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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

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The results of the laboratory assays of the Yugoslav typhoid vaccines used in the field trials at Osijek are disappointing when compared with the results of the field trials.

The tests carried out at the Lister Institute in England, the Central Institute of Hygiene in Zagreb, Yugoslavia, and the Walter Reed Army Institute of Research in the USA indicate that, except for the agglutination tests for H antigen, there is little or no demonstrable difference in the potency of the two Yugoslav vaccines in either active immunization tests or passive protection tests.

Although only a relatively small number of assays were carried out using the intracerebral route of challenge, the results indicate that there is no advantage in this method over the more usual intraperitoneal route. Since there was a difference between the potency of the vaccines in the field trails, it must be concluded that the mouse is not a suitable animal for typhoid assay or that the proper way of testing the mouse has not yet been found. The great variation in detail in nominally identical tests made in different laboratories and the differences in the results emphasizes the essential importance of at least one common assay, identical in detail between collaborating laboratories, in a study of this kind.

In 1954 the World Health Organization asked the Lister Institute of Preventive Medicine to take part in the laboratory testing of certain vaccines to be used in a controlled field study at Osijek, Yugoslavia, in 1954-55, the results of which have been reported elsewhere (Cvjetanovič, 1957; Yugoslav Typhoid Commission, 1957). Two kinds of typhoid vaccine, one heat-killed and preserved with phenol and the other killed and preserved with alcohol, were used.

Laboratory tests were also carried out at the Central Institute of Hygiene, Zagreb, Yugoslavia, (Ikić, 1956) and at the Walter Reed Army Institute of Research, Washington, D.C., USA (Edsall et al., 1959). Subsequently, at the instigation of the WHO Expert Committee on Biological Standardization (1955), the Department of Biological Standardization of the Statens Seruminstitut at Copenhagen, then under the direction of Dr O. Maaløe, arranged a detailed collaborative laboratory investigation.

The fact that this laboratory investigation of the potency of typhoid vaccines was to be run in close conjunction with a controlled field study offered a unique opportunity in the testing of typhoid prophylactics.

Current experience with pertussis vaccines (Great Britain, Medical Research Council, 1956; Standfast, 1958) had shown the importance of close collaboration between the laboratory and the field, and the importance of the selection of the best test by the laboratory workers—that is, the laboratory test that reflected the field results, not the laboratory test that gave the most clear-cut and statistically elegant measure of laboratory potency.

At the Lister Institute the test of these Yugoslav vaccines was made the opportunity for an extensive examination. The potency tests carried out were of three kinds: (*a*) active immunization tests in mice, (*b*) agglutination tests for H, O and Vi agglutinins in sera from rabbits immunized with the test vaccines, and (*c*) passive protection tests in mice with the rabbit antisera. In addition to the two Yugoslav vaccines, Lister Institute reference vaccines were included in each assay, and in a few tests the American acetone-killed vaccine (see p. 1020 of Edsall et al., 1959). All vaccines were tested in each assay and the results of each test were therefore strictly comparable.

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The reference vaccines were included as a check on the test and the challenge; but the main consideration of all assays was the direct comparison of the Yugoslav phenolized vaccine with the Yugoslav alcoholized vaccine.

MATERIALS AND METHODS

Vaccines

Two shipments of vaccine prepared in Zagreb were received; the first on 18 July 1954 from Zagreb, and the second on 18 June 1955 from Zagreb via Copenhagen, where it had been diluted and rebottled. The original Zagreb vaccine was stated to contain 3×10^9 bacteria/ml.

Two Lister Institute reference vaccines were used, one a fluid heat-killed, phenol-preserved vaccine, the other an alcohol-treated vaccine. These two vaccines proved to be of almost identical potency in most tests and a single set of results is given in most of the tables.

Mice

White mice, strain Cl, weighing 15-19 g were used. For each test, either active or passive, 200 mice of the same age, weight and sex were distributed at random into cages containing 10 mice.

Active immunization tests in mice

There is no standardized routine for active immunization tests in mice, various methods being used in different laboratories, but in all the procedure is based on a graded series of immunizing doses and a constant challenge dose. In the assays reported in this paper all vaccines were given by the subcutaneous route in order to avoid using the same route for both vaccine and challenge. In each assay three or four graded doses of each vaccine were given in a standard volume of 0.5 ml. Two injections of each dose were given, 14 and 7 days before the intraperitoneal challenge in saline and a single injection of each dose was given before either the intraperitoneal challenge in mucin or the intracerebral challenge.

