Controlled field trial of a typhoid vaccine prepared with a nonmotile mutant of *Salmonella typhi* Ty2 *

M. H. WAHDAN,[1] J. E. SIPPEL,[2] I. A. MIKHAIL,[2] A. E. RAHKA,[1] E. S. ANDERSON,[3] H. A. SPARKS,[2] & B. CVJETANOVIĆ [4]

A controlled field trial was performed in Egypt to evaluate a whole cell typhoid vaccine prepared with a nonmotile mutant of S. typhi *Ty2 (TNM1) devoid of flagellar antigen. This vaccine did not elicit an H antibody response, but significant Vi and O agglutinin responses were observed. There were 34 typhoid cases among 21 063 six- to seven-year-old children who received the TNM1 vaccine, and 44 cases among 21 017 children in the control group who received tetanus toxoid. These results suggest that TNM1 vaccine does not provide protection against typhoid fever, and that H antigen may be an important component of an effective vaccine.*

Immunization by subcutaneous inoculation with killed whole *S. typhi* cells provides lasting protection against typhoid fever (1–6), even when administered in one dose (7).[a] However, because they stimulate the synthesis of O, H, and Vi antibodies, currently used typhoid vaccines reduce the reliability of the Widal test as a means of diagnosing enteric fever in vaccinated subjects.

A whole cell vaccine was prepared with strain TNM1, a nonmotile mutant of *S. typhi* strain Ty2 (8). This TNM1 vaccine produces high Vi and O titres in rabbits but, being devoid of flagellar antigen, does not induce the formation of H antibody (9). Therefore, this vaccine would theoretically provide the protection characteristic of whole cell vaccines without interfering with the Widal test for typhoid H agglutinin.

This is a report of a controlled field trial carried out in Alexandria, Egypt, from December 1972 to December 1973 to study the effectiveness of TNM1 vaccine.

MATERIALS AND METHODS

Population

Newly admitted schoolchildren, 6–7 years of age, were chosen for the trial for the following reasons: they are a susceptible group, normally and naturally exposed to the risk of infection with typhoid and tetanus; they had not been vaccinated against typhoid, since it is only after school admission that immunization against enteric fever is carried out in Egypt; and all cases of infectious disease among schoolchildren in Egypt are brought to the attention of the health authorities, so that all cases of typhoid fever in the trial population should be detected. The parents of the children involved were thoroughly informed of the nature of the trial, and their free and informed consent to vaccination was obtained.

Vaccine

The TNM1 vaccine, together with the tetanus toxoid that was used as the control, were prepared by the Lister Institute of Preventive Medicine, London, England. Both vaccines were colour-coded so that neither the field workers nor the vaccinated subjects knew which was being given. The TNM1 vaccine was acetone-killed and lyophilized and contained 2×10^9

* This study was conducted under the auspices of the Ministry of Public Health of the Arab Republic of Egypt and of the World Health Organization and was supported by grants from the World Health Organization and Project MR041.20,01,0261A26X of the US Navy Bureau of Medicine and Surgery. The opinions expressed herein are those of the authors and do not necessarily reflect the official views of the sponsoring authorities and organizations.

[1] High Institute of Public Health, Alexandria University, Egypt.

[2] NAMRU-3, Abbassia Fever Hospital, Cairo, Egypt.

[3] WHO Enteric Reference Centre, Central Public Health Laboratory, Colindale, London, England.

[4] Division of Communicable Diseases, World Health Organization, Geneva, Switzerland.

[a] See also this issue p. 75.

Table 1. Cases of typhoid among immunized children, February-December 1973

Vaccine	Number vaccinated			Typhoid cases			Attack rate [a] per 10 000
	males	females	total	culture	Widal	total	
TNM1							
2 doses	8 761	7 918	16 679	19	9	28	16.8
1 dose	2 310	2 074	4 384	4	2	6	13.7
total	11 071	9 992	21 063	23	11	34	16.1
control							
2 doses	8 786	7 864	16 650	14	20	34	20.4
1 dose	2 295	2 072	4 367	7	3	10	22.9
total	11 081	9 936	21 017	21	23	44	20.9
total	22 152	19 928	42 080	44	34	78	

[a] The difference in attack rate between the vaccinated and control groups was not statistically significant.

bacterial cells per ml. Both vaccines were air-freighted to Egypt in dry ice and stored at 4°C or below until use. The TNM1 vaccine was reconstituted in the laboratory on the morning of the day it was to be used. Subcutaneous vaccination was performed using 1-ml disposable syringes. The first dose was 0.2 ml and the second, 3 weeks later, was 0.4 ml, so that the total dose contained 1 200 million organisms.

