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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland
AN EVALUATION OF VACCINES AND THE EFFECTIVENESS OF VACCINATIONS AGAINST TYPHOID FEVER

Following is a translation of an article by Paulina Heislowa, Felicja Raboszynska and Zygmunt Kudelski in Przegląd Epidemiologiczny (Epidemiologic Review), Vol 17, 1963, pages 115-56.

XII. Agglutinative Antibodies in the Serums of Rabbits Immunized with Anti-Typhoid Vaccines

(From the Serum and Vaccine Testing Plant of the State Hygiene Establishment: Director Prof. Dr. H. Heisel).

In the preceding papers we have given the results of experiments made on white mice with four anti-typhoid vaccines prepared in domestic factories, namely: from acetone vaccine, formal-phenol, Grassett-Slopek and endotoxin prepared according to Westphal.

The immunization, choice of rabbits and production of the agglutination reactions were done in accordance with the recommendations of the World Health Organization (1). The tetavalent antigens H, O and Vi were obtained from the Institute of Marine Medicine in Gdansk. The determination of the titer of Vi antibodies by haemagglutination was made according to the Landy and Lamb method (2). The antigen Vi for haemagglutination was obtained from Copenhagen in alkalized form. Human blood corpuscles of the O group, washed and preserved in modified Alsever liquid, were used for the haemagglutination reaction.

- 1 -

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Results

Before commencing the vaccinations, all the rabbits showed an antibody titer of 1:10 or 1:20; in some animals a titer of anti Vi 1:5 was noted. The serum of none of the rabbits used showed agglutinative properties with respect to antigen H.

Acetone Vaccine. Table I gives the results of tests of the sera of nine rabbits immunized with acetone vaccine. The rabbits of group I, i.e., those immunized with vaccine diluted to 1:10, showed, before administration of the second dose, a titre of anti H ranging from 1:40 to 1:2560; seven days after the fourth dose, from 1:640 to 1:5120. The agglutination titre of anti O before the second dose in all rabbits was determined to be 1:320; seven days after the fourth dose the titre in two rabbits remained unchanged, while in three it rose to 1:640. The anti Vi titers determined by the agglutination method varied before the second dose from 1:5 to 1:20; but seven days after the fourth dose, from 1:40 to 1:640. The anti Vi titre determined by hemagglutination varied before the second dose from 1:24 to 1:72; but seven days after the fourth dose, from 1:80 to 1:3800.

The rabbits of group II, immunized with vaccine diluted in a physiological NaCl solution in the proportion 1:1,000, showed before the second dose an anti H agglutination titre from 1:10 to 1:40 seven days after the fourth dose, it was between 1:320 and 1:5120. The anti O titre before the second immunizing dose fluctuated from 1:40 to 1:80; seven days after the fourth dose, from 1:80 to 1:320. Both before the second and after the fourth dose, the sera showed weak agglutinative properties with relation to antigen Vi, in dilutions of 1:5 or 1:10. No antigen Vi was revealed in this group of rabbits by means of hemagglutination. One rabbit of the second group died from causes not connected with immunization, so that our tests were made on four rabbits.

Results of the control agglutination reactions: the emulsions of O and H antigens showed no agglutination in sodium chloride physiological solution. Antigen H with positive serums showed agglutination to ++ + in dilutions from 1:320 to 1:2560; with known negative serum it produced no agglutination. Antigen O showed agglutination with known positive serum in dilutions from 1:320 to 1:640. With known negative serum it showed no agglutinative properties. Antigen Vi with standard serum produced agglutination.
... in dilutions of 1:2000. It showed no agglutination.

The level of control reactions in hemagglutination:

The adjuvant showed no hemagglutination with an

... of non-sensitized blood corpuscles. An emulsion of

... monocloned with antigen VI showed no

... in a physiological NaCl solution and with a

... antiserum. With standard serum hemagglutination

... obtained to ++ + in dilutions of 1:9600 or 1:10,000.

...-Pseud Vaccine. Table II gives the results of

... of the sera of ten rabbits immunized with formal-

... vaccine. In the first group of rabbits the level of

... before the second dose varied between 1:320

... 1:100 seven days after the fourth dose, it grew from

... 1:1280 both before the second dose and seven days

... the fourth immunizing dose. VI antibodies, found only

... by agglutination, amounted before the second
day to 1:140 and, seven days after the fourth dose, to 1:40,

... by hemagglutination: 1:15 and, seven days after

... dose, to 1:40, revealed by hemagglutination:

... anti-VI. With the sera of the remaining rabbits,

... reactions proved negative.

... the second group of rabbits, anti H agglutination

... 1:30 was found before the second dose; from

... 1:120 seven days after the fourth immunizing dose.

... before the second dose were found to range

... 1:30: seven days after the fourth dose an

... growth from 1:30 to 1:60 was noted, and in

... even a drop from 1:60 to 1:10. The VI anti-

... by agglutination showed in two rabbits a

... of level from 1:10 to 1:40 and from 1:5 to 1:10,

... the second and after the fourth dose.

