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Studies of Immunity in Typhoid Fever

Protection Induced by Killed Oral Antigens or by Primary Infection *

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Adult male volunteers were orally vaccinated with two "killed" antityphoid preparations. The recommended doses of both vaccines resulted in serum antibody development in only a few of the subjects. When the dose of the monovalent preparation (Taboral) was doubled, serological responses occurred more frequently, with a rise in O agglutinins in nearly one-fifth of the subjects, in H agglutinins in approximately one-fourth, and in Vi antibodies in nearly half.

When vaccinated volunteers were fed virulent typhoid organisms, disease occurred less frequently among those men vaccinated with Taboral at twice the recommended dose (38%) than among those not vaccinated (54%). This preparation did not confer protection at the recommended dose.

Volunteers who had previously recovered from an induced typhoid infection received a further challenge with virulent organisms. These persons developed typhoid fever less frequently (23%) than individuals without prior typhoid exposure (30%).

Immunity in typhoid fever is not clearly defined. Not only does the disease occur among well-vaccinated individuals (Benenson, 1964) but recurrences of clinical typhoid fever have been well documented (Marmion et al., 1953).

Two forms of cell suspensions of killed bacteria have been widely employed as antityphoid vaccines. The vaccines, an acetone-inactivated (K) and a heat-inactivated phenol-preserved (L) suspension of *Salmonella typhosa* (strain Ty2), are administered in the form of two or three subcutaneous or intramuscular injections. Cvjetanović & Uemura (1965) have summarized the results of various typhoid vaccine field trials conducted between 1954 and 1964. In a more recent field trial comparing the two vaccines, protection rates after 7 years were 88% for the acetone-killed and 65% for the heat-phenolized typhoid vaccine (Ashcroft et al., 1967). In the same study, the degree of protection showed little diminution until the fifth year.

Efficacy of these parenterally administered vaccine preparations has been quantified in adult male volunteers (Hornick et al., 1967). They were effective when the number of live virulent *S. typhosa* administered to vaccinated volunteers did not exceed an ID_{25} for the unvaccinated control group. The protection afforded by vaccines K and L in these volunteers following this low challenge dose was similar to that seen in various field trials. As a result of these studies, a better method of vaccination or else an improved vaccine seemed desirable.

Oral vaccination was one logical alternative. The oral route would probably minimize the local and systemic post-vaccinal reactions found with parenteral vaccines and might offer the additional advantage of direct stimulation of local intestinal immune mechanisms. The first line of defence against systemic invasion by typhoid bacilli must be the gastrointestinal tract. In many individuals the pathogen never multiplies after being swallowed. Also, as seen with typhoid carriers, intra-lumenal

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growth may occur without the development of disease: these carriers may excrete as many as 1000 million organisms per gram of faeces. Whether the ability of the gastrointestinal tract to prevent the development of systemic infection is a result of intestinal immune proteins (coproantibodies), inhibition by indigenous microflora, or other factors remains to be elucidated. However, it appears that local factors almost certainly play an important role in conferring protection against progressive disease. Through direct stimulation of intestinal immune factors, oral vaccination has been shown to be the most effective means of immunization against a variety of enteric pathogens (Sabin, 1957; Couch et al., 1963; Mel et al., 1968; Cvjetanović et al., 1970).

Preliminary work with an oral " killed " typhoid vaccine in children was encouraging in that humoral antibodies were produced and there were no post-vaccinal complications (Vlădoianu et al., 1965). The present report includes studies of the immunogenicity of two oral " killed " antityphoid preparations in man. Also reported is a study of the efficacy of one of the vaccines in which the frequency of induced clinical typhoid fever is compared among vaccinated and unvaccinated adult male volunteers. The protection afforded by oral vaccine is compared with that following a similar challenge study of men who had recovered from a previously induced typhoid infection.

MATERIALS AND METHODS

The first vaccine examined, Typhoral,[1] was an enteric-coated tablet containing 33×10^9 organisms of *S. typhosa* (strain Ty58) with equal numbers of paratyphoid A and B. During manufacture the organisms were harvested in a Sharples centrifuge and were inactivated in acetone. The suspension containing all three organisms was frozen and thawed 5 times, and then lyophilized, incorporated into tablets, and enteric-coated.

