Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1966, **34**, 211-222

Controlled Field Trials and Laboratory Studies on the Effectiveness of Typhoid Vaccines in Poland, 1961-64*

Final Report

POLISH TYPHOID COMMITTEE [1]

In recent years studies of the effectiveness of different typhoid vaccines have been sponsored by the World Health Organization in British Guiana, the USSR, and Yugoslavia. A similarly sponsored study has been made in Poland under the auspices of the Polish Typhoid Committee. In the controlled field trial four types of vaccine were used: (1) bacterial acetone-killed and -dried (vaccines K and P), (2) bacterial formol-killed phenol-preserved (vaccine N), (3) Westphal's endotoxin adsorbed on aluminium hydroxide (vaccine S), and (4) Grasset's vaccine (autolysate of typhoid bacilli adsorbed on aluminium hydroxide; vaccine T). The control vaccine was tetanus toxoid (vaccine O). Laboratory tests were also carried out.

In children aged 5-14 years who received two inoculations, vaccine N was the most effective, followed by K; vaccine T was distinctly less effective. In people aged 15-60 years the small number of typhoid cases made evaluation difficult; however, vaccines N and P inoculated once afforded protection, whereas vaccine S imparted none.

Further studies are desirable on the laboratory testing of typhoid vaccines: at present the H and O agglutination tests with sera of immunized rabbits, combined with an active mouse-protection test, can be recommended, provided that a standard typhoid vaccine is used for comparison.

In January 1959 the Polish Typhoid Committee was appointed to develop a programme of epidemiological and laboratory studies on the effectiveness of vaccination against typhoid fever (Kostrzewski, 1963), the aim being to provide a rational basis for

(1) selection of the most effective typhoid vaccine;

(2) determination of the duration of immunity conferred by typhoid vaccination and of optimum intervals of revaccination; and

(3) selection of a laboratory test for the routine control of typhoid vaccines that would give results conforming with the epidemiological observations.

VACCINES

Four different typhoid vaccines were used. Two were bacterial cell suspensions, namely, formol-killed phenol-preserved vaccine (code letter N) and acetone-killed and -dried vaccine prepared according to Landy (P). The other two were bacterial extracts, namely, heat-killed at 100°C, extracted with phenol according to Westphal, and adsorbed on aluminium hydroxide (S) and heat-killed at 100°C, extracted according to Grasset, and adsorbed on aluminium hydroxide (T). The vaccines were prepared in Poland (Polish Typhoid Committee, 1965) starting from the same suspension of *Salmonella typhi* Ty 2 received from the Statens Seruminstitut, Copenhagen (Kruczałowa & Schillerowa, 1963).

* The study reported here was financed by the Polish health authorities and was supported by the Polish Academy of Sciences and the World Health Organization.

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In order to ensure that the results of this study would be comparable with those of studies sponsored by the World Health Organization in Yugoslavia (Yugoslav Typhoid Commission, 1964) and British Guiana (Typhoid Panel, UK Department of Technical Co-operation, 1964), an acetone-killed and -dried vaccine (K) produced at the Walter Reed Army Institute of Research in Washington (Division of Immunology, Walter Reed Army Institute of Research, 1964) was also used.

For the control group, tetanus toxoid vaccine absorbed on aluminium hydroxide (O) was prepared.

In November 1960 the vaccines prepared in Poland were submitted to routine State control. In January 1961, 415 persons aged 5-31 years were inoculated with the vaccines to check their reactions to vaccination and to determine the appropriate dosage (Brzezinski & Gancarz, 1963; Magdzik & Milewska, 1963; Zołnierkowa & Przestalska, 1963). The doses determined upon (for children as well as adults) were 5×10^8 bacteria of the formol-phenolized vaccine or volumes of the extract in the Grasset and endotoxic Westphal vaccines corresponding to 5×10^8 bacteria. Since the acetone-killed vaccine (P) produced strong reactions, its dosage was reduced to 2.5×10^8 bacteria. All the vaccines were injected subcutaneously in the region of the lower insertion of the deltoid muscle in doses of 0.5 ml.

The epidemiological studies of the effectiveness of the vaccines were carried out in a controlled field trial in five provinces—Kielce, Lodz, Poznan, Warsaw and Wroclaw—in 25 counties or towns that had the highest typhoid incidence in Poland in 1953-57. In these areas, mass vaccination, which had been performed annually since 1947 on those aged 5-60 years with the Grasset-Slopek typhoid-paratyphoid vaccine, was discontinued in 1958.