... rabits the antibody level in both tests was

... remaining two rabbits no anti VI agglutina-

... discovered. It was not possible to detect any VI

... revealed by hemagglutination. The controls proceeded as

... of the acetone vaccine.

... according to Grasset-Slopek. Table III gives

... the results of agglutination and hemagglutination with the

... of ten rabbits immunized with Grasset-Slopek vaccine.

... whole cycle of immunization, the rabbits of

... group produced no H antibodies, with the excep-

... of one rabbit, in which anti H agglutinins were

... dilution of the serum seven days after the
done. Before the second immunizing dose, the O antibodies varied between 1:40 and 1:320; seven days after the fourth dose, the antibody level had grown from 1:160 to 1:320. VI antibodies in the agglutination reaction seven days after the fourth dose were found in two rabbits to be in the ratio of 1:10 and 1:40. The serums of the remaining rabbits showed no agglutinating properties for antigen VI. No anti VI agglutinins were discovered in this group of animals by means of the hemagglutination reaction.

The rabbits of the second group, with the exception of one, in whose serum anti H agglutinins were found in a 1:5 dilution, did not produce any H or VI antibodies. The O antibody level was just as high before the second dose as seven days after the fourth dose, and varied between 1:40 and 1:640. The controls proceeded as in the case of the acetone vaccine.

Endotoxin according to Westphal. Table IV shows the results obtained in reactions with the serums of nine rabbits immunized with Westphal endotoxin. One rabbit in the second group died from causes not connected with immunization. In none of the rabbits, either of the first or of the second group, were H or VI antibodies discovered. The height of the anti O titer in all the rabbits of both groups, both before the second, and seven days after the fourth immunizing dose, was 1:20 or 1:40. The controls proceeded as in the case of the acetone vaccine.

Discussion

The present paper presents the results of experiments made on rabbits immunized with four anti-typhoid vaccines. The level of the antacellular antibodies (anti H, O and VI) was tested in the animals in accordance with the instructions given by the Department for the Standardization of Biological Preparations of the World Health Organization. Animals were therefore selected which before commencement of immunization either contained in their serums no natural antibodies directed against the typhoid bacillus antigens or else showed activity in low dilutions. Two groups of rabbits of five each were immunized with each vaccine, the doses being appropriately chosen.

Extremely different results were noted in the rabbits immunized with acetone vaccine and Westphal vaccine. The acetone vaccine caused a regular appearance of all three kinds of antibodies, i.e., anti H, O and VI. On the contrary, the Westphal endotoxin infected in both small and large doses was unable to stimulate the organism of the rabbit to produce anti H and anti VI agglutinins. Only a slight growth in anti O agglutinin was noted.
The formol-phenol vaccine stimulates the rabbit to produce anti H and anti O agglutinins to a rather considerable degree, but anti VI agglutinins only slightly.

The Grasset-Slopek vaccine has a stimulating effect on the production of anti O agglutininns, but only sporadically causes production of H and VI antibodies.

It is evident from our experiments, the formation of VI antibodies under the influence of immunologically active vaccine (acetone vaccine) depends upon the size of the dose. Rabbits immunized with vaccine diluted in the proportion 1:100 (which corresponded to 50,000 bacterial cells in 0.5 ml) reacted considerably by stronger production of anti VI than the animals immunized with vaccine diluted in the proportion 1:1000 (50,000 bacterial cells in 0.5 ml). The experiments presented threw an interesting light on the dynamics of formation of antibodies. Especially instructive in this respect are the observations made on rabbits immunized with acetone vaccine, which was the only one to cause regular formation of antibodies sought. As the quantity of the immunizing doses was increased, the H antibodies showed a marked growth in activity. The O antibody level obtained after the first approximately large dose was not subject to any fluctuations as the immunizing doses were repeated.

VI antibodies behaved in this respect like the H antibodies, i.e., the sera were constantly active in the other dilutions as the immunizing doses were administered.

Another striking fact was the lack of influence by the natural antibodies found in the sera upon the intensity of formation of antibodies under the influence of immunization. The results of the tests of the sera of rabbits immunized with the four different antityphoid vaccines are interesting when compared with those of trials, of these vaccines in an active test on white mice.

