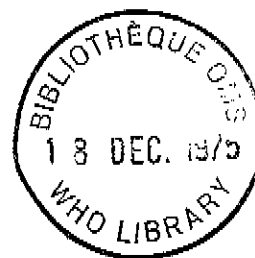


PRESENT STATUS OF
IMMUNIZATION AGAINST TYPHOID FEVER

by

Dr R. Germanier^a1. Introduction

The first vaccination of man against typhoid fever was performed some 80 years ago. In 1896 Wright³¹ inoculated two medical officers with killed typhoid bacilli. One of them was subsequently challenged with viable *Salmonella typhi*. No symptoms of illness occurred and the vaccine was considered to be protective. Similar studies were performed by Visokovich in the USSR. The numerous investigations that followed these initial studies, however, were not conclusive in demonstrating the protective value of similar typhoid vaccines (see reviews by Olitzki²¹ and Topley Wilson).³⁰ Doubts concerning the efficacy of vaccination against typhoid fever were corroborated by findings that even recovery from acute typhoid fever was not followed by an absolute protection against a second infection. Marmion¹⁹ noted a high incidence of recurrent cases of typhoid fever in British troops in the Middle East during World War II. Fifty-five men who had recovered from a first infection were exposed to *S. typhi* in a second epidemic 5 months later. Twenty percent of these men developed typhoid fever again, compared with an overall first disease attack-rate of 34%. Results obtained with human volunteers were similar.¹⁰ Twenty-two volunteers who developed typhoid fever after challenge with one ID₂₅ dose were rechallenged with an identical dose 2-12 months later. The attack rate was 23% compared to 30% in a previously unexposed control group. These findings, together with the fact that relapses occur in 10 to 20% of patients, indicate that immunity to typhoid fever is never absolute. Despite these observations numerous attempts were made to develop potent vaccines. Under WHO sponsorship, large-scale controlled field trials were carried out in Yugoslavia, Guyana, Poland and the USSR^{32, 27, 23, 15} which permitted a proper evaluation of these vaccines under field conditions. Studies with human volunteers yielded a further insight into the problems of immunity against typhoid fever.

2. Parenteral typhoid vaccines

One of the objectives of the above-mentioned field trials was to study the effectiveness of two typhoid vaccines that had been prepared on the basis of proposals by a WHO Expert Committee on Biological Standardization, namely an acetone-inactivated vaccine (code-letter K) and a heat-phenol-inactivated vaccine (code-letter L).^{8, 29} The results were summarized by Cvjetanović & Uemura⁶ and by Joo.¹⁸ As can be seen from Table 1, both vaccines conferred statistically significant protection. Vaccine K was slightly superior to vaccine L. Not only was the protection of a high degree but it was also longer lasting. For instance, in the Guyana trial the protection afforded by vaccine K was 93% during the first 3 years after vaccination and 81% in the following 4 years.

It was observed in the trials in Guyana and Poland that schoolchildren who received only a single dose of vaccine were as well protected as those who received two doses. Since the number of persons receiving only one dose was rather small this observation has to be interpreted with caution. Recently, in another trial carried out in Tonga with one and two doses of acetone-dried vaccine, it was demonstrated that primary immunization with two doses gave better and longer lasting immunity than a single dose.²⁶

^a Research Department, Swiss Serum and Vaccine Institute, 3001, Berne, Switzerland

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However, since all these trials were performed in areas where typhoid fever was endemic, it can be assumed that persons volunteering for the trials had previously undergone symptomless infections and thus had already acquired a certain degree of immunity, so that subsequent vaccination merely functioned as a booster. This would suggest that the observations in the various field trials are applicable only to persons living in endemic areas and that they can be applied only with caution to persons living in countries where typhoid fever is not endemic.

In addition to the two vaccines K and L, other types of typhoid vaccine were tested in field trials such as a whole-cell vaccine inactivated with alcohol and "chemical-type" vaccines consisting of extracted complete typhoid antigens or endotoxoid. None of these proved to be more effective than the K and L whole-cell vaccines.

The following conclusions can be drawn from these results:⁶ The acetone-inactivated vaccine (K) was found to be the best among the vaccines tested. The protection afforded was not only of a higher degree but it was also longer lasting. The heat-phenol-inactivated vaccine (L) was only slightly less effective than the vaccine K. The protection conferred by the alcohol-inactivated vaccine was lower than that of vaccines K and L. Some chemical-type vaccines composed of extracted complete typhoid antigens conferred relatively good protection when administered in high doses, while others did not. The efficacy of endotoxoid-type vaccines was low.

The efficacy of the vaccines was not improved by adjuvants.

