STUDY OF INDUCED TYPHOID FEVER IN MAN
I. EVALUATION OF VACCINE EFFECTIVENESS*

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TYPHOID fever is uniquely a human disease which is only partially reproduced in the chimpanzee. These primates develop less extensive lesions and mild, self-limiting typhoidal infections which do not require antibiotics for recovery. The lack of a suitable animal host has impeded the development of an understanding of pathogenesis and methods of therapy and control of this serious enteric disease. Because of this, typhoid vaccines have undergone numerous evaluations since their introduction over 60 years ago. Early British field trials implied that protection was afforded to vaccinees which subsequently led to routine administration to U. S. troops in 1913. Suspicion regarding the vaccine's usefulness arose during and after World War II because of outbreaks of typhoid fever in immunized troops. Unrecognized defects in the initial field trials may have led to unwarranted enthusiasm for use of vaccine. Most of these field trials were uncontrolled and were performed simultaneously with the application of environmental hygienic measures for the control of typhoid fever. Early in the 1950's plans were formulated by the World Health Organization to undertake extensive well-controlled field trials in areas of typhoid endemicity. These studies were conducted in Yugoslavia, British Guiana, Poland, and Russia.[1] These investigations showed that vaccines were protective, especially in children, regardless of whether one or two doses were given or the

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type of vaccine employed. The method of preparation of vaccines appeared to significantly alter subsequent protective value in adults. Acetone killed and dried vaccine, which preserves Vi antigen, was shown to be most effective in preventing adult infections. The realization of these variables, the lack of knowledge of the quantitative efficacy of typhoid vaccines, and the global incidence of the disease were prime considerations which prompted the study of typhoid fever in volunteers. Approval and guidance for these studies was provided by the Commission on Epidemiological Survey of the Armed Forces Epidemiological Board. Extensive prior experience with the naturally acquired disease, knowledge of the time of challenge, the dose of organisms employed, the opportunity for close clinical and laboratory monitoring, and the availability of effective chemotherapy gave us confidence that these studies could be conducted with minimal risk to volunteers.

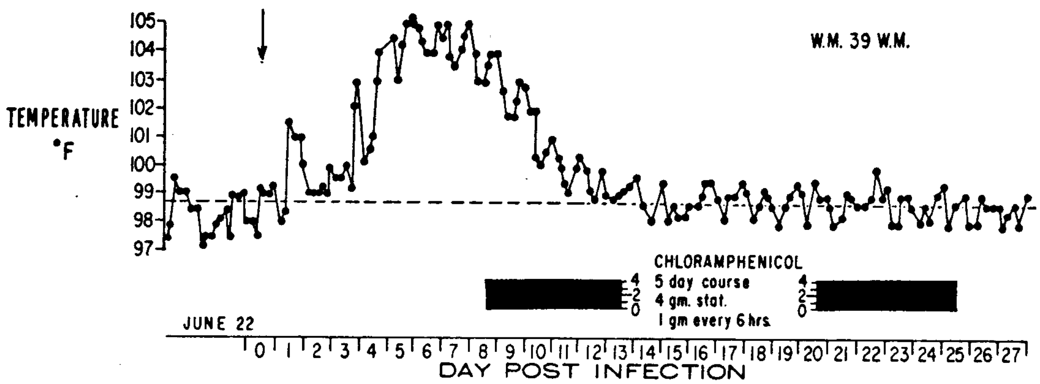
No serious complications, including the carrier state, have been encountered and all men have recovered fully. Volunteers were well informed, healthy adult inmates at the Maryland House of Correction, Jessup, Maryland. The strain of *Salmonella typhosa* used to infect was fully characterized and is pathogenic for man. It was obtained from a chronic carrier admitted to University of Maryland Hospital and is designated the Quailes strain. Following cholecystectomy for calculi, T-tube drainage provided the bile from which typhoid bacilli were isolated. The typhoid bacteria were incorporated in skimmed milk for storage at −70 degrees C which specimens served as the source for each challenge inoculum. The bacteria were transferred twice in liquid and solid media to insure uniformity of the challenge suspension. The number of typhoid bacilli used for challenge was estimated and 1.0 cc. aliquots were placed in 30 cc. of milk. Each volunteer gargled and then swallowed this contaminated inoculum. Subsequent plate counts verified the anticipated number of organisms in each inocula.

