LEPROMIN VS PURIFIED BACILLUS SUSPENSION
II. COMPARATIVE TESTS WITH A PURIFIED BACILLUS SUSPENSION

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INTRODUCTION

Reported in this article are the results of clinical tests, made in four widely-separated regions, comparing the effects of a relatively tissue-free “purified bacillus suspension” (PBS) and a Mitsuda-Hayashi-type lepromin. The results do not indicate that the findings with regular lepromin are significantly affected by the content of tissue elements; and they do not indicate any distinct advantage, but rather some disadvantage, in removing the bulk of those elements.

The preparation of the antigens used in these tests has been described in the preceding paper (7). One-half of a batch of leproma pulp was used to prepare a regular lepromin (7), and the other half was used for the suspension, thus ensuring equality of the two antigens with respect to the original condition of the bacilli involved.

For the suspension, the bacilli were harvested from the tissue pulp with chloroform, after which acetone was added in double the volume of the chlorofom suspension to permit the collection of the bacilli by centrifuging, relatively free from the considerable amount of lipids that the chloroform suspension had contained. They were then suspended in phenol-saline, in about 50 per cent greater concentration than the lepromin.

It is not claimed that the product was entirely free from elements

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extraneous to the bacilli themselves, for slight clouding occurred when
the acetone was added to the original chloroform extract (perhaps due
to the precipitation of phospholipids), and traces of adsorbed proteins
may possibly have accompanied the bacilli. If there were particulate
tissue elements among the bacilli, they were not seen in stained smears
after counterstaining with methylene blue. The light clouding of the
final product, which resembled a bacterial "vaccine," compared with
the density of the control lepromin, was evidence of the completeness
with which the bulk of the tissue elements had been eliminated. It could
therefore be expected with confidence that whatever late or Mitsuda
reactions the suspension might induce would be due practically entirely
to the bacillary bodies.

In then remained to test in suitable subjects the antigenicity of the
suspension, in comparison with the standard lepromin that had been
prepared for the purpose. The testing could not be done at Culion,
where the antigens had been prepared, for lack of suitable subjects. It
might have been entrusted entirely to the Cebu unit of the Leonard
Wood Memorial, in the charge of one of us (R.S.G.), but then there
would have remained the familiar question of whether or not similar
results would be obtained by other workers in other parts of the world.

Consequently, arrangements were made for trials by the other co-
authors, in New Guinea, East Africa and South Africa, and to all collab-
orators were sent sufficient quantities of the two antigens. This afforded
an opportunity for the long-desired trial of a given batch of lepromin
by different leprologists, which to our knowledge had never been done.

It was suggested that in the testing the antigens be given to separate
groups of individuals, because of the possibility that in simultaneous,
or parallel, testing the effects of the dose of a stronger antigen might
set up a condition of reactivity that would cause a greater response to
the weaker antigen than would occur if the latter were used alone. On
the other hand, there is uncertainty in the use of different groups in
that their reaction averages might be materially affected if they should
contain different proportions of individuals of different degrees of re-
activity. Both methods were actually used in the trials, and evidence
has been adduced that simultaneous testing did not significantly affect
the results.

Since the subjects chosen for the trials and the methods of reporting
differed to some extent, each report is dealt with individually and sum-
marized in the discussion. The comments and conclusions accompany-
ing the individual reports are not presented in full. In the main, there
was essential similarity of results.

The term "standard" is used here because it was made by the standard Mitsuda-Wade
method and was used as the standard for the comparison. The term does not imply that it
necessarily constitutes the much-talked of "standardized" lepromin, which is still to be settled
upon.
The main interest has been in the late, or Mitsuda, reaction, the one that really counts in practice. It has been our experience (4) that different batches of lepromin which give the same results with the late reaction may give very different ones with the early reaction.

Measurements of the reaction lesions were made in millimeters in all cases. In each trial the readings were all made by the same observer, thus eliminating the possible factor of observational error; there may of course have been differences in reading practices between the different observers. To lessen irregularities due to relatively limited numbers of cases, the data have been tabulated as of 2-millimeter intervals.

Only one of the reporters (R. S. G.) spoke of "positive" reactions and he analyzed his results with respect to "strong" (2+ and 3+) reactions. It has been found useful to apply that analysis to the other data; but because the 3 mm. criterion of positivity (and 6 mm. for 2+ reactions) recommended by the Madrid Congress (9) is commonly used, that standard has also been considered.

**Tests in the Philippines**

(R. S. Guinto)

The findings in the tests made in Cebu were analyzed in full detail, with extensive statistical evaluations, but only the principal findings are presented here. Where percentage of positives are discussed in this section, they are based on the WHO Committee's criteria of positivity.6

Equally matched groups of healthy adults and young school children, the latter 6.9 years of age, were tested with the two antigens separately, as originally planned. At the same time, a third group of such children was tested with both antigens simultaneously, to see if there might be any detectable difference in results. No definite difference on that account being evident, the figures for all the children tested have been combined. Furthermore, the tuberculoid leprosy cases were also tested by simultaneous injections. A few lepromatous cases tested were all nonreactive, and nothing more need be said about them.