The second European oral vaccine, Taboral,[2] was a monovalent preparation each tablet of which contained 100×10^9 acetone-killed *S. typhosa* (strain Ty2) organisms and 20% sorbitol. The tablets were coated with Keratine and were manufactured by the Swiss Serum Vaccine Institute.

The two vaccines were administered to healthy, informed, adult male, volunteer inmates of the Maryland House of Correction.[3] Three Typhoral tablets were administered on 3 successive days (total 9 tablets per man) to 94 volunteers. A total of 43 men received 2 Taboral tablets a day for 3 days (total 6 tablets), while 29 additional volunteers received the monovalent Taboral preparation in a dose of 4 tablets per day for 3 days (total 12 tablets). Reactions to the vaccine were assessed by recording subjective complaints. Serum agglutinins were determined by standard techniques on weekly blood specimens obtained over 6 months.

Challenge studies were performed only with those volunteers who ingested the more concentrated, monovalent oral vaccine (Taboral). The challenge with 100 000 virulent *S. typhosa* (Quailes strain) was given, in a manner previously described (Hornick et al., 1967), 8–10 weeks after vaccination, to 35 volunteers who had previously received the lower Taboral dose (6 tablets) and to 21 men who had received the higher dose (12 tablets). Reactions were compared with those seen among 52 unvaccinated volunteers who received the same virulent challenge.

Twenty-two volunteers who had previously recovered from induced typhoid fever with positive blood and stool cultures and rising serum agglutinins received a second typhoid challenge. Within 12 months of the initial infection, they were fed 100 000 *S. typhosa* (Quailes strain) and their reactions were compared with those in 34 control volunteers who received the same oral inoculum

In each of the studies, once induced typhoid fever had developed, the volunteers were admitted to a special research ward maintained by the Division of Infectious Diseases, University of Maryland School of Medicine, appropriate antimicrobial therapy was begun, and the patient's clinical course was carefully monitored by a staff of nurses and physicians. Criteria for specific therapy included fever of 103°F (for over 36 hours) and clinical signs and symptoms of typhoid fever, including abdominal pain, constipation, and presence of rose spots. The diagnosis was confirmed by recovery and culture of *S. typhosa* from blood and stools and by rising titres of typhoid agglutinins. In this

[1] Manufactured and kindly furnished by Behringwerke, Marburg-Lahn, Federal Republic of Germany.

[2] Manufactured and kindly furnished by Swiss Serum Vaccine Institute, Berne, Switzerland.

[3] Each volunteer agreed to participate after the nature of the study had been explained. No coercion was used and each man was free to withdraw at any time. The conditions relating to volunteer studies outlined in the Declaration of Helsinki were adhered to in these studies. See also the Postscript on p. 672.

study, only those individuals who received antibiotic treatment were considered to have typhoid disease and only the data from these individuals were included in the analysis of vaccine- or disease-induced resistance.

RESULTS

Table 1 shows the serological response of volunteers vaccinated with Typhoral and of those vaccinated with the 2 dosage levels of Taboral. In general, the serum agglutinin titres did not show a 4-fold rise consistently when the vaccines were administered in the recommended dosage schedules. Only 3 of 94 men (3%) experienced a significant rise of flagellar or H-agglutinin following vaccination with Typhoral, while 12% and 17%, respectively, showed 4-fold rises of the O and Vi agglutinins. With twice the recommended dose of Taboral (12 tablets) serological response occurred more frequently (Table 1).

Table 1. Serological response following oral typhoid vaccine

Vaccine	No. of tablets	No. of volunteers	No. with 4-fold rise in serum agglutinin		
			O	H	Vi
Typhoral	9	94	11 (12%)	3 (3%)	16 (17%)
Taboral	6	43	2 (5%)	3 (7%)	3 (7%)
Taboral	12	29	5 (17%)	7 (24%)	14 (48%)

In these studies, no intestinal or systemic reactions were found among the volunteers following oral vaccination with either vaccine or dosage schedule.