The vaccines K and L and a purified Vi-antigen vaccine were also tested in human volunteers.¹⁶ Each received three doses, the first two with an interval of one week and the third dose a month after the second. The volunteers were challenged 3 months to one year after vaccination by oral application of viable *S. typhi* (Table 2). No protection was demonstrated against a high challenge dose (ID₁₀₀ and LD₅₀). However, when the challenge dose was reduced to one ID₂₅, 70% protection (average of vaccines K and L) became evident. No significant difference between vaccine K and vaccine L was noted. The vaccine prepared from purified Vi-antigen was significantly less protective.

It can be concluded from the results of all these trials that parenteral application of typhoid vaccines now in use gives good and long-lasting protection against typhoid fever. This protection, however, is far from being as complete as that produced by certain viral or toxoid vaccines. Furthermore, the immunity induced by the vaccines is probably completely overwhelmed by a large infective dose of *S. typhi*.

3. Oral typhoid vaccines

3.1 Killed vaccines

The concept of oral vaccination is not new. It dates from the fundamental studies of Besredka in 1919.¹ Oral, killed typhoid vaccines have been commercially available for at least 50 years. They were introduced for practical reasons such as simplicity of preparation, ease of administration, lack of side reactions, and absence of any hazards. Their efficacy has, however, always been questioned. Progress in fundamental knowledge concerning the local immune mechanisms of the gastro-intestinal tract and the success of oral poliomyelitis vaccine strongly stimulated re-examination of the problem of oral vaccination. The subject was recently reviewed by a WHO Scientific Group.²²

Serological studies in man have indicated that antibody production is stimulated by oral killed vaccines which thus can possibly give some protection.²⁸ In 1948, 29 000 persons living in a highly infected area were immunized with an oral killed vaccine by Raettig.²⁴ In the following year he noted a decrease in contact infections in this area and concluded that oral immunization was effective. Findings from such uncontrolled observations cannot be taken as conclusive. This is also applicable to one of the more recent studies of Borgoño et al.² in Chile. In children vaccinated orally with 3 tablets for 3 consecutive days (total

dose: 5.4×10^8 typhoid bacilli) and observed for a period of $10^{1/2}$ months, the incidence in the vaccinated group was 12.6% while the non-vaccinated children had an incidence of 79.2%. This high protection rate of about 75% achieved with an unusually low dose of vaccine was not confirmed in a subsequent strictly controlled field trial in the same country.

Controlled field trials with killed oral typhoid vaccines in large child populations have also been performed in India. In the first of 3 trials³ the vaccine, administered in 3 doses of one tablet, each containing 100×10^9 killed S. typhi, showed an effectiveness of around 24%. This marginally encouraging result was not confirmed in the second⁴ and third trials in which vaccine tablets containing 300×10^9 and 400×10^9 killed organisms respectively were given on 3 consecutive days. The characteristics of the vaccines used in the first and second field trials are summarized in Table 3. The vaccine used in the third trial was prepared according to the recommendations of a WHO informal consultation;³³ this same vaccine was also tried in Chile and no significant protection was noted in 7 months of follow up (J.M. Borgoño, personal communication).

Comparable results were obtained in human volunteers.¹⁰ The vaccine (Taboral, Swiss Serum and Vaccine Institute) used in the first field trial in India was assayed in adult males, who received 6 tablets containing 100×10^9 acetone-killed S. typhi. No protection was demonstrated. However, when the same vaccine was given in twice the former dose (12 tablets), a significant reduction in the attack rate was observed. From previous experiments with parenteral vaccines¹⁶ (Table 2) it can be postulated that if the volunteers had been challenged with a weaker dose (ID_{25}) an even higher degree of protection would have been demonstrated.

3.2 Live vaccines

The causative agent of typhoid fever, S. typhi, is pathogenic only for man with perhaps the exception of some non-human primates such as chimpanzees. Accordingly, experimental work has mostly been done in animal models with other Salmonella species. The most frequently used model was the mouse and S. typhimurium or S. enteritidis. It is generally accepted that vaccination with living, attenuated Salmonella produces a more effective immunity in mice than suspensions of the killed virulent strain when given parenterally. The superiority of attenuated live vaccines is particularly well demonstrated when they are administered orally.^{13, 5}

Various attenuated S. typhimurium strains have been tested in animal models.

In man, so far, only two attenuated S. typhi strains, a streptomycin-dependent strain and a galactose-epimerase-deficient mutant, have been studied.