**Comparison of separate and simultaneous testing.**—The comparative data on positive Mitsuda reactions of the children tested in separate groups and in parallel are shown in Table 1. The differences, although they suggest that there might have been a little effect on the results of PIBS on simultaneous testing, are actually quite lacking in significance in the statistical sense.

**Fernandez reactions.**—Regarding positivity of the early reaction, in the tuberculoid cases both antigens gave an equally high rate, 62.7%

<table>
<thead>
<tr>
<th>Group</th>
<th>Separate tests</th>
<th>Parallel tests</th>
<th>Difference and SE of difference</th>
<th>Odds against chance occurrence of such a difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepromin</td>
<td>65</td>
<td>89.2</td>
<td>67</td>
<td>3.3 ± 5.9</td>
</tr>
<tr>
<td>PBS</td>
<td>71</td>
<td>69.0</td>
<td>67</td>
<td>8.0 ± 7.5</td>
</tr>
</tbody>
</table>

6 That reporter used the 4 mm. criterion for positivity recommended by the WHO Committee (10), and 8 mm. as the lower limit of 2+ reactions.
per cent, but there were twice as many strong reactions (moderately strong, 2+ only) in the lepromin group as in the other. In the adult normals, lepromin caused somewhat more positive reactions than PBS, 47.1 per cent against 36.8 per cent, and also the more strong reactions (again none more than 2+), but the differences in the reaction rates are not significant.

The same holds true of the immature and less reactive school children, although oddly enough the reverse relationship was found. With lepromin the early positives were 10.6 per cent, while with PBS they were 14.5 per cent. Statistically, this difference is completely without significance. However, in the lepromin group there were only 9 individuals with 2+ reactions, while in the other group 3 gave 3+ reactions, in addition to 4 who were 2+. This difference in the early reactivity of the children is probably a fortuitous occurrence; it did not appear in the Mitsuda reactions.

Considering the total of early positives (all test groups combined) there was, with few exceptions, the usual association of late reactions with the early ones (but, of course, not vice versa). Of 63 individuals Fernandez-positive to the lepromin 61 (96.8%) were also Mitsuda positive; and, similarly, of 66 early reactors to PBS, 60 (90.9%) were also Mitsuda positive, and the dissociation may be somewhat more frequent with the suspension.

Mitsuda reactions.—The essential data for the Mitsuda reactions in all groups tested are given in Table 2. The percentage distributions of reactions, at 2 mm. intervals, for each of the three case groups are shown graphically in Fig. 1 (A, B and C).

Tuberculoid cases: Among the 51 tuberculoid cases (first section of

<table>
<thead>
<tr>
<th>Table 2.—Philippine tests, in tuberculoid cases, healthy adults and healthy children 6-9 years of age.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of reaction (mn.)</td>
</tr>
<tr>
<td>Lepromin</td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>0-2</td>
</tr>
<tr>
<td>3-4</td>
</tr>
<tr>
<td>5-6</td>
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<tr>
<td>7-8</td>
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<td>9-10</td>
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<td>11-12</td>
</tr>
<tr>
<td>13-14</td>
</tr>
<tr>
<td>15-16</td>
</tr>
<tr>
<td>Ave. (mm.)</td>
</tr>
</tbody>
</table>

* Tested simultaneously.

** Tested in separate groups.

* Groups tested separately and in parallel combined.
Fig. 1. Distribution curves, by size of reactions, of Cebu cases, comparing standard lepromin and the purified bacillus suspension. A. Tuberculoid cases. B. Healthy adults. C. Healthy children.
Table 2), none gave a reaction less than 3 mm. in diameter with either antigen. In other words, none was negative by the Madrid criteria. By the WHO criteria, however, 20 PBS cases would be read negative against only 6 cases with lepromin. From the percentage columns it is seen that with lepromin the largest figure was for the 7-8 mm. group, whereas with the suspension the largest was for the 5-6 group. The averages, 7.7 and 6.8 mm. respectively, are distinctly, although not strikingly, different.

Considering the percentage of all the reactions in tuberculoids regarded as positive (WHO criteria; 96 for lepromin and 90 for PBS), the odds against chance occurrence of the difference (5.9 + 5.2%) was calculated to be only 3.3 to 1, so it is quite insignificant. There is a large difference in the percentages of the stronger reactions (2+ and 3+), 47.0 and 29.4, but although that may be significant it is not highly so.

Of the 51 cases in the tuberculoid group, 12 were bacteriologically positive reactional cases. Although this group was too small to justify citing percentages, a comparison of them with the nonreactional cases shows no notable difference in total positives, but there was a distinct tendency to weaker reactions, especially with the lepromin.

Healthy adults: Here again (second part of Table 2), no case was in the 0-2 mm. group with either antigen. The differences in percentages are most marked in the 3-4 mm. and the 9-10 mm. groups, the greater effect of lepromin being evident. The averages, 7.9 and 7.1 mm., respectively, were slightly larger than for the tuberculoid cases, showing—contrary to expectations—that the healthy individuals were fully as reactive as the infected persons. Both the total numbers of positives and the numbers of stronger reactions were somewhat the larger for the lepromin antigen, but in neither event was the difference statistically significant.