From February through June 1961, 331 617 children aged 5-14 years were vaccinated twice at an interval of four weeks and 39 303 others were vaccinated once with vaccines K, N, T or O. In the same period, 359 038 people aged 15-60 years were vaccinated twice and 113 680 once with vaccines N, P, S or O (Table 1).

Each group was vaccinated separately, following a scheme designed to ensure comparability of the groups of persons vaccinated with different vaccines (*Epidem. Rev.*, 1963).

TABLE 1

NUMBER OF PERSONS VACCINATED IN THE FIELD TRIAL [a]

Vaccine	Number in age-group 5-14 years		Number in age-group 15-60 years	
	1 dose	2 doses	1 dose	2 doses
K	9 136	81 534		
N	10 373	83 917	29 762	88 721
P			29 337	87 521
S			27 470	90 934
T	9 727	82 432		
O	10 067	83 734	27 111	91 862
Total vaccinated [b]	39 303	331 617	113 680	359 038

[a] According to the Census of December 1960, the population in the area of the field trial numbered 438 151 in the age-group 5-14 years and 1 089 683 in the age-group 15-60 years.

[b] The number of persons vaccinated with different vaccines includes 2925 persons under the age of 5 years and 513 over 60 years who were inoculated with vaccines K, N, P, S, T or O. The number inoculated with vaccines K, N, O and T includes 21 459 over the age of 15 years, who were vaccinated in schools together with younger pupils.

COMPARABILITY OF GROUPS INOCULATED WITH DIFFERENT VACCINES

When the vaccinations were complete, a statistical analysis was made of the comparability of the groups inoculated with the different vaccines on the basis of a 20% sample, choosing every fifth person in submitted lists. The analysis showed that the groups of persons inoculated with vaccines K, N, T or O, as well as of those inoculated with vaccines N, P, S or O, were comparable with respect to age and sex in the different counties or provinces, as well as in the entire territory of the controlled vaccinations. The best results, with regard to both the numbers inoculated in relation to the population in the age-group and the comparability of groups inoculated with different vaccines, were obtained in the group of vaccinated children aged 5-14 years (Magdzik, 1963; [1] *Epidem. Rev.*, 1963).

Further evaluation of the comparability of the groups was based on data from 11 counties pertaining to persons inoculated with the different vaccines

[1] See also Magdzik, W. [*Controlled trial vaccination against typhoid fever: epidemiological evaluation of comparability of groups of persons inoculated with different vaccines*]. (Unpublished paper, in Polish, available from the Library of the State Institute of Hygiene, Warsaw.)

TABLE 2

INCIDENCE OF TYPHOID FEVER AMONG VACCINATED PERSONS DURING THE PERIOD 1 JULY 1961–30 JUNE 1964

Vaccine	No. of doses	Incidence in children 5-14 years old in 1961			Incidence in adults 15-60 years old in 1961		
		Clinical manifestation and serological examination	Confirmed by culture of *S. typhi*	Total	Clinical manifestation and serological examination	Confirmed by culture of *S. typhi*	Total
K	1	1	1	2			
	2	3	6	9			
N	1	0	0	0	0	2	2
	2	5	4	9	5	9	14
P	1				3	0	3
	2				2	6	8
S	1				1	7	8
	2				5	17	22
T	1	1	3	4			
	2	17	21	38			
O	1	0	6	6	1	8	9
	2	9	39	48	6	13	19

among patients who had diphtheria, scarlet fever, dysentery or epidemic hepatitis during the period of observation, from July 1961 to June 1964. Statistical analysis demonstrated that the groups of children inoculated with vaccines K, N, T or O, as well as the groups of persons aged 15-60 years inoculated with vaccines N, P, S or O, were comparable (Magdzik, 1963).[1]

No statistically significant differences between the numbers of persons inoculated with the different vaccines were found among healthy persons who had contact with typhoid patients or who resided in epidemic environments during the period of observation (Magdzik, 1963).[1]

On the basis of these findings, it was concluded that, during the three years since the termination of trial vaccinations, no significant differences had arisen between the groups inoculated once or twice with different vaccines, and that the groups were comparable throughout the three-year period of the survey.

[1] See also Magdzik, W. [*Controlled trial vaccination against typhoid fever: epidemiological evaluation of comparability of groups of persons inoculated with different vaccines*]. (Unpublished paper, in Polish, available from the Library of the State Institute of Hygiene, Warsaw.)

