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DETERMINATION OF THE DEGREE OF ACTIVITY OF AN ANTIANTHRAX SERUM USED ON WHITE MICE

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DETERMINATION OF THE DEGREE OF ACTIVITY OF AN ANTIANTHRAX SERUM USED ON WHITE MICE

- USSR -

Following is the translation of an article by Professor I. V. Likhachev, State Scientific-Control Institute of Veterinary Preparations, in the Russian-language periodical Trudy (Proceedings ...), Vol 6, 1956, pages 252-264.

Summary

The author describes a series of experiments, aimed at discovering a more precise method for determining the strength or activity of antianthrax serum than the presently widespread method of testing with innoculation of rabbits, the author calibrated the strength of antianthrax serum of strain 317 on the basis of the number of white mice dying upon innoculation with various concentrations of the serum.

At the present time the active specific properties of antianthrax immunity serum are determined on rabbits. This method is very widespread, although it is accompanied by great imprecision because it does not enable establishment of the degree of activity of the biopreparation. In attempting to perfect the method of titration of anthrax serum, many scientists have tried to find another animal which would enable resolution of this task. White rats, white mice and other animals were tested. This path of investigation, however, was not substantiated.

In seeking a more perfected method of titration of...
Antianthrax serum the present author selected white mice as experimental animals most suitable for laboratory manipulation. Because of its particular biological properties, encapsulated anthrax vaccine of strain STI was used as anthrax culture. In white mice the STI anthrax strain evokes a gradually growing infection, first forming edema at the site of introduction of the culture. In our opinion these properties of the strain, in combination with the immunoserum, must enable smooth development of the infection process, and also must reveal the specificity and degree of activity of the biopreparation.

The initial, orienting experiments enabled certain conclusions to be made with respect to the specificity of control of antianthrax serum in white mice with anthrax vaccine of the STI strain, which does not encapsulate, because only the antianthrax serum prevented the death of white mice from lethal doses of the culture.

At the same time it was established that white mice 14 to 18 g in weight are the most suitable for the given purpose.

An outline of the dilution of various series of antianthrax serum used in titration is presented in Table 1. Our experiment included both highly active series, and serum series with reduced activity.

Table 1.

<table>
<thead>
<tr>
<th>Dilution on the Order of:</th>
<th>Amount of Serum, ml</th>
<th>Amount of Physiological Solution, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>0.45</td>
</tr>
<tr>
<td>3</td>
<td>0.001</td>
<td>0.499</td>
</tr>
</tbody>
</table>

For the purpose of simplification and ease of dilution of the serum, the amount of physiological solution and amount of serum used were approximately 10-fold greater. The diluted sera were injected subcutaneously in the back of the white mice, in dosages of 0.5 ml. The control white mice were inoculated with washes of one-day agar culture of the STI strain, diluted to 5 or 6 billion microbe-body. 
A dosage of 0.5 ml of this suspension was injected subcutaneously at the internal surface of the thigh 20 to 24 hours after injection of the serum. The inoculated white mice were observed for a period of 8 to 10 days, i.e. 3 or 4 days after the death of the control mice. After the period of observation the local reaction to injection of the culture was studied and evaluated in the inoculated white mice. The were classified as follows, depending upon the strength of the reaction:

(a) no reaction, 0;
(b) very weak, hardly noticeable edema of thigh, +;
(c) edema of thigh, not extending to the abdominal wall, ++;
(d) pronounced edema of thigh and part of the adjacent abdominal surface, +++.

A sample outline of the titration of the serum is shown in Table 2.

Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Serum Dilution</th>
<th>Evaluation of Results of Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of Series, No. of Total Dose (ml)</td>
</tr>
<tr>
<td>Control</td>
<td>--</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

In the control mice inoculated with 0.5 ml suspension of microbes of the above strain, containing 5 to 6 billion microbe bodies, edema often was lacking due to the rapid death of the animals.

On the basis of a great deal of material on the titration of antianthrax serum the following rules for evaluation of the activity of the biopreparation were established.
1. The serum was considered active if all white mice inoculated with doses of 0.1 and 0.05 ml survived, but the control mice died within 3 to 6 days after inoculation.

2. The serum was considered to have reduced activity if two or three of the white mice inoculated with 0.05 ml dosage died, but those inoculated with 0.1 ml survived.

3. The serum was considered to have very low activity and to be unsuitable for practical application if any of the white mice died upon inoculation with 0.1 ml dosage.