Laboratory Characteristics of Paratyphoid B Vaccines Tested in Controlled Field Trials

L. B. HEJFEC AND L. A. LEVINA

Laboratory for Study of the Efficacy of Bacterial Preparations, Moscow Mechnikov Institute for Research on Vaccines and Sera, Moscow, Union of Soviet Socialist Republics

Received for publication 19 February 1969

The immunization of human beings with paratyphoid B vaccines, in doses producing a marked protective effect in field trials, resulted in H-agglutinin titers that were significantly higher than those produced by ineffective doses. In immunization of rabbits and white mice, the same difference in doses had a significant effect on the ability of vaccines to stimulate the formation of H and O antibodies. A parallel was noted between the effectiveness of vaccine for human beings and the activity of sera of the corresponding group of people in the passive mouse-protection test, with a correlation between such activity of sera and the 19S O-hemagglutinin titers. The data obtained suggested as promising the determination of agglutinin dynamics in the sera of inoculated human beings and animals to evaluate the antigenic activity of paratyphoid B vaccines in the laboratory. The passive mouse-protection test with human sera holds promise for laboratory evaluation of the effectiveness of paratyphoid B vaccines. At the same time, none of the variation of the active mouse-protection test employed permitted a significant difference to be detected between the vaccines effective and ineffective for man. Comparison of data on the frequency and degree of febrile vaccine reactions in man with results of laboratory evaluation of vaccine toxicity showed that, provided a reference vaccine with known pyrogenic capacity for man was employed, the study of stress effect of vaccines on guinea-pigs (determination of 17-oxycorticosteroid content) may permit prediction of the reactive power of the vaccine. No correlation was found between the pyrogenic capacity of vaccines for man and their toxicity for mice.

Parallel field and laboratory studies carried out over the last decade have provided a critical approach to the evaluation of many commonly employed laboratory tests and have contributed to the development of scientifically justified methods of laboratory control of typhoid vaccines (1, 3, 5, 8, 10, 11, 15, 16, 19-21).

As a result of the controlled field trial carried out in the USSR to evaluate paratyphoid B vaccines (9), we now have a collection of preparations with assayed protective power for man (both effective and ineffective). This has offered new opportunities for the study of postvaccinal immunity to paratyphoid B and for the laboratory assessment of immunogenicity and toxicity of paratyphoid B vaccines. In the controlled field trials, the protective effect for man was in direct correlation with the dose of the paratyphoid B component; a dose equal to 25% of the effective dose of the typhoid component failed to protect from paratyphoid B infection. Increasing the dose four- to fivefold produced an effect similar to that produced by the administration of typhoid com-

ponent; i.e., it afforded protection for more than 30 months after two injections and for up to 10 months after a single injection.

The main objective of this study was to explore the use of laboratory tests to establish the dose-dependence of protective effect of paratyphoid B vaccines, within the range of significance for the effectiveness for man. Another objective was to assess any correlation between the data on reactive capacity of vaccines for man and those on their toxicity for animals.

MATERIALS AND METHODS

Vaccines. Four vaccines, which had been tested in field trials, and two experimental batches were studied. The alcohol-killed divalent vaccine (code-letter V) and heat-killed divalent vaccine (code-letter G) contained 0.2×10^9 organisms of *Salmonella paratyphi* B and 0.8×10^9 organisms of *S. typhi* per ml; one dose for children contained 0.12×10^9 paratyphoid B cells. These two vaccines had been tested in field trial no. 5, in which it was established that they failed to protect human beings against paratyphoid B infection (10). The monovalent heat-killed vaccines Z-I and Z-2

contained 10^9 organisms of *S. paratyphi* B per ml; one dose for children contained 0.6×10^9 organisms. The monovalent vaccines had been tested in the 1963–64 field trial, and a pronounced protective effect was revealed (9). The two experimental vaccines, the alcohol-killed monovalent vaccine W and the heat-killed monovalent vaccine F, adsorbed by aluminium hydroxide contained 10^9 organisms of *S. paratyphi* B per ml. All the vaccines were prepared from *S. paratyphi* B (strains 42 and 50602), grown in broth in reaction vessels under aeration (24), and preserved by 0.5% phenol.

