Typhoid Fever Vaccine—Yes or No?

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Despite numerous evaluations of typhoid vaccine, no other commonly used biologic preventative has raised as much controversy regarding efficacy. Shortly after its introduction by Wright in 1897, it was tested in British troops engaged in the Boer War. This nondefinitive appraisal was the first of many field trials that failed to be conclusive. The incidence of typhoid fever declined steadily owing to clarification of the modes of infection and application of better health practices rather than to the effect of vaccines.

The accumulated evidence of vaccine effectiveness suffered from lack of application of proper controlled techniques. Appropriately, beginning in 1954, the World Health Organization has sponsored well planned field trials in various areas of the world. Field trials are accepted media for vaccine evaluation and are required for typhoid vaccine since the disease is uniquely human. Experimentally produced infections in chimpanzees have clarified some features of the pathogenesis of typhoid fever and of immune relationships. Unfortunately, typhoid bacilli provoke only mild enteric fever in these animals which does not mimic human typhoid fever. Other animals are not susceptible.

WHO-sponsored field trials were initiated in Yugoslavia, Poland, and British Guiana, and induced typhoid studies utilizing healthy inmate volunteers were begun in 1959 at the University of Maryland School of Medicine under the aegis of the Armed Forces Epidemiological Board. Full details of the volunteer studies are presented elsewhere.[5] This presentation summarizes the results of these two differing approaches to the controversial issue of vaccine prophylaxis of typhoid fever.

MICROBIAL AND HOST FACTORS PERTINENT TO PATHOGENESIS

Two factors pertinent to the efficacy of vaccine are the antigenic composition of Salmonella typhosa and those host factors that inhibit or

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Table 1. *Relationship of Number of Typhoid Bacilli (Quailes Strain) to Disease in Healthy Male Volunteers*

NUMBER OF ORGANISMS	NUMBER WITH DISEASE PER NUMBER CHALLENGED
10^9	40/42 (95%)
10^8	8/9 (89%)
10^7	16/32 (50%)
10^5	32/116 (28%)
10^3	0/14 (0%)

enhance spread of the infection. The heat-stable O antigen is in the bacterial cell wall. A rising antibody titer to this antigen is of diagnostic importance in acute infections. Many strains of S. typhosa contain a heat-labile Vi, or virulence, antigen, which presumably envelops the O antigen and may prevent agglutination of the organisms by O antiserum. The Vi antigen is important in pathogenesis of mouse salmonellosis and is regarded as a component necessary for inclusion in an effective vaccine. The flagellar or H antigen has been considered significant for inducing protective antibodies in man.

The volunteer studies showed that typhoid bacilli invade only through the gastrointestinal tract and that organisms survive in the stomach for 30 minutes in spite of low pH. The upper oropharynx and the respiratory tract are unlikely sites of initial invasion; infection does not occur after aerosolization of pathogenic bacilli.

Table 1 shows the number of organisms required to produce attack rates of 25 to 100 per cent in healthy adult males. The ID_{25} and ID_{50} infectious doses of S. typhosa were 10^5 (100,000) and 10^7 (10,000,000), respectively. These rates were determined in those volunteers with disease, which is defined as illness associated with oral temperature of 103° F. or greater for over 36 to 48 hours. Specific treatment was then initiated. Once illness occurred, the clinical courses were identical, regardless of the dose of the infectious inoculum. Only the incubation period varied with size of the infecting dose. There was inverse correlation: longer incubation periods occurred with the smaller doses.

The size of the infectious inoculum was always ascertained in the volunteer trials. In naturally acquired sporadic or epidemic infections, the epidemiologic data must provide this information, which is a conjectural estimation only. In water-borne outbreaks, a pure culture of typhoid bacilli is not ingested. Other bacteria, viruses, or chemicals might be ingested that either enhance or depress the virulence of S. typhosa. Such enhancement has been demonstrated in mice and possibly humans. Bohnhoff and co-workers[2] showed that intestinal flora protect mice from invasion of the intestinal wall by S. typhimurium. Mice pretreated with streptomycin show a rise in pH because of a striking reduction of bacteroides in the colon. Susceptibility is increased 10,000 fold. Volunteers pretreated with oral neomycin or streptomycin were infected with 1000 S. typhosa, whereas no infection occurred without antibiotic administration. Bacterial antagonism in the intestine may be a significant defense mechanism.