Four cages of unvaccinated mice from the same group were set aside for the titration of the challenge dose.

Three routes of challenge were used: (*a*) intraperitoneal in saline, (*b*) intraperitoneal in mucin, and (*c*) intracerebral in saline.

Rabbits

Pure-bred New Zealand Red male rabbits from the Institute's stock were used, of the same age and weight. They were used only if in a preliminary test their sera were negative at 1/4 for Vi and at 1/100 for H and O antibodies.

Preparation of antisera in rabbits

Two sets of antisera were prepared: (*a*) with the first shipment of vaccines received direct from Zagreb, and (*b*) with the second shipment received via Copenhagen.

(*a*) In the first series, four groups each of five rabbits were immunized according to the Institute schedule giving a first dose of 500×10^6 organisms followed by three doses of 1000×10^6 organisms at 7-day intervals. Each dose was made up in 4 ml of saline and given intravenously. Each rabbit was bled on the sixth day after each injection, and the sera from each bleeding were kept separate. A pool was prepared of equal volumes of serum from the last bleeding from each rabbit in the group.

(*b*) In the second series, a WHO schedule was used on four groups, each of five rabbits, which were given four doses of 50×10^6 organisms in 4 ml intravenously at 7-day intervals. Each rabbit was bled six days after each injection. A sample from each bleeding of each rabbit was kept separate, and a pool was prepared from equal volumes of serum from the last bleeding of each rabbit in the group.

Passive protection tests in mice

Three or four serial dilutions in saline of the pooled sera were injected subcutaneously in a volume of 0.5 ml into groups of 10 mice. Groups of mice were challenged four hours later by each of the three challenge methods.

Intraperitoneal challenge in saline. A saline suspension of *Salmonella typhi*, strain Ty 2, was used. It was prepared from the growth from a slope of " double-strength " nutrient agar, made by adding 20 g Bacto nutrient broth (Difco Laboratories), 13 g New Zealand agar and 8.5 g sodium chloride per litre, incubated for 6 hours at 35° C, and diluted in saline to contain 100×10^6 organisms per ml. A challenge dose of 50×10^6 in 0.5 ml was used: this dose was invariably between 2 LD_{50} and 4 LD_{50}. The mice were observed for five days and the deaths and the survivors on each dose recorded. The Institute strain of Ty 2 obtained originally from Dr Felix was used. All the challenge suspensions agglutinated to titre with Vi serum and were inagglutinable by O serum.

Intraperitoneal challenge in mucin. The method used was that described by the United States Depart-

TABLE 1
PRELIMINARY MOUSE TESTS ON YUGOSLAV VACCINES: ACCUMULATED RESULTS OF SURVIVORS[a] AND PERCENTAGE SURVIVAL

Immunizing dose (millions)	Type of challenge	Yugoslav alcoholized vaccine		Yugoslav phenolized vaccine		Lister reference vaccine	
500	Intraperitoneal in saline	40/80	50 %	42/100	42 %	66/100	66 %
50		20/110	18 %	24/125	18 %	55/110	50 %
30	Intraperitoneal in mucin	68/95	72 %	73/96	76 %		
3		41/115	36 %	40/115	35 %	48/69	70 %
0.3						16/35	46 %
300	Intracerebral in saline	59/105	56 %	65/100	65 %		
30		36/105	34 %	35/99	35 %		
100						47/74	64 %
10						38/80	48 %

[a] Survivors/total number of mice.

ment of Health, Education, and Welfare, National Institutes of Health (1953), except that the vaccines were given subcutaneously, and the challenge was on the seventh day after immunization, not on the sixth day. A suspension of *Salmonella typhi*, strain T 63, in 0.5 ml sterile 5% hog gastric mucin in saline was used for the challenge. Strain T 63, supplied through the kindness of the Director of the Laboratory of Biologic Control, National Institutes of Health, Washington, D.C., USA, was O-agglutinable and so differed from strain Ty 2. The challenge suspension was grown for six hours at 35° C on "double-strength" nutrient agar. The challenge dose was 1000 organisms and varied between 5 LD_{50} and 37 LD_{50}.