Animals. Randomly selected white mice of both sexes, weighing 12 to 14 g, were used for the active and passive mouse-protection tests and for determining the toxicity of vaccines and their ability to stimulate antibody formation. Chinchilla rabbits of both sexes, weighing 2,500 g, were used for evaluation of antibody production. Male guinea-pigs, weighing 260 to 330 g, were used to investigate the stress-activity of the vaccines.

Active mouse-protection test. By use of the Luippold's scheme (14), the vaccines were each administered in a single dose brought to a volume of 0.5 ml with saline solution. Two routes of immunization were used, subcutaneous and intraperitoneal. The challenge dose (3 to 10 LD_{50} of 20-hr *S. paratyphi* B strain 42 culture; 0.07×10^9 to 0.15×10^9 organisms in saline solution) was administered 12 to 14 days later. Survivors were recorded 3 days after challenge.

Mouse toxicity. Twofold dilutions of the vaccines were inoculated intraperitoneally and survivors were recorded 3 days later.

Stress activity in guinea-pigs. Free 17-oxycorticosteroids in 1 ml of blood plasma were determined by the method of Silber and Porter [modification of Yudaev and Pankov (23)], 4 hours after intracardiac injection of 0.01×10^9 organisms (in 0.5 ml).

Passive mouse protection. Four serial twofold dilutions of human sera were injected intraperitoneally into 5 or 6 mice, 0.5 ml each. The challenge dose (3 to 7 LD_{50} of agar culture in saline solution) was also administered intraperitoneally 1 hr later. Survivors were recorded 4 days after challenge.

Antibody production in experimental animals. Mice were twice injected intraperitoneally with 0.5 ml of vaccine at an interval of 14 days. Each vaccine was tested on two groups of mice given either 0.02×10^9 or 0.10×10^9 organisms. Each mouse was bled after immunization, 7 to 14 days, and pooled sera from five mice were tested.

The rabbits were injected intravenously twice at intervals of 28 to 30 days. The effects of differing doses (0.05×10^9 and 0.20×10^9 organisms) were compared. Blood was taken at different periods after immunization.

Immunization of human beings. Two groups of persons, from 7 to 40 years old, were given a single inoculation; the first group received 0.120 to 0.125×10^9 organisms; the second group, 0.50 to 0.60×10^9 . Blood specimens were taken before immunization and from 20 to 30 days thereafter.

Preparation of sera and determination of H-agglu-

tinin and O-hemagglutinin titers. The fresh sera (8 to 10 ml from each man and each rabbit) were immediately lyophilized, and precautions were taken for sterility. The ampoules with lyophilized sera (0.5 or 1.0 ml) were kept at 2 to 10 C. The sera from mice were stored at -70 C without lyophilization.

The H(b) agglutinins were determined by reaction with the liquid H(b) antigen prepared in the Lenin-grad Institute of Vaccines and Sera. The O-antibody titers were determined in the hemagglutination test (13), employing antigen prepared from *S. paratyphi* B strain by Raistrick and Topley technique [modified by Kossova and Nechaeva (12)]. This antigen was adsorbed on O-group human erythrocytes. The titers of 7S-antibodies were determined by the method of Deutsch and Morton (4). To sera diluted 1:5 in buffer solution (pH 7.3 to 7.4) was added an equal volume of 0.2 M 2-mercaptoethanol buffer solution, or only buffer, for the control. After 2 hr at 37 C, mixtures were held in the refrigerator for 14 hr. To control the inactivation of 19S antibodies, standard rabbit serum containing both 19S and 7S antibodies was tested in each experiment.

Statistical methods. The results of the active mouse-protection test were analyzed by regression analysis (6, 7). When determining the toxicity of vaccines and the passive mouse protection with sera, the median doses (ED_{50}) and their 95% confidence limits were calculated by the method of Van den Waerden (22). Geometric mean titers were determined by the method of Spaun (17); the significance of the difference of the titers was determined by student criteria. Correspondence between the results of various tests was estimated by correlation coefficient.

RESULTS

Toxicity for mice compared with pyrogenic capacity for man. Comparison of the V and G divalent vaccines, which exhibited little difference in their pyrogenic capacity for men (Table 1), has shown that they also differ insignificantly with respect to mouse toxicity (Table 2). Comparative study of paratyphoid B monovalent vaccines has shown that vaccine F is significantly more toxic for mice than vaccine Z-1, whereas the latter was found to have the greater pyrogenic capacity for man.

