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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 Paula Heislowa, Felicja Rabozynska and Zygmunt Kudelski in Przegląd Epidemiologiczny (Epidemiologic Review), Vol 17, 1963, pages 61-86.

XII. Agglutinative Antibodies in the Serum 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, Grasset-Slopek and endo-
to... prepared according to Westphal.

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

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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 titer of anti H ranging from 1:40 to 1:2560; seven days after the fourth dose, from 1:640 to 1:5120. The agglutination titer of anti 0 before the second dose in all rabbits was determined to be 1:320; seven days after the fourth dose the titer 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 titer 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 titer from 1:10 to 1:40 seven days after the fourth dose, it was between 1:320 and 1:5120. The anti 0 titer 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 0 and H antigens showed no agglutination in sodium chloride physiological solution. Antigen H with positive sera showed agglutination to +++ in dilutions from 1:320 to 1:2560; with known negative serum it produced no agglutination. Antigen 0 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,
ly, reacted with known negative serum, as well as in a
well to the sodium chloride solution, in 5% sodium
sodium chloride solution, and in 2.5% sodium chloride solution and in

Results of control reactions in hemagglutination:
the red blood cells showed no hemagglutination with an
equivalent of non-sensitized blood corpuscles. An emulsion of
red blood cells sensitized with antigen VI showed no
hemagglutination in a physiological NaCl solution and with a
negative serum. With standard serum hemagglutination
was obtained to ++ + in dilutions of 1:9600 or 1:10,000.

Formaldehyde Vaccine. Table II gives the results of

samples of the sera of ten rabbits immunized with formol-

vaccine. In the first group of rabbits the level of
antibodies before the second dose varied between 1:320
and 1:160; seven days after the fourth dose, it grew from
1:160 to 1:1530. The level of antibodies was between
1:320 and 1:1280 both before the second and second doses
and during the fourth immunizing dose. VI antibodies,
found only in one rabbit by agglutination, amounted before the second
dose to 1:6 and, seven days after the fourth dose to 1:40,
amounted by hemagglutination: 1:15 and, seven days after
the fourth dose, to 1:40, revealed by hemagglutination:

1:10 and 1:34. With the sera of the remaining rabbits,
no reactions proved negative.

In the second group of rabbits, anti H agglutination
of 1:10 to 1:160 was found before the second dose; from
1:320 to 1:1280 seven days after the fourth immunizing dose.
Antibodies before the second dose were found to range
from 1:8 to 1:160; seven days after the fourth dose an
increase in titre of 1:30 to 1:120 was noted, and in
1:60 to 1:10. The VI antiserum was agglutinated by two rabbits a
level of level from 1:10 to 1:40 and from 1:5 to 1:10,
before the second and after the fourth dose.
In both rabbits the antibody level in both tests was
1:20. With the remaining two rabbits no anti VI agglutina-
tion was discovered. It was not possible to detect any VI
antibodies by hemagglutination. The controls proceeded as
in the acetone vaccine.

According to Grasset-Slopek, Table III gives
the results of agglutination and hemagglutination with the
sera of ten rabbits immunized with Grasset-Slopek vaccine.

Out the whole cycle of immunization, the rabbits of
the first group produced no H antibodies, with the excep-
tion of one rabbit, in which anti H agglutinins were
found in a 1:50 dilution of the serum seven days after the
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 1:510. 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 sera 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 1:6 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 sera 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 anticytoplasmic 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 sera 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 formal-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 agglutinins, 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 1:10 (which corresponded to 50,10^6 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 (10^9 bacterial cells in 0.5 ml). The experiments presented throw 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 the antibodies sought. As the quantity of the immunizing doses was increased, the H antibodies showed a gradual growth in activity. The O antibody level obtained after the first appropriately 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 lower 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 anti-typhoid vaccines are interesting when compared with those of trials of these vaccines in an active test on white mice.

In an 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 formal-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 formal-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 próbek krwi</th>
<th>Miana aglutynacyjna</th>
<th>Hemaglutynacyjne</th>
<th>Miana aglutynacyjna</th>
<th>Hemaglutynacyjne</th>
<th>Miana aglutynacyjna</th>
<th>Hemaglutynacyjne</th>
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<tr>
<td>I</td>
<td>Na krwi</td>
<td>H</td>
<td>O</td>
<td>Vi</td>
<td>H</td>
<td>O</td>
<td>Vi</td>
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<td>5</td>
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<tr>
<td>Przed II dawką</td>
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<td>2560</td>
<td>320</td>
<td>20</td>
<td>22</td>
<td>2560</td>
<td>820</td>
</tr>
<tr>
<td>Przed II dawką</td>
<td>7 dni po IV dawce</td>
<td>3500</td>
<td>640</td>
<td>10</td>
<td>20</td>
<td>3500</td>
<td>640</td>
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</tbody>
</table>

Miana przedstawia odwrotność rozcięczenia. Nie badane oznaczone kropką.

Legend (prettaining to all tables) appears on last page.
<table>
<thead>
<tr>
<th>Grupa</th>
<th>Czas po braniu próbek prób</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>
<th>H</th>
<th>O</th>
<th>VI</th>
<th>H</th>
<th>O</th>
<th>VI</th>
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<td>15</td>
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<tr>
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<td>7 dół po IV dawce</td>
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<td>640</td>
<td>640</td>
<td>320</td>
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<td>820</td>
<td>10</td>
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Table III

Vaccine according to Grasset-Slopek

<table>
<thead>
<tr>
<th>Grupa</th>
<th>Czas pobrania próbek krwi</th>
<th>Mięsa aglutynacyjne</th>
<th>Mięsa aglutynacyjne</th>
<th>Mięsa aglutynacyjne</th>
<th>Mięsa aglutynacyjne</th>
<th>Mięsa aglutynacyjne</th>
<th>Mięsa aglutynacyjne</th>
<th>Mięsa aglutynacyjne</th>
</tr>
</thead>
<tbody>
<tr>
<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>
</tr>
<tr>
<td>1</td>
<td>Przed I dawką</td>
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<td>10</td>
<td>10</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>Przed II dawką</td>
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<td></td>
<td>80</td>
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<td>80</td>
</tr>
<tr>
<td></td>
<td>7 dni po IV dawce</td>
<td>640</td>
<td>640</td>
<td></td>
<td>640</td>
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<td>640</td>
</tr>
</tbody>
</table>
Table IV

Endotoxin according to Westphal

<table>
<thead>
<tr>
<th>Grupa</th>
<th>Czas pobrania próbek krwi</th>
<th>Miana aglutynacyjne</th>
<th>Miana aglutynacyjne</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>H</td>
<td>O</td>
</tr>
<tr>
<td>1. Przed 1 dawką</td>
<td>51</td>
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<td>2. Przed II dawką</td>
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</tr>
<tr>
<td>3. Przed 1 dawkę</td>
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<td>20</td>
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<tr>
<td>4. Przed II dawkę</td>
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<td>20</td>
<td>40</td>
</tr>
</tbody>
</table>
Legend for the preceding tables:

1) Group
2) Time of collection of blood samples
3) No of rabbit
4) Agglutination titers
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 -