TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE, VOL. 74, NO. 5, 1980

## ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE

## Ordinary Meeting

**Manson House, 8th November, 1979**

*The President:* Dr. L. G. GOODWIN, *in the Chair*

# Volunteer studies of typhoid fever and vaccines

WILLIAM E. WOODWARD

*Division of Infectious Diseases, University of Maryland School of Medicine, Baltimore, Maryland, USA*

**Summary**

A series of studies evaluated the efficacy of three categories of typhoid vaccines: killed organisms given parenterally and orally and living attenuated mutants given orally. Vaccinees and unvaccinated controls were challenged with a single strain of virulent *Salmonella typhi*. Control individuals with prior military service (i.e. mandatory parenteral immunization) were significantly protected compared to non-veterans. Clinical protection of vaccinees was greatest (87%) following oral immunization with a mutant strain lacking epimerase.

## Introduction

In 1896, Sir A. E. Wright described the first known instance of typhoid immunization (WRIGHT, 1896), utilizing the method of Haffkine for cholera prophylaxis, in two British officers of the Indian Medical Service.

The following year WRIGHT & SEMPLE (1897) immunized an additional 11 men utilizing a 24-hour agar culture of typhoid bacilli emulsified in broth and heat-inactivated at 60°C for 5 min. Vaccine effects occurred in two to three hours with extensive local inflammation and later with the universal occurrence of fever, anorexia, nausea, and "faintness and collapse". Three individuals did not regain complete health for three weeks. Protective efficacy was demonstrable in vaccinated guinea-pigs, but immune sera did not inactivate virulent bacilli *in vitro*.

With this limited and somewhat ominous background, several thousand British soldiers in India and a further 100,000 in the Boer War were inoculated at the turn of the century (HAWLEY & SIMMONS 1934). In the latter instance, claims were made that vaccination led to a 50% reduction in disease incidence. But record keeping was poor, controls were lacking, and some insisted that the vaccine had actually increased the number of cases and deaths. (Undoubtedly, future generations will label our present evaluation of vaccine efficacy as suspect, due to a lack of rigorous methodology and analysis.)

Because of the controversy, vaccination of the British Army was temporarily halted and reinstituted in 1904 utilizing the Rawlins strain isolated from the spleen of a fatal case. The organism was selected for its alleged low toxicity to man and animal, its ability to stimulate circulating antibody, and its capacity to produce a uniform suspension in salt solution, one characteristic of smooth colonies. This strain was used continuously by the British Army and a substrain by the US Army in the production of parenteral vaccines.

Because of the confusion about results of parenteral vaccination during the Boer War, Lieutenant James Carroll, Director of Laboratories of the US Army Medical School, vaccinated volunteers in 1904 with a "killed" strain delivered orally (TIGERTT, 1959). The vaccine was prepared from the Dorset strain, isolated in 1898 from a fatal case and maintained on artificial media for six years.

A six-day broth culture of the organisms was heat-inactivated at 56°C for one hour, incubated for a further seven days at 36°C, sterility tested, stored in a refrigerator, and subsequently fed to 13 volunteers including Carroll himself. Ten (77%) men subsequently developed a disease compatible with typhoid fever after an average incubation period of 14 days (range 6 to 28 days). The actual incubation may have been shorter (average 10·5 days) since several men had received more than one dose of "vaccine" which was subsequently found to have been incompletely inactivated.

In view of the preceding unfortunate experiences, vaccination with killed organisms given by injection was delayed until 1911, at which time it became mandatory in the US Army and Navy. Again, vaccine efficacy was claimed, but analysis depended primarily upon a comparison with disease incidence in the unvaccinated civilian community. No military control population was available.

Any number of vaccine trials, utilizing parenteral and oral preparations, were conducted over the next half century. But it was not until the 1960s that the first controlled field trials were properly conducted (YUGOSLAV TYPHOID COMMISSION, 1964; POLISH TYPHOID COMMITTEE, 1965; HEFJEC *et al.*, 1966; ASHCROFT *et al.*, 1967). These studies of heat- and phenol-inactivated parenteral vaccines were carried out in hyperendemic areas in Russia,

Reprint Requests: Program in Infectious Diseases and Clinical Microbiology, University of Texas Health Science Center, Post Office Box 20708, Houston, Texas 77025, USA.

Yugoslavia, Poland and British Guyana. The most widely cited of these, conducted in Guyanese school children, demonstrated protection of 65 to 88% for up to seven years (ASHCROFT *et al.*, 1967). Since Guyana is hyperendemic with respect to typhoid fever, the investigation could not provide evidence as to vaccine effectiveness in a previously unexposed population.

