Delayed Reactions to Bouillon Medium of Tuberculin*

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In testing persons who are negative to weak dilutions of old tuberculin, it is frequently noted that a disproportionately large reaction may occur with the stronger dilutions. The suspicion has been voiced that these reactions to strong concentrations of old tuberculin may be non-specific. The problem is of special importance in testing children prior to BCG administration. Are those who react only to a 1:100 or 1:10 strength of old tuberculin to be looked upon as previously infected and not in need of BCG, or is this a non-specific reaction, not indicative of previous exposure?

Delayed reactions to old tuberculin, not caused by the tuberculin-protein per se, are theoretically possible from two causes principally:

1) Irritation of injected materials due to acidity or alkalinity, excessive concentration of salts, or chemicals causing an inflammatory response.

2) Allergic responses to substances derived from the bouillon medium.

This problem has been attacked by a number of workers. Aronson1 reported on sensitization to MA100; Furcolow2 on reactions to purified protein derivative in infants; Palmer3 on the geographic distribution of non-specific reactions; and Hart4 and Freund and Hart5 on reactions to substances in the medium.

This report concerns an investigation of hypersensitivity to bouillon medium.

Materials and Methods

Old Tuberculin (Parke, Davis and Company) diluted in a buffered diluent6 was used in the following strength: 1:10,000, 1:1,000, 1:100, and 1:10. Tuberculin PPD was used in the second strength and also the same diluted 1:10 and 1:100.

The bouillon medium generously furnished by Parke, Davis and Company was made in a manner identical with old tuberculin

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except that it was not inoculated with tubercle bacilli. The formula is as follows: Peptone 2 per cent, sodium chloride 0.5 per cent, glycerin 5 per cent, and beef infusion Q.S. The P.H. is adjusted to 7.6. The medium is sterilized at high steam pressure. The sterile bouillon is then evaporated over a steam bath to 1/10 the original volume. This batch was prepared in September 1949. This material was used in the same dilutions and diluent as old tuberculin (freshly made bouillon was tried, but was nonreactive).

A preparation of Long's medium was made and concentrated 10 times over a steam bath. It was used in a 1:10 dilution.

New glass was used for the bouillon and Long's medium.

All tests were done by the Mantoux method, using 0.10 cc. intracutaneously. The reactions were read at 72 hours and the transverse diameter of the indurated area was recorded. In one group the duration of the induration was studied by following it daily until it disappeared.

Tests were performed on the following groups of persons:
1) 34 tuberculous children.
2) 426 tuberculous adults.
3) 150 newly hired employees.
4) 88 affiliate student nurses.

The latter group came from a rural area, thus providing individuals who were completely insensitive to tuberculin.

Reactions were considered negative if there was no induration, doubtful if there was induration up to 5 mm. in diameter, and positive if over 5 mm. in diameter.

Control tests with diluent alone established the fact that this material caused no reactions.

The large majority of tests were done by giving simultaneous injections of old tuberculin 1:10,000 and bouillon. The latter was usually the 1:10 dilution. Tests with bouillon 1:100 and 1:1000 however were also carried out. All of the tuberculous patients had received tests with old tuberculin during the preceding several months.

Sixteen patients who were negative to the first two tests with bouillon 1:10 received 10 successive intracutaneous tests each at 48-hour intervals.

Seventy-four of the affiliate nurses were completely insensitive to tuberculin in four strengths including 10 mg. of old tuberculin. This series of tests was repeated at the end of two months and tests with bouillon 1:10 were included at the beginning and end of each series.

A number of variations of the above experiments were carried out. Some of the patients were tested with PPD instead of old tuberculin.
Results

Tests with bouillon medium gave positive reactions at 72 hours which were indistinguishable by inspection and palpation from the usual tuberculin reaction. The reactions were sometimes very firm and indurated and at others soft and puffy. They ranged in size up to 2 cm. in diameter of the induration. Additional erythema was frequent. Bleb formation occurred in two patients.

Observations on the duration of these reactions indicated that they last as long as 10 days, depending upon their size and intensity. They usually did not remain quite as long as the tuberculin test which was put on the skin simultaneously.

The large majority of the reactions were obtained with a 1:10 dilution of the bouillon. Forty-four per cent of the adult patients and 20 per cent of the tuberculin positive employees tested gave definitely positive reactions to this material. A small percentage gave doubtful reactions and the remainder were completely negative.

