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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 Weislowa, Felicja Rabozynska and
Zygmunt Kudelski in Przegląd Epidemiologiczny
(Epidemiologic Review), Vol 17, 1963, pages
81-86.]

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. Weisel).

In the preceding papers we have given the results
of experiments made on white mice with four anti-phoid
vaccines prepared in domestic factories, namely: from
acetone vaccine, formol-phenol, Grasset-Slopek and endo-
toxin 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
antigenic antigens H, 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.

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 serums 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 titer of anti O 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 O 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 serums 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 1:2500; 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 with known negative serum, as well as in a physiological sodium chloride solution, in 5% sodium chloride solution, in 2.5% sodium chloride solution and in distilled water.

Results of control reactions in hemagglutination: the sera tested showed no hemagglutination with an emulsion of non-sensitized blood corpuscles. An emulsion of blood corpuscles sensitized with antigen VI showed no agglutination in a physiological NaCl solution and with a known negative serum. With standard serum hemagglutination was obtained to + + + in dilutions of 1:9500 or 1:10,000.

Formol-Passol Vaccine. Table II gives the results of tests of the serums of ten rabbits immunized with formol-passol vaccine. In the first group of rabbits the level of antibodies before the second dose varied between 1:320 and 1:10; seven days after the fourth dose, it grew from 1:10 to 1:5120. The level of O antibodies was between 1:10 and 1:1280 both before the second dose and seven days after the fourth immunizing dose. Vi antibodies, found only in one rabbit by agglutination, amounted before the second dose to 1:5 and, seven days after the fourth dose, to 1:40, revealed by hemagglutination: 1:15 and, seven days after the fourth dose, to 1:40, revealed by hemagglutination: 1:15 and 1:24. With the serums of the remaining rabbits, the reactions proved negative.

In the second group of rabbits, anti H agglutination was 1:10 to 1:50 was found before the second dose; from 1:10 to 1:1280 seven days after the fourth immunizing dose.

Antibodies before the second dose were found to range from 1:5 to 1:150; seven days after the fourth dose an increase in growth from 1:80 to 1:320 was noted, and in one rabbit even a drop from 1:50 to 1:10. The Vi antibodies tested by agglutination showed in two rabbits a rise of level from 1:10 to 1:40 and from 1:5 to 1:10, respectively, before the second and after the fourth dose. In the third rabbit the antibody level in both tests was 1:10; in the remaining two rabbits no anti Vi agglutination was discovered. It was not possible to detect any Vi antibodies by hemagglutination. The controls proceeded as in the case of the acetone vaccine.

Results according to Grasset-Slopek. Table III gives the results of agglutination and hemagglutination with the sera of ten rabbits immunized with Grasset-Slopek vaccine. Throughout the whole cycle of immunization, the rabbits of the first group produced no H antibodies, with the exception of one rabbit, in which anti H agglutinins were found in a 1:10 dilution of the serum seven days after the fourth

dose. Before the second immunizing dose, the O anti-bodies 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 1:40 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 anticellular 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 serum 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 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 $50 \cdot 10^6$ bacterial cells in 0.5 ml) reacted considerably by stronger production of anti Vi than the animals immunized with vaccine diluted in the proportion 1:100 ($5 \cdot 10^6$ 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 marked growth in activity. The O antibody level obtained after the first appropriately large dose was not subject to large fluctuations as the immunizing doses were repeated. The Vi antibodies behaved in this respect like the H antibodies, i.e., the serums were constantly active in the higher dilutions as the immunizing doses were administered. Another striking fact was the lack of influence by the natural antibodies found in the serums upon the intensity of formation of antibodies under the influence of immunization. The results of the tests of the serums 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 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 serum. 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

Tabela I
Szczepionka acetonowa

Grupa	Czas po- brania próbki 2) kwil 3)	4) Miara aglutynacyjna			5) 4) Miara aglutynacyjna			6) 5) 4) Miara aglutynacyjna			7) 6) 5) Miara aglutynacyjna			8) 7) 6) Miara aglutynacyjna			Hemag- liza
		H	O	Vi	H	O	Vi	H	O	Vi	H	O	Vi	H	O	Vi	
I) rozcienczone nie 1:10	Przed I dawką	—	10	—	—	—	10	5	—	—	—	—	—	—	—	—	—
	Przed II dawką	21	40	320	20	2560	320	20	23	1280	320	10	24	25	1280	320	5
	7 dni po dawką	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	IV dawce	2560	320	320	1920	5120	640	320	3800	5120	640	320	3800	480	2560	640	40
II) rozcienczone nie 1:1000	Przed I dawką	—	10	5	—	—	20	5	—	—	—	—	—	—	—	—	—
	Przed II dawką	26	20	40	5	27	20	80	5	28	80	80	—	29	10	80	10
	7 dni po dawką	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	IV dawce	320	80	—	—	320	160	10	—	—	—	—	—	—	5120	320	10

Miara przedstawiają odwrotność rozcieńczeń.

Nie badane oznaczono kropką.

Legend (pretaining to all tables) appears on last page.

Tabela II

Formol-Dzokol Vaccine

		Tabela II Szczepionka formolowo-fenolowa											
Grupa	Czas po braniu próbki dni	Miana aglutynacyjna			Miana aglutynacyjna			Miana aglutynacyjna			Miana aglutynacyjna		
		H	O	Vi	H	O	Vi	H	O	Vi	H	O	Vi
I) I rozcieńczenie 1:10	Przed I dawką	—	10	—	—	10	—	—	10	—	—	10	—
	Przed II dawką	11	—	—	13	1280	640	—	—	—	15	640	—
	7 dni po dawką	—	—	—	—	—	—	—	—	—	—	—	—
	IV dawce	—	—	—	—	1280	1280	—	—	—	24	2560	640
II) II rozcieńczenie 1:1000	Przed I dawką	—	10	—	—	10	—	—	10	—	—	10	—
	Przed II dawką	16	—	—	18	80	80	—	—	—	20	40	80
	7 dni po dawką	—	—	—	—	—	—	—	—	—	—	—	—
	IV dawce	—	—	—	—	640	820	40	—	—	—	640	10

Waccine according to 'rasset-Slorek

[illegible]

Table IV
Endotoxin according to Westphal

Tabela IV
Endotoksyna według Westphala

Endotoksyna Wieding Westphalia																									
Grupa	Czas pobrania próbki krwi	Miana aglutynacyjna			Nr kropli	Miana aglutynacyjna			Nr kropli	Miana aglutynacyjna			Nr kropli	Miana aglutynacyjna			Nr kropli	Miana aglutynacyjna			Nr kropli	Miana aglutynacyjna			Nr kropli
		H	O	Vi		H	O	Vi		H	O	Vi		H	O	Vi		H	O	Vi		H	O	Vi	
I rozlewność	Przed I dawką	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Przed II dawką	—	20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Przed III dawką	—	20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Przed IV dawką	—	20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
II rozlewność	Przed I dawką	—	10	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Przed II dawką	—	40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Przed III dawką	—	40	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
	Przed IV dawką	—	20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	

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 -