TABLE 1. Temperature reactions to vaccination 18 to 22 hr after the first injection

Vaccine	No. examined	Persons with elevated temperature			Persons with temperature higher than 37.5 C		
		No.	Per cent	2 SE	No.	Per cent	2 SE
G	1,807	519	28.4	2.1	173	9.6	1.4
V	1,759	446	25.4	2.1	164	9.3	1.4
Z-1	2,958	907	30.6	1.7	272	9.2	1.0
F	1,580	245	15.5	2.3	55	3.4	1.1

Stress effect for guinea-pigs compared with pyrogenic capacity for man. In experiments on guinea-pigs (12 animals in a group), vaccines Z-1 and F were compared. In all cases, 17-oxycorticosteroid concentration was higher with the injection of Z-1. The average 17-oxycorticosteroid concentration was 85.2 ± 8.1 μg per ml after immunization with the F vaccine and 117.4 ± 8.2 μg per 100 ml after immunization with the Z-1 vaccine. Therefore, a correlation was found with the results of comparative study of these two vaccines with respect to man.

Active mouse protection. Experiments were carried out with immunization by the subcutaneous route or by the intraperitoneal route. In all of the trials (Tables 3 and 4), a well-defined dose-

effect dependence was noted (regression coefficients differing significantly from 0 at 0.99), the deviation from parallelism of regression lines being insignificant ($P > 0.05$). In the first series (subcutaneous immunization), no differences in the immunogenicity of divalent vaccines V and G were detected, both being inferior in this respect to the monovalent vaccine Z-2. Therefore, in this case a preparation effective for man proved to be more potent in the active mouse-protection test than the vaccines ineffective for man. In the other series of experiments with subcutaneous immunization, the vaccine Z-1 (effective in man) was investigated in addition to the previously studied Z-2 (effective) and V (ineffective) vaccines. Z-1 was found to differ from Z-2 in its immunogenic-

TABLE 2. *Mouse toxicity of paratyphoid B vaccines*

Test series	Vaccine	Death ratios based on doses ^a				Dose ^b		
		12.5 (10)	6.25 (5)	3.12 (2.5)	1.56 (1.25)	TD ₅₀	L _{max}	L _{min}
1	G	23/28	32/37	22/38	2/38	2.98	3.39	2.61
	V	29/29	40/41	21/40	10/31	2.54	2.91	2.21
2	Z-1	34/42	36/58	14/59	1/10	4.15	5.03	3.42
	F		46/57	35/48	11/55	2.12	2.43	1.85

^a Dosage is expressed in billions of organisms. Numbers in parentheses indicate dosage for test series 2. Values represent the ratios of the number of mice that died (numerator) to the number of mice in the experiment (denominator).

^b Values represent the median doses of the vaccines (in billions of organisms) and their 95% confidence limits.

TABLE 3. *Comparative study of paratyphoid B vaccines in active mouse protection*

Test series ^a	Vaccine	Survival ratios based on doses ^b					Dose ^c		
		500	100	20	4	0.8	ED ₅₀	L _{max}	L _{min}
1	Z-2	58/87	48/80	28/85	17/85	15/81	91.84	247.2	34.13
	V		29/87	17/87	14/81	8/89			
	G		20/84	10/87	12/85	10/95			
2	Z-2	68/102	59/116	48/115	39/113	28/119	50	150	20
	V		35/110	34/118	34/120				
	Z-I	52/117	37/117	28/115	32/114				
3	Z-2		109/125	92/126	67/129	45/129	1.68	9.38	0.3
	V			109/126	80/132		1.52	2.97	0.08
	Z-I		70/127	44/125	32/128		112.76	552	23.02
4	Z-2		64/67	57/70	41/68	20/67	2.15	4.8	0.96
	V			58/65	50/68		1.63	2.58	1.03
	Z-I		50/74	32/76	15/76		40	105.2	15.21

^a Routes of immunization were subcutaneous for test series 1 and 2 and intraperitoneal for 3 and 4.

^b Dosage is expressed in millions of paratyphoid B cells. Values represent the ratios of the number of mice that lived (numerator) to the number of mice in the experiment (denominator).

