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Evaluation of Typhoid Vaccines in the Laboratory and in a Controlled Field Trial in Poland *

Preliminary Report

POLISH TYPHOID COMMITTEE [1]

In 1961 a controlled field trial of anti-typhoid vaccines was carried out in 25 regions in Poland. Four types of vaccine were studied: (1) bacterial acetone-killed and -dried vaccine (two kinds), (2) bacterial formol-killed phenol-preserved vaccine, (3) Westphal's endotoxin adsorbed on aluminium hydroxide, and (4) Grasset's vaccine (autolysate of typhoid bacilli adsorbed on aluminium hydroxide). The control vaccine was tetanus toxoid. A total of 690 655 persons received two inoculations. Prior to the field trial, laboratory tests were carried out on the vaccines, postvaccinal reactions were studied, and a serological examination was made of randomly selected blood samples. The vaccination was followed by a two-year survey of cases of typhoid and other diseases. Among children aged 5-14 years, the formol-killed phenol-preserved vaccine was found to be the most effective and Grasset's vaccine the least. Among adults aged 15-60 years, no conclusive evidence for the effectiveness of the vaccines could be obtained owing to the small number of cases. This may be due to the maintenance of immunity through repeated annual vaccination with bacterial vaccines.

INTRODUCTION

In Poland during the past 12 years, the typhoid morbidity rate has decreased from 24 per 100 000 to 8 and the mortality rate from 3.3 per 100 000 to 0.1. In spite of this improvement, the typhoid incidence is still comparatively high, and the public health service is continuing its attempt to eliminate the endemic foci of the disease.

The mass vaccination inaugurated during the years 1940-44 was continued after the war. Since 1947, vaccination against typhoid fever has been obligatory for all people between the ages of 5 and 60 in selected areas of the country, designated annually according to the epidemic situation. During the years 1950-60, about 10 million people were vaccinated every year.

The TAB vaccine used until 1948 was made of whole typhoid and paratyphoid A and B bacteria, killed by heat or formol. Two inoculations were given at an interval of 7-10 days. Since 1948, Grasset's vaccine as modified by Slopek (lysate of typhoid and paratyphoid A and B bacilli with added aluminium hydroxide) has been used for mass immunization. A single dose of the vaccine is given and the procedure repeated every year.

From 1946 to 1949, there was a decrease in the incidence of typhoid fever among adults of 20-50 years and to a lesser extent among adolescents of 10-20 years. There was practically no change in the incidence of typhoid among children under 10 years of age. The same trend was observed during the period 1951-60—a gradual decline in the crude typhoid-incidence rate and a rather high incidence persisting in children of up to 10 years.

A comparison of the typhoid-fever incidence among vaccinated and non-vaccinated people during epidemics at Lodz (1950) and Cracow (1957) failed to provide evidence of the effectiveness of the immunization carried out 1-3 years prior to the outbreaks. It therefore became doubtful whether Grasset's vac-

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[1] Members of the Committee: Z. Buczowski, B. Kassur, J. Kostrzewski, H. Kucharzyk, K. Lachowicz, H. Meisel, K. Neyman, W. Prażmowski, L. Rzucidło, S. Slopek, H. Wiór, and H. Załęska. The participants in the field trial and the working arrangements are given in the Annex.

cine was sufficiently immunogenic or whether the vaccination procedure was appropriate.

A committee appointed in January 1959 to review the situation recommended the evaluation of the effectiveness of four types of typhoid vaccine: bacterial formol-killed phenol-preserved vaccine (code letter N)[1]; bacterial acetone-killed and -dried vaccine according to Landy (P); Westphal's endotoxin with added aluminium hydroxide (S); and Grasset's vaccine as modified by Slopek (T). It was decided to use tetanus toxoid vaccine (O) for the control group.

With the assistance of WHO, the Polish study was related to those carried out in Yugoslavia and British Guiana by the use of an acetone-killed vaccine (K) produced at the Walter Reed Army Institute of Research in Washington, D.C.

According to the plan, about a million persons from 25 districts in 5 provinces were to be immunized (Kostrzewski, 1963). Children in the age-group 5-14 years were to receive vaccines K, N, T or O, and persons in the age-group 15-60 years vaccines N, P, S or O. In this way a comparison could be made between the field trials in Poland and those in Yugoslavia with respect to the groups inoculated with vaccines K, N, T or O. It would also be possible to compare the effects of different types of vaccine within particular age-groups (5-14 or 15-60) in Poland.

VACCINES

Vaccines N, P and T were prepared by the Serum and Vaccine Production Laboratories, Cracow (Kruczałowa & Schillerowa, 1963), vaccine S by the United Serum and Vaccine Production Laboratories, Warsaw, and the tetanus toxoid by the Warsaw Production Laboratory of Sera and Vaccines. Vaccine K was obtained from the Walter Reed Army Institute of Research.

The vaccines N, P, S and T were prepared from typhoid strain Ty 2 received from the Statens Serum-institut, Copenhagen (this strain was also used in the Walter Reed Army Institute of Research for the production of vaccines K and L for the Yugoslav field trial).