## Materials and Methods

In 1959 members of the University of Maryland School of Medicine began a series of studies of experimental typhoid vaccines. Since no adequate laboratory or animal model was (or is) able to predict vaccine efficacy, the research was conducted in volunteers who received an immunizing agent and who, along with appropriate controls, were subsequently challenged with virulent typhoid bacilli. The design of the studies, which have been reported individually (DUPONT *et al.*, 1970, 1971a, 1971b; GILMAN *et al.*, 1977; GREISMAN *et al.*,1961; HORNICK & GREISMAN, 1978; HORNICK & WOODWARD, 1966; HORNICK *et al.*, 1967, 1970, 1971; LEVINE *et al.*, 1976, 1978; SPRINZ *et al.*, 1966; SNYDER *et al.*, 1964), was not to induce serious illness for the purpose of following its course but rather to evaluate the protection afforded by the experimental vaccine in question. If illness occurred, it was treated promptly and thoroughly. Over 16 years, 1,886 volunteers were vaccinated and 762 were challenged without a single life-threatening complication or death.

Volunteers were male inmates of the House of Correction, Jessup, Maryland. They were screened by thorough medical and laboratory examination and were fully informed as to the nature of the research on at least two occasions. The research protocol, approved in advance by multidisciplinary review committees, emphasized that volunteers could withdraw at any time.

The present report summarizes the entire volunteer experience with three categories of vaccines, inactivated preparations given parenterally and orally and living attenuated mutants given orally. All challenges were conducted with a single typhoid strain (Quailes) isolated in 1958 from a chronic civilian carrier. The strain was preserved at $-70°C$ and never developed resistance to antibioitcs.

## Results

Before evaluating various vaccines, it was necessary to establish a reliable human model of illness. Based on an earlier unpublished experience in which $6 \times 10^6$ organisms (not Quailes) had not caused disease, the earliest Jessup studies utilized $10^8$ to $10^9$ organisms. At this dose, 90% of control volunteers developed illness, obviously a much higher attack rate than ordinarily occurs under natural circumstances.

Lowering the dose to $10^6$ to $10^7$ bacteria resulted in 65% of men becoming ill. And at this challenge level, no protection could be demonstrated in volunteers who had been given the same vaccines utilized in the Guyanese field trials.

At the lowest dose used, $10^3$ organisms, no disease was caused in 12 recipients. Most subsequent studies employed a challenge inoculum of $10^5$ organisms, at which level disease occurred in 40% of more than 300 control volunteers. At this level, vaccine efficacy was demonstrable.

Challenges occurred from one and a half to nine months following vaccination. It was not possible to locate sufficient numbers of vaccinated volunteers beyond this interval so that an evaluation of long-term protection is not possible.

Detailed methods of preparation, dosage, and administration of the various vaccines have been published (HORNICK & WOODWARD, 1966; HORNICK *et al.*, 1967; DUPONT *et al.*, 1971a, 1971b; GILMAN *et al.*, 1977; HORNICK *et al.*, 1971; LEVINE *et al.*, 1976). The first studies employed two parenteral preparations, one acetone-inactivated and the other phenol-heat-inactivated. These had been used in the Guyanese studies and are equivalent to what is available commercially. Given in one to three doses, these vaccines induced serum antibody levels to H, O, and Vi antigens that were four to 20-fold greater than in control individuals. The protective effect for the two vaccines amounted to 67% and 75%, respectively. Both results were significant statistically, $p = 0{\cdot}02$ and $0{\cdot}009$.

Another set of studies utilized a purified Vi antigen administered parenterally. This antigen is felt to account for the virulence of typhoid bacilli. While vaccine recipients demonstrated a 13-fold increase in Vi antibody titres, the immunogen was entirely incapable of inducing protection in our experimental situation. In fact, a slightly greater percentage of vaccinees developed illness than did controls.

The second category of experimental vaccination employed a German product, then and still being marketed to the public. This consisted of organisms inactivated by acetone and freeze-drying, placed in enteric coated capsules, and given orally. Even given to volunteers in twice the recommended dose, no serum antibody response was provoked and clinical protection was minimal (15%).

The final group of vaccines studied consisted of living organisms given orally in multiple doses. The two strains had been derived artificially, were attenuated mutants and caused no adverse effects in laboratory animals. The first of these was dependent upon the presence of streptomycin in the medium. When given in a freshly prepared form to volunteers, this vaccine protected 74% from subsequent challenge ($p = 0{\cdot}0002$). However, protection was virtually obliterated when a lyophilized preparation was employed.