Healthy children: The data (third part of Table 2) show that of nonreactors (or virtual nonreactors), even by the lower Madrid limit, there was 1 to lepromin but 4 to PBS. Strong reactors were few, none above the 9-10 mm. group, but there was considerable difference in favor of lepromin at the +4 mm. level. The averages, 5.5 and 4.8 mm., are low—as would be expected in children so young and immature. However, in this lot of subjects, and only in this one, the difference of percentages of positives with the two antigens, 90.9 and 73.2 = 17.7 ± 4.69 per cent, is highly significant.

It is evident that, whereas the purified bacillus suspension is only slightly less effective than the lepromin in tuberculoid cases and healthy adults, and that with its use few if any cases among adults would be read doubtful or negative which with lepromin would be positive, the

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It may be that this result is affected to some degree by the tendency to weaker reactions on the part of the cases which were, or recently had been, in the state of reaction. —R.S.G.
PBS would be much less suitable for practical work involving young children.

Ulceration.—It was observed, regarding ulcerations in adults, that PBS caused fewer than lepromin. The rates of ulceration in the positively reacting adults were as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Lepromin</th>
<th>PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculoid cases</td>
<td>(8/49)</td>
<td>(5/46)</td>
</tr>
<tr>
<td>Healthy adults</td>
<td>(14/53)</td>
<td>(8/55)</td>
</tr>
</tbody>
</table>

In this respect the healthy adults were strikingly more reactive than the tuberculoid cases, a matter considered in connection with the late reaction.

**Tests in Netherlands New Guinea**

(D. L. Liiker)

Here the testing of the two antigens was done entirely in separate groups of individuals. After trying them out in small groups of lepromatous and borderline patients, to make sure that they would not give anomalous results, two balanced groups of each of the populations to be tested were chosen for the trial. These consisted of tuberculoid cases, 50 and 52 in numbers; apparently normal adults, 60 and 64; and normal children between 9 and 15 years of age, 34 and 32. Readings were made on the 21st day; the early reactions, difficult to read accurately in the dark-skinned subjects, were observed but not recorded.

The normals were of the Wamena Bay area, where the prevalence of leprosy has been found by survey to be 85 per thousand. Under the existing conditions it did not seem sensible to attempt to distinguish between contacts and noncontacts. It is safe

<table>
<thead>
<tr>
<th>TABLE 3.—New Guinea tests, made on separate groups of tuberculoid cases, healthy adults, and healthy children 8-15 years of age; Mitsuda reactions, read on the 21st day.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of reaction (mm.)</td>
</tr>
<tr>
<td>Lepromin (50)</td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>0.2</td>
</tr>
<tr>
<td>0.4</td>
</tr>
<tr>
<td>5.6</td>
</tr>
<tr>
<td>7.8</td>
</tr>
<tr>
<td>9.10</td>
</tr>
<tr>
<td>11.12</td>
</tr>
<tr>
<td>13.14</td>
</tr>
<tr>
<td>15.16</td>
</tr>
<tr>
<td>17.18</td>
</tr>
</tbody>
</table>

Ave. (mm.) 8.6 7.1 8.8 7.0 8.1 6.0

* Numbers of ulcerating reactions given in parentheses.
to assume that many had had contact with open cases, and it is possible that some of the adults may even have abortive infections, with lesions that had cleared up without trace. Apart from this unknown and indeterminable factor of contact, it was attempted to make the groups of normals as comparable as possible.

Mitsuda reactions.—The results in terms of measurements of the Mitsuda reactions are shown in Table 3. The figures in parentheses represent the numbers of reactions which ulcerated.

Tuberculous cases: Here, as in the Philippine cases, none of the 50 patients tested with lepromin was entirely negative (according to the Madrid criteria), and only 3 were in the 3-4 mm. range. On the other hand, with the PBS antigen 5 of 52 cases tested would be called negative anywhere, having had reactions less than 3 mm., and another 9 were less than 5 mm. The average sizes of the reactions were 8.6 and 7.0, respectively, a difference which is statistically significant.

The percentages of strong reactions were: (a) by the Congress criteria, 82 and 63, respectively; and (b) by the WHO Committee criteria, 60 and 48. It seems safe to say that the PBS was distinctly the less effective of the two antigens in the leprosy patients concerned.

Healthy adults: This group proved to be quite as reactive as the tuberculous cases, as in the Philippine trials. With the lepromin, none had reactions less than 3 mm. (3, again, were in the 3-4 mm. group), but with the suspension 1 was less than 3 mm. (and 10 others were less than 5 mm.). The averages were 8.8 mm. and 7.0 mm., respectively, the difference also statistically significant.

The percentages of strong reactions were: Madrid criterion, 83 and 67, respectively; WHO Committee criterion, 62 and 48. The similarity of these figures to those of the tuberculous group is striking.

Healthy children: These two groups, as was to be expected, were somewhat less reactive than the others, 4 of those tested with lepromin being quite negative, and 7 of those tested with the suspension. However—presumably by chance in the selection of subjects—4 of the latter group had larger reactions than any in the former. Because of these probably fortuitous cases, the difference between the averages, which were 6.4 mm. and 6.0 mm., respectively, was relatively small.

The percentages of strong reactions were: Madrid criterion, 65 and 53, respectively; WHO criterion, 42 and 31. The corresponding percentages for the considerably younger Philippine children were: Madrid criterion, 58 and 22; WHO criterion, 7.6 and 2.9. Even allowing for the effect of an age difference, it would seem that some reaction-stimulating factor was operative in New Guinea that was absent, or materially less effective, in the Philippines.