[1] The letter symbols designating the vaccines produced in Poland are an alphabetic continuation of the symbols for the vaccines used in WHO studies, of which vaccines K and L were employed in controlled trials in Yugoslavia and British Guiana in 1960 (Yugoslav Typhoid Commission, 1964; Typhoid Panel, UK Department of Technical Co-operation, 1964).

The four vaccines were prepared from the same parent suspension, of which (to ensure sufficiency) there were seven lots, each consisting of 500-600 Roux bottles. For inoculations on a given day, a new ampoule of Ty 2 strain was used. Eighteen-hour cultures of this strain were seeded into broth medium, and four-hour broth cultures were subinoculated on agar medium (double calf-meat extract, 2% Witte peptone and 2.5% Korean agar).

After 18-20 hours' incubation, the growth was washed off with phosphate buffer (pH 7.2). The suspension from 100 Roux bottles was pooled into one container and considered as one harvest. Each harvest was divided volumetrically into four parts: 35.55% was assigned for the preparation of the formol-killed phenol-preserved vaccine; 28.9% for the acetone vaccine; 28.9% for the vaccine of Westphal's endotoxin; and 6.65% for the Grasset-Slopek vaccine. Samples of the stock suspension from each harvest were examined serologically and under the microscope, and were used for determining the density of the suspension and the nitrogen content. After discarding the harvests that failed to fulfil requirements, the remaining harvests were combined into four starting suspensions, assigned for the production of the four types of vaccine. In all, 41.5 litres of suspension of a medium density of 58×10^9 bacteria per millilitre were collected.

Vaccine N

Vaccine N was a formol-killed phenol-preserved vaccine. To a measured volume of suspension from each harvest, 1% of formol was added. The suspension was preserved for 24 hours at 36°C and then stored in a refrigerator at 4°C. The density of the suspension was determined in three laboratories, the mean value being 60×10^9 bacteria per millilitre. The suspension was then diluted by adding 170 ml of it to 10 litres of buffered saline solution containing 0.45% phenol, so that a concentration of 1×10^9 bacteria per millilitre was obtained. The vaccine was dispensed into 50-ml flasks.

Vaccine P

Vaccine P was an acetone-killed and -dried vaccine. It was prepared by Landy's method, according to the instructions of Dr A. Benenson of the Walter Reed Army Institute of Research (Division of Immunology, Walter Reed Army Institute of Research, 1964).

The resulting suspension was divided into 1.5-ml quantities and dried for 36 hours. The dry mass in

particular flasks, corresponding to 50 ml of vaccine of a density of 10^9 bacteria per millilitre, varied from 0.02 to 0.03 g and the moisture content from 5% to 13.8%. The total nitrogen was 0.072 mg/mg dry mass. For suspending the acetone vaccine a phosphate-buffered physiological saline solution (pH 7.2) containing 0.45% phenol was prepared, the solution being dispensed in bottles in 50-ml quantities.

Vaccine S

Vaccine S was an endotoxin prepared by Westphal's method (Westphal, Luderitz & Bister, 1952) and adsorbed on aluminium hydroxide.

The calculation of the amount of vaccine to be prepared from the endotoxin was based on the density of the starting suspension used for vaccine N. It was assumed that the amount of endotoxin contained in 1 ml of vaccine S should be equivalent to the amount of endotoxin that could be extracted from 1×10^9 typhoid bacilli.

For the preparation of 570 litres of vaccine, 6.77 g of endotoxin were used, the remaining amount of endotoxin (i.e., 0.71 g) being left aside for control purposes. The endotoxin was dissolved in 5.7 litres of phosphate-buffered saline solution containing 0.45% phenol. This solution was Seitz-filtered and, after being checked for sterility, diluted with buffered saline to give the desired amount of vaccine. Sterile 2% aluminium hydroxide was then added in an amount sufficient to give a final concentration in the vaccine of 0.03% $Al(OH)_3$. The vaccine was dispensed in 50-ml flasks.

Vaccine T

Vaccine T was prepared by the Grasset-Slopek method (Slopek, 1947; Slopek & Abgarowicz, 1948) and adsorbed on aluminium hydroxide.

From the lysate obtained from 6.65% of starting suspension, 145 litres of vaccine were prepared, the density then being 1×10^9 bacteria per millitre. The lysate was diluted with phosphate-buffered saline solution containing 0.45% phenol, and an adsorbent of 2% aluminium hydroxide solution was added in an amount sufficient to give a final concentration in the vaccine of 0.03% $Al(OH)_3$. The vaccine was dispensed in 50-ml bottles.

LABORATORY EVALUATION

The immunizing potencies of vaccines N, P, S and T were evaluated in various laboratories. Agglutination tests on the sera of rabbits immunized with the four vaccines and active mouse-protection tests were performed in the Department of Sera and Vaccines, State Institute of Hygiene. Active mouse-protection tests with zymosan were carried out in the Department of Epidemiology, State Institute of Hygiene, and, in the Department of Medical Microbiology of the Medical Academy of Wroclaw, an opsonocytophagic test was carried out on the sera of persons immunized with the vaccines.

