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EDITORIALS

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FURTHER REGARDING THE LEPROMIN REACTION

It is practically thirty years since Fumio Hayashi brought the Mitsuda test to general attention, yet it is still being studied in efforts to improve upon it in one way or another, and to understand the reaction induced by it. Last year THE JOURNAL had four articles on the subject, and four others appear in the present issue. Their high lights are summarized briefly, with comments.

Azulay and associates,¹ studying the histopathology of reaction lesions, found that 25 per cent of 73 clinically positive reactions did not show the tuberculoid granuloma expected of truly positive reactions, while 38 per cent of 21 clinically doubtful reactions did so. It was therefore concluded that the macroscopic readings of the lepromin reaction is undependable.

It would seem to be a question whether the microscopic findings in biopsied reaction lesions—if biopsy were a generally practicable procedure—would be any more dependable. In that connection it may be noted that Bechelli *et al.*² pursuing the primary question of whether or not a

¹ AZULAY, R. D., DE ANDRADE, L. M. C., SILVA, C., RABELLO NETO, A. V., AZULAY, J. D., NEVES, R. G. and ALONSO, A. M. Comparison of the macroscopic readings and microscopic findings of the lepromin reaction. *Internat. J. Leprosy* **28** (1960) 38-43.

² BECHELLI, L. M., RATH DE SOUZA, P. and QUAGLIATO, R. Correlação entre os resultados da leitura clínica e do exame histopatológico da reação de Mitsuda. *Rev. brasileira Leprol.* **27** (1959) 172-182 (see abstract in this issue, p. 134). There are other pertinent abstracts in this issue to which attention should be called, but it would be impracticable to attempt to review them here.

clinically positive reaction always corresponds to a histologically positive response, have recently extended their study [see THE JOURNAL **26** (1958) 426] to a total of 293 specimens, from 231 patients and 62 contacts. No clear-cut answer has been arrived at. For example, among 92 reactions of +1 grade, only 45 per cent were definitely positive histologically by the criteria used, and 25 per cent were clearly negative (the others "favored" positive); while of 34 ulcerated (3+) reactions only 56 per cent were definitely positive.

Among 52 cleared-up (*branqueada*) lepromatous cases there were 12 reactions of 1+ grade none of which was definitely positive histologically, but 5 favored positivity.

Pertinent in this connection is the report of Mukerjee and Kundu,³ who tested 17 long-subsided lepromatous cases and found histologically positive changes not only in the 2 low-degree clinical reactions seen but also in the injection site of 1 of the clinically negative cases. In all three cases, control specimens failed to show the tuberculoid changes.

The question of the effect of repeated injections of lepromin in infants was dealt with by Rosemberg and associates.⁴ They found that lepromin reactivity was induced in their subjects much less frequently and strongly by this means than by BCG vaccination. It would be interesting to know whether the results in older children (e.g., schoolchildren) or adults would be similar.

Two articles by Taylor and associates were based on laboratory experiments in sensitized guinea-pigs. First,⁵ they found that sensitizing by lepromin injections caused enhanced responses to both tissue and bacillus components of lepromin, and that there was less immune response than after injections of heat-killed BCG. It was concluded that the tissue components should be separated from the bacilli in the preparation of skin-test reagents, both for epidemiologic investigations and for studies of the usefulness of different antigenic preparations (soluble elements and bacillary bodies).

Later⁶ they found that repeated injections of guinea-pigs with the whole lepromin aggravated only the early reaction to that antigen. The late reactions to the whole antigen and to purified bacillus suspensions

³ MUKERJEE, N. and KUNDU, S. The late lepromin reaction in subsided lepromatous cases. *Internat. J. Leprosy* **29** (1961) 14-28.

⁴ ROSEMBERG, J., DE SOUZA CAMPOS, N., AUN, J. N. and DA ROCHA PASSOS, C., JR. Immunobiologic relation between tuberculosis and leprosy. X. Comparative study of the results of the lepromin test in subjects submitted to serial injections of Mitsuda's antigen and to oral BCG vaccination. *Internat. J. Leprosy* **28** (1960) 271-283.

⁵ TAYLOR, C. E., HANKS, J. H., MOSES, H., MITTAL, M. C. and KANT, L. The antigenic components of lepromin as assayed in guinea-pigs. *Internat. J. Leprosy* **28** (1960) 284-299.

⁶ TAYLOR, C. E., HANKS, J. H. and KANT, L. The effect of partial purification of lepromin on the Fernandez- and Mitsuda-type responses in guinea-pigs. *Internat. J. Leprosy* **28** (1960) 423-427.

were similar, the treatment to which the bacilli had been subjected in the purification not having affected their antigenicity in that respect. It was concluded that the late-phase reaction requires a capacity for localizing and destroying the injected bacilli at a significant rate.

In a consolidation article Kinnear Brown, with Stone,⁷ relates what had been done since 1955 with his economical multipuncture depot test.⁸ The antigen, made up in a vehicle composed largely of light liquid paraffin, is retained in the injection sites longer than an ordinary saline suspension would be. A little of the preparation (0.1 cc. is sufficient for 25 tests) is placed on the skin and driven in with a 6-needle Heaf multipuncture instrument. Positive reactions can be graded satisfactorily, and there are never any ulcerations.

A similarly-made suspension of normal skin caused no reactions by this method, either in lepromin-positive patients or in healthy children, while a suspension of a tuberculoid-lesion tissue caused reactions in only a few of the patients but in none of the normals. It is concluded that, with this method, the response to lepromin is independent of any tissue component.