Table 2 shows the frequency of development of typhoid fever following a virulent challenge among volunteers vaccinated with two dosage levels of Taboral as compared with that among unvaccinated controls. No evidence of protection against disease was seen when only 6 Taboral tablets were administered. Fewer stool isolations were positive for *S. typhosa* from adult males given 12 tablets and clinical typhoid fever was less frequent in this group than in the unvaccinated controls, although the difference in frequency of fever was not statistically significant. It may be that absence of pathogen multiplication is a measurable index of intestinal immunity following oral vaccination. The frequency of development of typhoid agglutinins following the virulent challenge did not differ between either vaccine group or its corresponding control group.

Table 2. Clinical and bacteriological findings among vaccinated volunteers and unvaccinated controls following ingestion of 100 000 virulent *S. typhosa*

Group	No. of volunteers	No. with positive stool isolation	No. with clinical typhoid fever	No. with relapse following therapy
Vaccinated with 6 Taboral tablets				
Vaccinees	35	21 (60%)	14 (40%)	3 (21%)
Controls	28	14 (50%)	12 (43%)	3 (25%)
Vaccinated with 12 Taboral tablets				
Vaccinees	21	7 (33%)	8 (38%)	0
Controls	24	15 (63%)	13 (54%)	2 (15%)

The data presented in Table 3 show that a 4-fold rise in serum agglutinins following oral vaccination could not be correlated with subsequent protection against virulent challenge. Note that the development of clinical typhoid fever could not be related to the presence or absence of prior humoral antibody development.

Table 3. Correlation of 4-fold serum agglutinin rise following vaccination with Taboral and typhoid fever development following virulent *S. typhosa* challenge

Serum agglutinin	No.	No. with clinical typhoid fever
4-fold rise		
O	7	3 (43%)
H	5	2 (40%)
Vi	11	4 (36%)
O, H, and Vi	2	1 (50%)
No agglutinin rise	34	13 (38%)

The results of the second challenge in volunteers who had fully recovered from clinical typhoid fever are given in Table 4. Following the ingestion

Table 4. Clinical findings among volunteers with prior induced typhoid fever and non-typhoid-exposed controls following ingestion of 100 000 virulent *S. typhosa*

Group	No. of volunteers	No. with clinical typhoid fever	No. with relapse following therapy
Prior disease	22	5 (23%)	0
Controls	34	11 (30%)	2 (18%)

of 100 000 virulent *S. typhosa* the attack rate among 22 volunteers who had the disease between 2 months and year previously was 23%, compared with a rate of 30% among 34 controls with no prior typhoid exposure.

DISCUSSION

The presence of typhoid organisms in the gastrointestinal tract does not ensure disease progression. These bacteria must enter the body from the intestinal lumen probably via the intestinal lymphoid system. In chimpanzees orally infected with *S. typhosa* there was early invasion of the intestinal epithelial lining but this site was cleared of bacilli within a short time; multiplication then occurred in the intestinal lymph follicles and draining mesenteric lymph nodes (Gaines, Sprinz et al., 1968). The gastrointestinal tract appears to act as a barrier to infection and is probably not essential to disease development. Gaines, Tully & Tigertt (1968) have shown that disease can be successfully established by intravenous or intramesenteric lymph node challenge in chimpanzees. In chimpanzees that had recovered from typhoid illness, after a second challenge bacterial multiplication and invasion in the intestine was suppressed and there was a less intense lymphoidal reaction (Gaines, Sprinz et al., 1968).

Because the major barrier to progressive disease appears to be the gastrointestinal tract, we have turned our attention to this organ and to local immune factors in relation to the development and evaluation of typhoid vaccines.

When two killed preparations were orally administered in the recommended doses to adult males in the present investigation, humoral antibody development resulted in only a small proportion of subjects. The absence of H antibodies following Typhoral vaccination was probably due to the sensitivity of the flagellar antigens to the action of digestive enzymes (Vlădoianu et al., 1965) and to the elimination or destruction of H antigen during centrifugation. That a higher percentage of individuals developed humoral antibody when the dose of Taboral was increased may have been due to increased absorption of typhoid antigen from the gastrointestinal tract.

The 12-tablet dose of Taboral (12×10^{11} killed organisms) may have conferred a limited degree of antityphoid immunity (Table 2) but this appeared to be unrelated to agglutinin development following vaccine administration. This is not surprising, however, as antibody titre cannot be correlated with immunity in typhoid fever and relapse is known to occur when the antibody level is at its peak (Hornick et al., 1967). With the small numbers of men employed in this study and the dose of virulent *S. typhosa* used, the incidence of typhoid fever among controls and vaccinees was not significantly different. Similar results have recently been obtained using Taboral in a field trial in India (Chuttani, 1971).