^c Values represent the median doses of the vaccines (in millions of paratyphoid B cells) and their 95% confidence limits.

TABLE 4. *Statistical analysis of data from Table 3*

Test series	Vaccine	Significance of		
		Regression	Deviation from parallelism	Mean difference
		<i>P</i>	<i>P</i>	<i>P</i>
1	V and G	<0.1	>0.4	>0.9
	V and Z-2	<0.001	>0.01	<0.05
2	V and Z-2	<0.001	>0.05	<0.02
	V and Z-1	<0.02	>0.07	>0.80
	Z-2 and Z-1	<0.001	>0.1	<0.05
3	V and Z-2	<0.001	>0.5	>0.1
	V and Z-1	<0.001	>0.2	<0.01
	Z-2 and Z-1	<0.001	>0.6	<0.001
4	V and Z-2	<0.01	>0.3	>0.05
	V and Z-1	<0.001	>0.3	<0.001
	Z-2 and Z-1	<0.001	>0.9	<0.001

ity for mice, but it showed no difference from vaccine V in this respect.

In the experiments with intraperitoneal immunization, no differences between vaccines Z-2 and V were found; i.e., in contrast to experiments with subcutaneous immunization the vaccine V exhibited a greater activity, being superior in immunogenicity to vaccine Z-1. Vaccine Z-2 was more immunogenic than vaccine Z-1, as in experiments with subcutaneous immunization.

Thus, no correlation was detected between immunogenic activity of the vaccines for mice and their effectiveness for man. With intraperitoneal administration, the activity of all the vaccines increased; there were also changes in the ratio

between their activities, yet there was no increase in the degree of correlation of active mouse-protection by the vaccines with their effectiveness for man.

Antibody titers in mice. The titers of 7S and 19S H(b) agglutinins showed a direct dependence on the vaccine dose for all the preparations studied (Table 4).

No 7S O-hemagglutinins were detected in mice after two intraperitoneal injections with paratyphoid B vaccines (Table 5). A direct dependence between the dose and 19S O-hemagglutinin titers was found for all vaccines tested. At the same time, the sera of mice immunized with the vaccine Z-1 exhibited lower 19S O-hemagglutinin titers than did the sera of mice immunized with the other vaccines.

Thus, although a dose-titer dependence was found with fivefold dilution of vaccines, this test cannot be recommended for evaluating paratyphoid preparations, since the injection into mice of the two preparations effective for man resulted in the formation of differing antibody titers.

Antibody titers in rabbits. When sera of rabbits inoculated with different doses of Z-1 and Z-2 vaccines were studied (Table 6), well-defined differences were noted in the titers of H(b) agglutinins. Those rabbits immunized with 50×10^6 organisms showed lower H(b)-agglutinin titers than did the rabbits immunized with 200×10^6 organisms (Table 6, Fig. 1). H- and O-antibody titers increased after the second injection. When overall results were considered (Fig. 2), differences in O-hemagglutinin titers between rabbits given two injections with varying doses of paratyphoid B vaccines were significant.

Thus, the correlation between the intensity of

TABLE 5. *Antibody titers (7S and 19S) in mouse sera after intraperitoneal immunization (two injections)*

Dose per injection ^a	Vaccine	7S			7S and 19S			
		H(b) agglutinins		O hemagglutinins	H(b) agglutinins		O hemagglutinins	
		Geometric mean titer	SE	(Geometric mean titer)	Geometric mean titer	SE	Geometric mean titer	SE
20	Z-1	1.26	0.10	<0.85	1.41	0.14	<0.85	0
	Z-2	1.32	0.07	<0.85	1.55	0.09	<0.85	0
	F	0.85	0	<0.85	1.40	0.11	<0.85	0
	W	1.48	0.12	<0.85	1.85	0.05	<0.85	0
100	Z-1	2.04	0.17	<0.85	2.67	0.15	1.11	0.05
	Z-2	1.79	0.03	<0.85	2.11	0.03	1.85	0.03
	F	2.30	0.08	<0.85	2.49	0.04	1.64	0.16
	W	2.29	0.08	<0.85	2.36	0.09	1.55	0.07

^a Expressed as millions of bacterial cells.