All stools were cultured; specimens of blood for culture were usually obtained daily from the fourth through the tenth post-challenge day. Chloramphenicol therapy was started when the temperature exceeded 103 degrees F for over 24 to 36 hours. Usually this was the third febrile day and the expected clinical manifestations of typhoid fever were present. These criteria were interpreted as indicating disease and only those volunteers with disease (that is, who required therapy) were used for analysis of the vaccine's effectiveness.

Initially the studies were designed to gain experience with the induced typhoid model and to determine the number of organisms necessary to produce disease with the infecting strain. The clinical

and laboratory features of induced typhoid fever were compatible with naturally acquired disease.

Figure 1 depicts the characteristic clinical course of a volunteer with typhoid fever. The severity of illness in those patients requiring specific treatment was identical once the fever reached 103 degrees F (as shown here) whether the initial challenge dose was low or high. It was generally not possible to differentiate the signs and symptoms whether infection was caused by 100,000 or one



Culture S. typhosa	BLOOD	0 0 + + + + + + 0 0 0
	STOOL	+ + + 0 + 0 + + + + 0 0 0 + 0 0 0 0 0 0 0 0 0 0 0
	BONE MARROW	0 0 0
Agglutinations S. typhosa	O	40 40 1280 1280 1280 1280 1280
	H	40 80 640 2560 2560 5120 5120
WBC (THOUSANDS)	WBC	10.9 11.0 11.85 9.65 5.4 5.7 5.5 4.55 3.5 5.75 4.1 9.55 9.05 8.0 6.05 5.1

FIG. 1.—The temperature course and pertinent laboratory findings in a patient with induced typhoid fever.

billion organisms. Incubation periods varied directly with the size of inoculum but not severity of disease. Bacteremia appeared with onset of fever; stool cultures were inconstantly positive and this variability appeared to be dose related. The higher the infecting dose the earlier the stool cultures became positive for *S. typhosa*. Occasionally typhoid bacilli persisted in asymptomatic patients longer than in treated patients, especially when the number of ingested organisms was small.

Therapy with chloramphenicol was uniformly associated with subjective improvement within 24 hours, definite beginning defervescence within 48 hours, and normal temperature in four to five days. Rose spots were rarely observed in view of early specific therapy. The most constant early physical finding was palpable gas-fluid filled loops of quiescent bowel. Leukopenia was a frequent finding which appeared at the height of the disease about the time antibiotic therapy was started.

The incidence of typhoid fever following the administration of varying numbers of organisms is shown in Figure 2. Accumulated cases are indicated for each dose of typhoid bacilli which ranged from one billion to one thousand organisms. At the upper end of the range, the attack rate was almost 100 per cent. Two men did not fulfill our criteria of clinical disease. For instance, one had prolonged bacteremia without fever, and the other had a self-limiting mild infection. Neither was treated. Following ingestion of 7 logs

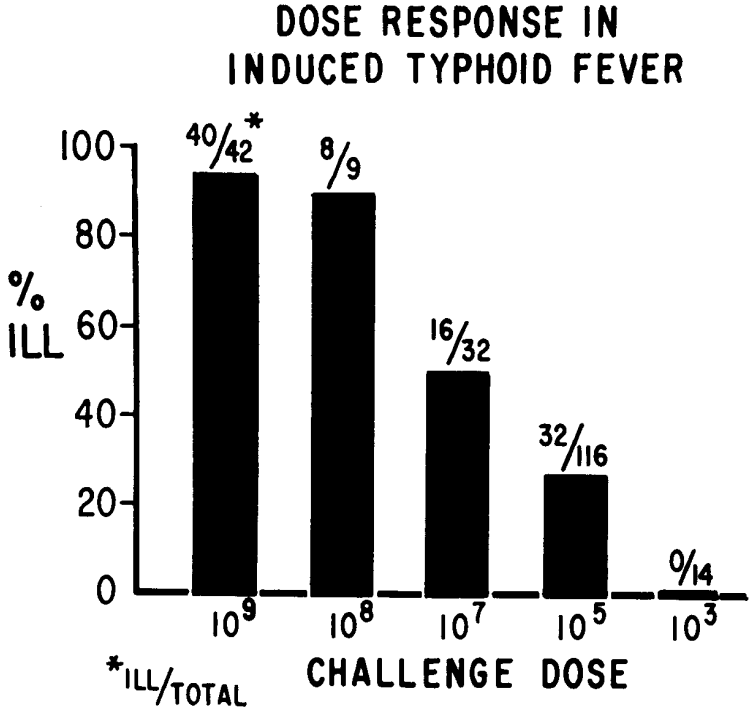


FIG. 2.—Incidence of typhoid fever in healthy volunteers following challenge with varying doses of the Quailes strain of *S. typhosa*.

or 10 million organisms, the attack rate was 50 per cent. In the majority of our vaccine trials either this ID_{50} dose or the ID_{25} dose of approximately 100,000 cells was employed.