Intracerebral challenge in saline. The method of Norton & Dingle (1935) was used. *Salmonella typhi*, strain 4904—a Vi-agglutinable, O-inagglutinable strain of high intracerebral virulence—was used for the challenge. The challenge suspension was grown for six hours at 35° C on "double-strength" nutrient agar standardized, and diluted so that 10 000 organisms, estimated by opacity, were contained in 0.03 ml. The mice were anaesthetized lightly with ether and the challenge was injected at a point midway between eye and ear and just lateral to the dorsal midline with a No. 27 needle on a 0.25-ml tuberculin syringe. The challenge varied between 14 LD_{50} and 135 LD_{50}. The mice were observed for 14 days.

Calculation of results. In these tests the ID_{50} for each vaccine, the PD_{50} for each antiserum and the LD_{50} for each challenge suspension were calculated by the Reed & Muench (1938) method.

Agglutination tests for H, O and Vi agglutinins

The geometric mean agglutination titre was calculated from several tests on each serum. All first-bleeding sera were tested together, all second-bleeding sera, etc.; at another time all the bleedings of one rabbit were tested simultaneously, so that although the results are given in titres and not in terms of a standard serum the results are strictly comparable.

Agglutinating suspensions and reference sera were obtained from the Standards Laboratory, Colindale, London, through the courtesy of the then Director, Colonel H. J. Bensted.

H agglutinations were read after two hours' incubation at 37° C, the O and Vi agglutinations after two hours' incubation at 55° C plus 20 hours at room temperature. Each test suspension was checked with H, O and Vi reference sera at each test.

RESULTS

Active immunization tests

The results of preliminary mouse tests with the Yugoslav vaccines and the Lister reference vaccines with the three types of challenge suggested that there was no great difference between the two Yugoslav vaccines, as will be seen from Table 1. These results

also indicate that the immunizing doses necessary to protect 50% of the mice against the different challenges are very different.

Table 2 shows the second series of active immunization tests in which each vaccine was put up at four dose levels and the ID_{50} accurately titrated. All these assays were on the first vaccine sample, received direct from Zagreb. The potency ratios and their fiducial limits of error for the Yugoslav vaccines in the three tests were 0.86 (0.53-1.39) for the intraperitoneal challenge in saline, 0.67 (0.28-1.62) for the intraperitoneal challenge in mucin, and 0.84 (0.3-2.35) for the intracerebral test. There was no demonstrable difference between the two vaccines by the active immunization tests.

TABLE 2

ACTIVE IMMUNIZATION TESTS OF MICE WITH YUGOSLAV TYPHOID VACCINES

Date of assay	Challenge		Vaccine ID_{50} (millions)		
	Route	LD_{50}	Yugoslav alcoholized [a]	Yugoslav phenolized [a]	Lister reference
2.2.55	Intraperitoneal in saline	4	200	103	48
10.2.55		4	307	266	76
3.3.55		3	200	100	41
20.9.55		2	114	122	33
4.10.55		2	146	192	29
11.10.55		2	147	192	13
	Geometric mean		176	152	35
16.2.55	Intraperitoneal in mucin	37	11	3.4	8
16.3.55		37	18	6.2	1.6
29.3.55		15	20	12	0.7
		22	13	8.4	1.0
27.9.55		5	3	4.3	0.8
18.10.55		5	1.6	2.6	0.3
	Geometric mean		8	5	1
30.3.55	Intracerebral in saline	31	72	32	26
28.7.55		100	54	150	50
21.9.55		10	14	8	5
28.9.55		15	40	28	18
12.10.55		14	54	35	11
19.10.55		135	54	60	45
	Geometric mean		43	36	20

[a] Both Yugoslav vaccines were from shipment No. 1.

Passive protection tests

Preliminary tests carried out at one dose level of individual rabbit sera before pooling suggested that, though there was little difference between the antisera to each of the vaccines demonstrable by the intraperitoneal test in saline, the Yugoslav alcoholized vaccine might be better than the Yugoslav phenolized by the challenge with mucin. The opposite appeared to be the case with the intracerebral challenge (Table 3).

These sera were therefore assayed at four dose levels and the PD_{50}'s calculated (Table 4). There was no difference between the two sera in the mucin test (No. 4 and 5). The intracerebral tests (No. 6 and 7) showed a rather smaller PD_{50} for the alcoholized serum than for the phenolized serum—the opposite of the preliminary tests. The differences in the assays fall within the expected experimental scatter and the single-dose-level test could not do more than give an indication of activity. Since it appeared to be impossible to distinguish these two sera, and, by implication the two vaccines, by passive protection tests, further tests of this sort were not made.