TABLE 6. *Antibody titers in rabbit sera after immunization with different doses of vaccines*

No. of injections	Dose per injection ^a	Vaccine	H(b) antibody		O hemagglutinins	
			Geometric mean titer	SE	Geometric mean titer	SE
1	50	Z-1	1.03	0.18	1.83	0.35
		Z-2	1.55	0.22	2.53	0.17
	200	Z-1	1.87	0.09	2.51	0.29
		Z-2	2.56	0.20	2.73	0.15
2	50	Z-1	2.67	0.33	2.73	0.23
		Z-2	2.08	0.17	3.07	0.17
	200	Z-1	3.47	0.24	3.38	0.28
		Z-2	2.90	0.21	3.46	0.12

^a Expressed as millions of bacterial cells.

antibody titer and the vaccine dose in rabbits was well defined with respect to H agglutinins and, although somewhat less consistently, with respect to O hemagglutinins, provided the effects of two different doses of the same vaccine were compared. Along with this, differences were noted in antibody titer after injection of the two preparations effective for man (Table 6). This finding

makes it impossible to recommend this test for comparative evaluation of the effectiveness of paratyphoid B vaccines.

Antibody titers in man. Comparative study of

FIG. 1. *H(b)*-antibody titers in human and rabbit sera 1 month after the first injection and in mouse sera 7 to 14 days after the second injection with paratyphoid B vaccine.

FIG. 2. *Paratyphoid B* O-haemagglutinins in human sera 1 month after the first injection, in rabbit sera 7 days after the second injection, and in mouse sera 7 to 14 days after the second injection with paratyphoid B vaccine.

human sera obtained in field trials revealed no significant differences in H- and O-antibody titers between the sera obtained from men inoculated with ineffective (V and G) and effective (Z-1 and Z-2) paratyphoid B vaccines (Table 7).

However, analysis of these data is extremely difficult because of the differences detected in the initial titers of paratyphoid B O-hemagglutinins between the groups inoculated with paratyphoid B vaccines in the dose of 120×10^6 organisms (vaccines G and V) and in the dose of 600×10^6 organisms (vaccines Z-1 and Z-2). This difference may be due to the fact that in 1962 the sera were taken from persons aged over 18, whereas in

1963-64 they were taken from children of school age.

To ascertain the dependence of antibody titer on the dose of vaccine, additional studies were carried out. A group of volunteers aged 17 to 20, randomly divided into subgroups that showed no difference in antibody titers prior to immunization, was inoculated once with monovalent vaccine. Subsequently, no differences were found in the titers of 7S and 19S O-hemagglutinins, which seemed related to the different doses of paratyphoid B vaccine injected (Table 8). At the same time, significant differences were established between the titers of 7S and 19S H(b)-agglutinins,

TABLE 7. Antibody titers in human sera collected during mass campaign of vaccination^a

Antibody	Vaccine	Dose per injection ^b	Before immunization							After immunization								
			No. examined	Number of persons with sera in ranges of titers					Geometric mean titer	SE	No. examined	Number of persons with sera in ranges of titers					Geometric mean titer	SE
				< 1.00	1.00 to 1.67	1.75 to 1.97	2.05 to 2.27	2.35 and over				< 1.0	1.0 to 1.67	1.75 to 1.97	2.05 to 2.27	2.35 and over		
H(b)-agglutinins	V	120	28	24	1	3			0.98	0.06	47	8	2	18	7	12	1.86	0.09
	G	120	32	26	5		1		1.01	0.06	41	10	4	7	13	7	1.83	0.11
	Z-1	600	51	48	2	1			0.88	0.04	47	20	3	2	8	14	1.67	0.11
	Z-2	600	22	20	2				0.90	0.03	24	7	5	4	2	6	1.73	0.15
O-hem-agglutinins	V	120	40	1	3	9	16	11	2.07	0.06	46		2	7	9	28	2.31	0.06
	G	120	42	4	3	6	12	17	2.05	0.08	46		1	4	11	30	2.45	0.06
	Z-1	600	51	5	10	10	17	9	1.83	0.07	45		2	1	5	37	2.64	0.07
	Z-2	600	22	1	3	9	6	3	1.86	0.08	22			1	1	20	2.75	0.08

^a Expressed in logarithmic values.

^b Expressed as millions of paratyphoid B bacterial cells.