3.3 Streptomycin-dependent S. typhi strains

The fact that a streptomycin-dependent strain of Shigella flexneri has been shown to be protective against dysentery caused by the homologous type has stimulated studies with similar mutants of Salmonella. A streptomycin-dependent strain of S. typhi was developed long ago by Reitman.²⁵ A derivative of this original strain (19V) designated 20SD has been shown in chimpanzees to be protective when administered daily together with streptomycin (7). Since the original streptomycin-dependent strain 19V was insufficiently stable, a one-step mutant derived from this strain, designated 27V, was developed and found to be safe, stable, and immunogenic when administered to mice.²⁵ This strain was safely given to adult volunteers in doses of up to 100×10^9 living cells. Challenge studies in volunteers vaccinated with this strain showed conflicting results. In the first study⁹ (Table 4), disease occurred in 13% of the persons vaccinated with freshly prepared vaccine as compared with 46% in the unvaccinated men (efficacy 71%). This degree of protection was not obtained in a second trial in which the volunteers were vaccinated with the identical strain preserved in lyophilized form.¹⁷ Further streptomycin-dependent strains have been developed by Mel et al.²⁰ and shown to be safe in volunteers.

3.4 Galactose-epimerase deficient *S. typhi*

The avirulence and protective capacity of *S. typhimurium* mutants lacking the enzyme UDP-4-galactose-epimerase (gal E mutants) have been studied in mice.¹² These gal E mutants were shown in animal tests to be superior to all other cell-wall mutants when used as live vaccines.¹¹ The outstanding protective capacity of such mutants was particularly well demonstrated when they were administered orally.¹³ The block in the enzyme UDP-4-galactose-epimerase prevents the normal synthesis of cell-wall lipopolysaccharide. However, *in vivo*, in the presence of exogenous galactose, complete smooth-type LPS is synthesized. This production distinguishes the gal E mutants from other rough mutants and provides them with an outstanding immunizing capacity. The avirulence of gal E mutants is due partially to the incomplete cell-wall LPS but chiefly to the strong bacterial lysis that follows the uptake of galactose and its cellular accumulation in the form of galactose-1-phosphate and UDP-galactose. Both the avirulence and the antigenicity of gal E mutants thus depends on the activity of all the enzymes required for galactose metabolism.¹⁰ On the basis of (a) the activity of these enzymes, (b) the distribution of the galactose within the cells, and (c) the sensitivity of the cells to galactose, assumptions can be made regarding the virulence and immunizing capacity of *Salmonella* gal E mutants.

Based on the results of previous studies with *S. typhimurium* mutants, various *S. typhi* gal E mutants were isolated and one strain designated Ty 21a was selected for further investigation.¹⁴ This mutant was found to be stable. A reversion to the wild type was not observed either *in vitro* after prolonged cultivation in highly selective galactose-containing media or *in vivo* following intraperitoneal inoculation into mice. The stability of strain Ty 21a was also confirmed by Dr E.S. Anderson,^a who examined it by different agglutination and phage-sensitivity tests.

Strain Ty 21a has been shown to be markedly less virulent for the mouse than the parent strain, the intraperitoneal LD₅₀ being above 10⁸ for both when the cells are suspended in saline and mucin. The reduced virulence of *S. typhi* Ty 21a was also demonstrated by following the bacterial counts in the liver and the spleen of intraperitoneally-infected animals (Fig. 1). The *S. typhi* Ty 21a cells were eliminated much faster than Ty 2 cells, even when the latter were given in a thousand-fold lower dose. Parenteral immunization of mice with live strain Ty 21a cells leads to a significant rise in O, H, and Vi antibodies. Such mice are protected against lethal doses of intraperitoneally- or intravenously-administered Ty 2 cells. However, from the previously-mentioned studies sponsored by WHO it is known that O, H, and Vi antibodies do not reflect immunity and accordingly cannot be used as a measure of the efficacy of a typhoid vaccine.

The safety of strain Ty 21a was demonstrated by oral administration in doses of up to 3 to 5 x 10¹⁰ in 220 adult volunteers in Baltimore, USA,¹⁷ in 36 volunteers in Berne (Switzerland), and in 370 children in Mexico City (B. Fernandez de Castro, personal communication) (Table 5). Stool examinations showed that approximately 10% of the vaccinated persons excreted the vaccine strain for 2 to 3 days following vaccination. In no case was a revertant found. Seroconversion was not very frequent (40%-H, 15%-O, 6%-Vi), but, as already mentioned, a seroconversion is not indicative of the potency of a typhoid vaccine.

The protective efficacy of *S. typhi* Ty 21a as a freshly prepared live oral typhoid vaccine was demonstrated by Hornick et al. in human volunteers.¹⁷ Adult males were given 6 to 8 oral doses of 3 to 5 x 10¹⁰ Ty 21a cells. Six weeks later they were challenged with virulent Ty 2 cells. This challenge provoked typhoid fever in 57% of the unvaccinated control group and in 8% of the vaccinated individuals. Thus, in this first trial the effectiveness of the vaccine was 86%. It was 55% in a subsequent identical second trial. In a third trial using galactose-grown Ty 21a cells the good results of the first trials were confirmed; the efficacy was 87%.

^a Director, Enteric Reference Laboratory, Colindale, London, (personal communication).

The need for further trials with lyophilized vaccine and with smaller and more practicable dosage schedules cannot be overemphasized.