Tests with bouillon 1:100 and 1:1000 were positive quite frequently among the children. Fifty per cent of the tuberculous children reacted to the 1:100 dilution as compared to 16 per cent of adults. Forty per cent of the tuberculous children reacted to

<table>
<thead>
<tr>
<th>TABLE I</th>
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<tr>
<td>Reactivity to Bouillon of Various Groups Tested</td>
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<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Patients</th>
<th>Reaction to Bouillon</th>
<th>Percentage Positive to Bouillon 1:10</th>
<th>Percentage Positive to Bouillon 1:100</th>
</tr>
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<tbody>
<tr>
<td>Student Nurses</td>
<td>88</td>
<td>14 positive</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>74 negative</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Employees</td>
<td>150</td>
<td>18 negative</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>39 positive</td>
<td>17</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>57 positive (not previously tested)</td>
<td>15</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>36 positive (previously tested)</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Tuberculous Children</td>
<td>34</td>
<td>24 positive</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 positive</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Tuberculous Adults</td>
<td>426</td>
<td>307 positive</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>86 positive</td>
<td>16</td>
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<tr>
<td></td>
<td></td>
<td>33 positive</td>
<td>12</td>
<td></td>
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<tr>
<td>Tuberculous Adults Over 60 years old</td>
<td>18</td>
<td>positive</td>
<td>55</td>
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the 1:1000 dilution as compared to 12 per cent of the adults. Reactions to the 1:10,000 dilution were either negative or doubtful. (The total number of children tested [see Table 1] was too small to be statistically significant.)

Tests were performed on a series of moribund patients who required a 1:10 dilution of old tuberculin to react. Ten of these were simultaneously tested with 1:10 bouillon and it was found that the reaction to the bouillon was about two-thirds the size of the reaction to old tuberculin.

Eighty-eight affiliate student nurses were tested, of whom 14 were tuberculin positive. Four of these were positive to bouillon 1:10. Of 74 tuberculin negative nurses, none reacted to bouillon. Thirty-four of these nurses received 12 injections each of old tuberculin and bouillon over a two-month period, but remained completely negative to both.

The 16 tuberculin positive patients who received 10 successive injections of 1:10 bouillon all remained negative to this material.

Of 36 tuberculin positive employees who stated that they had previously had tuberculin tests, 33 per cent were positive to the bouillon. Of 57 similar employees who stated that they had not been previously tested, 15 per cent were positive. Eighteen tuberculin negative employees did not react to bouillon. Retests two to 12 weeks later revealed that some of those negative at first became positive to the bouillon later.

Tests with Long’s medium were uniformly negative, including patients repeatedly tested with PPD.

Discussion

It is evident from the above results that the bouillon medium is capable of giving reactions very similar to tuberculin. Strong concentrations are required, usually 1:10, although sometimes 1:100 or 1:1000 react. No reaction was observed to this material among non-reactors to tuberculin. Among those sensitive to tuberculin, a considerable proportion react to the bouillon.

It is apparent moreover that this reaction is not due to irritation, since it is absent among non-reactors to tuberculin. It is an allergic response to a specific substance in the bouillon and is limited to a portion of the tuberculin reactors. The specific substance is probably beef protein, perhaps altered chemically during the process of concentration over the steam bath, by aging, or by combination with one or more of the other ingredients of the medium.

Many of the tuberculin reactors who were negative to the bouillon could not be sensitized to the latter even after numerous intradermal injections. We were unable to note any basic dif-
ference otherwise between those who were sensitive to bouillon and those who could not be sensitized.

It appears probable that hypersensitivity to bouillon is artificially produced by the injection of old tuberculin (which of course contains bouillon). This follows from the fact that repeated tests of various groups produced successively larger percentages of reactors to bouillon. Also, employees who stated that they had previously had tuberculin tests gave a higher percentage of positives to bouillon than those not previously tested. Although some of the latter group were positive to bouillon, we assume that they may have forgotten a previous test, perhaps performed when they were children.

Since many reactors to old tuberculin do not react to bouillon, it is evident that sensitivity to the latter does not depend upon cross-reactivity, that is, a chemical similarity between bouillon and tuberculoprotein.

Raffel\textsuperscript{7} has demonstrated that sensitization to tuberculoprotein and other substances can be accomplished in the non-tuberculous animal by using the wax from the tubercle bacillus as an adjuvant. The presence of the wax apparently is necessary to establish the delayed type of hypersensitivity. The absence of this wax from the bodies of those who have never had a primary infection with tubercle bacilli may be the reason it is impossible to artificially sensitize them with the bouillon. To explain our inability to sensitize some of the tuberculous patients we may speculate that the wax, though present in their bodies, is unavailable because it is chemically bound or changed.

