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AN EVALUATION OF VACCINES AND THE EFFECTIVENESS OF VACCINATIONS AGAINST TYPHOID FEVER

[The following is a translation of an article by Paula Meislowa, Felicja Rabczynska and Zygmunt Kudelski in Przeglad 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. M. Meisel).

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 bacterial 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 1:480 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 agglutinability with known negative serum, as well as in a [illegible] sodium chloride solution, in 5% sodium [illegible] solution, in 2.5% sodium chloride solution and in [illegible] water.

[illegible] of control reactions in hemagglutination: [illegible] tested showed no hemagglutination with an [illegible] of non-sensitized blood corpuscles. An emulsion of [illegible] bacilli sensitized with antigen Vi showed no [illegible] in a physiological NaCl solution and with a [illegible] negative serum. With standard serum hemagglutination [illegible] obtained to + + + in dilutions of 1:9600 or 1:10,000.

Formol-Phenol Vaccine. Table II gives the results of [illegible] of the serums of ten rabbits immunized with formol-phenol vaccine. In the first group of rabbits the level of [illegible] antibodies before the second dose varied between 1:320 and 1:[illegible]0; seven days after the fourth dose, it grew from [illegible] to 1:5120. The level of O antibodies was between 1:[illegible]0 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: [illegible] 1:4. With the serums of the remaining rabbits, [illegible] reactions proved negative.

In the second group of rabbits, anti H agglutination [illegible] 1:10 to 1:80 was found before the second dose; from [illegible] to 1:1280 seven days after the fourth immunizing dose. [illegible] bodies before the second dose were found to range [illegible] 0 to 1:160; seven days after the fourth dose an [illegible] growth from 1:80 to 1:320 was noted, and in [illegible] even a drop from 1:80 to 1:10. The Vi antibodies tested by agglutination showed in two rabbits a [illegible] rise of level from 1:10 to 1:40 and from 1:5 to 1:10, [illegible] respectively, before the second and after the fourth dose. [illegible] third rabbit the antibody level in both tests was [illegible] the remaining two rabbits no anti Vi agglutinations [illegible] discovered. It was not possible to detect any Vi [illegible] by hemagglutination. The controls proceeded as [illegible] case of the acetone vaccine.

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

dose. 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:[illegible]. 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:[illegible]0 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.

As 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 50.10^6 bacterial cells in 0.5 ml) produce considerably by stronger production of anti Vi than the animals immunized with vaccine diluted in the proportion 1:1,000 (5.10^6 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 gradual growth in activity. The O antibody level obtained after the first appropriately large dose was not subject to later 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 immunisation 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

1) Grupa	2) Czas pobrania próbek krwi	3) Nr króla	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja	(1) Nr króla	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja	6) Nr króla	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja	6) Nr króla	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja	(1) Nr króla	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja
7) I rozcieńczenie 1:10	Przed I dawką	21	—	10	—	·	22	—	10		·	23	—	10	5	·	24	—	20	—	·	25	—	10	5	·
	Przed II dawką		40	320	20	·		2560	320	20	72		1280	320	10	24		320	320	10	48		1280	320	5	24
	7 dni po IV dawce		2560	320	320	1920		5120	640	640	3800		5120	640	320	3800		640	320	40	480		2560	640	40	480
8) II rozcieńczenie 1:1000	Przed I dawką	26	—	10	5	·	27	—	20	5	·	28	—	10	5	·	29	—	20	5	·	30	—	10	5	·
	Przed II dawką		20	40	5	—		20	80	5	—		80	80	—	—		10	80	10	—		20	40	5	—
	7 dni po IV dawce		320	80	—	—		320	160	10	—		padł					5120	320	10	—		2560	320	10	—

Miana przedstawiają odwrotność rozcieńczeń. Nie badane oznaczono kropką.