The second oral attenuated vaccine consisted of a mutant strain lacking epimerase, an enzyme necessary for the completion of the O side chain of the bacterial lipopolysaccharide cell well. In our hands, this vaccine has proved to be the most successful.

Prepared in media containing 0·1% galactose, this vaccine was given orally in increasing doses to a succession of volunteers. Virtually no side effects were encountered. In 99 men given five to eight doses each containing $3 \times 10^7$ bacteria the organism had virtually disappeared from stool cultures within two days of administration. Serum antibody was poorly stimulated and averaged no more than twice that of controls. And reversion of the mutant to the parent strain was not observed in 958 stool isolates

tested. Following subsequent virulent challenge, clinical protection of 87% could be demonstrated. Furthermore, the stool carriage of virulent organisms was markedly diminished in vaccinees.

*Military Service*

Whether a volunteer had previously been in the armed service (and therefore had received mandatory parenteral typhoid immunization) was recorded in 93% of individuals who served as controls. Following experimental challenge with $10^5$ bacilli, 200 men without military background experienced a clinical attack rate of 48%, whereas this figure was 20% among 105 military veterans. This highly significant reduction had persisted for an average of 12 years following discharge of veterans from the military. In an additional group of 51 veterans who received one of our effective experimental vaccines, the attack rate was further lowered to 6%.

## Discussion

The field trials (YUGOSLAV TYPHOID COMMISSION, 1964; POLISH TYPHOID COMMITTEE, 1965; HEFJEC *et al.*, 1966; ASHCROFT *et al.*, 1967) of the 1960s first demonstrated that parenteral typhoid vaccines were reasonably successful in inducing immunity. And these vaccines continue to be used commonly throughout the world. At least two limitations should be noted, however. Firstly, the field trials were conducted in areas hyperendemic for typhoid fever where prior natural exposure to disease may well have occurred. Any protective effect in previously unexposed populations could not be evaluated. Secondly, use of parenteral preparations is attended by appreciable side effects and requires some skill in sterile technique.

The research studies at the University of Maryland have helped to solve these limitations. American volunteers whose only known exposure to typhoid antigen consisted of mandatory vaccination while in military service were considerably protected by parenteral immunization. This protection of 58% ($\% \text{ effect} = \frac{100\,(b-a)}{b}$ where b = control and a = vaccinee) is comparable to that observed in the field trials and had persisted for over a decade. We feel that these results constitute the first properly controlled trial of standard vaccines in a non-endemic or virgin population.

The use of oral attenuated vaccines has provided encouragement. The preparations were easily administered. Side effects were nil. Reversion to virulence was not observed. Stool carriage of virulent *Salmonella typhi* was markedly reduced in vaccinees (from 60% to 11%). And, most importantly, the afforded clinical protection was superior, 87% in the case of the epimerase-less mutant.

A field trial of this latter vaccine has lent further encouragement. The trial utilized vaccine prepared by the Swiss Serum and Vaccine Institute and was conducted by the Pasteur Institute and the Egyptian Ministry of Health. With appropriate controls, 15,000 schoolchildren in Alexandria were given three doses of a lyophilized preparation. Preliminary unpublished results have indicated clinical protection of 100% after one year of observation.

While this vaccine is not yet commercially available, the future looks bright. We must be aware, however, that protective capacity of similar typhoid vaccines can only be evaluated by carefully conducted trials in a natural setting or in volunteers.

## Acknowledgements

The summary of research reported herein would not have been possible without the contributions of many persons. Special recognition is extended to Dr. Richard B. Hornick, Director of the Division of Infectious Diseases, the late Dr. Fred R. McCrumb, former Director, Dr Theodore E. Woodward, Chairman of the Department of Medicine, University of Maryland School of Medicine, more than 100 investigators who participated in the original research, and the administrative officials and prison volunteers of the Maryland House of Correction, Jessup, Maryland.

Research support was received from the US Army Research and Development Command Contract No. DA 49-193-MD-2867.

## References

Ashcroft, M. T. Singh, B., Nicholson, C. C., Ritchie, J M., Sobryan, E. & Williams, F. (1967). A seven-year field trial of two typhoid vaccines in Guyana *Lancet*, **ii,** 1056-1059.

DuPont, H. L., Hornick, R B., Snyder, M. J., Dawkins, A. T., Heiner, G G & Woodward, T. E. (1971). Studies of immunity in typhoid fever. Protection induced by killed oral antigens or primary infection. *Bulletin of the World Health Organization,* **44,** 667-672

DuPont, H. L., Hornick, R. B., Snyder, M. J., Libonati, J. P. & Woodward, T. E. (1971). Immunity in typhoid fever: evaluation of live streptomycin-dependent vaccine. *Antimicrobial Agents and Chemotherapy* 1970, pp. 236-239.