TYPHOID MORBIDITY IN THE AREA OF THE FIELD TRIAL: JULY 1961 TO JUNE 1964

During the three years of observation after the termination of the controlled vaccinations, 605 cases of typhoid fever were notified in the territory studied, 510 occurring in persons within the age-groups covered in the field trial.

Among children who had been inoculated once or twice and whose age in 1961 was 5-14 years, 116 cases of typhoid fever were notified, in 80 of which *S. typhi* was isolated from the blood, stools or urine. Among persons inoculated once or twice, whose age in 1961 was 15-60 years, 85 cases of typhoid fever were notified, in 62 of which *S. typhi* was isolated (Table 2).

In persons not inoculated during the trial, 393 cases of typhoid fever were notified, and there were

TABLE 3

INCIDENCE OF TYPHOID FEVER AMONG VACCINATED PERSONS DURING THE PERIOD 1 JULY 1961 - 30 JUNE 1964 [a]

Vaccine	No. of doses	Incidence in children 5-14 years old in 1961						Incidence in adults 15-60 years old in 1961					
		No. of persons vaccinated	Incidence [b]	July 1961 to June 1962	July 1962 to June 1963	July 1963 to June 1964	Total July 1961 to June 1964	No. of persons vaccinated	Incidence	July 1961 to June 1962	July 1962 to June 1963	July 1963 to June 1964	Total July 1961 to June 1964
K	1	9 136	cases			1	1						
			rate			1.1	0.37						
	2	81 534	cases	2	2	2	6						
			rate	0.25	0.25	0.25	0.25						
N	1	10 373	cases					29 762	cases	1	1		2
			rate						rate	0.34	0.34		0.22
	2	83 917	cases	1	2	1	4	88 721	cases	2	4	3	9
			rate	0.12	0.24	0.12	0.16		rate	0.23	0.45	0.34	0.34
P	1							29 337	cases				
									rate				
	2							87 521	cases		4	2	6
									rate		0.46	0.23	0.23
S	1							27 470	cases	5	2		7
									rate	1.82	0.73		0.85
	2							90 934	cases	3	6	8	17
									rate	0.33	0.66	0.88	0.62
T	1	9 727	cases	2	1		3						
			rate	2.06	1.03		1.03						
	2	82 432	cases	5	7	9	21						
			rate	0.61	0.85	1.09	0.85						
O	1	10 067	cases	2	1	3	6	27 111	cases	1	4	3	8
			rate	1.99	0.99	2.98	1.99		rate	0.37	1.48	1.11	0.98
	2	83 734	cases	20	11	8	39	91 862	cases	2	7	4	13
			rate	2.39	1.31	0.96	1.55		rate	0.22	0.76	0.44	0.47

[a] Only those cases confirmed by isolation of *S. typhi* are included.

[b] Rate is per 10 000 persons per year.

TABLE 4

SIGNIFICANCE OF DIFFERENCES IN INCIDENCE OF TYPHOID FEVER IN PERSONS INOCULATED WITH VACCINES K, N, P, S, T OR O [a]

Vaccines compared	No. of doses	Values of χ^2 for: Children 5-14 years old in 1961 — July 1961 to June 1962	July 1962 to June 1963	July 1963 to June 1964	July 1961 to June 1964	Adults 15-60 years old in 1961 — July 1961 to June 1962	July 1962 to June 1963	July 1963 to June 1964	July 1961 to June 1964
K : O	1	2.0	1.0	0.8	3.0				
	2	14.3 P<0.001	6.0 P<0.02	3.4	23.3 P<0.001				
N : O	1	2.0	1.0	3.0	6.2 P<0.02	0.0	2.0	3.0	4.2 P<0.05
	2	17.2 <0.001	6.2 P<0.02	5.4 P=0.02	28.5 P<0.001	0.0	0.7	0.1	0.6
P : O	1					1.0	4.3 P<0.05	3.0	8.7 P<0.01
	2					2.0	0.7	0.5	2.4
S : O	1					2.6	0.7	3.0	0.1
	2					0.2	0.1	1.3	0.5
T : O	1	0.0	0.0	3.0	0.9				
	2	8.8 P<0.01	0.8	0.1	5.1 P<0.05				
K : N	1			1.0	1.0				
	2	0.3	0.0	0.3	0.5				
K : T	1	2.0	1.0	1.0	0.8				
	2	1.3	2.8	4.4 P<0.05	8.2 P<0.01				
N : P	1					1.0	1.0		2.0
	2					2.0	0.0	0.2	0.5
N : S	1					2.9	0.5		3.0
	2					0.2	0.3	2.1	2.2
N : T	1	2.0	1.0		3.0				
	2	2.8	2.8	6.5 P<0.02	11.9 P<0.001				
P : S	1					5.3 P<0.05	2.0		7.4 P<0.01
	2					3.0	0.3	3.3	4.9 P<0.05

[a] Calculated from the data in Table 3. At the 95 % probability level and one degree of freedom, the limiting value of $\chi^2 = 3.841$. Where the differences are significant the value of P is given.