Graphs of the distribution curves of the reactions according to size, based on 3-millimeter intervals, shown in Fig. 2, are fairly regular, and they illustrate the fact that the PBS antigen generally induced smaller reactions than the lepromin.
Fig. 2. Distribution curves, by sizes of reactions, of New Guinea cases, comparing standard lepromin and the purified bacillus suspension. A. Tuberculoid cases. B. Healthy adults. C. Healthy children.
Ulcration.—The individuals in this study seem to have been especially prone to ulceration of the reaction lesions. In some instances this occurred when the lesions measured only 6 or 7 mm. in diameter. The totals are as follows:

<table>
<thead>
<tr>
<th></th>
<th>Lepromin</th>
<th>PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuberculous cases</td>
<td>(19/50)</td>
<td>38%</td>
</tr>
<tr>
<td>Healthy adults</td>
<td>(31/60)</td>
<td>55%</td>
</tr>
<tr>
<td>Healthy children</td>
<td>(5/54)</td>
<td>15%</td>
</tr>
</tbody>
</table>

It is unexpected that there should be no difference whatever in this respect between the two antigens in the leprosy cases, and no real difference in the children (the PBS figure slightly the higher because there were more of the largest reactions). Why, in these circumstances, the healthy adults had materially more ulcerations than the tuberculoid cases with lepromin, but not with the PBS, we are not prepared to say. The lower figure for the suspension is closely similar to those of the leprosy cases—which are close to the figure in the East African patients tested with lepromin (30%), but not to those tested with the suspension (18%).

Tests in East Africa

(J. Ross India)

This is a report of reactions in tuberculous cases only, tested in separate groups. Because the patients in the Iteso (now Alupe) leprosarium attached to the Research Centre had all been tested with lepromin, the work was done at the nearby Tororo Hospital in Uganda, a general hospital where leprosy cases are treated as outpatients. Of these, 100 tuberculoid cases, not previously injected with lepromin, were selected for the trial. Besides them, several lepromatous cases were injected with one or the other of the antigens to try them out, but since none of these cases gave a positive reaction with either antigen, they are not considered further.

For the comparison, the tuberculous cases were divided into two clinically balanced groups of 50 each, it being expected that in groups of that size the effects of individual variations in reactivity would be minimized in the averages. Readings were made after 2 days for the Fernandez reaction, and on the 21st day for the Mitsuda reaction.

Fernandez reactions.—The measurements of the early reactions were not recorded in detail. It can only be said that those caused by the two antigens were fairly similar, and that the suspension seemed to be at least as active in this respect as the regular lepromin.

Mitsuda reactions.—The data on the late reactions, at 2 mm. intervals, are summarized in Table 4, with (in parentheses) the numbers of reactions that ulcerated. In no case was the reaction lesion less than 3
mm, in diameter, and thus by the commonly-used scale all were positive.

There were 9 reactions larger than the 15-16 mm. limit observed in the Cebu cases, and 5 larger than the 17-18 mm. limit of the New Guinea cases. The largest reaction to lepromin was 22 mm., while 2 of the suspension reactions reached the extraordinary sizes of 25 and 32 mm.

In spite of the extra large PBS reactions referred to, the average for the lepromin lesions was 10.5 mm., and that for the suspension lesions 8.8 mm., the difference—1.7 mm.—being the largest one seen in this study. A better idea of the differences between the two antigens is seen in the percentages of the strong reactions: on the Congress basis, 90 and 74, respectively; on the WHO Committee basis, 71 for lepromin and 46 for the suspension. The lepromin figures are larger than the suspension figures by about 22 per cent and 61 per cent, respectively.

The graph showing size distribution of the reactions in these cases is, because of the wide spread for so limited a group of cases, unusually irregular and is not reproduced.

Ulcerations.—Ulcerations occurred in 15 (30%) of the lepromin cases and only 9 (18%) of the suspension cases, which is what might have been expected in view of the weaker antigenicity of the suspension. However, in each antigen group there was ulceration of 1 reaction—lesion less than 10 mm. in diameter. For comparison with the Cebu data for positives (WHO basis), there was ulceration in 32 per cent (15/47) of the positives in the lepromin group and in 20 per cent (9/44) of those of the suspension group—which rates are practically twice as high as those in the Cebu tuberculoids (16 and 11, respectively).
Tests in South Africa

(R. Kooij)

The tests at Pretoria were made entirely in parallel, in 21 tuberculoïd cases and 22 lepromatous cases, besides a few labelled "borderline and indeterminate" cases of which no more need be said. In addition to the antigens supplied, two others were used for comparison, each case being tested with all four simultaneously. The two extra antigens were: (1) a regular Mitsuda-Wade lepromin made locally from earlobe material, and (2) a Dharmendra-type antigen also prepared locally from earlobes.

On comparison of the results with those in the East African cases there is found no indication that the results were affected by the multiple testing. No note was made of previous lepromin testing of the cases; probably many of them had had one or more tests. However, from the experience reported by Garrod and Wade (3) it is unlikely that the findings were affected by previous testing.