In the active test on white mice immunized with the above-mentioned four vaccines, the highest immunization activity was found in the acetone vaccine and minimum immunizing properties in the Westphal endotoxin. The Grasset-Slopek vaccine and the formol-phenol vaccine occupy an intermediate position, and their immunization values are of like order. Comparing these results with those of the present study, we see a far-reaching agreement. The acetone vaccine best protects mice from infection and stimulates the rabbit most to produce H, O and VI antibodies, which are discoverable even with a considerable dilution of the sera. On the contrary, the Westphal endotoxin hardly protects the mice at all and does not affect the production of H, O or VI antibodies by rabbits. The Grasset-Slopek and formol-phenol vaccines are both tests occupy an intermediate position between the acetone vaccine and the Westphal endotoxin.
Table I
Acetone Vaccine

<table>
<thead>
<tr>
<th>Grupa</th>
<th>Czas pobrania próbki krwi</th>
<th>Przed I dawką</th>
<th>Przed II dawką</th>
<th>I rozmiesz. 110</th>
<th>II rozmiesz. 1100</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N krøla</td>
<td>H</td>
<td>O</td>
<td>Vi</td>
<td>H</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>21</td>
<td>40</td>
<td>320</td>
<td>20</td>
</tr>
</tbody>
</table>

Miana przedstawia odwrotność rozmieszczenia. Nie badane oznaczone kropką.

Legend (prettaining to all tables) appears on last page.
<table>
<thead>
<tr>
<th>Group</th>
<th>1st day</th>
<th>2nd day</th>
<th>3rd day</th>
<th>4th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. O.</td>
<td>50</td>
<td>25</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>H. O.</td>
<td>50</td>
<td>25</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>H. O.</td>
<td>50</td>
<td>25</td>
<td>12.5</td>
<td>6.25</td>
</tr>
<tr>
<td>H. O.</td>
<td>50</td>
<td>25</td>
<td>12.5</td>
<td>6.25</td>
</tr>
</tbody>
</table>

Note: H. O. stands for horse serum.
Table III

Vaccine according to Grasset-Slopek

# Table III

<table>
<thead>
<tr>
<th>Grupa</th>
<th>Czas pobrania próbki krwi</th>
<th>Nr</th>
<th>Miana aglutynacyjna</th>
<th>Nr</th>
<th>Miana aglutynacyjna</th>
<th>Nr</th>
<th>Miana aglutynacyjna</th>
<th>Nr</th>
<th>Miana aglutynacyjna</th>
<th>Nr</th>
<th>Miana aglutynacyjna</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>H</td>
<td>O</td>
<td>VI</td>
<td>H</td>
<td>O</td>
<td>VI</td>
<td>H</td>
<td>O</td>
<td>VI</td>
</tr>
<tr>
<td>1)</td>
<td>Przed I dawką</td>
<td>1</td>
<td>10</td>
<td></td>
<td></td>
<td>2</td>
<td>10</td>
<td></td>
<td>3</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Przed II dawką</td>
<td>2</td>
<td>40</td>
<td></td>
<td></td>
<td>3</td>
<td>220</td>
<td>6</td>
<td>4</td>
<td>320</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>7 dni po IV dawce</td>
<td>3</td>
<td>80</td>
<td>320</td>
<td></td>
<td>8</td>
<td>160</td>
<td></td>
<td>9</td>
<td>320</td>
<td>40</td>
</tr>
<tr>
<td>2)</td>
<td>Przed I dawką</td>
<td>4</td>
<td>10</td>
<td></td>
<td></td>
<td>5</td>
<td>10</td>
<td></td>
<td>6</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Przed II dawkę</td>
<td>5</td>
<td>40</td>
<td></td>
<td></td>
<td>8</td>
<td>40</td>
<td></td>
<td>9</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 dni po IV dawce</td>
<td>6</td>
<td>640</td>
<td>220</td>
<td></td>
<td>10</td>
<td>80</td>
<td></td>
<td>8</td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>
Table IV
Endotoxin according to Westphal

<table>
<thead>
<tr>
<th>Grupa</th>
<th>Czas pobrania próbek krwi</th>
<th>H</th>
<th>O</th>
<th>Vi</th>
<th>H</th>
<th>O</th>
<th>Vi</th>
<th>H</th>
<th>O</th>
<th>Vi</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Przed I dawką</td>
<td>31</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>32</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>I.</td>
<td>Przed II dawką</td>
<td></td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>33</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>I.</td>
<td>Po dniach po dawkach</td>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
<td>34</td>
<td>20</td>
</tr>
<tr>
<td>II.</td>
<td>Przed I dawką</td>
<td>36</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>37</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>II.</td>
<td>Przed II dawką</td>
<td></td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td></td>
<td>38</td>
<td>40</td>
</tr>
<tr>
<td>II.</td>
<td>Po dniach po dawkach</td>
<td></td>
<td></td>
<td></td>
<td>40</td>
<td>40</td>
<td>40</td>
<td></td>
<td>39</td>
<td>40</td>
</tr>
</tbody>
</table>

Legend:
- H: Hémolysine
- O: Oxydase
- Vi: Viability

*Note:* The table contains data on endotoxin levels according to Westphal's method, with columns for different time points after administration of samples.
Legend for the preceding tables:

1) Group
2) Time of collection of blood samples
3) No of rabbit
4) Hemagglutination titer
5) Hemagglutination
6) No of rabbit
7) I dilution 1:10
8) II dilution 1:1,000
9) Before 1st dose
10) Before 2nd dose
11) 7 days after 4th dose

- END -