Agglutination tests

The results of the agglutination tests are shown in Table 5. In Series 1, the rabbits were immunized with vaccines from shipment 1, and in Series 2 with vaccines from shipment 2. The same batches of Lister reference vaccine were used in both series. Similar results were obtained in both series but the titres in Series 1 are in practically every case higher than those in Series 2. Each bleeding of each rabbit was kept separate and tested repeatedly.

The two Yugoslav vaccines differ little in Vi antigenicity, not at all in O antigenicity, but markedly in H antigenicity. In Series 1, which had the larger scheme of dosage, 2/5 rabbits responded with a low H titre after the third injection and 4/5 after the fourth injection. In Series 2, with the smaller dosage, 1/5 responded after three doses and 2/5 after the fourth dose, and these two gave a minimal response. With the Yugoslav phenolized vaccine 5/5 responded maximally with the big dosage. The H response to the low dosage was poor but all five rabbits reacted to the fourth dose of antigen. The response

TABLE 3

PRELIMINARY PASSIVE PROTECTION TESTS OF MICE WITH ANTISERA FROM RABBITS IMMUNIZED WITH YUGOSLAV VACCINES OF SHIPMENT NO. 1: ACCUMULATED RESULTS OF SURVIVORS[a] AND PERCENTAGE SURVIVAL

Type of challenge	Dose of serum (ml)	Serum from rabbits immunized with					
		Yugoslav alcoholized vaccine		Yugoslav phenolized vaccine		Lister reference vaccine	
Intraperitoneal in saline	0.4	51/250	20 %	39/280	14 %	126/310	41 %
Intraperitoneal in mucin	0.1	110/120	92 %	73/120	61 %	127/160	79 %
Intracerebral	0.05	37/80	46 %	60/80	75 %	65/110	59 %

[a] Survivors/total number of mice. All mice were given serum 4 hours before the challenge.

to the H antigen in the Yugoslav alcoholized vaccine was very much poorer than the response to the phenolized vaccine and this remains the main difference between these vaccines.

TABLE 4

PASSIVE PROTECTION TESTS OF MICE WITH ANTISERA FROM RABBITS IMMUNIZED WITH YUGOSLAV VACCINES OF SHIPMENT No. 2

Type of challenge	No. of test	PD_{50} (ml) of antisera from rabbits immunized with		
		Yugoslav alcoholized vaccine	Yugoslav phenolized vaccine	Lister reference vaccine
Intraperitoneal in saline	1	0.70	0.60	0.63
	2	0.40	0.28	0.50
	3	0.40	0.23	0.50
	Geometric mean	0.50	0.37	0.54
Intraperitoneal in mucin	4	0.052	0.055	0.034
	5	0.09	0.09	0.011
	Geometric mean	0.071	0.072	0.023
Intracerebral in saline	6	0.032	0.065	0.086
	7	0.043	0.053	0.057
	Geometric mean	0.037	0.059	0.072

The American acetone vaccine was included in Series 2 only and gave the following titres: Vi, 60; O, 5 600; H, 3 200. All rabbits responded to the acetone vaccine.

DISCUSSION

A comparison of the laboratory results obtained at the Lister Institute with those from Washington and Zagreb is interesting but somewhat confusing. First, it should be noted (Table 6) that though the three laboratories were nominally carrying out the same test, these tests differed in detail; the route of vaccination, the number of doses and the time between the last vaccination and challenge varied in each case.

The potency ratios with fiducial limits of error were calculated from the paired experiments of Edsall et al. (1959) and from the examples in this paper (Table 2). In the last column of Table 6 the fiducial limits are given as percentages, which suggest that the level of accuracy of testing in the two laboratories is similar. It is not possible to calculate the potency ratio and limits from Ikić's tables (1956), but we may assume a level of accuracy of the same order, or perhaps rather better as more mice were used for each vaccine. The chief difference in the results with the intraperitoneal challenge in saline between the three laboratories (Table 6) is the good result with the Yugoslav alcoholized vaccine at Washington and the good result with the phenolized vaccine at Zagreb. Ikić (1956), however, has pointed out that the Zagreb results, even if the tests which did not show a graded response to dose increase are discarded, are at the limit of statistical

TABLE 5

H, O, AND Vi AGGLUTINATION TESTS ON SERA OF RABBITS IMMUNIZED WITH YUGOSLAV VACCINES [a]