16 cases in persons who had been vaccinated in other territories with other vaccines, but who contracted typhoid fever in the region under study.

The epidemiological evaluation of the effectiveness of vaccination was based only on clinically diagnosed cases proved by isolation of *S. typhi* from the blood, stools or urine. The incidence of typhoid fever among persons immunized with different vaccines is indicated in Table 3.

In children who had been inoculated twice, 70 bacteriologically confirmed cases of typhoid fever were recorded, but there were distinct differences between groups immunized with different vaccines. The lowest incidence was observed in persons immunized with the formol-phenolized vaccine (N), and the highest in vaccinees who had received the Grasset endotoxic vaccine (T).

The small number of cases of typhoid fever occurring over a period of three years in persons inoculated once does not allow any firm conclusions to be drawn. Nevertheless, the incidence of typhoid fever in persons inoculated once with vaccines K, N or P was significantly lower than that in those inoculated once with vaccines S, T or O.

EVALUATION OF EFFECTIVENESS OF THE VACCINES IN HUMANS

The differences in typhoid incidence between the various groups inoculated with the different typhoid vaccines or with the control vaccine (O) are statistically analysed in Table 4. Table 5 shows the effectiveness of different vaccines in children whose age in 1961 was 5-14 years and who were inoculated with two doses of the vaccines.

The formol-killed phenol-preserved vaccine (N) proved to be the most effective. Its effectiveness (K), i.e., the ratio of the number of cases of the disease in persons in the control group to the number in persons immunized with a given vaccine, in the three years following termination of the vaccinations was 20, 5 and 8, and the mean index for the three-year period was 10. The American acetone-killed vaccine (K) was somewhat less effective; its mean effectiveness for the three-year period was 6. However, the difference in effectiveness of vaccines N and K is not statistically significant.

The small number of cases of typhoid fever in the group of people aged 15-60 years does not allow firm conclusions to be drawn about the effectiveness of the other acetone-killed vaccine (P). In people who received one dose (in comparison with the control group and those inoculated with vaccine S) and in those who received two (compared with those inoculated with vaccine S), vaccine P proved effective, the differences in the incidence of typhoid fever being statistically significant (Tables 3 and 4).

The protection afforded by the Grasset vaccine (T) was slight and of short duration. Protective action and an effectiveness of 4 were observed only during the first year after the vaccinations. In the second year only 35% of those vaccinated were protected and in the third year protection was no longer evident.

The Westphal endotoxin adsorbed on aluminium hydroxide (S) gave no protection in these trials.

TABLE 5

EFFECTIVENESS OF K, N AND T VACCINES IN CHILDREN 5-14 YEARS OLD [a]

Vaccine	July 1961–June 1962		July 1962–June 1963		July 1963–June 1964		Average July 1961–June 1964	
	Effectiveness [b]		Effectiveness		Effectiveness		Effectiveness	
	K	*E*(%)	*K*	*E*(%)	*K*	*E*(%)	*K*	*E*(%)
K	9.6	90	5.2	81	3.8	74	6.2	84
N	19.9	95	5.5	82	8.0	88	9.7	90
T	3.9	74	1.5	35	0.9	0	1.8	45

[a] Only those cases confirmed by isolation of *S. typhi* are included.

[b] Effectiveness is defined by:

$$K = \frac{\text{incidence per 10 000 in the control group } (b)}{\text{incidence per 10 000 in the vaccinated group } (a)}$$

$$E = 100(b-a)/b \ (\%).$$

DURATION OF IMMUNITY AFTER VACCINATION

In order to assess the duration of immunity after vaccination, the incidence of typhoid fever in persons inoculated once or twice with the formol-phenolized vaccine N, which was the most effective of those studied, was compared with the incidence in the control group. For this analysis, the group of children was combined with the group of adults (Table 6).

The incidence of typhoid fever in persons inoculated twice with the formol-phenolized vaccine (N) in the three successive years of observation was lower than that in the corresponding control group. The percentage effectiveness (E) was 87% in the first year, 65% in the second, and 66% in the third.