The TNM1 vaccine and the tetanus toxoid were given to alternate classes of schoolchildren. Altogether, 42 080 children were vaccinated. Some children who were absent on the day of vaccination received only one dose (Table 1).

Identification of cases

Children admitted to hospital with suspected fever were checked for age and school. In addition, school and class absenteeism was inquired into by school health visitors, and any absence due to sickness was investigated. When a first year primary school child was reported as having suspected enteric fever, his school and class were identified and his card was obtained. His colour-code, date of vaccination, and doses received were then noted.

Subjects with clinical symptoms consistent with typhoid, and who either yielded *S. typhi* from blood, urine, or stool cultures or whose Widal tests were positive, were identified as cases of typhoid fever. A subject who had received TNM1 vaccine and who subsequently developed typhoid fever could be diagnosed simply by the presence of TH agglutinins.

RESULTS

Typhoid morbidity

Cases of typhoid among vaccinees are listed in Table 1. There was little difference in the sex distribution and numbers of individuals immunized in the TNM1 and control groups, an indication that the selection was random and that the two groups were comparable. Although there were more cases in the control group than in the TNM1 group, the difference was not significant ($P = 0.258$). Moreover, there were more bacteriologically confirmed cases in the TNM1 group.

Serology

A single 0.2-ml dose of TNM1 vaccine was administered to 55 nursing students after their free and informed consent had been obtained. A second dose was not given owing to the occurrence of systemic and local reactions in 14 of the students. Blood samples were obtained from each student on the day of immunization and after 3 weeks, 3 months, and 5 months. Sera were prepared and assayed for *S. typhi* agglutinins by the method of Anderson (9). One of the students who experienced an adverse reaction to the vaccine had initial O and H titres of 1–200 and presumably had recently been exposed to *Salmonella* antigen either as an infection or as vaccine. However, the average initial Vi, O, and H agglutinin titres in the students who reacted were about the same as those in the students who had no adverse reactions; the serologic responses in the two

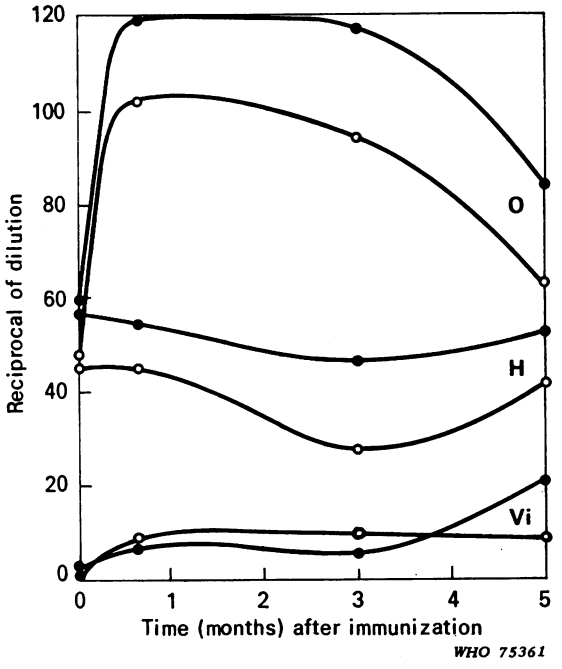

Fig. 1. Widal responses in nursing students. Those who showed adverse reactions are indicated by open circles, those who showed no reaction by closed circles.

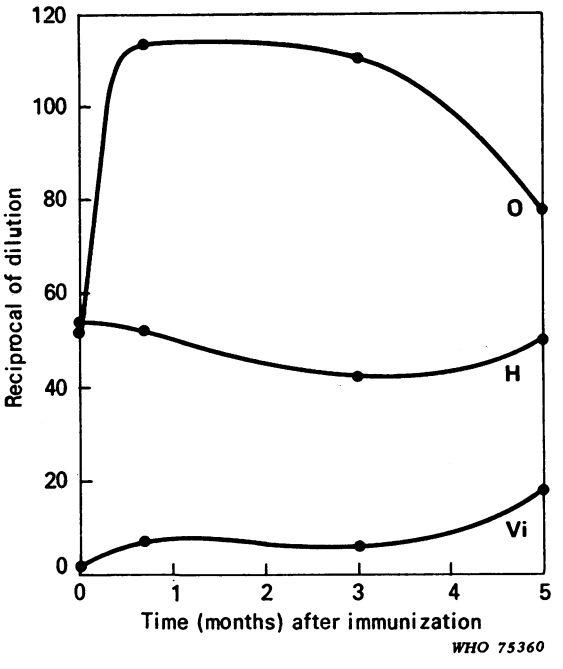

Fig. 2. Average Wida reactions in 55 nursing students.

