ENHANCEMENT OF POSITIVITY OF THE MITSUDA REACTION
OBTAINED WITH DILUTED ANTIGEN

H. Floch
Institut Pasteur de la Guyane Française
Cayenne, French Guiana

For several years now I have used the Mitsuda-Hayashi antigen diluted 1:750, resulting in a great saving of lepromin, which nowadays is not easy to obtain (7). Decrease of the intensity of positive reactions does not run parallel to the degree of the dilution; instead, it is stepwise, for which reason certain dilutions are of much more interest than others. This is especially so with the 1:150 and 1:750 dilutions (3). The former of these gives results which do not differ essentially from those of the normal antigen, but at that low dilution the saving in lepromin is not very great. That is why it has seemed to me better to set the dilution at 1:750 (4).

Diniz and Neto (1), in comparing normal and diluted antigens, have seen decrease in the number of positive results with dilution, but not the diminution of intensity of the positive reactions that I myself have seen. Their figures have been used in making the graph here shown (Text-fig. 1), the curve of which is quite similar to my own (4). One could not better demonstrate the interest of the 1:750 dilution: the decrease in the number of positive results between 1:20 and 1:750 (increase of dilution 37.5 times) is really of little importance. On the other hand, between 1:750 and 1:1000 (increase of dilution only one-third) the decrease in

![Text Fig. 1](image-url)
positive results is much greater. Wade (8) has emphasized the importance of these investigations.

The decrease of intensity of the positive reactions has led me to attempt to overcome this regrettable effect of dilution by incorporating in the dilute antigens certain substances designed to augment the degree of positivity of the reactions—this effect being obtained without affecting their specificity, which obviously is the primary requirement. General use of dilute Mitsuda antigen would obviously be more practical if it were possible to overcome, at least in large part, this diminution of intensity of positivity.

The purpose of this study, then, was to ascertain if the intensity of positive reactions to 1:750 lepromin could be increased without loss of specificity.

For this purpose I have performed, in the same patients, intradermal Mitsuda tests with two different antigens, one the 1:750 dilution of normal lepromin, the dilution made with 0.5 per cent phenolized saline, the other the same dilution but with the addition of 12 per cent glycerin and 2 per cent paraffin oil. I wished to see if the presence of these foreign substances would augment the intensity of the specific positive responses, without provoking a nonspecific nodular reaction as is known to be done by paraffin oil in high concentration.

It has been reported (5) that glycerin itself seems to augment the positivity of the intradermal reaction to killed BCG, when it is present in 30 per cent concentration in the test antigen. It is also known that tubercle bacilli suspended in paraffin oil provoke an allergy and a specific immunity much more intense than do the bacilli alone (6). It would seem logical, therefore, to expect that the paraffin oil, by an analogous mechanism, might also increase the antigenic power of the Hansen bacillus, but in this case in the detection of allergy (6).

These two preparations, 1:750 diluted normal lepromin (lepromin 1:750 DN), and the glycerin-paraffin oil preparation of the same basic dilution (lepromin 1:750 GP), were tested in 114 cases. The results are shown in the first part of Table 1.

With 37 per cent of the cases giving 1+ positive reactions to both antigens, and with 10 per cent negative to both, there was agreement in the reactions in a total of 47 per cent of the group (64 cases). In 69 instances (55%) there were differences. In all but a few cases (51 times out of the 60, or 85%), the reinforced antigen gave stronger reactions than the control, thus confirming my hypothesis.

If for the comparison only positive results are considered, which is logical, it is to be said that in no case was there positivity to one antigen and negativity to another. This shows that the additives had not affected the specificity of the lepromin.

In summary, the figures are as follows: out of 102 positive results, in 42 instances the responses to the two antigens were of essentially the
same intensity; in 51 instances the response to the preparation with additive (GP) was stronger than to the normal control dilution (DN); in only 9 instances was the positivity of the response to the control antigen the greater.

Table 1—Comparison of responses to diluted normal lepromin (DN) and the same with additives: A, glycerine-paraffin oil (GP), and B, normal skin extract (NS).