TABLE 8. Antibody titer (7S and 19S) in human sera as related to immunization with paratyphoid B monovaccine^a

Antibody	Dose per injection ^b	No. examined	Before immunization						After immunization							
			Number of persons with sera in ranges of titers:					Geo-metric mean titer	SE	Number of persons with sera in ranges of titers					Geo-metric mean titer	SE
			< 1.00	1.00 to 1.67	1.75 to 1.97	2.05 to 2.27	2.35 and over			< 1.0	1.00 to 1.67	1.75 to 1.97	2.05 to 2.27	2.35 and over		
H(b) agglutinins 7S	125	70	65	4	1			0.90	0.02	43	12	6	7	2	1.22	0.06
	500	144	131	9	2	2		0.95	0.04	49	28	22	29	16	1.59	0.08
7S and 19S	125	70	50	13	7			1.05	0.04	15	10	25	12	8	1.68	0.06
	500	144	88	30	13	9	4	1.20	0.06	8	16	39	41	40	2.03	0.06
O hemagglutinins 7S	125	70	54	11	4	1		1.03	0.04	48	11	6	4	2	1.16	0.06
	500	144	121	13	5	4	1	0.98	0.04	91	23	22	7	1	1.17	0.05
7S and 19S	125	70	1	4	14	11	40	2.42	0.08		3	7	10	50	2.60	0.07
	500	144	3	8	21	29	83	2.39	0.06		3	4	24	113	2.65	0.06

^a Expressed in logarithmic values.

^b Expressed in millions of bacterial cells.

which were higher in the subgroup inoculated with the dose of 500×10^6 paratyphoid B organisms than in the subgroup inoculated with 125×10^6 organisms.

Thus, a correlation was found between the intensity of H(b)-agglutinin formation in man and the dose of paratyphoid B vaccine.

Passive mouse protection with human blood sera. In one series of experiments, a study was made of sera obtained in field trials (Table 9), and significant differences were detected in protective activity as between human beings immunized with effective (Z-1 and Z-2) and ineffective (G and V) vaccines. In the second series, in which the sera of those individuals simultaneously inoculated with different doses of paratyphoid B monovalent vaccine were investigated, a higher activity was detected in those receiving a greater (effective) dose of the vaccine (Table 10).

Thus, a direct correlation was found between the effectiveness of vaccines and the mouse-protective activity of sera from inoculated humans.

DISCUSSION

Comparison of data on the pyrogenic capacity of paratyphoid B vaccines for man with results of study of the toxicity of the preparations injected intraperitoneally into white mice revealed no correlation. At the same time, results of study of the stress effect of paratyphoid B vaccines (comparative determination of 17-oxycorticosteroid concentrations) on guinea-pigs were very close to data on the pyrogenic capacity of the vaccines for man. Thus, the test for determining the stress activity in experiments on guinea pigs seems to be promising for laboratory evaluation and prediction of pyrogenic power of paratyphoid B vaccines.

TABLE 9. *Mouse-protective properties of human sera before and after immunization with different vaccines (during mass campaign of vaccination)*

Vaccine	Dose per injection ^a	Before immunization						After immunization					
		Survival ratios based on doses ^b				Dose ^c		Survival ratios based on doses ^b				Dose ^c	
		0.025	0.0125	0.0062	0.0031	PD ₅₀	L _{max} ^d	0.025	0.0125	0.0062	0.0031	PD ₅₀	L _{max} ^d
						ml	ml					ml	ml
V	120	54/85	47/85	31/85	21/85	0.0101	0.0117 (0.0088)	55/80	52/80	32/80	36/80	0.0078	0.0090 (0.0067)
G	120	102/161	77/161	54/161	42/161	0.0109	0.0120 (0.0098)	115/161	103/161	79/161	58/161	0.0077	0.0084 (0.0069)
Z-1	600	135/207	98/207	67/207	47/207	0.0111	0.0121 (0.0101)	155/207	144/207	129/207	100/207	0.0060	0.0065 (0.0055)
Z-2	800	53/72	43/72	42/72	23/72	0.0075	0.0088 (0.0064)	57/72	56/72	53/72	35/73	0.0050	0.0058 (0.0048)

^a Expressed as millions of paratyphoid B bacterial cells.

^b Dosage is expressed in milliliters. Values represent the ratios of the number of mice that lived (numerator) to the number of mice in the experiment (denominator).