The agglutination tests (Meisel, Rabczyńska & Kudelski, 1963) on the sera of immunized rabbits [1] showed that:

(1) vaccine P had the highest immunizing potency, as indicated by the increase in O, H and Vi antibody levels,

(2) vaccine N gave rise to a similar increase in O and H levels and to a slighter one in Vi antibody titre,

(3) vaccine T was significantly less potent, and

(4) vaccine S did not produce any significant increase in the O, H or Vi agglutinin levels.

Using the mouse-protection test in mice immunized with two doses of the vaccines, with an interval of four weeks between inoculations, and infected with a suspension of bacteria in saline solution or in zymosan solution, the same workers (Kudelski, Meisel and Rabczyńska, 1963) again found that vaccine P had the highest immunogenic potency and vaccine S the lowest.

A further active mouse-protection test was carried out (Kostrzewski, Płachcińska & Grużewski, 1963) in which the mice were each given a single injection of one of the vaccines K, N, P, S and T at a dosage of 4×10^5, 4×10^6, or 4×10^7 typhoid bacilli (or the corresponding doses of endotoxin). Seven days after inoculation the mice were divided into three groups and infected with live typhoid bacilli in zymosan at dosages of 5000, 25 000 or 125 000 bacteria, according to the size of the vaccine dosage. The test indicated that:

(1) vaccine N had the highest immunogenic potency,

(2) vaccine P was less potent, but the differences between vaccines N and P were not significant,

(3) vaccines K and T were less potent than the two preceding ones, but the differences between vaccines K, P and T were not significant,

[1] The rabbits were inoculated intravenously four times, at intervals of seven days, with 0.5 ml of the vaccines in dilutions of 1:10 and 1:1000. Blood samples were collected after the second and fourth inoculations.

(4) vaccine S was significantly less potent than all the others.

The phagocytic test (Ładosz, 1963), using the sera of men inoculated twice at an interval of four weeks with a dose of 0.5 ml of vaccines N, P, S or T, showed that vaccine P was the most effective and vaccine S the least. The potencies of vaccines N and T were equal to each other, and both were less potent than vaccine P.

Serological tests on human sera were carried out (Kopacka & Słubicka, 1963) to determine the increase in O, H and Vi antibody levels. The samples of sera were obtained from 301 persons vaccinated twice at an interval of four weeks with a dose of 0.5 ml of vaccines N, P, S and T. Of these 301 people, 68 had been inoculated with vaccine N, 81 with vaccine P, 69 with vaccine S and 83 with vaccine T. It was ascertained, on the basis of the arithmetic mean titre calculated before and after vaccination, that vaccines N and P stimulated the production of O and H antibodies, while Vi antibodies rose only slightly. Vaccine T produced a lower rise in O antibodies than vaccines N and P, only a very slight increase in H antibodies and about the same increase in Vi antibodies. Vaccine S proved to be the least effective and stimulated only a slight increase in O antibodies.

REACTION TO VACCINATION

Before the mass immunization campaign was inaugurated, the vaccination reactions were investigated in a group of people who had not been vaccinated against typhoid fever during the two preceding years.

The vaccines were administered subcutaneously by means of a deep injection into the external side of the arm. Two injections were given at an interval of one month. Observations were made on:

(1) general reactions—temperature of the body, headache, muscle or joint ache, chill, nausea, vomiting and diarrhoea, and

(2) local reactions—the extent of redness and swelling at the site of the injection of the vaccine, and swelling of the lymph-nodes.

The vaccinations were carried out on 125 pupils of a secondary school, 15-18 years of age. The dose consisted of 1 ml of vaccines N, P, S or T, containing (or equivalent to) 10^9 bacilli (Żołnierkowa & Przestalska, 1963). The vaccines were injected in rotation as the children presented themselves for vaccination. The inoculation was followed by some severe reactions, especially in the children who received vaccines P and N, of whom 21%-48% had temperatures of 38°C or more and 57%-67% had local redness and swelling more than 10 cm in diameter.

In consequence it was decided to reduce the injection dose of the vaccine to 0.5 ml. In this volume of vaccines K, N, S or T were some 5×10^8 bacteria or a corresponding quantity of antigen. A similar volume of vaccine P contained 2.5×10^8 bacteria. The vaccines were administered in this dosage during the continuation of the study of postvaccinal reactions as well as in the controlled mass-vaccination campaign conducted in 1961. In the decreased dosages, vaccines N and P still provoked some severe reactions, and 9%-12% in the 3-20-year age-group had temperatures of 38°C or more. Among adults of 19-31 years, vaccine N provoked temperatures of 38°C or more in 23% of cases. Less reaction was observed after inoculation with vaccine T and least with vaccine S. The differences between the post-vaccinal reactions obtained with vaccines N and P were not statistically significant; neither were the differences between the reactions obtained with vaccines T and S. However, the differences between the reactions obtained with vaccines N and P on the one hand and T and S on the other were significant.