<table>
<thead>
<tr>
<th>Reactions</th>
<th>A. Diluted normal vs GP preparation</th>
<th>Reactions</th>
<th>B. Diluted normal vs NS preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases</td>
<td>Per cent</td>
<td>No. of cases</td>
</tr>
<tr>
<td>L/750 DN-</td>
<td>12</td>
<td>10</td>
<td>L/750 DN-</td>
</tr>
<tr>
<td>L/750 GP-</td>
<td>42</td>
<td>37</td>
<td>L/750 GP-</td>
</tr>
<tr>
<td>Agreement</td>
<td>54</td>
<td>47</td>
<td>Agreement</td>
</tr>
<tr>
<td>L/750 DN 2+</td>
<td>9</td>
<td>8</td>
<td>L/750 DN 2+</td>
</tr>
<tr>
<td>L/750 GP 1+</td>
<td>51</td>
<td>45</td>
<td>L/750 NS 1+</td>
</tr>
<tr>
<td>Disagreement</td>
<td>60</td>
<td>53</td>
<td>Disagreement</td>
</tr>
<tr>
<td>Total</td>
<td>114</td>
<td>100</td>
<td>Total</td>
</tr>
</tbody>
</table>

Another thing to be considered in this connection is that, hypothetically, the components of the leprous tissues (more particularly the lipoids) in the normal Mitsuda-Hayashi antigen may perhaps have a rôle analogous to what we have just seen, that is, an effect of a foreign element. If so, this might explain why the Dharmendra "bacillary" antigen usually gives reactions less strong than (although unquestionably quite as specific as) those of the Mitsuda-Hayashi antigen, without resorting to the explanation (although it is also highly possible and logical) of possible modifications of the bacillary components of the Dharmendra antigen resulting from the treatment with chloroform and ether during its preparation.

Various authors, from the time this test was first investigated, have studied the behavior of extracts of normal tissues used as controls. Lopes De Faria (7) has stated that such an antigen of normal skin made by the Mitsuda-Hayashi method may give positive late reactions in a good number of tuberculoid patients, although the reactions he observed were weaker than those provoked in the same patients by an integral lepromin.

Such positive reactions to a normal-skin antigen can obviously have nothing specific about them, and it is important to note that the same author obtained only negative results in 8 lepromatous cases in which he used that antigen, a fact which I myself have verified in 15 such patients.
De Faria concluded that in the Mitsuda (i.e., late) reaction three elements act as antigens: the Hansen bacilli, the special components of the leprous tissues, and the normal skin-tissue elements. 

Because of this fact it occurred to me that it might perhaps be possible to use an antigen containing for economy few bacilli (e.g., our 1:750 lepromin), but reinforced by a preparation of normal skin tissue made like ordinary lepromin. The question was whether this normal-skin extract used as an additive could augment the positivity of the late reaction—the specific positive response, elicited by the presence of the Hansen bacilli—without affecting specificity, as was found to be the case with the glycerine-paraffin oil preparation.

In any event, one would not run any risk of seeing a negative response become positive in lepromatous patients, because as has been said the normal-skin antigen alone does not cause reactions in such cases. That—to repeat—is obviously the primary concern, that a leprosy antigen should as a general rule give negative results in lepromatous cases, because of their specific anergy.

To investigate this matter I prepared an antigen made up of equal parts of lepromin diluted to 1:375 and of normal-skin extract prepared according to the Hayashi technique, 1:20. The final preparation, therefore, was 1:750 with respect to the Hansen-bacillus element, and 1:40 with respect to the added skin preparation. One could, of course, prepare an antigen of 1:20 concentration of the latter element, which would thus be nearer, theoretically, to the "normal" lepromin, but I believe that to be too concentrated.

This antigen was tested in 115 patients, in parallel with the "diluted normal" (DN) lepromin as control. After the proper intervals the Fernandez reaction was recorded for 111 of them, and the Mitsuda reaction for 109.

The results are given in the second section of Table 1, not including the readings of the Fernandez reaction. That reaction showed precisely the same trends as did the late one, except that this early one was negative in 56 per cent of the 111 cases (to both antigens), against 27 per cent negatives for the Mitsuda reaction.