^c Values represent the median doses of the vaccines and their 95% confidence limits.

^d Values in parentheses represent L_{min}.

TABLE 10. *Mouse-protective properties of human sera before and after immunization with different doses of paratyphoid B vaccine*

Sera obtained	Dose per injection ^a	Survival ratios based on doses ^b				Dose ^c		
		0.025	0.0125	0.0062	0.0031	PD ₅₀	L _{max}	L _{min}
Before immunization	125	27/50	13/50	20/50	2/30	0.0147	0.0174	0.0144
	500	49/100	39/100	21/100	6/60	0.0155	0.0190	0.0127
After immunization	125	39/50	31/50	12/50	13/50	0.0095	0.0113	0.0080
	500	83/100	72/100	52/100	29/100	0.0069	0.0073	0.0061

^a Expressed as millions of bacterial cells.

^b Dosage is expressed in milliliters. Values represent the ratios of the number of mice that lived (numerator) to the number of mice in the experiment (denominator).

^c Values represent the median doses of the vaccines and their 95% confidence limits.

No correlation was found between the effectiveness of paratyphoid B vaccines for man and their immunogenicity in active mouse-protection tests, with single subcutaneous or intraperitoneal immunization of animals. Results of the present experiments support those reported (15, 16, 18) on the presence, in typhoid-paratyphoid B vaccines, of factors that alter the resistance of white mice immunized by the intraperitoneal route. Thus, the alcoholized divalent vaccine, which failed to protect human beings from paratyphoid B in field trials, was found to contain certain components that stimulated the immunogenic effect in experiments on mice. This circumstance made it extremely difficult to use the active mouse-protection test for comparative evaluation of paratyphoid B preparations administered intraperitoneally.

Study of antigenic activity of paratyphoid B vaccines, in experiments aimed at determining the dynamics of antibody formation in immunized human beings and animals, revealed a direct correlation between the vaccine dose and the intensity of response.

Thus, the immunization of human beings with paratyphoid B vaccines, in doses producing a marked protective effect in field trials, resulted in H-agglutinin titers that were significantly higher than with the injection of ineffective doses of these vaccines. In immunization of rabbits and white mice, the same difference in doses strongly affected the ability of vaccines to stimulate the formation of both H and O antibodies.

At the same time, the two vaccines effective for man gave different antibody titers in animals; also, no well-defined dependence was found between the intensity of antibody formation and the dose of paratyphoid B vaccines when sera from human beings vaccinated in different years were studied. Although the data obtained testify to the fact that this test is insufficiently sensitive to be used for evaluating the protective effectiveness of paratyphoid B preparations, it can be recommended for determining the antigenic activity of paratyphoid B vaccines.

The most promising test for determining the effectiveness of paratyphoid B vaccines appears to be the determination of the passive mouse-protective activity of blood sera of inoculated human beings. In this test, the best-defined correlation was noted between the protective effectiveness of vaccines and the activity of the sera studied. The protective activity of human sera inoculated with effective paratyphoid B vaccines was higher than that of sera from individuals inoculated with doses that proved ineffective in field trials. A correlation was found between the activity of individual sera in the passive mouse-

protection test and 19S O-hemagglutinin titers ($r = 0.51$, $m_r = 0.15$). The fact that there were substantial differences in the protective activity of human sera, whereas differences in O- and H-antibody titers were not consistent, suggests that there are some additional substances present in paratyphoid B vaccines responsible for their protective effectiveness for man.

ACKNOWLEDGMENTS

We are grateful to N. A. Oserezkovskij for his help in determining the stress activity of the vaccines and to A. K. Drosd for help in the work with volunteers.