Readings were made the second day after the injections for the Fernandez effect, and subsequently at weekly intervals on the 7th, 14th, 21st, and 28th days. The occurrence of ulceration was not recorded. Of the later readings, it suffices to consider only those made in the 21st day. The results with lepromin and the PBS are given in Table 5. The averages for all of the antigens used at the times of reading are shown in Fig. 3.

Tuberculoïd cases.—(a) Fernandez reactions: Of the 21 standard lepromin tests, only 6 gave positive early reactions (over 10 mm.) at 48-hours, and only 5 of the suspension tests—not always in the same persons. Whatever the previous vicissitudes of these cases—and despite high rates of late reactions—the frequencies of the early reaction were low, only around 25 per cent compared with over 60 per cent in the Cebu cases.

Striking inconsistencies were seen. For example, one lepromin case with a strongly positive (20 mm.) early reaction had only a 7 mm. early
reaction to the suspension. On the other hand, one case gave a 10-mm. reaction to the suspension whereas that to lepromin was only 4 mm. The early-reaction averages were: lepromin, 7.5 mm.; suspension, 6.0 mm. It is to be seen from Fig. 3 that the PBS average at 2 days was the lowest for the four antigens, indicating a relative paucity of the immediately-available antigenic element (free proteins).

The Westfort lepromin also gave 6 early positives, with the relatively low average of 6.2 mm. The Dharmendra-type antigen gave only 3 early positives, although the average was slightly larger, 6.5 mm. It is obvious that, with this particular batch of the Dharmendra-type antigen, the early reactions would not serve to pick out the tuberculoid cases which would give the late reactions to lepromin, as was Dharmendra's intention.

(b) Mitsuda reaction: The late results with the two antigens under test are shown in Table 3, mostly by 2 mm. intervals. As said, with lepromin all were positive by the usual criterion, with only 1 less than 5 mm. With the suspension, one case was quite negative, and 6 were less than 5 mm. The averages were 9.6 and 7.2 mm.—less than those in the East African cases, but more than those from the other side of the world, and with a material difference between the two.

Table 3.—South African cases, tuberculoid (21 cases) and lepromatous (22), tested in parallel with lepromin and the purified bacillus suspension; readings at 21 days.

<table>
<thead>
<tr>
<th>Reading (mm.)</th>
<th>Tuberculoid (21 cases)</th>
<th>Lepromatous (22 cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lepromin PBS</td>
<td>Lepromin PBS</td>
</tr>
<tr>
<td>0</td>
<td>— 1</td>
<td>6 12</td>
</tr>
<tr>
<td>1</td>
<td>— —</td>
<td>3 6</td>
</tr>
<tr>
<td>2</td>
<td>— —</td>
<td>11 3</td>
</tr>
<tr>
<td>3-4</td>
<td>1 6</td>
<td>— —</td>
</tr>
<tr>
<td>5-6</td>
<td>8 5</td>
<td>— 1</td>
</tr>
<tr>
<td>7-8</td>
<td>2 2</td>
<td>— —</td>
</tr>
<tr>
<td>9-10</td>
<td>4 2</td>
<td>— —</td>
</tr>
<tr>
<td>11-12</td>
<td>2 3</td>
<td>— —</td>
</tr>
<tr>
<td>13-14</td>
<td>1 0</td>
<td>— —</td>
</tr>
<tr>
<td>15-16</td>
<td>— 1</td>
<td>— —</td>
</tr>
<tr>
<td>&gt;16</td>
<td>3 1</td>
<td>— —</td>
</tr>
<tr>
<td>Ave. (mm.)</td>
<td>9.6 7.2</td>
<td>1.2 0.7</td>
</tr>
</tbody>
</table>

Comparing the 21-day reactions to the two antigens in individual cases, in 3 cases the readings were identical. In 14 cases the PBS reading was the lower, usually materially so, in certain instances as much as 6 to 8 mm. less; and in one case it was quite negative. On the other hand, there were four instances in which the PBS reaction was the larger, but none by more than 1 or 2 mm.

Although the groups are small for the calculation of percentages, it
may be noted that by the Madrid criteria the stronger reactions were 75 for the lepromin and 50 for the suspension; by the WHO criteria they were 48 and 36, respectively.

Regarding the 28-day readings, they were slightly larger than the 21-day readings in a few instances, but not enough to make that later reading significantly different. In fact they were usually slightly the smaller, as were the averages—9.3 vs 9.6 for lepromin and 6.8 vs 7.2 for PBS.

Lepromatous cases.—In none of the 22 lepromatous cases was either the early or late reaction to lepromin positive, even with the 3 mm. limit for the latter, although one-half of them were read 2 mm. In one case of the suspension group the late reaction was distinctly positive, 5 mm., against only 2 mm. for the lepromin—why cannot be said. The averages were 1.2 and 0.8 mm. The data for these cases is included in Table 5—by single millimeters in the subpositive range, where more activity of the lepromin than of the suspension is evident. Nevertheless, no cause for dissatisfaction with the regular lepromin is seen.

Other antigens.—In the tuberculous cases both of the other antigens were slightly but not significantly more effective in inducing the early reaction than was the suspension, which gave by a little the lowest average of all.

Regarding the Mitsuda reactions, those to the locally-made lepromin measured 3 mm. or more in all cases (17 of them 5 mm. or more), but with the Dharmendra antigen 7 cases were less than 3 mm. The averages were 7.3 and 4.6 mm., respectively.