In interpreting these results, account is taken only of the late (Mitsuda) readings. In the first place, in no case was there the discordance of positivity with one of these antigens and negativity with the other. The specificity of the 1:750 NS (normal-skin) antigen is therefore certain, because—as has already been proved—the 1:750 DN (dilute normal) is specific.

Further, of the 79 positive Mitsuda reactions, only 19 (24%) of the responses to the two antigens were of the same value, whereas 60 (76%) were stronger with the 1:750 NS antigen than with the control. These results are definitely better than those previously obtained by means of a 1:750 NS antigen with 2% paraffin oil and 12% glycerin.
It has also been possible to compare, but unfortunately in only 13 patients, the results of the intradermal reaction to the 1:750 NS lepromin and the normal 1:20 lepromin. In 10 of them the results were fairly similar; in 2 others the reactions to the normal lepromin were slightly the stronger; while in the last one the reaction to the reinforced dilute antigen was definitely the more marked.

From these results I believe that an additive consisting of 2 per cent paraffin oil and 12 per cent glycerin, or, better, one of a phenolized extract of normal skin, in lepromin diluted to 1:750 will overcome to a large extent the weakening of the positive responses due to the dilution. This they will do without modifying the specificity of the preparations. The preparation of the “1:750 NS” lepromin is easy.

SUMMARY

For economy, because of scarcity of good material to make lepromin, the author in practice uses a 1:750 dilution of the Mitsuda-Hayashi antigen instead of the normal 1:20 concentration. To overcome the commonly weaker response that this dilution elicits in positively reacting cases, he has attempted to enhance his preparation. To be useful, any additive that would enhance reactivity would have to do so without affecting such specificity as normal lepromin has.

One preparation tried was the 1:750 dilution containing 12 per cent glycerine and 2 per cent paraffin oil (L/750 GP of Table 1). Compared in 114 cases with the diluted normal lepromin (L/750 DN), the only disagreements were with respect to strength of positive reactions. Out of 102 positives, the GP antigen gave the stronger results in 51 (50%), while the DN control was the stronger in only 9; in the other cases both gave the same results.

The other preparation was a 50:50 mixture of a 1:375 dilution and an extract of normal skin prepared by the Mitsuda-Hayashi technique (1:20). The results in 109 cases were essentially similar to but better than those obtained before. Of 79 positive reactions, 60 (76%) were stronger with the reinforced antigen, while the control did not give the stronger reaction in any instance.

The author concludes that either of these preparations can be used without any loss of specificity, the second being the better one.

RESUMEN

Por razones de economía, debido a la escasez de buen material para preparar lepromina, el A. emplea en la práctica una dilución al 1:750 del antiguo de Mitsuda-Hayashi, en vez de la concentración normal de 1:20. Para superar la respuesta generalmente más débil que esta dilución provoca en los casos que reaccionan positivamente, ha tratado el mismo de reforzar su preparación. Para que resulte útil, todo aditivo que acreciente la reactividad tendría que hacerlo sin afectar la especificidad que posee la lepromina normal.

Una preparación ensayada contenía 12 por ciento de glicerina y 2 por ciento
de aceite de parafina (L/750 GP de la Table 1). Comparada en 114 casos con la lepromina normal diluida (L/750 DN), los únicos desacuerdos fueron con respecto a la intensidad de las reacciones positivas. De 102 positivas, el antígeno con GP obtuvo los resultados más poderosos en 51 (50 por ciento), en tanto que la DN testigo fue más intensa únicamente en 2; en los demás casos ambas arrojaron los mismos resultados.

La otra preparación fue una mezcla al 50:50 de una dilución al 1:375 y un extracto de piel normal preparado con la técnica de Mitsuda-Hayashi (1:20). Los resultados en 109 casos fueron en el fondo semejantes, pero mejores que antes. De 79 reacciones positivas, 60 (76 por ciento) fueron más intensas con el antígeno reforzado, en tanto que la preparación testigo no produjo la reacción más poderosa en ningún caso.

Deduce el A. que puede usar en una u otra de estas preparaciones sin la menor pérdida de especificidad, siendo la segunda la mejor.

REFERENCES