The conclusions reached from the study of post-vaccinal reactions was that vaccines N, S and T used in doses containing 5×10^8 bacteria (or the amount of antigen obtained from 5×10^8 bacteria) and vaccine P used in doses containing about 2.5×10^8 bacteria did not provoke excessive reactions and in consequence could be used in mass vaccination.

AREA AND POPULATION SELECTED FOR THE FIELD TRIAL

The controlled vaccination trial was organized in five provinces—Kielce, Lodz, Poznan, Warsaw and Wroclaw—and comprised 25 towns and counties. In these areas, the typhoid morbidity rate between 1953 and 1960 varied from 2.0 to 3.8 per 100 000 compared with rates of between 1.5 and 2.8 per 100 000 for the country as a whole.

In the towns and counties where the field trial was to take place, mass immunization with the Grasset-Slopek typhoid-paratyphoid vaccine had been performed almost every year up to 1958, but since that time the procedure had been discontinued.

Before the inauguration of the controlled vaccination trial, a serological survey of O, H and Vi antibodies was performed in the population, which had not been vaccinated against typhoid fever during the two years prior to the survey. Agglutination and

TABLE 1

O, H AND Vi AGGLUTININS IN 417 PERSONS SELECTED AT RANDOM BEFORE THE FIELD TRIAL

Age (years)	Number of persons examined	Percentage of persons showing the following reactions to tests for the presence of antibodies		
		Negative	O antibodies only	O, H and Vi antibodies
7-19	296	2.7	86.5	10.8
20-62	121	2.5	28.1	69.4

passive haemagglutination tests were applied. By means of random selection in 25 towns and counties, 417 blood samples were collected from persons aged 7-60 years. Of these, 296 samples were from persons in the age-group 7-19 years, and the remaining 121 were from those of 20 years and over.

Differences were found in the pattern of *Salmonella typhi* antibodies in relation to age (Table 1). In 69.4% of the individuals over 20 years of age, O, H and Vi antibodies were found, while in the 7-19-years age-group only 10.8% of the persons examined showed the presence of all three antibodies, but 86.5% showed the presence of O antibodies alone. In the older age-group, moreover, the level of antibodies, although generally low, was higher than in the younger age-group (Table 2). The strongly positive serological reactions were more frequently met in older than in younger persons.

VACCINATION CAMPAIGN

The controlled field trial with the typhoid vaccines lasted from February to June 1961. Three groups of children aged 5-14 years were vaccinated with three types of typhoid vaccine—K, N and T—and a fourth group was immunized with tetanus toxoid vaccine O. Three groups of people aged 15-60 years were vaccinated with typhoid vaccines N, P and S, and a fourth group with control vaccine O.

The complete vaccination took the form of two injections at an interval of four weeks, each injection (for children as well as adults) containing 0.5 ml of vaccine. Vaccines K and P, being dry preparations, required suspension in a fluid before injection. Vaccine K was suspended in the amount of fluid necessary to give 10^9 bacteria per millitre. Vaccine P was suspended at half this concentration. Suspensions of vaccines K and P were always used within 48 hours of their preparation.

The vaccination was carried out in dispensaries, out-patient clinics, nurseries, kindergartens, schools and places of work by the health personnel of the establishment concerned or by specially organized teams. In outlying villages, the vaccination was carried out mainly by such teams. In most counties, children under 14 years of age and persons 15 years of age and over were vaccinated separately on different days. However, since parents often came accompanied by children of pre-school age, each team had a supply of vaccines for children. When this was not the case, the children were vaccinated only with the N or the O vaccine, both of which were used for both adults and children. As a result, a somewhat higher number of pre-school children were vaccinated with N or O than with K or T vaccines. The various vaccines were administered alternately in each population group by every team on successive days. This system was intended to ensure comparability between groups of different age, sex and environment, inoculated with a given vaccine.

Records of the vaccination were entered on the same forms as are used in the annual typhoid vac-

TABLE 2

O, H AND Vi ANTIBODIES PRESENT IN PERSONS BEFORE THE FIELD TRIAL

Age (years)	No. of persons examined	Test	Percentage of persons showing the following O antibody titres						Percentage of persons showing the following H antibody titres					Percentage of persons showing the following Vi antibody titres			
			Negative	1/10	1/20	1/40	1/80	1/160	Negative	1/10	1/20	1/40	1/80	Negative	1/10/	1/20	1/40
7-19	296	Agglutination	25.3	42.9	26.4	5.1	0.3	—	95.5	0.7	2.4	1.4	—	97.0	3.0	—	—
		Haemagglutination	2.7	—	16.6	56.4	23.3	1.0	—	—	—	—	—	93.2	5.4	1.4	—
20-62	121	Agglutination	6.6	40.5	43.8	9.1	—	—	44.6	19.0	21.5	14.1	0.8	79.4	16.5	4.1	—
		Haemagglutination	3.3	—	5.8	31.4	57.0	2.5	—	—	—	—	—	69.4	19.0	10.8	0.8