In Fig. 3 it is seen that the Dharmendra antigen, alone of the four, gave relatively low average readings at 7 days (4.1 mm.) and the curve never went much higher—striking evidence of the relatively low antigenicity of the Dharmendra antigen—whereas the lepromin curve rose steadily to the 21st day, dropping a little thereafter. The curves of the suspension and the Westfort lepromin lie closely in parallel between the others.

DISCUSSION

The work here reported had a secondary purpose, besides the comparison in practice of the purified bacillus suspension and the standard lepromin. That was to compare results obtained with the same lots of antigens by different workers in different peoples in different parts of the world, which had not been done before. The trials were planned primarily for tuberculous cases, and groups of such cases were tested in all four centers. In two of the centers, with populations of very different racial composition, the work was extended to include healthy persons, both adults and children.

For a comparison of the effects of the two antigens in tuberculous cases, we may consider either average reaction sizes, which is useful, or
the percentages of positives. If the Madrid criteria of positivity should be used (i.e., $1^+ = 3-5$ mm.), little information would be gained because so many of the test groups would be 100 per cent positive. It is more informative to use the figures for the stronger reactions ($2^+$ and $3^+$), and for that we choose to regard 7 mm. as the lower limit of $2^+$ reactions. Of necessity, the factor of ulceration is ignored. The results are shown in Table 6.

<table>
<thead>
<tr>
<th>Place, group</th>
<th>Lepromin</th>
<th>Suspensions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average</td>
<td>Strongly positive</td>
</tr>
<tr>
<td></td>
<td>reaction</td>
<td>(percentages)</td>
</tr>
<tr>
<td></td>
<td>(mm.)</td>
<td></td>
</tr>
<tr>
<td>Philippines</td>
<td>7.7</td>
<td>(34/51) 67%</td>
</tr>
<tr>
<td>New Guinea</td>
<td>8.6</td>
<td>(37/50) 74%</td>
</tr>
<tr>
<td>East Africa</td>
<td>10.5</td>
<td>(42/50) 84%</td>
</tr>
<tr>
<td>South Africa</td>
<td>9.6</td>
<td>(12/21) 57%</td>
</tr>
<tr>
<td>Totals</td>
<td>—</td>
<td>(125/172) 73%</td>
</tr>
</tbody>
</table>

Comparison of the averages for each group shows that in all instances those for the PBS are the lower. Although the difference in the Philippine figures (0.9 mm.) was regarded as of uncertain significance, that of the New Guinea figures (1.5 mm.) was declared to be significant. The East African difference (1.6 mm.) for the same number of cases must be no less so.

The regional differences in percentages of strongly positive reactions to lepromin are interesting, giving indications not seen in the percentages of total positives. The New Guinea group exceeded the Philippine group; and the East Africa exceeded the New Guinea one by a wider margin (but not in the PBS figures). The fact that the South African figures should be so low seems anomalous.

The data on healthy adults and children tested in the Philippines and New Guinea are consolidated in Table 7, in which are included percentages for the stronger reactions (7 mm. and above).

Besides the usual differences between the lepromin and PBS data—the latter regularly the lower—perhaps the most striking feature of this table is the absence of any reactions beyond the 15-16 group, whereas in the tuberculinoid cases there had been 10 larger reactions with lepromin and 7 with the suspension. The averages for the adults were therefore somewhat smaller than in the tuberculinoid group, 8.4 mm. and 7.0 mm., respectively, and the percentages of strong reactions, 70 and 52 per cent.

8 By the Madrid criteria $2^+$ is "larger than 5 mm." and $3^+$ is any lesion with ulceration. By the WHO criteria $2^+$ is "larger than 7 mm." One table is not set up for division at either of these points, hence our choice of 7 mm. as the limit.
somewhat lower. The general run of healthy adults, however, were quite as reactive as the tuberculoid cases.

Leiker, in his report, stressed the point that, because of the high endemicty of leprosy in the region, the majority of normal adults might be considered contacts. That, however, is not the case in the community from which the Philippine subjects were drawn, and yet the findings were essentially similar. It seems likely that some other factor than leprosy contact is responsible for the almost universal reactivity of adults in the communities concerned—and that factor cannot be tuberculous infection except perhaps to a limited extent.

For the children, who because of their lower ages are naturally less reactive, the situation is different. The percentages of stronger reactions to lepromin by the Madrid criterion in the Philippine children, only 6-9 years of age, and in the New Guinea children, 8-15 years old, are fairly similar, but otherwise—in the lepromin tests by the WHO criteria, and in the PBS tests by both criteria—there are considerable differences, indicating the greater reactivity of the older group:

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Criterion</th>
<th>Philippine (6-9 yrs)</th>
<th>New Guinea (8-15 yrs)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepromin:</td>
<td>Madrid</td>
<td>57%</td>
<td>63%</td>
<td>59%</td>
</tr>
<tr>
<td></td>
<td>WHO</td>
<td>8%</td>
<td>47%</td>
<td>16%</td>
</tr>
<tr>
<td>PBS:</td>
<td>Madrid</td>
<td>23%</td>
<td>43%</td>
<td>29%</td>
</tr>
<tr>
<td></td>
<td>WHO</td>
<td>3%</td>
<td>21%</td>
<td>8%</td>
</tr>
</tbody>
</table>

The conclusion that few positive reactors would be missed by using the
PBS antigen obviously does not apply to young children, and young contacts are involved in much of the practical testing work that is done.