In order to determine vaccine effectiveness, volunteers were randomly assigned to one of several vaccine groups. Two monovalent, whole organism vaccines were identical to those tested in the WHO field trials. These were intensively investigated for their ability to protect against induced disease. The vaccines, both derived from the same strain of typhoid Ty-2, were designated K and L and prepared at WRAIR. Vaccine K was prepared by killing and preserving the bacterial mass with acetone. Vaccine L was heat killed and phenol preserved, a preparation similar to the com-

mercial typhoid vaccine. Acetone does not remove much of the Vi antigen. Antibodies to this antigen have been shown to prevent infection in mice. Phenol inactivates most of the Vi antigen. Volunteers received three doses of these vaccines; the first two doses a week apart and the third one month after the second injection. Challenge with viable bacteria was performed three to eighteen months later.

Results of the challenge studies are depicted in Figure 3. Early in the trials, it was obvious that the one billion cell challenge was

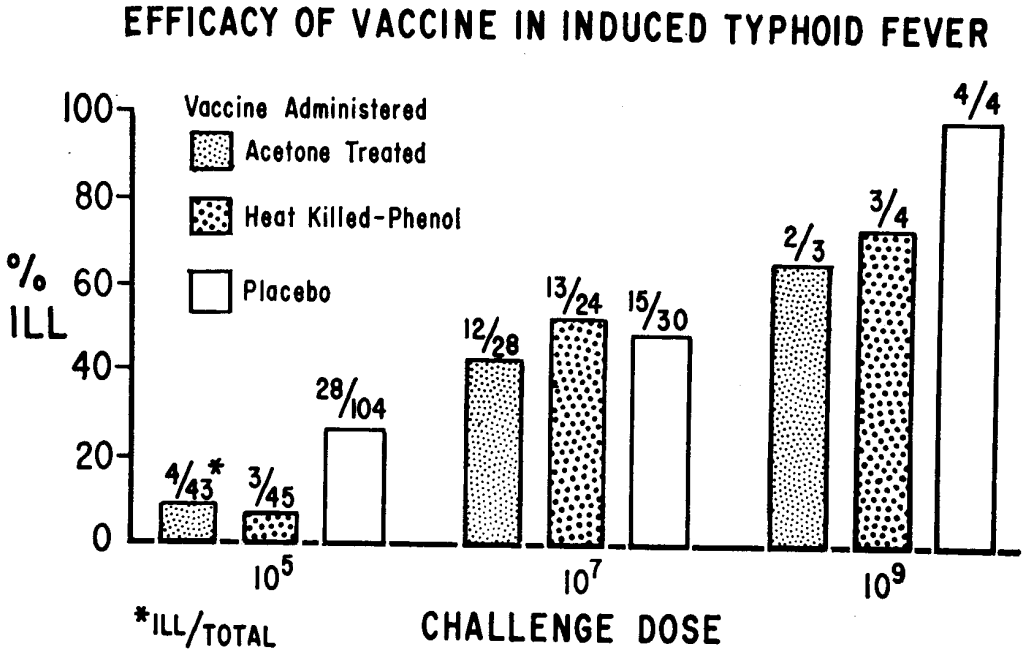


FIG. 3.—Incidence of disease in controls and vaccinees following challenge with varying doses of *S. typhosa*.

overwhelming and an ID_{50} dose was next employed. Our failure to show that either vaccine induced protection following this challenge prompted further reduction in the number of organisms administered. Sufficient evidence has now been accumulated from the study of 192 additional volunteers to indicate that protection to an ID_{25} dose was manifest by the majority of vaccinated volunteers. Results with acetone and phenol vaccines (K and L) were equally good. Purified Vi antigen has been used as a test vaccine. The results, not shown on this figure, have shown it to afford a level of protection similar to the other vaccines, K and L. This evidence, plus the failure to demonstrate superiority of vaccine K over vaccine L, indicates that the Vi antigen may not be of major importance in inducing protective antibodies in man. The exact protective antigen

of the typhoid bacillus remains to be identified. There was no correlation between resistance to disease and the presence of high titers of circulating agglutinins to O and H antigens. Indeed, further data are needed for clarification of those mechanisms involved in immunity to typhoid fever. Present evidence indicates that typhoid vaccines will generate resistance to small challenges of *S. typhosa*.