DuPont, H. L., Hornick, R. B., Weiss, C. F. Snyder, M. J. & Woodward, T. E. (1970). Evaluation of chloramphenical acid succinate therapy of induced typhoid fever and Rocky Mountain spotted fever. *New England Medical Journal,* **282,** 53-57.

Gilman, R. H., Hornick, R. B., Woodward, W. E., DuPont, H. L,. Snyder, M. J., Levine, M. M. & Libonati, J. P. (1977). Evaluation of UDP-glocuse-4-epimeraseless mutant of *Salmonella typhi* as a live oral vaccine *Journal of Infectious Disease*, **136,** 717-723.

Greisman, S. E., Woodward, T. E., Hornick, R. B. & Snyder, M. J. (1961). Typhoid fever: a study of pathogenesis and physiologic abnormalities. *Transactions of the American Clinical and Climatological Association,* **73,** 146-161.

Hawley, P. R. & Simmons, J. S. (1934) The effectiveness of vaccines used for the prevention of typhoid fever in the United States Army and Navy. *American Journal of Public Health,* **24,** 689-709.

Hornick, R. B., DuPont, H. L., Dawkins, A. T., Snyder, M. J. & Woodward, T. E. (1971). Evaluation of typhoid vaccines in man. Symposia Series In: *Immunobiological Standardisation,* **15,** 143-150 Basel: Karger

Hornick, R. B. & Greisman, S. E. (1978). On the pathogenesis of typhoid fever. *Archives of Internal Medicine,* **138,** 357-359.

Hornick, R. B., Greisman, S. E., Woodward, T. E., DuPont, H. L., Dawkins, A. T. & Snyder, M. J. (1970). Typhoid fever: pathogenesis and immunological control. *New England Journal of Medicine,* **283,** 686-691; 739-746.

Hefjec, L. B., Salmin, L. V., Lejtman, M. Z., Kuz'minova, M. L., Vasil'eva, A. V., Levina,. Benciznova, T. G., Pavlova, E. A. & Antonova, A. A. (1966). A controlled field trial and laboratory study of five typhoid vaccines in the U.S.S.R. *Bulletin of the World Health Organization,* **34,** 321-339.

Hornick, R. B. & Woodward, T. E. (1966). Appraisal of typhoid vaccine in experimentally infected human subjects. *Transactions of the American Clinical and Climatological Association,* **78,** 70-78.

Hornick, R. B., Woodward, T. E., McCrumb, F. R. Jr., Snyder, M. J., Dawkins, A. T., Bulkeley, J. T., de la Macorra, F. & Corazza, F. A. Jr. (1967). Typhoid fever vaccine—yes or no? *Medical Clinics of North America,* **51,** 617-623.

Levine, M. M., DuPont, H. L., Hornick, R. B., Snyder, M. J., Woodward, W. E., Gilman, R. H. & Libonati, J. P. (1976). Attenuated streptomycin-dependent *Salmonella typhi* vaccine: potential deleterious effects of lyophilization. *Journal of Infectious Diseases,* **133,** 424-429.

Levine, M. M., Grados, O., Gilman, R. H., Woodward, W. E., Soliz-Plaza, R. & Waldman, W. (1978). Diagnostic value of the Widal test in areas endemic for typhoid fever. *American Journal of Tropical Medicine and Hygiene,* **27,** 794-800.

Polish Typhoid Committee (1965). Evaluation of typhoid vaccines in the laboratory and in a controlled field trial in Poland. *Bulletin of the World Health Organization,* **32,** 15-27.

Snyder, M. J., Hornick, R. B., McCrumb, F. R., Jr., Morse, L. J. & Woodward, T. E. (1964). Asymptomatic typhoidal bacteremia in volunteers. *Antimicrobial Agents and Chemotherapy-1963,* pp. 604-607.

Sprinz, H., Gangarosa, E. J., Williams, M., Hornick, R. B. & Woodward, T. E. (1966). Histopathology of the upper small intestines in typhoid fever. *American Journal of Digestive Diseases,* **11,** 615-625.

Tigertt, W. D. (1959). The initial effort to immunize American soldier volunteers with typhoid vaccine. *Military Medicine,* **124,** 342-349.

Wright, A. E. (1896). On the association of serous haemorrhages with conditions of deficient blood-coagulability. *Lancet,* **ii,** 807-809.

Wright, A. E. & Semple, D. (1897). Remarks on vaccination against typhoid fever. *British Medical Journal,* **i,** 256-259.

Yugoslav Typhoid Commission (1964). 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.