TABLE 3
NUMBERS OF PERSONS VACCINATED IN THE FIELD TRIAL

Classification	Numbers of persons inoculated						
	Total	Vaccines					
		K	T	N	O	P	S
First inoculation							
Age-group 5-14 years	370 920	90 670	92 159	94 290	93 801		
Age-group 15-60 years	472 718			118 483	118 973	116 858	118 404
Totals	843 638	90 670	92 159	212 773	212 774	116 858	118 404
Second inoculation							
Age-group 5-14 years	331 617	81 534	82 432	83 917	83 734		
Age-group 15-60 years	359 038			88 721	91 862	87 521	90 934
Totals	690 655	81 534	82 432	172 638	175 596	87 521	90 934
First inoculation only							
Age-group 5-14 years	39 303	9 136	9 727	10 373	10 067		
Age-group 15-60 years	113 680			29 762	27 111	29 337	27 470
Totals	152 983	9 136	9 727	40 135	37 178	29 337	27 470

cination campaign. The data entered on the forms included the vaccinee's name, sex, year of birth, address and place of employment or school, the date of the vaccination, and the serial number of the vaccine. Persons vaccinated were entered on separate lists according to the vaccine administered. At the first injection, each person vaccinated was given a card on which the code symbol of the vaccine and the date of the first injection were noted, as well as the date of the next injection. After the second injection had been entered, the card served as a certificate of vaccination.

The numbers of people vaccinated are given in Table 3.

COMPARABILITY OF GROUPS OF PERSONS VACCINATED WITH DIFFERENT VACCINES

The differences in the numbers of persons vaccinated with the different vaccines (Table 3) were due principally to three factors.

(1) In some areas, children of pre-school age were not vaccinated on a separate day but at the time assigned for the inoculation of adults. This led to many of the children being inoculated with N and O only.

(2) Persons injected with vaccines K, P and N, which gave strong postvaccinal reactions, evaded the second injection more often than those inoculated with the vaccines giving milder reactions. Clearly, postvaccinal reactions exert an influence on the number of persons vaccinated for the second time.

(3) Vaccines K and P were prepared in amounts expected to be sufficient for a day's injections. If the amount was inadequate, the remaining people were injected with the other vaccines.

However, it is safe to assume that the data regarding the groups of persons vaccinated with the various vaccines of the sets K, N, O, T and P, N, O, S are adequately comparable within their respective age-groups and permit a reliable epidemiological evaluation of the effectiveness of the vaccination.

In order to ascertain whether, after the completion of vaccination (1 July 1961), any changes occurred in the comparability of the groups inoculated with the different vaccines, the following studies were performed:

(1) In many towns or counties in the area of the field trial, an analysis was made of the number of inoculated persons suffering from diphtheria, scarlet

TABLE 4

INCIDENCE OF DIPHTHERIA, SCARLET FEVER, DYSENTERY AND INFECTIOUS HEPATITIS AMONG VACCINATED PERSONS IN SELECTED REGIONS OF THE FIELD-TRIAL AREA FROM JULY 1961 TO JUNE 1963

Vaccine	No. of doses	Attack rate per 100 000 per year									
		Age-group 5-14 years					Age-group 15-60 years				
		Diph-theria	Scarlet fever	Dysen-tery	Infectious hepatitis	Total incidence	Diph-theria	Scarlet fever	Dysen-tery	Infectious hepatitis	Total incidence
K	1	34	251	11	80	376					
	2	12	175	3	133	324					
T	1	41	83	21	83	228					
	2	19	180	7	134	340					
N	1	54	135	9	99	298	0	0	8	57	65
	2	20	209	2	130	360	0	3	14	54	71
O	1	75	150	0	159	384	0	0	4	53	58
	2	16	196	8	134	354	1	3	8	57	69
P	1						8	0	4	45	57
	2						0	4	14	58	75
S	1						4	0	9	26	39
	2						0	2	11	60	72

fever, dysentery and infectious hepatitis. The results are given in Table 4.

(2) In the whole area of the field trial, an analysis was made of the vaccination status of persons who had been in contact with typhoid-fever patients (Table 5).

(3) An investigation was made into the vaccination status of persons from different environments and localities where typhoid-fever cases were reported (Table 6).

These studies showed that, during the period of epidemiological observation, no changes of essential significance occurred among the persons inoculated with the different vaccines.

FOLLOW-UP STUDY IN THE FIELD-TRIAL AREA

From 1 July 1961, every reported case of typhoid fever was investigated clinically, bacteriologically and epidemiologically. The results were entered on the survey card that carried the vaccination status of the patient. The survey card also contained information regarding the vaccination of persons who were in contact with the patient.

Tables 7 and 8 record the number of typhoid cases notified during the period 1 July 1961 to 30 June 1963 in individual provinces within the area of the field trial. Typhoid cases in which clinical diagnosis was confirmed by positive blood, stool or urine culture are separated from those in which diagnosis was based on clinical manifestations, serological and/or epidemiological evidence only, without positive bacteriological confirmation.