Once typhoid bacilli penetrate the intestinal epithelium, they reside intracellularly; this provides protection from effective antibiotic action. Infected mouse fibroblast tissue culture cells withstand the effect of

chloramphenicol in the bathing menstruum for as long as 21 days. Following removal of this antibiotic, the organisms again reproduce and are cytotoxic.[4] Pertinent to this point is the observation that human subjects given 3 gm. of chloramphenicol daily for seven days beginning 24 hours after challenge with an ID_{100} dose were not protected from disease; only the incubation period was prolonged. When the antibiotic regimen was extended to 28 days, there was no clinical illness but bacteremia and antibody formation were demonstrated at the same time that they were found in control subjects. Thus, effective chemotherapy for seven days will not prevent typhoid fever if the organism has had 24 hours to localize intracellularly.

ROLE OF HUMORAL ANTIBODIES IN TYPHOID IMMUNITY

There was no correlation in control subjects (nonvaccinated) between the prechallenge titers of O, H, and Vi antibodies and the subsequent clinical course. This was especially true of those volunteers without demonstrable antibodies at the time of challenge with the ID_{25} dose; 36 to 56 men without O antibodies failed to become ill. Nineteen of 38 men with negative H titers failed to manifest disease, whereas only three of 15 with negative Vi antibody titers developed typhoid fever. Obviously humans resist challenge in the absence of conventional humoral antibodies. A search for other circulating immune substances or additional cellular defense mechanisms in noninfected volunteers is warranted.

Immunity following acute typhoid fever is incomplete. Relapses occurred in about 10 per cent of patients before effective chemotherapy was available in spite of high titers of typhoid agglutinins. With antibiotic treatment, this rate has subsequently doubled. This suggests that chloramphenicol inhibits protein synthesis and subsequent production of protective antibody. There is no confirmatory evidence in man for or against this concept. Marmion studied two epidemics of typhoid fever occurring in a British Air Force camp in Egypt.[6] Fifty-four men became reinfected during the second epidemic, which occurred five months after the first. The attack rates were similar regardless of whether chloramphenicol was given in the initial outbreak.

Second episodes of illness in rechallenged volunteers correlated with the dose of typhoid bacilli (Table 1) employed. The role of chloramphenicol in depressing immune responses could not be accurately assessed since all volunteers were treated initially early in their disease. Reinfection did occur, suggesting that the host was unable to establish a high level of immunity consequent to the original infection.

RESULTS OF VACCINE TRIALS

It was anticipated that inactivated vaccines would not produce complete immunity since relapses and reinfections occur in both untreated and antibiotic-treated typhoid patients. Evidence from the World Health Organization trials supported this contention and the volunteer studies provided additional quantitative data.

The test vaccines were produced at WRAIR* from the standard

*Walter Reed Army Institute of Research, courtesy of Dr. Joseph Lowenthal.

Table 2. *Results of WHO Field Trials with Vaccines K and L—Two Doses**

COUNTRY AND YEARS	AGE AND COMPOSITION	VACCINE GROUPS†			EFFECTIVENESS‡	
		K	L	CONTROL	K	L
Yugoslavia 1960–1963	2–50 years Mainly children	16/5028	37/5068	75/5039	79%	51%
British Guiana 1960–1964	5–15 years School children	6/24046	26/23431	99/24241	94%	73%
Poland 1961–1963	5–14 years School children	4/81534		31/83734	87%	
USSR 1962–1963	School children and young adults		13/36112	50/36999		73%
	One Dose of Either Vaccine K or L					
British Guiana 1960–1964	5–15 years School children	0/3319	3/3371	14/3515	100%	78%
Poland	5–14 years	0/9136		3/10067	100%	–

*Modified from data of Cvjetanovic and Uemura.[3]

†Number of cases of typhoid per persons in trial.

‡Effectiveness $\frac{100\ (b - a)}{b}$; a = incidence in vaccinated group, b = incidence rate in controls.

Ty[2] strain. Two types of vaccines, designated K and L, were employed. K vaccine consisted of acetone-inactivated typhoid bacilli which preserved most of the Vi antigen. Vaccine L was produced by conventional heat-phenol inactivation which destroys much Vi antigen. Results of the field trials conducted in Yugoslavia and British Guiana are shown in Table 2.[1, 3] Vaccine K appeared to be more effective than vaccine L in protecting adults. In children, vaccine L was more immunogenic than in adults. Vaccine K again was best in children. These controlled field trials involved large population groups and were subjected to the double-blind technique. The protection reported in these groups did not wane during the three to four year follow-up and one dose of vaccine appeared to give as much protection as two.

