacilar al 1:5000, estudiando la reacción precoz y tardía; y en base a los resultados observados llegó a las siguientes conclusiones:

1) Los antígenos diluidos solamente tienen valor o aplicación práctica en los fuertes reactivos (positividad de 2+ y 3+ con la lepromina clásica) ya que dan el mismo porcentaje de positividad que el antígeno normal aunque la intensidad es menor.

2) Los antígenos diluidos no deben utilizarse en los reactivos débiles (positividad de 1+ con el antígeno normal) porque sus resultados son siempre negativos y podría inducir a error.

3) En todo individuo en quien se aplica por primera vez la lepromina o sea cuando se desconoce su capacidad de responder a la misma, debe utilizarse siempre la lepromina normal.

4) Los antígenos diluidos deben utilizarse únicamente en los fuertes reactivos, para las investigaciones periódicas que suele hacerse con el fin de estudiar la curva inmunológica. Con ello se ahorra material antigenico y se evita al mismo tiempo las cicatrices residuales que suelen dejar las fuertes lepromino reacciones.

## REFERENCES

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## DESCRIPTION OF PLATE

## PLATE (9)

FIGS. 1-3. Strong reactors, tested with the indicated dilutions of the Mitsuda-Hayashi lepromin. In Figs. 1 and 2 there is evident lessening of intensity with dilution, an effect not seen in Fig. 3, which is exceptional. Note residual scars of previous reactions.

FIG. 4. A weak reactor, similarly tested with dilutions of the Mitsuda-Hayashi antigen. Although there is a 1+ reaction to the normal (1:20) suspension, there is none to the higher dilutions. Those reactions are frankly negative.

FIG. 5. Strong reactor, tested with the Dharmendra bacillary antigen in the concentrations indicated. Although the 1:5000 reaction is positive, with ulceration, it is less strong than that to the normal 1:1000 suspension.

FIG. 6. Weak reactor, tested with the Dharmendra antigen dilutions. The normal 1:1000 suspension has caused a 1+ reaction, but the 1:5000 site is negative.

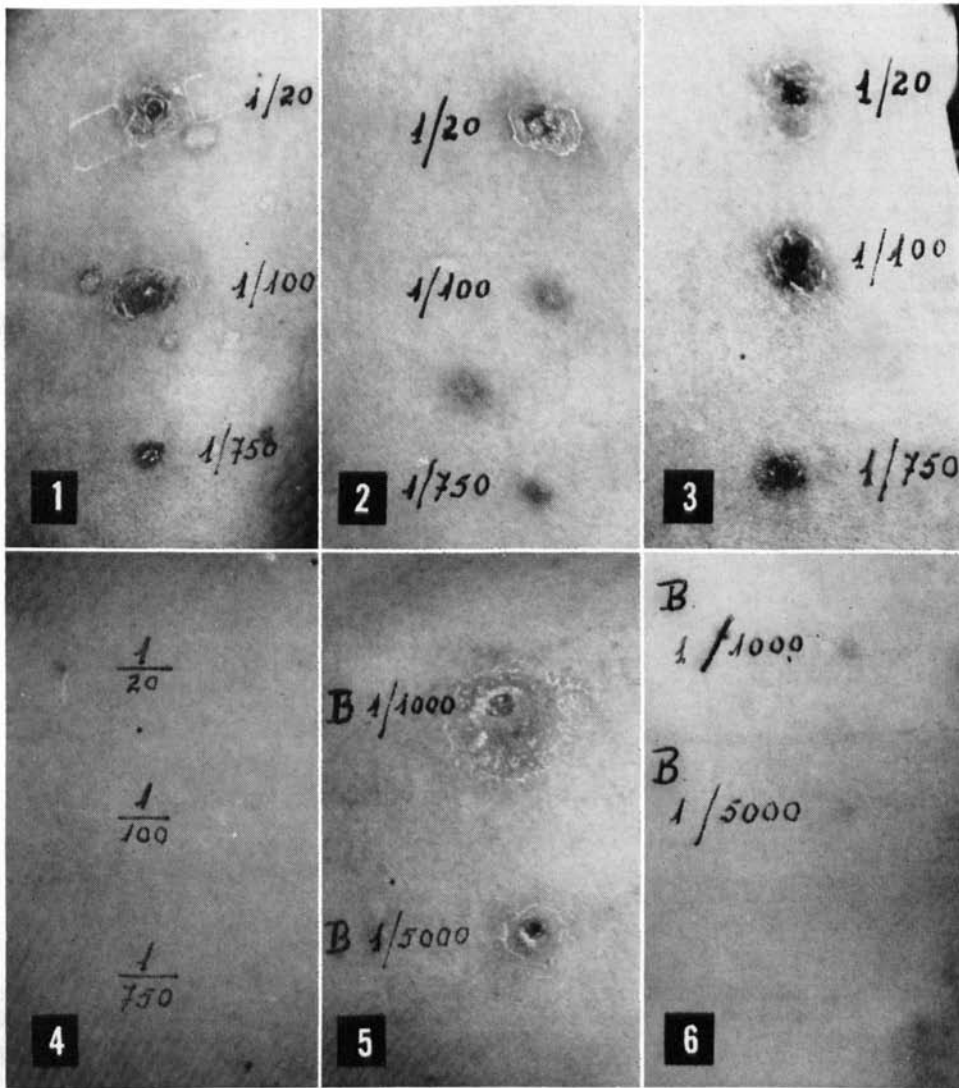


PLATE 9