The incidence of typhoid fever in groups of persons vaccinated with vaccines K, N, P, S or T, compared with the control group immunized with vaccine O, is shown in Table 9. This table contains

TABLE 5. DISTRIBUTION ACCORDING TO VACCINATION STATUS OF PERSONS IN CONTACT WITH TYPHOID-FEVER CASES IN THE FIELD-TRIAL AREA

Vaccine	No. of doses	Age-groups			
		5-14 years		15-60 years	
		July 1961-June 1962	July 1962-June 1963	July 1961-June 1962	July 1962-June 1963
K	1	2	4		
	2	27	27		
T	1	3	4		
	2	33	25		
N	1	2	5	4	8
	2	21	29	25	20
O	1	6	0	6	2
	2	25	39	20	28
P	1			8	3
	2			25	19
S	1			6	7
	2			18	22

TABLE 6 DISTRIBUTION ACCORDING TO VACCINATION STATUS OF PERSONS FROM DIFFERENT ENVIRONMENTS WHERE TYPHOID-FEVER CASES WERE REPORTED

Environment	Time of examination	Vaccine					
		K	T	N	O	P	S
Age-group 5-14 years							
School at Zyrardow	April 1962	179	179	179	178		
Farm at Siedlce	December 1962	3	3	4	5		
Town of Glinojeck	January 1963	114	109	101	104		
Town of Kolbiel	April 1963	44	37	42	54		
School at Kielce	April 1963	55	47	59	45		
Age-group 15-60 years							
Town of Zyrardow	April 1962			176	196	180	166
Farm at Siedlce	December 1962			3	7	9	4
Agricultural Technical School at Siedlce	December 1962			17	13	13	16
Town of Glinojeck	January 1963			86	78	90	105
Sugar factory at Glinojeck	January 1963			23	25	27	31
Town of Kolbiel	April 1963			62	51	53	54
Factory at Legionowo	April 1963			61	54	53	60

TABLE 7

DISTRIBUTION ACCORDING TO VACCINATION STATUS OF TYPHOID-FEVER CASES AMONG CHILDREN AGED 5-14 YEARS DURING THE PERIOD 1 JULY 1961 TO 30 JUNE 1963

Basis of diagnosis	Province	Number of cases in following vaccine groups							
		K		N		T		O	
		1 dose	2 doses	1 dose	2 doses	1 dose	2 doses	1 dose	2 doses
Confirmed by blood, stool and/or urine culture of *S. typhi*	Kielce						2	1	5
	Lodz		1						2
	Poznan				1		2		2
	Warsaw		3		1	3	4	1	16
	Wroclaw				1		4	1	6
Total			4		3	3	12	3	31
Clinical manifestations and serological examination	Kielce					1	1		
	Lodz				1				1
	Poznan								1
	Warsaw	1	1		2		11		5
	Wroclaw						2		
Total		1	1		3	1	14		7

only cases bacteriologically confirmed. A significant difference in the typhoid-incidence rate appears between the persons vaccinated twice with vaccine N and those in the control group ($P < 0.0001$).

Among children in the age-group 5-14 years vaccinated twice with vaccines K, N or T, the typhoid-incidence rate was lower than in the control group. The difference is significant (Table 10). The difference in the incidence of typhoid fever between the groups of persons immunized with vaccines K or T and the difference between the groups immunized with vaccines N or T are also significant. On the other hand, the difference in the incidence of typhoid between the groups of persons immunized with the vaccines K or N is not significant.

The difference in the typhoid-incidence rate between the persons in the age-group 15-60 years vaccinated twice with N, P or S vaccine and the control group is not significant.

Table 11 compares the incidence of typhoid fever in the group of children of up to 14 years of age who received vaccine N with that in the control group during four consecutive periods of six months and two periods of one year after vaccination. During the first year after vaccination, the two doses of vaccine N conferred protection on 95% of the group, and the proportion of typhoid fever cases was 1:20. During the second year the protection amounted to 82%, and the proportion of cases was 1:10. Only the typhoid cases confirmed by a positive culture of *Salmonella typhi* from blood, stool or urine are recorded.

DISCUSSION

People in Poland may have a history of immunization against typhoid fever different from that of people elsewhere. One must assume that juveniles

TABLE 8

DISTRIBUTION ACCORDING TO VACCINATION STATUS OF TYPHOID-FEVER CASES AMONG ADULTS AGED 15-60 YEARS DURING THE PERIOD 1 JULY 1961 TO 30 JUNE 1963

Basis of diagnosis	Province	Number of cases in following vaccine groups							
		N		P		S		O	
		1 dose	2 doses	1 dose	2 doses	1 dose	2 doses	1 dose	2 doses
Confirmed by blood, stool and/or urine culture of *S. typhi*	Kielce	—	—	—	—	2	—	1	—
	Lodz	—	—	—	—	1	—	—	2
	Poznan	—	3	—	2	1	3	—	4
	Warsaw	2	1	—	1	2	4	3	2
	Wroclaw	—	2	—	1	1	2	1	1
Total		2	6	—	4	7	9	5	9
Clinical manifestations and serological examination	Kielce	—	—	1	1	—	—	—	—
	Lodz	—	—	—	—	—	1	—	1
	Poznan	—	—	—	—	—	—	—	—
	Warsaw	—	1	2	—	—	2	1	2
	Wroclaw	—	1	—	1	1	—	—	1
Total		—	2	3	2	1	3	1	4

up to the age of 20, if vaccinated at all, have been given the Grasset vaccine. On the other hand, many adults in the age-group 20-60 years were immunized with bacillary vaccine during the years 1940-47, and men serving in the Army have been subject to this procedure even in recent years.