In persons inoculated once with the formol-phenolized vaccine (N), the incidence of typhoid fever (compared with that in the control group) was significantly different only in the third year after termination of the vaccinations. However, the difference in the number of typhoid cases for the three-year period was statistically significant ($\chi^2=9.6$; $P < 0.01$).

On the basis of the data in Tables 4, 5 and 6, it can be concluded that vaccination against typhoid fever with a sufficiently immunogenic vaccine, such as the formol-phenolized (N) or the acetone-killed (K) vaccine, administered twice at an interval of four weeks, affords protection for at least three years. In the third year after termination of the vaccinations, from 65% to 90% of persons exposed to infection, depending on the age-group, were protected.

LABORATORY EVALUATION OF IMMUNOGENICITY OF TYPHOID VACCINES

Vaccines N, P, S and T were also submitted to laboratory tests in several research centres in Poland

TABLE 6

SIGNIFICANCE OF DIFFERENCES IN INCIDENCE OF TYPHOID FEVER AMONG PERSONS 5-60 YEARS OF AGE VACCINATED WITH VACCINE N OR VACCINE O (CONTROL)

Vaccine	No. of doses	No. of persons vaccinated	Incidence	July 1961 to June 1962	July 1962 to June 1963	July 1963 to June 1964	Total July 1961 to June 1964
N	1	40 135	Cases	1	1	0	2
			Rate	0.25	0.25	0	0.17
	2	172 638	Cases	3	6	4	13
			Rate	0.17	0.35	0.23	0.25
O	1	37 178	Cases	3	5	6	14
			Rate	0.81	1.34	1.61	1.26
	2	175 596	Cases	22	18	12	52
			Rate	1.25	1.03	0.68	0.99
Significance of differences	1		χ^2	1.0	2.9	6.4	9.6
			P	NS [a]	NS [a]	<0.02	<0.01
	2		χ^2	14.1	5.8	3.8	22.7
			P	<0.001	<0.02	0.05	<0.001

[a] NS = not significant.

and elsewhere. When the studies were completed, the results were submitted to the Polish Typhoid Committee before the results of the controlled field trial were known.

Laboratory studies on the immunogenic potency of the vaccines were carried out in Poland at:

(*a*) the Department of Epidemiology of the State Institute of Hygiene in Warsaw, using the active mouse-protection test with zymosan (Kostrzewski et al., 1963);

(*b*) the Department of Serum and Vaccine Studies of the State Institute of Hygiene, by the modification of the active mouse-protection test used in international co-operative studies undertaken by WHO (Kudelski et al., 1963);

(*c*) the Department of Epidemiology of the Medical Academy in Warsaw [1] and the Department of Serum and Vaccine Studies of the State Institute of Hygiene, by the agglutination test with sera of rabbits immunized with the vaccines under study (Meisel et al., 1963; Naruszewicz-Lesiuk, 1964).

In addition, various serological tests were performed with the sera of humans immunized with different vaccines—namely:

(*a*) agglutination and haemagglutination tests at the Department of Bacteriology of the State Institute of Hygiene (Kopacka & Słubicka, 1963b);

(*b*) determination of the opsonizing power of the serum by the phagocytic test in humans and rabbits immunized with the vaccines, at the Department of Medical Microbiology of the Medical Academy in Wroclaw (Ładosz, 1963);

(*c*) the Coombs antiglobulin test with O, H and Vi antigens, at the Provincial Sanitary-Epidemiologic Station in Cracow [1] (Lutynski, 1964);

(*d*) passive protection test with human sera inoculated in chick embryos, at the Department of Epidemiology of the Medical Academy in Warsaw (Naruszewicz-Lesiuk; [2] Płachcinska, 1963).

In centres outside Poland, the vaccines were studied by active mouse-protection tests at the Lister Institute, Elstree, England; the Walter Reed Army Institute of Research, Washington, D.C., USA; the Division of Biologics Standards of the National Institutes of Health, Bethesda, Md., USA; and the Institute of Immunology, Zagreb, Yugoslavia. The results obtained (unpublished) indicated that the immunogenic potency of the P vaccine is higher than that of the K vaccine, which was used as a standard. The N and T vaccines were less immunogenic than the K vaccine, and the S vaccine did not exhibit any appreciable protective action.

The active mouse-protection test

In two Polish research centres, the active mouse-protection test was performed in two modifications (Kostrzewski et al., 1963; Kudelski et al., 1963).