In experiments with BCG it was found that, while 57 per cent of unvaccinated children were lepromin positive (the rate not affected by tuberculin testing), 86 per cent were positive after the tuberculin negatives had been vaccinated. A comparison of lepromin reactivity in persons with (*a*) natural tuberculin hypersensitivity and (*b*) BCG-induced hypersensitivity showed 73 per cent reactivity in the former and 92 per cent in the latter. Furthermore, reactions were strongest in the vaccinated individuals.

A point of interest brought out in BCG experiments concerns the timing of induced reactivity. A group of children negative to a first depot lepromin test were each vaccinated with BCG and at the same time given a second lepromin test. The new lepromin sites had reacted 4 weeks later. The sites of the original lepromin tests also reacted (showing the depot effect)—but not for another 2 weeks, a delay about which one can only speculate.

As for the matter of dilutions, it was concluded that while a 1/100 depot lepromin gave fewer positives than did the usual 1/20 preparation (on the order, in one observation, of 45:57), it would nevertheless distinguish between those who would react satisfactorily to normal lepromin and those who would react weakly or not at all.

In view of the small amount of lepromin used per test, there would ordinarily be no need to use so high a dilution in practical work. It

⁷BROWN, J. A. K. and STONE, M. M. The multipuncture depot lepromin test: I. Technique and advantages. II. Application to the study of BCG-induced lepromin reactivity. *Internat. J. Leprosy* **29** (1961) 1-13.

⁸Two abstracts of previous reports appear in this issue.

would be interesting to know the effect of repeated testing by this method, which introduces so little of the antigen into the skin, or to what extent dogs—which are so readily affected by ordinary testing—would be sensitized. It is to be hoped that other workers will take up this method experimentally, and that that will not be hindered by the need of procuring the special instrument used. A simpler, home-made multipuncture instrument might be devised that in practice would serve the purpose.

Turning now to the reports of Leiker, of studies made with the regular test while in Netherlands New Guinea, he first⁹ attempts to evaluate the effects of the two main constituents of lepromin, the bacilli and the tissue elements. First, he found that a phenol-saline suspension of normal skin will give “positive” reactions in normal persons (as well as in tuberculoid leprosy patients). Adults showed more reactivity to that antigen than did younger persons, and tuberculin positives more than negatives; there was no such response in any of the lepromin-negative lepromatous patients tested. At most, the reactions were much weaker than those caused by a 1/150 dilution of regular lepromin, and it is concluded that the tissue element plays a smaller part in reactions to lepromin than the leprosy bacilli, although its effect cannot be entirely ignored.

With a 1/90 dilution of lepromin, 87 per cent of 124 tuberculoid patients gave reactions measuring 3 mm. or more, and more than 50 per cent of the healthy persons tested also were positive. On the other hand 1/90 dilution of the normal skin suspension had very slight—but not entirely negligible—effects. This led to the conclusion that somewhat higher dilutions of lepromin than usual can be used, to minimize the effect of the tissue component, without materially affecting the results of the tests.

Leiker questions the idea that in the production of positive lepromin reactions both the bacilli and the tissue elements act merely as foreign bodies. Against that idea are the differences in reactivity to the antigens used, the specificity of nonreactivity to lepromin of lepromatous cases, and the effects of tuberculosis infection and BCG vaccination. The “allergic paralysis” explanation of the nonreactivity of lepromatous cases is likewise not in accord with actualities.

Regarding the reaction to intradermal injections of normal tissue suspensions—the components of which, incidentally, have never to our knowledge been described in detail—nobody seems to have raised the question whether or not any special reactivity, or sensitivity, is involved in the positive reactions. Would second injections of the same group of subjects elicit more positives than first injections? Or would

⁹ LEIKER, D. L. Studies on the lepromin test. I. The influence of the bacillary and tissue components in dilutions of lepromin. *Internat. J. Leprosy* **29** (1961) In press.

those reactive to the first testing react more strongly and/or quickly to second tests than originally? Would persons who have reacted to lepromin (a combination bacillus-tissue element suspension) later react to a tissue-suspension antigen any more frequently, or strongly, or quickly, than persons who had not been injected with lepromin? That there is something more than a foreign-body effect involved in this phenomenon is indicated by the fact that lepromatous cases do not react to such antigens, and also the influence of the age and tuberculin-positivity factors found by Leiker.

Leiker's second article¹⁰ has to do with the time of reading of the late reaction, and ulceration, based on observations with the 1/90 lepromin dilution. With respect to size of reaction lesions, there was little difference between 3-week and 4-week readings, but the numbers of ulcerations increased materially in the interval—rather more, incidentally, in women than males, and in children than adults. He disagrees with the recommendation of the Madrid congress to place all ulcerating reactions in the strongest-reaction category regardless of size of the reaction lesions; grading should be based on size.

It may be agreed that a 1/90 dilution should be economical of lepromin, but its effectiveness would depend on how well the lepromas are selected for bacillus content, and how well they are cleansed of extraneous tissues which only add to the weight of the specimens and to the connective-tissue content of the suspension. With that proviso, it is likely that many practical users of lepromin would agree that in the dilution specified the tissue element of the antigens would have negligible if any effect on the reactions. Whether or not grading should depend entirely on the size of the reaction lesion, without regard to the presence or absence of ulceration, will likely remain a matter of opinion, but the suggestion should be considered. The question here would seem to be whether violence of a tissue reaction, leading to ulceration, signifies a corresponding degree of resistance to infection.

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¹⁰ LEIKER, D. L. Studies on the lepromin test. II. Time of reading and ulceration of the lepromin reaction. *Internat. J. Leprosy* **29** (1961) In press.