groups were also similar (Fig. 1). The overall Vi and O response (Fig. 2) resembled that for the Ty2 vaccine reported by Benenson (10). Fifty-three of the students showed a rise in Vi titre and 51 a rise in O titre; all 55 students responded to at least one of these antigens. Only 4 students, however, showed a higher H antibody level.

Reaction after vaccination

Because of the reactions among the nursing students, it was decided to make an indirect estimation of reactions in the vaccinated children. The names of the children absent the day after vaccination were obtained from all schools. The rate of absence after the first dose was 7.4% among TNM1 vaccinees and 4.5% in the control group ($P = <0.001$). The corresponding rates after the second dose were 4.2% and 3.8% ($P = 0.290$).

DISCUSSION

The TNM1 vaccine is theoretically identical to other *S. typhi* whole cell vaccines prepared with the Ty2 strain, except that it is devoid of H antigen and therefore does not interfere with the Widal test for H antibody. Indeed, the TNM1 vaccine produced high levels of Vi and O antibody in the immunized nursing students, but no significant increase in H antibody level was detected. The vaccine preparation used was therefore antigenic, and should have provided immunity to typhoid fever if the H antibody were not involved in protection. However, the morbidity data gave no indication that the TNM1 vaccine protected against typhoid. These results support the thesis that H antibody plays a role in protection.

The laboratory data currently available indicate that flagellar antigen is not an essential component of effective typhoid vaccines (9, 11, 12). However, laboratory tests may not reliably reflect vaccine efficacy (3, 13, 14), since field trial data suggest that effective typhoid vaccines do elicit a good H antibody response.

Benenson (10) discussed agglutinin responses found in several field trials. When phenol- and alcohol-preserved Ty2 vaccines were compared in Yugoslavia, the phenol-preserved vaccine was significantly more protective (70% as against 26%). However, the only outstanding serologic difference between these vaccines was that the phenol-preserved preparation evoked a greater H agglutinin response.

Acetone-inactivated and dried Ty2 vaccine (K) and heat-inactivated and dried Ty2 vaccine (L) were compared in Guyana, where vaccine K produced 94% protection and vaccine L 71% protection (4), and in Yugoslavia, where they afforded 79% and 51% protection, respectively (15). The H agglutinin response was significantly greater with the K vaccine. There was no difference in response to the Vi antigen, and more O agglutinin was produced with the L vaccine.

Three vaccines prepared from the same Ty2 suspension were tested in Poland (2). Two were whole-cell suspensions: a formalin-killed phenol-preserved vaccine (N), and an acetone-killed and dried vaccine (P). The other vaccine (T) was an extract of bacterial antigens. Vaccine N provided 83% protection in children whereas vaccine T gave only 22% protection. In adults, vaccine N gave 43% and vaccine P 61% protection. The anti-H response with vaccine T was less than 5% of the anti-H response observed with vaccines P and N. The O and Vi responses with the 3 vaccines were, however, similar (10).

Hejfec et al. (3) tested four vaccines prepared from Ty2 in the USSR. Vaccine G, which was 86% effective, was a heat-killed phenol-preserved vaccine. Vaccine V was an alcohol-preserved dried vaccine and was 72% effective. Vaccines A and K were bacterial extracts and afforded 58% and 22% protection, respectively. There was an increase in O and Vi antibody titres in subjects immunized with each of the four vaccines, but increases in H antibody occurred only in individuals who received G or V vaccine, which also gave the greatest protection. However, vaccine A provided some protection without increasing H agglutinin titres in vaccinees.

The evidence currently available strongly suggests that there is a correlation between protection and high H antibody levels. The results of this study support this view. It is unlikely, however, that H antibody is itself responsible for protection, since human relapse has often been noted in patients with high H agglutinin titres. It seems more probable that a property other than the synthesis of flagellar antigen determines immunogenicity and is absent from this non-motile mutant.