In Pristina, Yugoslavia,[9] a high incidence of disease occurred from heavily contaminated water. Those persons *not* volunteering for the typhoid vaccine study had six times the attack rate of the tetanus toxoid vaccine control group (Table 3). These data suggested that those highly motivated persons who volunteered for the study were more concerned with preventive measures and were able to avoid typhoid fever in spite of their failure to receive specific vaccine (placebo group). Despite this artifact, some protection was afforded those persons who received typhoid vaccines.

The volunteer studies have permitted more definitive quantitative estimation of vaccine effectiveness. One strain obtained from a carrier was used, and the exact number of organisms required to infect and

the time of challenge were known. Volunteers received the same vaccines employed in the WHO trials. Each received three doses (rather than two), the first two at weekly intervals and the third a month after the second. Oral challenge of viable S. typhosa occurred three months to one year post vaccination. Initially the ID_{100} dose of one billion organisms was employed and no protection was noted, as shown in Table 4. Vaccine-induced resistance to an ID_{50} dose was not demonstrated. By contrast, the infecting ID_{25} dose differentiated the vaccinees from the control group. No difference between vaccine K or L was noted in this study involving adult males. A purified form of Vi antigen was given to a few volunteers who did not show any greater immunity than with the K or L vaccines. Analysis of the base line antibody titers of these vaccinees failed to show any correlation with subsequent immunity. There was no evidence that the course of typhoid fever was mitigated in those vaccinated volunteers who developed disease. The currently available vaccines established protection in volunteers with a vaccine effectiveness of 67 per cent but only against a low dose of typhoid bacilli, 10^5 (based on 27 per cent attack rate in the control group at this challenge level versus 9 per cent in vaccinees).

Table 3. *Typhoid Fever Among the Volunteers and Nonparticipants in a Controlled Field Trial in Pristina, Yugoslavia**

TYPE OF VACCINE	CASES OF TYPHOID NO. OF PERSONS	RATE PER 1000	EFFECTIVENESS
K	13/3346	3.9	70%
L	20/3386	5.9	53%
Control	43/3340	12.9	0
Total volunteers	76/10,072	7.5	
Nonparticipants	777/9500	81.8	

*Modified from data of Yugoslavia Typhoid Commission.[9]

Table 4. *Results of Vaccine K- and L-Induced Immunity in Volunteers Challenged with Varying Doses of S. typhosa*

	CHALLENGE DOSE		
VACCINE	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

DISCUSSION

Assuming that the results of controlled field trials and volunteer studies are comparable, one can speculate that infecting doses encountered in nature approximate 100,000 organisms, especially in water-borne outbreaks. Since this number of typhoid bacilli causes disease in the minority of exposed persons, perhaps the available vaccines enhance the resistance of this susceptible group so that fewer contract the disease.

Host resistance in infectious processes depends on the interaction of many factors. In typhoid fever the importance of the ecology of the gastrointestinal tract when salmonella are ingested may be of prime importance. The best supporting evidence is derived from the fact that 75 per cent and 50 per cent of unvaccinated control subjects who ingested 100,000 and ten million organisms, respectively, did not become ill. Since little or no evidence of infection occurred in these volunteers, it appeared that the ingested bacteria could not overcome the local defenses of the intestine and subsequently enter the systemic circulation. The mechanisms responsible for this inhibition require clarification. Possible contributory factors operative in the intestine are changes in pH, bacterial antagonism, lysozyme, the effect of colicins or similar substances, tissue and coproantibody, and phagocytosis by epithelial cells.

In some viral infections the presence of circulating antibodies is not necessarily equated with resistance; local antibodies in nasal secretions appear to be most important in protecting the respiratory tract from parainfluenza type I infection.[8] Similar immune substances may be operative in typhoid fever. These and other mechanisms may explain the paradox of the typhoid carrier in whose biliary and intestinal tracts there are large numbers of virulent organisms,[7] yet disease does not occur. Conceivably, children have a less well adapted homeostatic intestinal environment which makes them more susceptible to infection. With increasing age, repetitive nonspecific antigenic and immunochemical experiences may enhance the resistance of the bowel.

SUMMARY

The contribution of vaccines in stimulating host defense against typhoid fever is of minor significance. In certain susceptible populations, it lowers the incidence of disease. There is need for clarification of the mechanisms responsible for defects of acquired resistance in persons who are prone to the disease.

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