It may be pointed out that the encouraging results with the multiple doses of oral live vaccine were seen when the challenge dose was ID₅₀ and not ID₂₅. In earlier studies a comparable protection after parenteral vaccination with killed vaccine was not demonstrated against such a high challenge dose. In field trials, however, the same parenteral vaccines proved to be effective. Thus oral vaccination with live attenuated *S. typhi* Ty 21a vaccine can be expected to be even more effective in the field. This hypothesis merits to be tested in controlled field trials.

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TABLE 1. EFFECTIVENESS OF PARENTERAL KILLED TYPHOID VACCINES IN VOLUNTEERS GIVEN TWO DOSES
FOUR WEEKS APART

Country and Reference	Date	Duration of observation (years)	Effectiveness %	
			Vaccine K acetone-inactivated	Vaccine L heat-phenol-inactivated
Yugoslavia (32)	1960-1963	3	79	51
Guyana (27)	1960-1963	3	93	77
	1963-1967	4	81	47
	1960-1967	7	88	65
Poland (23)	1961-1964	3	85	-
USSR (15)	1962-1963	1	-	73
	1962-1965	3	-	66

TABLE 2. EFFECTIVENESS OF VACCINES K, L AND Vi IN VOLUNTEERS
CHALLENGED WITH VARYING DOSES OF S. TYPHI

Vaccine	Challenge dose		
	10^9	10^7	10^5
K	2/3*	12/28 (43%)	4/43 (9%)
L	3/4	13/24 (54%)	3/45 (7%)
Vi	6/7	10/14 (71%)	2/13 (15%)
Control	4/4	15/30 (50%)	28/104 (27%)

*Cases of typhoid fever per number of persons challenged.

TABLE 3. CHARACTERISTICS OF ORAL KILLED VACCINES USED IN FIELD TRIALS

Manufacturer	Swiss Serum and Vaccine Institute	Behringwerke
Reference	Chuttani et al. ³	Chuttani et al. ⁴
Strain of <u>S. typhi</u>	Ty 2	Ty 58
Dose	3 x 100 x 10 ⁹	3 x 300 x 10 ⁹
Drying Method	Lyophilization	35°C Vacuum
Killing Method	acetone	acetone
Effectiveness	24%	0%

TABLE 4. CHARACTERISTICS OF LIVING TYPHOID VACCINE STRAINS

Strain designation	20SD / 27V / SmD	Ty 21a
Characteristic	Streptomycin-dependent	Lacks enzyme UDP-4-galactose-epimerase
Origin (ref.)	M. Reitman ²⁵	R. Germanier ¹⁴
Safety tests	chimpanzees (20SD) ⁷ adults (27V) ¹⁷ 1104 adults & 622 children (SmD) ²⁰	209 adults ¹⁷ 370 children (Mexico)
Efficacy of freshly prepared vaccine in human volunteers	71% (27V) ⁹	87% ¹⁷

TABLE 5. SAFETY AND EFFICACY TESTS WITH THE ORAL S. TYPHI Ty 21a VACCINE

Trial	Date	Number of Volunteers	Dosage (no. of live cells)	Reactions	Stool isolations		Seroconversions			Protection against LD50 challenge
					Vaccine Strain	Wild Strain	H	O	V1	
Univ. of Maryland, Baltimore	1973 Summer	43	6-8 doses ¹⁰ (3-5 x 10 ¹⁰)	12% diarrhoeas	24%	0	33%	12%	3%	86%
Swiss Serum & Vaccine Institute, Berne	1973 Autumn	8	(2 x 10 ⁷) to (2 x 10 ¹⁰)	none	-	-	-	-	-	-
Swiss Serum & Vaccine Institute, Berne (Biel)	1974 Spring	20	2 doses ⁸ (5 x 10 ⁸) and (5 x 10 ⁹)	none	10%	0	30%	25%	15%	-
Univ. of Maryland, Baltimore	1974 Summer	58	8 doses ¹⁰ (3-8 x 10 ¹⁰)	none	36%	0	60%	4%	0	55%
Institut National de Hygiene, Mexico	1974 Autumn	370 (Children)	3 doses ¹⁰ (1-2 x 10 ¹⁰)	light diarrhoeas as in control children	a few	0	-	-	-	-
Swiss Serum & Vaccine Institute, Berne	1975 Spring	8	(1 x 10 ⁹) and (2 x 10 ¹⁰)	none	-	-	-	-	-	-
Univ. of Maryland, Baltimore	1975	119	(3-5 x 10 ¹⁰)	none	19%	0	54%	15%	2%	87%

Fig. 1. GROWTH CURVES OF S. TYPHI Ty 2 AND Ty 21a IN LIVERS AND SPLEENS OF MICE AFTER INTRAPERITONEAL ADMINISTRATION OF 10^4 OR 10^8 CELLS