Agglutination	Bleeding	Yugoslav vaccine				Lister reference vaccine			
		Alcoholized		Phenolized		Alcoholized		Phenolized	
		Series 1 [b]	Series 2 [c]	Series 1 [b]	Series 2 [c]	Series 1 [b]	Series 2 [c]	Series 1 [b]	Series 2 [c]
H	1	0	0	0	0	0	1 600	2 000	300
	2	0	0	800	0	1 000	3 000	10 000	2 000
	3	300	200	6 000	800	8 000	3 000	30 000	6 000
	4	1 500	200	20 000	800	14 000	5 000	51 000	6 000
O	1	3 000	500	4 000	300	2 500	2 000	3 000	1 600
	2	10 000	3 000	12 000	3 000	7 000	3 000	5 000	3 000
	3	16 000	5 000	25 000	6 000	14 000	2 500	12 000	3 000
	4	25 000	6 000	25 000	6 000	14 000	5 000	8 000	5 000
Vi	1	15	20	20	15	0	10	0	15
	2	60	30	30	30	20	15	10	25
	3	120	30	100	30	60	30	60	20
	4	130	50	120	60	80	40	70	20

[a] Titres shown are arithmetic means.
[b] Series 1 = Rabbits immunized by Lister schedule.
[c] Series 2 = Rabbits immunized by WHO schedule.

TABLE 6

COMPARISON OF ACTIVE IMMUNIZATION TESTS OF THE SAME VACCINES IN THREE LABORATORIES

Challenge	Laboratory [a]	Route of Vaccine [b]	Approx. no. of mice used for each vaccine	No. of vaccinations	Time to challenge (days)	Mean ID_{50} (millions) from Ikić (1956), Edsall et al. (1959) and Table 2				Potency Ratio A: P	Fiducial limits of error	Fiducial limits as percentages
						American acetone vaccine	Yugoslav alcoholized vaccine (A)	Yugoslav phenolized vaccine (P)	Lister alcoholized vaccine			
Intraperitoneal in saline	L	Sc	250	2	7		176.0	152.0	35.0	1: 0.86	0.53-1.39	62 %-160 %
	W	Ip	600	1	6	1.3	12.7	36.7		1: 2.81	0.92- 8.62	33 %-310 %
	W	Ip	300	1	14	7.6	36.9	76.0		1: 2.06		
	Z	Sc	1 200	1	14		41.0	16.0	11.0	1: 0.39		
Intraperitoneal in mucin	L	Sc	250	1	7	0.94	7.9	5.4	0.9	1: 0.67	0.28- 1.62	42 %-240 %
	W	Ip	550	1	6	0.7	0.8	5.4		1: 6.6	3.02-14.45	46 %-220 %
	W	Ip	250	1	14	1.1	5.0	6.2		1: 1.25	0.59- 2.65	47 %-212 %
Intracerebral	L	Sc	250	1	7	20.0	43.0	36.2	20.0	1: 0.84	0.30- 2.35	36 %-280 %
	Z	Sc	540	1	14		23.0	22.0		1: 0.96		

[a] Laboratories: L = Lister Institute; W = Washington; Z = Zagreb. [b] Route of vaccine: Sc = subcutaneous; Ip = intraperitoneal.

TABLE 7

COMPARISON OF PASSIVE PROTECTION TESTS IN THREE LABORATORIES

Route of challenge [a]	Route of serum	Time after serum (hours)	Laboratory [b]	Approx. no. of mice used for each serum	Geometric mean PD_{50} (ml) of sera from rabbits immunized with		Potency Ratio A : P	Fiducial limits of error	Fiducial limits as percentages
					Yugoslav alcoholized vaccine (A)	Yugoslav phenolized vaccine (P)			
Ip	Sc	4	L	120	0.48	0.34	1: 0.71		
Ip	Ip	1	W	250	0.029	0.098	1 : 3.33	1.9 -5.82	57 %-175 %
Ip	Sc	4	Z	300	0.116	0.147	1: 1.27		
Ip	Sc	48	Z	550	0.117	0.228	1: 1.95		
IpM	Sc	4	L	80	0.071	0.072	1: 1.01		
IpM	Ip	1	W	550	0.00024	0.00018	1 : 0.74	0.32-1.73	43 %-234 %

[a] Route of challenge: Sc = subcutaneous; Ip = intraperitoneal; IpM = intraperitoneal in mucin.
[b] Laboratories: L = Lister Institute; W = Washington; Z = Zagreb.

