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EDITORIALS

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LEPROMIN TESTING WITH DILUTED ANTIGEN

It is now a well-recognized fact that, imperfect as modern treatment of leprosy may be, the results of it are making it more and more difficult in most places to obtain bacillus-rich lepromas suitable for making lepromin. And at the same time more and more stress is being laid on the use of the Mitsuda test, for which that antigen is required. Thus has arisen a new and unprecedented problem.

One way of partially relieving the situation is to improve the technique of preparing lepromin and lessen the wastage involved in the classical Mitsuda-Hayashi technique. Thus the WHO Expert Committee incorporated in its report[1] an otherwise unpublished method by which the yield of lepromin of normal concentration can be materially increased. A lot of 1,300 cc. prepared by that method was recently supplied to the South Pacific Commission, and it is reported as giving satisfactory and even superior results.[2]

Another approach, initiated by Floch, is to use high dilutions of lepromin.[3] It has been known practically from the first that wide variations of bacillus concentration may occur without vitiating the results, and Hayashi himself once referred to the reaction as of "all or nothing" type. Some of the preparations described in the early days, when attempts were being made to standardize the antigen by bacillus counts, varied tremen-

[1] W.H.O. Expert Committee on Leprosy. First report. World Hlth. Org. Tech. Rep. Series No. 71, September 1953.

[2] DR. G. LOISON, research officer for health, South Pacific Commission; personal communication.

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

dously. Indeed, there is reason to believe that lepromins as regularly prepared today by different workers differ widely in this respect.

Pardo-Castelló and Tiant[4] once wrote that a lepromin-positive individual will react to dilutions as high as 1:3000, "although with less intensity." We venture to say, however, that it would be a strong reactor indeed who would respond to so weak a dilution, and that many weak reactors would probably remain completely unresponsive.

Floch is convinced that the "normal" antigen (which he holds to be 1:30, incidentally whereas Hayashi's technique makes a 1:20 preparation) is too concentrated.[5] He finds that the lessening of intensity of the response does not parallel the degree of dilution of the antigen, but occurs more or less stepwise. The results with a 1:50 dilution do not differ much from those with the normal dilution, and he recommends its use. He finds a second zone in the region from 1:300 to 1:750. Further dilution, to 1:1000, again affects the results considerably. In the intermediate zone the positive results, he states, can be correlated with those obtained with the normal dilution by adding one plus (1+) to the reading.

Floch has not, to our knowledge, published data to show whether or not the percentages of reactors with normal and dilute antigens are the same. In the absence of any statement to the contrary the reader assumes they are, but that may not be so, especially among persons who are not strong reactors. Nor has evidence been seen that he has inquired whether the introduction of a full dose in one arm may perhaps influence the reaction to a dilute dose in the other arm of the same person. That might be the case if any sort of antibody effect is involved in the reaction.

Diniz and Neto, in Belo Horizonte, interested by Floch's reports, presented at the Madrid Congress findings which are now made available.[6] They took into account the factor just referred to, and they also considered the possibility that previous BCG vaccination might affect the results; nothing is said of any possible effect of previous lepromin testing, which evidently had been done at least once in every case. In one group of BCG-vaccinated children they gave each individual three test injections, namely, normal lepromin, and 1:750 and 1:1000 dilutions. To another BCG group they gave only the two high dilutions. To one small group that had not been BCG-vaccinated they gave only 1 injection, of the 1:750 dilution, and to another similar group they gave only an injection of the 1:1000 dilution. Nothing was done in this study with stronger, intermediate concentrations.

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[4] PARDO-CASTELLO, V. and TIANT, F. R. Leprosy: The correlation of its clinical, pathologic, immunologic and bacteriologic aspects. *J. American Med. Assoc.* **143** (1943) 1264-1268; *reprinted* in *Internat. J. Leprosy* **15** (1947) 202-213.

[5] FLOCH, H. Discussion sur les résultats obtenus dans la réaction de Mitsuda a l'aide d'antigenes dilués. *Internat. J. Leprosy* **22** (1954).

[6] DINIZ, O. and NETO, H. A. Results of the use of dilute Mitsuda antigen. *Internat. J. Leprosy* **22** (1954) 144-146.

Actually, because they injected 0.2 cc. per test, their injections were practically equivalent to 1:375 and 1:500 dilutions given in the usual dose of 0.1 cc. This is especially so because most of the bacilli of a suspension are concentrated near the point of injection by the filtering effect of the dermal tissue web, and relatively few spread with the fluid to the limit of the wheal which it produces.

The very brief statement about their results merely says that the reactions to both of the dilutions used were "closely comparable to those obtained with the 1:20 control," with slight lessening of the positivity with increase of the dilution. Some of the reactions obtained were surprisingly strong, as shown by the pictures illustrating the paper, even in unvaccinated cases and with only the single injections. That the matter is not so simple, however, is to be seen from the table, which shows nearly 80 per cent positives with the 1:20 antigen, but only 65 per cent with the 1:750 dilution and barely 50 per cent with the 1:1000 dilution. And any papule above 3 mm. in diameter was recorded as positive.

In this respect the tests with the different dilutions are decidedly not comparable. There is therefore good reason for the authors' suggestion that persons not reactive to the weaker antigens should be retested with stronger ones, up to full strength. How practicable that would be in field work may be a question. Obviously, to apply repeatedly a test that takes three weeks for readings would be very different from repeated testing where readings are made after 48-72-hours.

This matter is an interesting one, from more than one point of view. It would take large-scale tests to determine, with reasonable certainty, how reactions to very small doses (i.e., high dilutions) are affected by factors such as previous testing, multiple doses given simultaneously in different sites, and BCG vaccination. From the practical point of view, it should be determined to what extent lepromins, prepared by a standardized technique and reasonably alike in concentration, can be diluted without affecting the results percentagewise. If instead of the normal concentration one could use even a 5 times dilution (i.e., 1:100) and get dependable results, the effects of the increasing shortage of material for making lepromin would be correspondingly lessened. —H. W. WADE