SUMMARY

Typhoid fever has been induced in volunteers to study the effectiveness of vaccines. Protection was afforded when healthy adult males were challenged with an ID_{25} dose of a pathogenic strain of *Salmonella typhosa*. No difference in efficacy was noted between two monovalent vaccines, one acetone killed and dried and the other heat phenol prepared. These investigations indicate that typhoid vaccines may be useful in preventing illness to small inocula in endemic areas which might follow water-borne exposure. However, epidemics, even in an immunized population, may occur when caused by large inocula such as in food-borne outbreaks or those due to breakdown in sewage disposal.

We gratefully acknowledge the willing participation and cooperation of the inmates of the Maryland House of Correction who have made these studies possible. Their eagerness to enroll in these and other studies attests to the acceptability of such investigations. Officials of the Maryland Penal System deserve special credit for their support and interest.

REFERENCE

1. CVJETANOVIC, B. and UEMURA, K.: The present status of field and laboratory studies of typhoid and paratyphoid vaccines. Bull. Wld. Hth. Org., *32*, 29–36, 1965.

DISCUSSION

DR. FRANCIS M. RACKEMANN (Boston): I would like to observe that in the treatment of individuals with vaccines, whether they be bacterial or protein extracts, there is a wide variation in the response of individual patients, not only in their immunity but in the local response to the individual doses.

I would like to ask Dr. Hornick whether he has observed any variation in immunity in accordance with the local reaction which the doses produce.

DR. HORNICK: The majority of the volunteers receiving the phenolized vaccine had the expected local and systemic reactions that you are talking about. This usually was most severe following the second and third doses. The acetone vaccine also initiated a high incidence of local reactions. We could not correlate local or systemic reactions to subsequent immunity.

DR. GEORGE GEE JACKSON (Chicago): In view of the fact that there is no relationship between the serum agglutinating antibodies and protection, I wonder if you have observations on whether or not there is maturation to a 7S antibody or whether or not these are all 19S antibodies throughout the postvaccine course.

Secondly, I am curious as to whether or not there is a difference in the protective value of the vaccine in relation to the time after vaccination. If I understood you, all of your rechallenge studies were conducted between 6 and 18 months, which is probably beyond the period of stimulation of factors which occurs with some of these vaccines, such as stimulation with the reticuloendothelial system which can give protection early, related to the antibody from the vaccine.

Can you see a difference in relation to the time after vaccination, and is there maturation from 19S to 7S antibodies?

DR. HORNICK: Dr. Jackson, we have no definitive data as yet on the type of antibodies present. These studies are currently underway.

We decided to use at least three to six months post vaccination as the time interval for challenge because of the evidence for prolonged protection noted in field trials. In addition, we have been unable to demonstrate any significant changes in RES activity or levels of serum complement following the administration of various forms of endotoxin. This would suggest that these parameters of resistance may not be altered by vaccine administration.

DR. WILLIAM B. BEAN (Iowa City): Why do you have these people gargle ahead of time?

DR. HORNICK: Dr. Bean, some suggestions have been made that because of the angiitis noticed in people early in the course of typhoid that one portal of entry may be the pharyngeal area. In order to ensure this area was exposed we had them gargle and swallow. Subsequent studies have shown that the pharynx is not an important site of penetration. It is not necessary to gargle.

DR. JOHNSON MCGUIRE (Cincinnati): At one time at the Walter Reed Hospital it was the thought that intracutaneous vaccination was more effective than subcutaneous vaccination. I wonder if in your opinion that is true.

DR. HORNICK: We have not used this route but there is evidence in the literature that at least agglutinin levels are equivalent by this route.

DR. FRANCIS C. WOOD (Philadelphia): I think possibly I ought to quote Dr. Austrian. When he first heard these results he said, "That's just what I have always said. You should be vaccinated when you are going to go into a typhoid area, but then you should act as though you hadn't been."

May I ask you one other question about the relative values of ampicillin and chloramphenicol in the treatment of typhoid fever?

DR. HORNICK: Yes, we have tried ampicillin against our particular strain and it doesn't work. The response is much delayed.