Testing with serial dilutions of old tuberculin thus introduces the possibility that an increasingly large portion of the reaction is due to the bouillon rather than to the tuberculoprotein. Even though this is true, such reactors are definitely tuberculin positive because, as noted above, non-reactors are invariably negative to the bouillon. This means that those children and others being tested prior to BCG inoculation who give delayed but so-called non-specific reactions to old tuberculin in the stronger concentrations probably must be considered reactors to tuberculin and, therefore, do not require BCG.

We believe that hypersensitivity to bouillon accounts for many, but not all, so-called delayed non-specific reactions to old tuberculin.

When sensitivity to bouillon is present, it seems to increase following repeated injections of either old tuberculin or bouillon. If this is true, then the anamnestic reaction to old tuberculin will require reinvestigation to determine whether it is due to the bouillon or the tuberculoprotein.
In the case of PPD, which is made by growing tubercle bacilli on Long's medium, the non-specific reactions which have been reported must be explained on some other basis, since we were unable to obtain any reactions with Long's medium.

Thus, it appears that the medium of growth plays an important role in the production of reactions to old tuberculin but not PPD.

SUMMARY

1) Certain preparations of bouillon medium, but not all, are capable of eliciting delayed reactions very similar to those of old tuberculin.

2) These reactions do not occur in persons completely insensitive to tuberculin. They occur in a considerable proportion of, but not all, tuberculin reactors.

3) Those tuberculin reactors who were negative to bouillon on the first two tests could not be sensitized by multiple injections.

4) Preparations of Long's medium did not produce delayed reactions.

5) The theoretical implications and possible practical applications of these findings are discussed.

Acknowledgment: We are very appreciative of the help of Miss Wilma Cannemeyer, medical technician.

RESUMEN

1) Ciertas preparaciones por medio de caldo son susceptibles de provocar reacciones similares retardadas muy similares a las de la tuberculina antigua.

2) Esas reacciones no ocurren en personas completamente insensibles a la tuberculina. Acontecen en una considerable parte de los reactores a la tuberculina, pero no en todos.

3) Los reactores de tuberculina que fueron negativos al caldo en las dos primeras pruebas, no pudieron ser sensibilizados por inyecciones múltiples.

4) Las preparaciones del medio de Long no produjeron reacciones retardadas.

5) Las consecuencias teóricas y probablemente prácticas de estos hallazgos son discutidas.

RESUME

1) Certaines préparations de bouillon de culture, et non toutes, sont susceptibles de créer des réactions tardives, absolument comparables à celles que peut déterminer la tuberculine brute.

2) Ces réactions ne surviennent pas chez des gens qui sont
parfaitement insensibles à la tuberculine. Elles apparaissent dans une proportion considérable, mais non pas exclusive, chez des individus allergiques.

3) Les individus allergiques qui ne réagissaient pas au simple bouillon ne purent être sensibilisés par des injections multiples.

4) Les préparations faites sur le milieu de Long ne provoquent pas de réactions tardives.

5) Les auteurs discutent les applications théoriques et pratiques de ces constatations.

REFERENCES


Discussion

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Doctors Lichtenstein and Cilella are to be congratulated on their original presentation regarding delayed reactions.

Certainly the specificity of the tuberculin reaction once considered to be absolute continues under scrutiny and the observations of Dr. Lichtenstein and Cilella warrant further work to determine, if possible, the reason for the reaction to the bouillon medium. It may be noted that non-specific reactions are seen in other forms of skin testing. People who have previously had Schick or Dick tests may subsequently develop false positives on re-testing in the same skin area apparently due to local allergic sensitivity.

When Schick tests became popular, it was first recommended that they be read in 48 hours. It was soon learned that a high percentage of positive reactions were false positives; now Schick
tests are read a week following the test and false positives are eliminated by this delay in reading. This, of course, is not comparable to the tuberculin reaction but probably does represent an induced local hypersensitivity which is not possible with tuberculin. In view of this I would like to ask if multiple or single sites were used to attempt to sensitize the negative bouillon reactors. Also of interest are the grooved cases of tuberculosis with a negative tuberculin reaction as recently re-stated by Mascher in the American Review of Tuberculosis for May, 1951.

I hope that the authors will continue to interest themselves in the tuberculin reaction as they have done for many years.