LITERATURE CITED

1. Bencianova, T. G. 1967. A study of the antigenic structure of typhoid monovaccines by diffuse precipitation in gel. *Zh. Mikrobiol.* 3:51-56.
2. Bencianova, T. G. 1967. A study of the antigenic structure of a typhoid-paratyphoid B divaccines, tested in epidemiological trial no. 5, 1965. *Zh. Mikrobiol.* 12:43-47.
3. Cvjetanovic, B., and K. Uemura. 1965. The present status of field and laboratory studies of typhoid and paratyphoid vaccines with special reference to studies sponsored by W.H.O. *Bull. World Health Organ.* 32:29-36.
4. Deutsch, H. F., and J. J. Morton. 1957. Dissociation of human serum macroglobulins. *Science* 125:600-601.
5. Edsall, G., M. J. Carlson, S. B. Formal, and A. S. Benenson. 1959. Laboratory tests of typhoid vaccines used in a controlled field study. *Bull. World Health Organ.* 20:1017-1032.
6. Finney, D. 1962. *Probit analysis*. Cambridge Univ. Press, New York.
7. Hald, H. 1956. *Mathematical statistic with technical appendix*. Moscow.
8. Hejfec, L. B. 1965. Comparative study of typhoid vaccines in active mouse protection test, p. 107-146. *In* T. A. Nicolaeva (ed.), *Typhoid vaccines*, Medicina, Moscow.
9. Hejfec, L. B., L. A. Levina, M. L. Kuzminova, et al. 1968. Strictly controlled epidemiological trial to assess the prophylactic activity of heated paratyphoid B monovaccine. *Zh. Mikrobiol.* 2:113-119.
10. Hejfec, L. B., L. V. Salmin, M. Z. Leitman, et al. 1966. A controlled field trial and laboratory study of five typhoid vaccines in the USSR. *Bull. World Health Organ.* 34:321-339.
11. Ikic, D. 1956. Problems of standardisation and control of typhoid vaccines. *Atti Congr. Intern. Standard. Immunol.*, 2nd, Rome, p. 311-327, 421-427.
12. Kossova, A. K., and A. S. Nechaeva. 1956. Preparation of enteric antigens from culture, cultivated in broth under aeration, vol. 8, p. 215. *Trudy Instituta Mecnikova*, Moscow.
13. Levina, L. A. 1967. Dynamics of antibody formation in rabbits, immunized with paratyphoid B monovaccines and associated preparations. *Zh. Mikrobiol.* 8:84-92.
14. Luippold, G. F. 1945. A proposed typhoid immunogenic unit for evaluation of antityphoid immunising substances. *Am. J. Public Health* 53:153-158.
15. Melikova, E. N., S. V. Lesnjak, L. D. Peremitina, J. J. Lifanova, T. S. Sedova, W. S. Sezgeeva, and T. G. Bencianova. 1965. Comparative study of immunogenic properties of the typhoid vaccines, tested in the field trial no. 5, p. 40-50. *In* *Typhoid vaccines*, Medicina, Moscow.
16. Pittman, M., and H. J. Bohner. 1966. Laboratory assays of different types of field trial typhoid vaccines and relationship to efficacy in man. *J. Bacteriol.* 91:1713-1723.
17. Spaun, J. 1956. Determination of S. typhi O- and Vi-antibodies by haemagglutination. *Acta Pathol. Microbiol. Scand.* 39:469-480.

18. Spaun, J. 1964. Studies on the influence of route of immunization in the active mouse protection test using intraperitoneal challenge for potency assay of typhoid vaccines. *Bull. World Health Organ.* 31:793-798.
19. Spaun, J., and K. Uemura. 1964. International reference preparation of typhoid vaccines. A report on international collaborative laboratory studies. *Bull. World Health Organ.* 31:782-812.
20. Standfast, A. F. 1960. A report on the laboratory assays carried out at the Lister Institute of Preventive Medicine on the typhoid vaccines used in the field study in Yugoslavia. *Bull. World Health Organ.* 23:37-45.
21. Taylor, A. and T. G. Bencianova. 1968. Vi antigen content in typhoid vaccines used for the controlled field trials in 1961, in the USSR. *Przegląd Epidemiologiczny* 1:57-69.
22. Van der Waerden, B. L. 1960. *Mathematical statistik*. Inostrannja Literatura, Moscow.
23. Yudaev, N. A., and J. A. Pankov. 1958. Modification of Silber and Porter's method for determination of 17-oxy-corticosteroid content in blood. *Prob. Endokrinol.* 2:35-42.
24. Zamuchovskaja, A. N., Z. V. Razumova, V. M. Lavrovskaja, and N. I. Kovaleva. 1965. Preparation of typhoid vaccines for the field trial no. 5, p. 33-39. *In* L. Levina (ed.), *Typhoid vaccines*, Medicina, Moscow.