In the evaluation of typhoid vaccines, it is useful to compare their efficacy with the degree of immunity following recovery from the disease. It would be difficult to understand how an inactivated vaccine against typhoid fever could offer more protection than the disease itself. The present studies indicate that the immunity conferred by an attack of typhoid fever is not complete even when the infecting dose is approximately ID_{25-30} (Table 4). It is interesting to note that the passage of virulent organisms through the intestinal barrier did not necessarily immunize the individual. Perhaps in typhoid fever the organisms that reach the intestinal lymphoid system and the systemic circulation quickly do not stimulate locally produced antibodies, or perhaps the early administration of chloramphenicol in such patients interferes with the synthesis of protective antibodies (Ambrose & Coons, 1963). In the present study clinical typhoid fever did not appear to be as protective as parenterally administered commercial vaccine (Hornick et al., 1967). It is probable that host factors are of prime importance in susceptibility to typhoid bacilli and that those who develop disease following a low-dose inoculum represent the more susceptible individuals. Even following clinical infection, such a population may be more susceptible than most immunized persons

without prior experience of the disease. Also in a study of naturally occurring typhoid fever, only a moderate degree of protection was found when patients with confirmed typhoid fever were exposed a second time (Marmion et al., 1953).

As the use of various parenteral killed vaccines has given protection in field trials against naturally occurring typhoid fever, while only low immunity to a challenge dose was found among prison volunteers vaccinated with the same vaccines (Hornick et al., 1967), it may be that most outbreaks of naturally occurring disease result from the ingestion of low numbers of organisms. The disparity of vaccine efficacy in natural and experimental conditions could also be explained by the possibility of a low degree of immunity among populations who may have been exposed to typhoid antigens in the past. Vaccination of such individuals may thus represent a " booster " to this low degree of existing immunity.

RÉSUMÉ

ÉTUDE DE L'IMMUNITÉ ANTITYPHOÏDIQUE: PROTECTION CONFÉRÉE PAR L'ADMINISTRATION ORALE D'ANTIGÈNES TUÉS OU PAR UNE INFECTION PRIMAIRE

On a administré par la voie orale à des volontaires deux vaccins antityphoïdiques tués. Dans le sérum des sujets qui avaient reçu les doses recommandées on a observé une faible fréquence d'agglutinines antityphoïdiques, mais des anticorps humoraux sont apparus plus régulièrement lorsque l'une des préparations avait été administrée à dose supérieure. Parmi les volontaires qui avaient reçu du vaccin monovalent tué à base de *S. typhosa* (souche Ty2) et auxquels on avait ensuite fait ingérer 10^5 germes typhoïdiques virulents, la fréquence d'apparition de la maladie a été moindre que chez les sujets non vaccinés. Ces données donnent à penser que la vaccination orale par un antigène typhoïdique tué confère un certain degré d'immunité mais, compte tenu de la dose de contre-épreuve utilisée et du nombre des volontaires étudiés, la protection obtenue n'était pas statistiquement supérieure à celle qui a été observée chez les sujets non vaccinés.

Parmi les volontaires remis d'une fièvre typhoïde cliniquement induite, et qui ont été soumis à une contre-épreuve virulente à base de *S. typhosa*, la maladie n'a pas été beaucoup moins fréquente que chez les sujets indemnes de toute exposition antérieure à la typhoïde. L'immunité conférée par l'infection typhoïdique dans ce groupe expérimental est apparue inférieure à celle qui, d'après les études précédentes, résulte de la vaccination par des suspensions de cellules bactériennes tuées.

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POSTSCRIPT

All individuals who developed typhoid fever were promptly hospitalized and given chloramphenicol therapy. Close clinical and laboratory control was maintained on all volunteers, and all stools were collected and examined to ensure that no carrier states were induced. Patients were discharged from hospital only after the complete antibiotic schedule had been given. This study was part of a long-term investigation into the pathogenesis and prophylaxis of typhoid fever and during the 10 years that these studies have been in progress no serious complications have occurred and no carriers have been revealed.