Why lepromin should in total be so much more reactive in children than PBS is not understood, but presumably it must be ascribed to an effect of the tissue element in the former. It might be of interest if those who are concerned with the effects of injections of "normal tissue" antigens should compare those effects in children and adults.

Not a few workers, especially South Americans, who use the Madrid congress criteria regard the weak (1+) reactions as of dubious significance, and tend to emphasize the stronger (>5) reactions. On that basis the differences between the results of standard lepromin and the suspension are certainly not negligible.

The factor of frequency of ulceration, which is of course an undesirable effect, is on the whole—but not regularly—in favor of the suspension. That, however, is the only point in its favor, and it cannot be regarded as compensating for the lower proportions of stronger reactions it induces, especially in young children.

Ulceration depends primarily on size of reaction. In Ross Innis' tuberculoid cases they began to appear in the 7-8 mm. group, and counting instances of that size and larger they occurred in 15 of 42 cases (36%) with lepromin and in 9 of 31 (29%) with the suspension—essentially similar proportions. In Leiker's data they began at the 5-6 mm. level, and in the healthy adults the percentages were 51 for lepromin and 42 for PBS; but, curiously, only PBS gave ulcerations at the 5-6 mm. level (4 of 18 cases). Furthermore, in the total of tuberculoid cases with reactions of that size or larger only 40 per cent had ulcers with lepromin while 50 per cent of the PBS cases had them (19 of 47 and 19 of 38), and in the children the percentages—less likely due to chance—were 19 with lepromin and 44 with PBS (5 of 26 and 8 of 18). This is not in favor of PBS.

Little can be said about the matter of acceleration of reactions in tuberculoid cases, reported by Olmos Castro (9) and called by Fernandez the "Olmos Castro phenomenon" (1). Most of the observers followed the usual custom and read the Mitsuda reactions on the 21st day. Kooij, however, measured the reaction lesions at 7-day intervals to the 28 day, and his graph based on periodic averages (Fig. 3) shows the maximum for the standard lepromin to have been reached at the time of the 2-week reading, although the curves for the three other antigens used, somewhat less antigenic, reached their maxima on the 14th day. There are no data for normal persons for comparison.

To return to the question of why the lepromin was on the whole the more active of the two antigens, and to obvious possibility that the tissue elements have some influence on the Mitsuda reaction, it is also obvious that it cannot be great, the bacilli are the main cause of positive reactions.
Kooij believes, on the basis of his work with normal tissue particles, that positivity of the Mitsuda reaction depends chiefly on the concentration of particles (leprosy bacilli and tissue). The standard lepromin, as usual, contained a great deal of particulate tissue elements, while the purified suspension contained virtually none, and yet the former induces — on the whole — only slightly more reactivity, and not constantly. Among Kooij’s 21 tuberculoid cases the suspension, tested in parallel with lepromin, gave as large or even slightly larger reactions in 7 instances. The view that the lepromin reaction is essentially a non-specific foreign-body reaction is not widely accepted. Lepromin is antigenic, as shown by the results of repeated tests in man, and in dogs this effect is particularly marked.

Regarding the desirability of removing the tissue elements from lepromin, Floch (2) argued against it, saying:

“C’est une erreur de vouloir éliminer à tout prix les éléments de tissus de la lepromine, car ils jouent, indiscutablement, un rôle dans la lepromine-réaction tel que nous le connaissons et l’interprétons.”

Wade (8) — writing after the observations here reported had been made — supported this opinion, saying, however, that the role of the tissue elements is decidedly secondary. He pointed out that the tissue elements of the leproma proper are more or less saturated with the products of metabolism and breakdown of the bacilli which they contain, to the extent of making them demonstrably acid-fast under certain circumstances of fixation and staining, and he held that “it is difficult to believe that such bacterial products in the tissue cells are without some influence in the antigenicity of lepromin.” The following passage appears in the Second Report of the WHO Expert Committee on Leprosy (11):

Certain workers believe that there is no need to remove the tissue elements, particularly in view of the fact that the lepra cells are impregnated with the products of metabolism of the active bacilli and of disintegration of the old ones. It is, however, recommended that further investigation of this matter be carried out, in order to arrive at a final conclusion.

The fact that in none of the tests of lepromatous patients reported in this study did the standard lepromin used cause false positive results, even by the Madrid (3 mm.) criterion, indicates that it is satisfactory for practical use. The suspension, more laborious and expensive of reagents to prepare, despite its greater content of bacillary bodies (9) regularly caused on the whole somewhat less reactivity (and usually but not always less ulceration), with an almost negligible proportion of false negatives except in young children; but the lesser proportions of reactions in them above 1+ level of the Madrid scale are not negligible. In general, the proportions of stronger reactions are considerably the less.

Since the findings of the different workers concerned, in different
parts of the world, were of the same tenor, and since none of the investigators evidenced interest in acquiring more of the suspension for personal use, it was concluded that there would be no profit in pursuing further this line of investigation. Although the results of this work, which was done several years ago, led to no change in our practice, it is nevertheless put on record because there has been no similar study in this field.