The first method consisted in a single subcutaneous inoculation, with one of the vaccines, of three groups of 32 mice, each with different doses (4×10^5, 4×10^6 and 4×10^7) of bacteria or with corresponding amounts of bacterial extract. After seven days the mice were challenged by intraperitoneal injections of different doses of living *S. typhi* suspended in zymosan solution (5×10^3, 2.5×10^4 and 1.25×10^5 bacteria). Statistical analysis, taking into account both variables (i.e., immunizing dose and challenging dose), showed that, in five out of six experiments, the N vaccine was the most effective, and in the remaining experiment its effectiveness was equalled by that of the P vaccine. In all six experiments the S vaccine was the least effective. The effectiveness of the N and S vaccines was reproducible in all six experiments. Statistical analysis of the results of four experiments in which the K, N, P, S and T vaccines were tested revealed significant differences between the weakest vaccine (S) and the remaining vaccines, and between the most effective vaccine N and vaccines K and T. The differences between P and the other vaccines were not significant (Kostrzewski et al., 1963).

The second method, which has been used in the WHO-sponsored international studies, consisted in inoculation of four groups of mice twice, first with a dose of 2×10^6 to 250×10^6 bacteria (or a corresponding amount of bacterial extract) and then with 4×10^6 to 500×10^6 bacteria. In five successive experiments the mice were challenged on the fourteenth day after the second inoculation with living typhoid bacilli suspended in sodium chloride solution in doses ranging from 5 LD_{50} to 25 LD_{50}. Vaccine P exhibited the highest immunogenic potency, vaccines N and T a weaker and equal potency, and vaccine S gave only very weak protection (Kudelski et al., 1963).

[1] Performed after publication of the preliminary results of the epidemiological survey.

[2] Naruszewicz-Lesiuk, D. [*Laboratory evaluation of the immunogenic potency of typhoid vaccines used in controlled trial vaccinations*]. (Unpublished paper, in Polish, available from the Library of the Medical Academy, Warsaw.)

AGGLUTINATION AND HAEMAGGLUTINATION TESTS

Agglutination tests with the sera of immunized rabbits demonstrated that vaccine N best stimulated the production of H and O agglutinins; the P and K vaccines were the next most effective. Vaccine T produced a smaller rise in the H-agglutinin titres than vaccines N, P and K, but a similar rise in the O agglutinins. Vaccines P and K gave the most distinct, although moderate, rise in the titres of Vi agglutinins and haemagglutinins. In all the tests the S vaccine was the weakest (Naruszewicz-Lesiuk, 1964).[1]

Serological reactions in human subjects

Blood samples of human subjects obtained 14 days after the second of two inoculations separated by an interval of four weeks showed a greater rise in the H-agglutinin titres when the formol-phenolized (N) or acetone (P) vaccine was used than when the Grasset (T) vaccine was inoculated. However, vaccines N and P were only slightly more effective than vaccine T in stimulating the production of O agglutinin and haemagglutinin, and the three vaccines did not differ in their ability to stimulate the production of Vi agglutinins and haemagglutinins. The titres of O, H and Vi agglutinins were not raised by the Westphal (S) vaccine (Kopacka & Słubicka, 1963b).

In the opsono-phagocytic test employing *S. typhi* Ty 2 or O-901, no significant differences between the N, P and T vaccines were found. Exceptionally, the P vaccine gave higher values of the phagocytic index with the sera of humans immunized with the P and T vaccines and the *S. typhi* Ty 2 strain. The highest phagocytic indices were observed after immunization with the P vaccine and the lowest after immunization with the S vaccine (Ładosz, 1963).

When the sera of human subjects immunized with N, P, S or T vaccines were studied by the Coombs antiglobulin test, the greatest differences between the vaccines related to the rise in H-antibody titres. The P vaccine was the most effective in this respect and the difference between the P and N vaccines was not significant. Both were distinctly more potent than the T and S vaccines, the effects of which were also not significantly different. With regard to the increase in O and Vi antibodies in the Coombs test, no difference was observed between vaccines N, P and T, but vaccine S was weaker (Lutynski, 1964).

The passive protection test with chick embryos and the sera of immunized human subjects proved to be of little use in the laboratory evaluation of typhoid vaccines (Naruszewicz-Lesiuk, 1964).[1]

The results of the laboratory and epidemiological observations on the effectiveness of vaccines K, N, P, S and T are summarized in Table 7.

DISCUSSION AND CONCLUSIONS

The results of the laboratory and epidemiological studies on the effectiveness of typhoid vaccines afford a basis on which to answer the questions that were put to the Polish Typhoid Committee.