RÉSUMÉ

ESSAI CONTRÔLÉ SUR LE TERRAIN D'UN VACCIN ANTITYPHOÏDIQUE PRÉPARÉ À PARTIR D'UN MUTANT NON MOBILE DE *SALMONELLA TYPHI* Ty2

Les auteurs ont procédé, en Egypte, à un essai contrôlé sur le terrain pour évaluer un vaccin antityphoïdique préparé à partir de cellules complètes d'un mutant non mobile de *Salmonella typhi* Ty2 (TNM1) ne possédant pas d'antigène flagellaire. Ce vaccin n'a pas suscité la production d'anticorps H, mais des réponses importantes en agglutinines Vi et O ont été observées. La population d'essai avait été répartie par tirage au sort en deux groupes analogues dont l'un a reçu le vaccin antityphoïdique et l'autre, servant de témoin, une injection d'anatoxine tétanique. Parmi les 21 063 écoliers de première année qui avaient reçu le vaccin TNM1, il s'est produit 34 cas de typhoïde contre 44 parmi les 21 017 enfants témoins auxquels avait été administrée l'anatoxine tétanique. La différence de morbidité entre les deux groupes n'est pas statistiquement significative. Ces résultats semblent indiquer que le vaccin TNM1 ne protège pas contre la typhoïde et que, pour être efficace, un vaccin antityphoïdique doit contenir l'antigène H.

REFERENCES

1. YUGOSLAV TYPHOID COMMISSION. A controlled field trial of the effectiveness of phenol and alcohol typhoid vaccines. *Bulletin of the World Health Organization*, **26**: 357-369 (1962).
2. POLISH TYPHOID COMMITTEE. Controlled field trials and laboratory studies on the effectiveness of typhoid vaccines in Poland, 1961-1964. *Bulletin of the World Health Organization*, **34**: 211-222 (1966).
3. HEJFEC, L. B. ET AL. A controlled field trial and laboratory study of five typhoid vaccines in the USSR. *Bulletin of the World Health Organization*, **34**: 321-339 (1966).
4. ASHCROFT, M. T. ET AL. A seven-year field trial of two typhoid vaccines in Guyana. *Lancet*, **2**: 1056-1063 (1967).
5. HEJFEC, L. B. Duration of postvaccination antityphoid immunity according to the results of strictly controlled field trials. *Journal of hygiene, epidemiol-*

ogy, microbiology and immunology, **13**: 154-165 (1969).

6. CVJETANOVIĆ, B. & UEMURA, K. The present status of field and laboratory studies of typhoid and paratyphoid vaccines with special reference to studies sponsored by the World Health Organization. *Bulletin of the World Health Organization*, **32**: 29-36 (1965).
7. HEJFEC, L. B. ET AL. Controlled field trials of paratyphoid B vaccine and evaluation of the effectiveness of a single administration of typhoid vaccine. *Bulletin of the World Health Organization*, **38**: 907-915 (1968).
8. ANDERSON, E. S. & GUNNELL, A. A suggestion for a new antityphoid vaccine. *Lancet*, **2**: 1196-1200 (1964).
9. ANDERSON, E. S. Proposed use of a non-motile variant of *Salmonella typhi* for the preparation of vaccine against typhoid fever. *In:* Proceedings of the International Symposium on Enterobacterial Vaccines, Berne, 1968. Basel, S. Karger, 1971, pp. 79-86. (*Symposia series in immunobiological standardization*, Vol. 15).
10. BENENSON, A. S. Serological responses of man to typhoid vaccines. *Bulletin of the World Health Organization*, **30**: 653-662 (1964).
11. TULLY, J. G. & GAINES, S. H antigen of *Salmonella typhosa*. *Journal of bacteriology*, **81**: 924-932 (1961).
12. TULLY, J. G. ET AL. Studies on infection and immunity in experimental typhoid fever. *Journal of infectious diseases*, **112**: 118-124 (1963).
13. RAŠKA, K. ET AL. A contribution to the laboratory assay of typhoid vaccines. *Bulletin of the World Health Organization*, **26**: 381-388 (1962).
14. SPAUN, J. & UEMURA, K. International reference preparations of typhoid vaccine. *Bulletin of the World Health Organization*, **31**: 761-791 (1964).
15. YUGOSLAV TYPHOID COMMISSION. A controlled field trial of the effectiveness of acetone-dried and inactivated and heat-phenol-inactivated typhoid vaccines in Yugoslavia. *Bulletin of the World Health Organization*, **30**: 623-630 (1964).