significance. The same criticism can be made of the Washington results; those for the sixth-day challenge are at the limit of statistical significance and those for the fourteenth-day challenge are not significant. All one can say of these results is that there is a suggestion in the Washington figures that the alcoholized vaccine is better than the phenolized, the very opposite to the trend of the Zagreb results. There was no demonstrable difference between these two vaccines in the Lister Institute results (Table 2); on three occasions the alcoholized vaccine was better than the phenolized and on three occasions worse. No explanation can be offered for the much greater ID_{50} of the vaccines at the Lister Institute than at Zagreb, particularly as the larger ID_{50}'s were not produced when the challenge was given intraperitoneally in mucin or intracerebrally.

Rather strange results were obtained at the Lister Institute and Washington with the intraperitoneal challenge in mucin. With a challenge on the sixth or seventh day the results are similar at the two laboratories for American acetone and Yugoslav phenolized vaccines, but completely different for Yugoslav alcoholized vaccine. The results at Washington for the challenge on the fourteenth day resemble the results at the Lister Institute for the challenge on the seventh day. The marked superiority of the alcoholized vaccine in the tests with the challenge on the seventh day carried out at Washington may well be due to a transient nonspecific protective effect of the alcohol vaccines as suggested by Edsall et al. (1959); this effect was only obvious at Washington, where both vaccine and challenge were given by the same route, i.e., intraperitoneally.

It was in order to avoid such effects that vaccines were always given subcutaneously at the Lister Institute, although the subcutaneous route was known to be less efficient in that larger doses are necessary to achieve the same results. A mouse can survive 250×10^6 *Salmonella typhi* strain Ty 2 subcutaneously, while the intraperitoneal LD_{50} of the same suspension is about 10×10^6. These results underline the essential importance of at least one common assay identical in detail between collaborating laboratories in any study of this type.

The results from all three laboratories for the passive protection tests are shown in Table 7. The differences are obvious, but the most remarkable feature is the very small PD_{50} of all sera in the Washington tests with mucin, of a quite different order from that in the Lister Institute tests. This, of course, could indicate a real difference in the immunogenic power of the American sera, but this does not seem likely from the similarity of the mean agglutination titres shown in Table 8.

In view of the varied schemes of dosage (Table 9), there is a surprising uniformity in agglutination

TABLE 8
COMPARISON OF H, O, AND Vi AGGLUTINATING TITRES FROM THREE LABORATORIES

	Laboratory	Shipment [a]	Scheme of dosage [b]	Vaccine used for rabbit immunization				
				American acetone	Yugoslav alcoholized	Yugoslav phenolized	Lister alcoholized	Lister phenolized
H	Lister Institute	A	Lister		1 500	20 000	14 000	52 000
	,,	B	WHO 1st	3 200	200	800	5 000	6 000
	Washington	3	WHO2nd	1 280	160	640		
	,,	3	WHO 3rd	80	<80	160		
	,,	1	WRAIR		<80	640		
	,,	4	WRAIR	2 560	<80	2 560		
	Zagreb	—	Zagreb		2	19 500	8 300	
O	Lister Institute	A	Lister		25 000	25 000	14 000	8 000
	,,	B	WHO 1st	6 000	6 000	6 000	5 000	6 000
	Washington	3	WHO2nd	2 560	2 560	2 560		
	,,	3	WHO 3rd	1 280	2 560	2 560		
	,,	1	WRAIR		5 120	5 120		
	,,	4	WRAIR	10 240	10 240	5 120		
	Zagreb	—	Zagreb		7 800	23 000	7 100	
Vi	Lister Institute	A	Lister		130	120	80	70
	,,	B	WHO 1st	55	50	60	40	20
	Washington	3	WHO2nd	320	80	20		
	,,	3	WHO 3rd	40	40	<10		
	,,	1	WRAIR		80	40		
	,,	4	WRAIR	160	160	<10		
	Zagreb	—	Zagreb		52	8	28	

[a] Shipment Lister Institute B was the same as Washington 3 (see p. 1020 and Table 1 of Edsall et al. (1959) for details).
[b] See Table 9 for scheme of dosage. WRAIR = Walter Reed Army Institute of Research.