The serological survey carried out before the field trial showed that the percentage of persons having agglutinins for all three types of antibody—O, H and Vi—is lower in children than in adults. One may assume that this phenomenon is related to the type of typhoid vaccine that has been used for mass immunization in the past.

In the field-trial area, children aged 5-8 years had not as a rule been immunized against typhoid fever prior to 1961. A majority of older children aged 8-14 years had probably been vaccinated once or even several times prior to 1958 with the typhoid-paratyphoid vaccine of the Grasset type. A large number of juveniles and adults aged 15-60 years had been vaccinated several times prior to 1958. Hence, for the purpose of evaluating the effectiveness of the different types of vaccine, the group of children aged 5-14 years is of the utmost importance. In this age-group, the formol-killed phenol-preserved vaccine and the acetone-killed vaccine proved to be more effective than the Grasset vaccine. Similar results have been obtained in laboratory tests.

Among persons aged 15-60 years immunized with N, P or S vaccines and with tetanus toxoid, the total number of bacteriologically proved cases was too small to draw any final conclusion. The highest number of cases occurred in the group vaccinated with the endotoxic vaccine S. However, the differences between the typhoid-incidence rates of the control group and the three groups immunized with vaccines N, P or S were statistically insignificant.

It appears on the basis of these observations that

TABLE 9. INCIDENCE OF TYPHOID FEVER AMONG VACCINATED PERSONS DURING THE PERIOD 1 JULY 1961 TO 30 JUNE 1963

Vaccine	Number of doses	Children 5-14 years old			Adults 15-60 years old			Total		
		Number of persons vaccinated	Number of cases	Attack rate per 100 000 per year	Number of persons vaccinated	Number of cases	Attack rate per 100 000 per year	Number of persons vaccinated	Number of cases	Attack rate per 100 000 per year
K	1	9 136	0	0						
	2	81 534	4	2.5						
N	1	10 373	0	0	29 762	2	3.4	40 135	2	2.5
	2	83 917	3	1.8	88 721	6	3.4	172 638	9	2.6
P	1				29 337	0	0			
	2				87 521	4	2.3			
S	1				27 470	7	12.7			
	2				90 934	9	4.8			
T	1	9 727	3	15.4						
	2	82 432	12	7.3						
O	1	10 067	3	14.9	27 111	5	9.2	37 178	8	10.8
	2	83 734	31	18.5	91 862	9	4.9	175 596	40	11.4

under our conditions the vaccines on trial do not afford protection to persons in the age-group 15-60 years. Further observations are necessary for the solution of this problem. The lack of difference between the groups of persons inoculated with the different types of vaccine and the control group may be due to the maintenance of the immunity conferred by bacterial vaccines that had been repeatedly administered for several years. This fact, and the epidemiological observations collected in the field-trial area in 1959 and 1960, when mass vaccination was discontinued for two years prior to the field trial, seem to indicate that annual vaccination is unnecessary in regions in which the population has acquired protection against typhoid fever through multiple vaccination.

Further study is necessary in order to ascertain how long the protection conferred by immunization against typhoid fever lasts.

TABLE 10. SIGNIFICANCE OF THE DIFFERENCES BETWEEN THE TYPHOID-INCIDENCE RATES IN VARIOUS GROUPS OF CHILDREN AGED 5-14 YEARS VACCINATED WITH TWO DOSES OF VACCINES K, N, T AND O

Typhoid-fever cases in vaccines [a]	x^2	P
K(4) : N(3)	0.2	not significant
K(4) : O(31)	20.2	<0.0001
K(4) : T(12)	4.0	<0.05
N(3) : O(31)	23.0	<0.0001
N(3) : T(12)	5.6	<0.02
T(12) : O(31)	8.1	<0.01

[a] Figures in parentheses represent the number of cases in each vaccine group.