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

Table II

Formol-Phenol Vaccine

Tabela II

Szczepionka formolowo-fenolowa

1) Grupa	Czas pobrania próbek krwi 2)	Nr króla 3)	4) Miana aglutynacyjne H	O	Vi	Hemaglutynacja 5)	Nr króla 6)	4) Miana aglutynacyjne H	O	Vi	Hemaglutynacja 5)	Nr króla 6)	4) Miana aglutynacyjne H	O	Vi	Hemaglutynacja 5)	Nr króla 6)	4) Miana aglutynacyjne H	O	Vi	Hemaglutynacja 5)	Nr króla 6)	4) Miana aglutynacyjne H	O	Vi	Hemaglutynacja 5)
7) I roczlenienie 1:10	Przed I dawką	11	—	10	—	·	12	—	10	—	·	13	—	10	—	·	14	—	10	—	·	15	—	10	—	·
	Przed II dawką		640	320	—	—		320	320	—	—		1280	640	—	—		1280	640	5	15		640	640	—	—
	7 dni po IV dawce		2560	640	—	—		640	320	—	—		1280	1280	—	—		5120	640	40	24		2560	640	5	7,5
8) II roczlenienie 1:1000	Przed I dawką	16	—	10	—	·	17	—	10	5	·	18	—	10	—	·	19	—	10	—	·	20	—	10	—	·
	Przed II dawką		40	80	—	—		80	160	5	—		80	80	—	·		10	160	10	—		40	80	5	—
	7 dni po IV dawce		320	80	—	—		640	320	40	—		640	160	—			1280	320	10	—		640	10	10	—

Table III

Vaccine according to Grasset-Slopek

Tabela III

Szczepionka według *Grasseta-Słopka*

1) Grupa	2) Czas pobrania próbek krwi	3) Nr królika	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja	3) Nr królika	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja	3) Nr królika	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja	3) Nr królika	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja	3) Nr królika	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja
7) I rozcieńczenie 1:10	Przed I dawką	1	—	10	—	·	2	—	10	—	·	3	—	10	—	·	4	—	10	5	·	5	—	10	—	·
	Przed II dawką		—	40	—	—		—	320	5	—		—	80	5	—		—	320	—	—		—	320	—	—
	7 dni po IV dawce		80	320	—	—		—	320	—	—		—	160	—	—		—	320	40	—		—	320	10	—
8) II rozcieńczenie 1:1000	Przed I dawką	6	—	10	—	·	7	—	10	—	·	8	—	10	—	·	9	—	10	—	·	10	—	10	—	·
	Przed II dawką		—	640	—	—		40	80	—	—		—	40	—	—		—	80	—	—		—	80	—	—
	7 dni po IV dawce		—	640	—	—		—	320	—	—		—	80	—	—		—	80	—	—		—	80	—	—

Table IV

Endotoxin according to Westphal

Tabela IV

Endotoksyna według *Westphala*

1) Grupa	2) Czas pobrania próbek krwi	3) Nr królika	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja	3) Nr królika	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja	3) Nr królika	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja	3) Nr królika	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja	3) Nr królika	4) Miana aglutynacyjne H	O	Vi	5) Hemaglutynacja
7) I rozcieńczenie 1:10	9) Przed I dawką	31	—	10	—	·	32	—	20	—	·	33	—	20	—	·	34	—	10	—	·	35	—	10	—	·
	10) Przed II dawką		—	20	—	—		—	40	—	—		—	40	—	—		—	40	—	—		—	40	—	—
	11) 7 dni po IV dawce		—	20	—	—		20	20	—	—		—	20	—	—		—	40	—	—		—	20	—	—
8) II rozcieńczenie 1:1000	9) Przed I dawką	36	—	10	—	·	37	—	20	—	·	38	—	10	—	·	39	—	40	—	·	40	—	20	—	·
	10) Przed II dawką		—	40	—	—		—	40	—	—		padł					—	20	—	—		—	20	—	—
	11) 7 dni po IV dawce		—	20	—	—		—	—	10	—							—	20	—	—		—	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 raboit
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 -