TABLE 9
SCHEDULES FOR RABBIT DOSAGE [a] USED IN THE THREE LABORATORIES

Days	0	4	7	14	21	28	35
Zagreb	500		1 000	Bleed			
Lister	500		1 000	1 000	1 000	Bleed	
WHO 1st	50		50	50	50	Bleed	
WHO 2nd	50		50	50	50	50	Bleed
WHO 3rd	0.5		0.5	0.5	0.5	0.5	Bleed
WRAIR [b]	1 500	1 500	3 000	Bleed			

[a] Millions of organisms in each dose given intravenously.
[b] Walter Reed Army Institute of Research.

results except for the considerably higher H titre obtained at the Lister Institute from the Yugoslav alcoholized vaccine with the Lister scheme of rabbit dosage. This relatively high figure was not obtained at Zagreb, although there was agreement between the H titres of the other vaccines at these two laboratories.

Although the low H antibodies elicited by the Yugoslav alcoholized vaccine compared with the high H antibodies elicited by the Yugoslav phenolized vaccines was the only uniform difference between these vaccines, the Yugoslav alcoholized vaccine may not have been quite so free of H antigen as appears at first sight. This supposition is strengthened by the results shown in Table IX of the report of the

Yugoslav Typhoid Commission (1957); 95.9% of 200 volunteers given the Yugoslav alcoholized vaccine produced H agglutinins with a geometric mean titre of 500.3 compared with 68.0% of the 200 controls, who had a geometric titre of 71.6.

Three points emerge from this collaborative laboratory assay:

(1) The active mouse protection tests with or without mucin will distinguish between certain vaccines (e.g., American acetone and Yugoslav phenolized) but not between others (Yugoslav alcoholized and Yugoslav phenolized).

(2) Man used as a test animal appeared to distinguish vaccines which the mouse cannot (Yugoslav alcoholized and Yugoslav phenolized). This may be because man is a much more sensitive test animal; if this is so, had the American acetone vaccine been tested in the field, it would have been still better than the tested vaccines. Or, it may be due to the fact that protection in the mouse depends on quite different factors from protection in man, and the test, as we understand it at the moment, is not even measuring the immunizing antigens. A similar discrepancy was found in intranasal testing of pertussis vaccines. This test consistently arranged vaccines in an order of potency quite different from the order of potency indicated by field trials in children (Standfast, 1958).

(3) It is hoped that the field trail at Osijek will be only the first of a series of trials in which there will be the closest co-operation between the various laboratories. On this occasion the Vi antigen has failed in the field and the laboratory assay has not proved of any value; we must now find a laboratory test, practical, consistent and reproducible, which can be correlated with the field results.

RÉSUMÉ

Les essais pratiques, strictement contrôlés, de vaccination contre la fièvre typhoïde effectués en Yougoslavie en 1954-55 ont été les premiers d'une série d'études destinées à comparer le pouvoir protecteur de divers vaccins, et à mettre au point un test de laboratoire permettant d'évaluer sur l'animal l'activité potentielle d'un vaccin sur l'homme. Une question connexe, qui s'est posée dès le début des recherches, est celle du rôle respectif des antigènes H, O et Vi de *Salmonella typhi*.

Cet article expose les résultats obtenus au Lister Institute, comparés à ceux du Central Institute of Hygiene de Zagreb et du Walter Reed Army Institute of Research, aux Etats-Unis d'Amérique, chargés d'essais comparatifs de vaccins antityphoïdiques. Il ressort de ces essais que la souris, proposée comme animal d'expérience en raison de sa sensibilité dans les tests de virulence, ne se prête pas à l'évaluation des vaccins, ou du moins, on n'a pas encore découvert une technique appropriée. L'homme établit, par ses réactions immunologiques, une distinction entre certains vaccins, à laquelle la souris est insensible, ou bien faut-il admettre que la protection de l'homme dépend d'autres facteurs que celle de la souris. Il semble que le test sur la souris tel qu'il est conçu actuellement ne mesure pas l'antigène essentiel au processus d'immunisation de l'homme.

On a utilisé pour ces essais des vaccins alcoolisés, phénolés ou acétonés, en suspension soit dans le soluté salin soit dans la mucine. On a procédé à l'immunisation active de la souris, par voie intrapéritonéale et intracérébrale, et à son immunisation passive par du sérum préparé sur le lapin. Les tests d'agglutination ont montré que les vaccins alcoolisés et les vaccins phénolés différaient fortement par leur antigénicité H, beaucoup plus élevée dans le vaccin phénolé.

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