TABLE 11

INCIDENCE OF TYPHOID FEVER AMONG CHILDREN AGED 5-14 YEARS IMMUNIZED WITH VACCINE N COMPARED WITH THE INCIDENCE IN THE CONTROL GROUP (VACCINE O)

Period	Vaccine O				Vaccine N			
	1 dose		2 doses		1 dose		2 doses	
	Cases	Attack rate per 100 000 per year	Cases	Attack rate per 100 000 per year	Cases	Attack rate per 100 000 per year	Cases	Attack rate per 100 000 per year
July to December 1961	1	20	9	22	0	0	0	0
January to June 1962	1	20	11	26	0	0	1	2
July to December 1962	0	0	9	22	0	0	1	2
January to June 1963	1	20	2	5	0	0	1	2
July 1961 to June 1962	2	20	20	24	0	0	1	1
July 1962 to June 1963	1	10	11	13	0	0	2	2
July 1961 to June 1963	3	15	31	19	0	0	3	2

Annex

PARTICIPANTS IN THE FIELD TRIAL AND WORKING ARRANGEMENTS

The general management of the field trial was carried out by J. Kostrzewski, Chief, Department of Epidemiology, State Institute of Hygiene, Warsaw.

The collection, elaboration and statistical analysis of compiled material was performed by W. Magdzik, Assistant, Department of Epidemiology, State Institute of Hygiene, Warsaw.

The following epidemiologists of provincial sanitary-epidemiological stations were responsible for the epidemiological observations and collection of primary material: M. Kasprzak (Lodz), M. Krajewska (Poznan), K. Neyman (Poznan), S. Pęska (Warsaw), H. Przestalska (Wroclaw), J. Szeląg (Warsaw), Z. Utracka (Kielce), T. Walter (Poznan), D. Żołnierkowa (Wroclaw).

Supervision and co-ordination on behalf of the Ministry of Health and Social Welfare were effected by H. Załęska, Vice-Director, Sanitary Epidemiological Department, Ministry of Health and Social Welfare, Warsaw.

The serological testing of blood samples of the population was done by B. Kopacka and A. Słubicka, Department of Bacteriology, State Institute of Hygiene, Warsaw.

The vaccines were produced by M. Kruczałowa, Sera and Vaccines Production Laboratory, Cracow, and B. Schillerowa, Sera and Vaccines Technological Laboratory, Warsaw.

The vaccines were laboratory tested by J. Kostrzewski, Z. Kudelski, P. Meisel, J. Płachcińska and F. Rabczyńska, State Institute of Hygiene, Warsaw, and by J. Ładosz, Department of Medical Microbiology, Medical Academy, Wroclaw.

The postvaccinal-reaction studies were made by J. Zołnierkowa and H. Przestalska, Provincial Sanitary-Epidemiological Station, Wroclaw, Z. Brzeziński and Z. Gancarz, Department of Hygiene, Medical Academy, Warsaw, and R. Lutyński, Provincial Sanitary-Epidemiological Station, Cracow.

RÉSUMÉ

Quatre types de vaccins antityphoïdiques ont été étudiés en Pologne en 1961, au laboratoire et sur le terrain au cours d'essais contrôlés: *a*) deux lots de vaccins à l'acétone, l'un préparé aux Etats-Unis (K), l'autre en Pologne (P); *b*) un vaccin tué par le formol et conservé dans le phénol (N); *c*) un vaccin à base d'endotoxine préparée selon la méthode de Westphal (S), et *d*) un vaccin obtenu par la méthode de Grasset modifiée par Slopek (T).

Le pouvoir immunisant de ces préparations a été déterminé au laboratoire par différents procédés: épreuve d'agglutination sur sérums de lapins immunisés, test de protection active chez la souris, test phagocytaire et recherche des anticorps chez les sujets vaccinés. Les vaccins à l'acétone et le vaccin N ont fait la preuve de leur supériorité sur les vaccins S et T.

Quant aux réactions post-vaccinales provoquées par les différentes préparations, elles ont été étudiées avant de procéder aux opérations de vaccination. A des doses représentant respectivement 5×10^8 bactéries et $2{,}5 \times 10^8$ bactéries, les vaccins N, S, T et le vaccin P ne provoquent pas de réactions post-vaccinales exagérées et peuvent servir pour la vaccination de masse.

Dans le courant de 1961, 690 655 personnes reçurent deux injections de l'une des cinq préparations, et 152 983 une seule injection. Un groupe témoin reçut de l'anatoxine tétanique. Une enquête fut ensuite menée dans la région, de 1961 à 1963, pour dépister les cas de fièvre typhoïde. Chez les vaccinés âgés de 5 à 14 ans, l'immunité la plus forte fut obtenue avec le vaccin N en deux injections (95% de protection la 1re année, 82% la 2e année), et la plus faible avec le vaccin de Grasset (T). On n'observa aucune différence significative entre le pouvoir protecteur des vaccins N et K. Chez les personnes âgées de 15 à 60 ans, le nombre de cas de fièvre typhoïde fut trop peu élevé et n'autorisa aucune comparaison entre l'efficacité des vaccins. Dans cette catégorie d'âge, on ne constata aucune différence significative entre l'incidence de la fièvre typhoïde chez les vaccinés et chez les non vaccinés, ce qui plaide en faveur de la persistance dans cette population d'une immunité acquise à la suite des nombreuses vaccinations antérieures.

D'autres recherches seront nécessaires pour déterminer la durée de la protection conférée par la vaccination.

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