Distinct differences in the effectiveness of the vaccines studied were observed in children aged 5-14 years. The best results were obtained with the formol-phenolized (N) vaccine, followed by the Walter Reed acetone-killed (K) vaccine; the Grasset (T) vaccine proved to be the least effective. The small number of cases of typhoid fever that occurred in persons aged 15-60 years makes evaluation of the vaccines difficult. Nevertheless, the N and P vaccines injected once afforded protection. In people inoculated twice, the incidence of the disease in those immunized with the P and N vaccines was lower than that in the control group and in the group immunized with the Westphal endotoxin (S), but the differences were not statistically significant, with the exception of the difference between the groups inoculated with the P and S vaccines over the complete three-year period of observation.

The serological survey of the population carried out before the controlled vaccination trial indicated the presence of H agglutinins of titre 1/10 or higher in 55% of persons aged 20-60 years, O-agglutinin titres of 1/40 or higher in 9.1%, and Vi-agglutinin titres of 1/10 or higher in 21%. In those less than 20 years old, H agglutinins were found in 5%, O agglutinins in 75%, and Vi agglutinins in 3% of those examined (Kopacka & Słubicka, 1963a). It may be assumed that most of the adults had had contact with typhoid bacilli in the past, either through vaccination or through natural infection. Hence, inoculation with vaccine N, P or S acted only as a " booster " dose in persons previously immunized against typhoid fever. Even under these conditions, the Westphal endotoxin (S) failed to impart any protection.

Statistical analysis of the epidemiological observations indicated that it was not possible to determine

[1] See also Naruszewicz-Lesiuk, D., *op. cit.*

TABLE 7

RESULTS OF CONTROLLED FIELD TRIALS AND LABORATORY EVALUATION OF EFFECTIVENESS OF TYPHOID VACCINES

Test	Order of effectiveness of vaccines from 1 (most) to 5 (least effective)					Remarks
	1	2	3	4	5	
Controlled field trial	N	K	T	S		Vaccine P could not be evaluated
Agglutination tests with human sera [a]						
H agglutination	P	N	T	S		
O agglutination	N	P	T	S		
Vi agglutination	NTP	—	—	S		N, P, T vaccines could not be differentiated
O haemagglutination	N	P	T	S		
Vi haemagglutination	TPN	—	—	S		N, P, T vaccines could not be differentiated
Coombs test with human sera [b]						
and H antigen	P	N	T	S		
and O antigen	NPT	—	—	S		N, P, T vaccines could not be differentiated
and Vi antigen	NPT	—	—	S		
Phagocytic test with human sera [c]	PNT	—	—	S		N, P, T vaccines difficult to differentiate; highest index after P
Agglutination and haemagglutination tests with rabbit sera [d]						
H agglutination	N	P	K	T	S	
O agglutination	NPKT	—	—	—	S	K, N, P, T vaccines could not be differentiated
Vi agglutination	PKNT	—	—	—	S	
Vi haemagglutination	P	K	T	NS	—	Low titres
Active mouse-protection test WHO modification [e]	P	NT	—	S		N and T vaccines could not be differentiated
with zymosan [f]	NP	TPK	—	—	S	N and P, as well as K, P and T, vaccines could not be differentiated

[a] Kopacka & Slubicka, 1963b
[b] Lutynski, 1964
[c] Ładosz, 1963
[d] Meisel et al., 1963
[e] Kudelski et al., 1963
[f] Kostrzewski et al., 1963

whether the N or the P vaccine was the better, although six cases of typhoid fever occurred among those who received the P vaccine and 11 among those who received the N vaccine; the difference is not, however, statistically significant. Nevertheless, the epidemiological observations do suggest that the P vaccine is the most effective, followed by the N and K vaccines, although it is possible that the difference in effectiveness of the vaccines is due to chance. The Grasset (T) vaccine is distinctly less effective.

Immunity after two inoculations with the K or N vaccine lasts at least three years. The duration of immunity after one or two inoculations with the P vaccine is presumably similar. It follows that children and young persons in Poland should be

inoculated twice in order to give them immunity for three years. In adults, considering the vaccination as a " booster " dose, a single inoculation may be adequate.

The results of the serological tests with H antigen are those in closest agreement with the epidemiological observations. The results of the agglutination tests with human sera and with the sera of rabbits immunized with the vaccines, as well as the Coombs reaction, also agree well. The results of O agglutination and haemagglutination with human and rabbit sera show that this test is less reliable than the H agglutination test for differentiating between the vaccines. The Vi agglutination and haemagglutination tests are not suitable for determining the effectiveness of typhoid vaccines in humans.