SUMMARY
Comparative tests of Wade’s “purified bacillus suspension” and the standard lepromin prepared from the same pool of leproma have been made in the Philippines, Netherlands New Guinea, East Africa and South Africa.

Consistently in the comparisons the standard lepromin gave more positive Mitsuda reactions, or more strong reactions, than the suspension; and in the healthy children tested the PBS sometimes gave negative results when the reactions to lepromin were positive. For this reason alone it would not be satisfactory for use in field work in which children are involved.

Lepromin gave more early reactions than the suspension in the cases for which that effect was reported (Philippines), suggesting that in the preparation of the latter the immediately-available antigenic elements had partly been eliminated.

The suspension on the whole caused somewhat less ulceration of the reaction lesions, which is a point in its favor. However, contrary findings were not infrequent, so use of the product could not be advocated strongly on that ground.

The results in tuberculoid cases permit comparison of the reactivities of the four different regional groups, whether considering average sizes of the reaction lesions or the percentage of the stronger reactions. Despite the high percentages of positive reactors in the Philippine subjects, the New Guinea people proved to be distinctly more so, and the East African cases the most reactive of all. The South African cases were much less reactive in comparison. The question of why people living in different environments differ materially in reactivity remains an intriguing problem.

RESUMEN
En las Filipinas, Nueva Guinea Holandesa, Africa Oriental y Sud-Africa, se han verificado pruebas comparadas de la “suspensión bacilar purificada” (SBP) de Wade y la lepromina estándar preparada del mismo lote combinado de lepromas.

Consistamente en la comparación la lepromina estándar rivalizó reacciones de Mitsuda más positivas o reacciones más intensas que la suspensión; y en los niños sometidos a pruebas de PBS dio algunos casos resultados negativos cuando eran positivas las reacciones a lepromina. Bastaría con esta razón para que no fuera satisfactoria la suspensión en obras en campaña en que figuran niños.

La lepromina acusó reacciones más tempranas que la suspensión en los casos para los
cual es consigna tal efecto (Filipinos), indicando esto que en la preparación de la
última se habían eliminado parcialmente los elementos antígenicos inmediatamente disponibles.
En conjunto, la suspensión ocasionó alguna menor alteración de las lesiones de la
reacción, lo cual constituye un punto en pro suyo. Sin embargo, no son raros los hallazgos
opuestos, de modo que no debe recomendarse obstinadamente sobre esa base el uso del producto.
Los resultados conseguidos en los casos tuberculosos permiten comparar las reac-
tividades de los cuatro distintos grupos regionales, ya que considerando los tamaños medios
de las lesiones de las reacciones o el porcentaje de las reacciones más intensas, a pesar de
los altos porcentajes de reacciones positivas en los sujetos filipinos, los de Nueva Guinea
mostraron una proporción decididamente mayor y los casos del África Oriental fueron los
más reactivos de todos. En comparación con éstos, los sudafrikanenses fueron mucho menos
reactivos. La cuestión de por qué la gente que vive en diversos medios ambientales discurso
sustancialmente en su reactividad sigue siendo un problema intrigante.

RESUMÉ

Une étude sur la comparaison des réactions à la lépromine standard et à la
"suspension basophile purifiée" de Wade (PBS), préparée à partir des sujets léproses,
à été menée aux Philippines, en Nouvelle-Guinée, en Afrique Orientale et en
Union Sud-Africaine.
Au cours de ces comparaisons, la lépromine standard a, de manière consistante,
entraîné un plus grand nombre de réactions de Mitsuda positives, ou bien des réactions
plus accentuées, que la suspension. De plus, chez les enfants sales, la PBS a parfois
donné des résultats négatifs, alors que les réactions à la lépromine étaient positives.
Pour cette raison, l’usage de cette suspension ne donnerait pas satisfaction sur le
terrain, lorsque des enfants sont en cause.
Lorsqu’on a tenu compte du délai de réaction (aux Philippines), la
lépromine a donné des réactions plus précoces que la suspension, suggérant ainsi que dans ce
dernier produit les éléments antécérents immédiatement accessibles ont été éliminé
au cours de sa préparation.
Dans l’ensemble, la suspension entraîne une ulceration quelque peu diminuée de
lesions réactionnelles. Ceci est un élément en sa faveur. Toutefois, comme des observa-
tions opposées ne sont pas rares, on ne peut se baser sur cet argument pour recommander
fermement l’usage de ce produit.
Les résultats obtenus chez les malades tuberculeux permettent de comparer la
réactivité des quatre groupes régionaux étudiés, soit d’après les dimensions moyennes
lesions réactionnelles, soit d’après le pourcentage des réactions plus accentuées. Malgré
l’importante proportion d’individus qui réagissent positivement aux Philippines, les sujets
de Nouvelle-Guinée se sont révélés les déposer nettement, et les malades d’Afrique
Orientale sont ceux qui, parmi tous, réagissent le plus. En comparaison, les sujets
d’Afrique du Sud réagissent beaucoup moins. La question de savoir pourquoi des
sujets vivant dans un environnement différent témoin de variations objectives de
réactivité reste un problème intrigant.

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