The active mouse-protection test with zymosan allows differentiation to be made between effective and ineffective vaccines and between highly and slightly effective vaccines, but does not indicate slight differences in effectiveness. In the modification which utilizes only a single inoculation, this test gives reproducible results in the evaluation of moderately and slightly effective vaccines (Kostrzewski et al., 1963). Results obtained with the test in the modification used in the WHO-sponsored international collaborative trials were similar, but less reproducible.

The opsono-phagocytic test and the passive protection test with chick embryos were not found suitable for the laboratory evaluation of the effectiveness of vaccines.

As routine laboratory tests of the effectiveness of typhoid vaccines, the H and O agglutination tests with sera of immunized rabbits, combined with the active mouse-protection test with zymosan (or in other modifications), can be recommended. In the laboratory evaluation of vaccines, a standard vaccine with immunogenic potency determined on the basis of epidemiological observations should be used for comparison.

The results of this study indicate, contrary to the opinion held for many years, that the Vi antigen plays a secondary role in immunity to typhoid fever, the H and O antigens being the main factors.

RÉSUMÉ

Les résultats d'une vaccination de masse antityphoïdique ont été étudiés en Pologne, de 1961 à 1964, sous les auspices de l'OMS et du Comité polonais de la Typhoïde. Quatre types de vaccins ont été utilisés: 1) vaccin inactivé à l'acétone et desséché; 2) vaccin inactivé au formol et phénolé; 3) endotoxine de Westphal adsorbée sur hydroxyde d'aluminium; et 4) vaccin de Grasset: autolysat de bacilles typhiques adsorbé sur hydroxyde d'aluminium. Un groupe témoin a reçu de l'anatoxine antitétanique. Au total, 152 983 personnes ont reçu une inoculation et 690 655 personnes en ont reçu deux.

Plusieurs laboratoires polonais et étrangers avaient effectué des épreuves de laboratoire sur les vaccins avant l'expérimentation. Les réactions postvaccinales ont été étudiées et une étude sérologique a été faite sur des échantillons de sang choisis au hasard. La vaccination a été suivie d'une surveillance de trois ans des cas de typhoïde et d'autres maladies.

Cent seize cas de fièvre typhoïde ont été enregistrés chez les enfants qui avaient été inoculés une ou deux fois et qui, en 1961, appartenaient au groupe d'âge 5-14 ans; chez 80 d'entre eux, *Salmonella typhi* a été isolé du sang, des selles ou de l'urine. Parmi les personnes inoculées une ou deux fois et qui, en 1961, appartenaient au groupe d'âge 15-60 ans, on a enregistré 85 cas de fièvre typhoïde; dans 62 de ces cas, *S. typhi* a été isolé. Seuls les cas cliniquement diagnostiqués et prouvés par l'isolement de *S. typhi* ont été retenus pour l'évaluation épidémiologique de l'efficacité de la vaccination.

Chez les enfants du groupe d'âge 5-14 ans, le vaccin inactivé au formol et préservé par le phénol s'est montré le plus efficace puis, ensuite, le vaccin inactivé à l'acétone; l'efficacité du vaccin de Grasset a été nettement inférieure. Le petit nombre de cas de fièvre typhoïde apparus chez les adultes de 15-60 ans n'a pas permis de se rendre pleinement compte de l'efficacité des vaccins. Néanmoins, une inoculation de vaccin inactivé au formol ou à l'acétone a procuré une protection contre la fièvre typhoïde. Deux inoculations de l'un ou l'autre de ces vaccins ont réduit l'incidence de la maladie, dans les groupes intéressés, par rapport à celle du groupe témoin et du groupe vacciné par l'endotoxine de Westphal; la différence n'était cependant pas significative. Aucune action protectrice n'est apparue chez les individus inoculés avec l'endotoxine de Westphal.

L'immunité procurée par deux inoculations de vaccin au formol ou à l'acétone a persisté au moins trois ans. Après deux inoculations du vaccin de Grasset, la durée de la protection a été inférieure à un an.

Les épreuves de laboratoire destinées à évaluer l'efficacité des vaccins antityphoïdiques nécessitent de nouvelles études. Actuellement, on peut recommander les épreuves d'agglutination H et O au moyen de sérums de lapins immunisés, en même temps que l'épreuve de protection active de la souris. Un vaccin antityphoïdique standard dont l'efficacité a été déterminée par une expérimentation de masse contrôlée devrait être utilisé comme référence au cours des épreuves de laboratoire.

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