STUDIES TO CONTROL ENDEMIC TYPHOID FEVER IN CHILE

ANNUAL/FINAL REPORT

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The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.
A multi-faceted program of applied research has been undertaken in collaboration with the Ministry of Health of Chile intended to lead to control of endemic typhoid fever in Santiago, Chile. These studies include: 1) Maintenance of prospective field trials evaluating the efficacy of Ty21a live oral typhoid vaccine given in various formulations and immunization schedules. 2) The first evaluations of Ty21a vaccine in infants and pre-school children. 3) Development of a new enzyme-linked immunosorbent assay (ELISA) to measure Vi antibody and its use as a serologic screening test to identify chronic typhoid carriers.
4) Evaluation of a new oral antibiotic regimen to eradicate the chronic typhoid carrier state without resort to surgery. **Keywords:**
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SUMMARY

A multi-faceted program of applied research was undertaken in collaboration with the Ministry of Health of Chile intended to lead to control of endemic typhoid fever in Santiago, Chile. Information derived from these studies is directly applicable to the prevention of typhoid fever in United States military personnel deployed in endemic areas. During the life of the contract, activities that were emphasized included:

1) Studies of the epidemiology of endemic typhoid fever in Santiago, including descriptive epidemiological analyses, case/control studies, family-based studies, seroepidemiologic studies and studies to quantitate the occurrence of Salmonella typhi bacteremia in young children.

2) A quantitation of the magnitude of the reservoir of chronic S. typhi carriers in Santiago.

3) Evaluation of a serologic screening test to detect chronic S. typhi carriers in an endemic area (Santiago), based on measurement of passive hemagglutination antibody to highly purified Vi polysaccharide antigen.

4) Development of a new enzyme-linked immunoorbent assay (ELISA) to measure Vi antibodies capable of processing large numbers of sera and its use as a serologic screening test to identify chronic typhoid carriers.

5) Evaluation of oral antibiotic regimens to eradicate the chronic typhoid carrier state without resort to surgery.

6) Environmental bacteriologic studies to detect the presence of S. typhi in irrigation waters and other surface waters incriminated epidemiologically in the transmission of typhoid fever.

7) Clinical bacteriology studies comparing the sensitivity of blood, bone marrow and duodenal string cultures in the isolation of S. typhi from patients with suspect typhoid fever.
8) Molecular analyses of *S. typhi* strains from Chile for the presence of plasmids and examination of the electrophoretic patterns after cutting the plasmids with restriction endonucleases.

9) Initiation of four large-scale field trials of live oral typhoid vaccine Ty2la to assess the efficacy of various formulations and immunization schedules.

10) The first evaluations of Ty2la vaccine in infants and pre-school children.
For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.
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Typhoid fever remains an important public health problem in many less-developed regions of the world and poses a risk for travelers from industrialized countries who visit such endemic regions. In virtually all endemic areas the incidence rates for typhoid fever are highest in children 5-19 years of age, i.e. school children. This is of potential relevance in the control of typhoid, since school children represent a "captive" population amenable to school-based immunization programs.

For United States military personnel who are stationed in less-developed areas or who must be prepared at short notice to operate in less-developed areas of geopolitical importance, typhoid fever represents an important potential health risk. The current vaccine utilized by the U.S. military forces to prevent typhoid fever, an acetone-inactivated preparation of whole *Salmonella typhi* inoculated parenterally, requires at least two doses given several weeks apart to immunize and causes high rates of significant adverse reactions. Therefore, a high priority has been given to identifying alternative typhoid vaccines that will provide significant protection without causing notable adverse reactions.

In areas where typhoid fever is endemic, the prevalence of chronic gallbladder carriers of *S. typhi* is often quite high. Thus, a particularly onerous risk of transmission of typhoid fever to U.S. military personnel in less-developed areas comes from foodhandlers from the indigenous population who may be chronic typhoid carriers and who unknowingly are involved in preparation of food. Under these circumstances, unwittingly, the potential exists for large epidemics to
occur. Furthermore, the size of the inocula of \textit{S. typhi} present in food vehicles may be sufficiently high to overcome the protective efficacy of the current acetone-inactivated parenteral vaccine. Consequently, a simple, practical yet sensitive and specific screening test is required to screen large groups of individuals for the presence of suspected chronic typhoid carriers.

Dependents, including children, who accompany U.S. military personnel stationed on tours of duty in less-developed countries must also be protected against typhoid fever. In young children the subject of adverse reactions to the current parenteral typhoid vaccines is even more pertinent.

Since 1980, with support from the U.S. Army Medical Research and Development Command, the World Health Organization and the Pan American Health Organization, the Center for Vaccine Development of the University of Maryland has conducted an applied research program on the control of typhoid fever in Santiago, Chile, a highly endemic area. This applied research program has included epidemiological studies, environmental bacteriologic studies, comparison of methods for diagnosis of acute typhoid fever, development of new diagnostic and treatment methods for carriers, and large-scale field studies with Ty21a live oral typhoid vaccine. Results of these studies have direct relevance for the improved prevention of typhoid fever in U.S. military personnel.

Wherever possible, the results of the various components of the research carried out under this program will be provided in this FINAL REPORT by attaching scientific manuscripts that have been published or submitted.
II. EPIDEMIOLOGIC STUDIES OF ENDEMIC TYPHOID FEVER

A. Descriptive Studies

A detailed summary of the descriptive epidemiology of endemic typhoid fever in Chile, and in particular in Santiago, is contained in APPENDIX A.

B. Case/Control Study

A case/control study was carried out to identify risk factors, protective factors and vehicles of transmission. Results are contained in APPENDIX B. Prior to this study, it had been considered dogma among local epidemiologists that typhoid fever was transmitted within the home by food handlers (relatives or domestic servants) who were chronic typhoid carriers. The stool culture data obtained in this study were the first to demonstrate that it is rare to find chronic carriers among the domestic foodhandlers in homes of index cases of typhoid fever in Santiago. These observations implied that typhoid fever was contracted largely outside the home. Family studies were carried out to enlarge on these initial observations.

C. Family-Based Studies

Results of family-based epidemiological studies are contained in APPENDIX C. These studies corroborated that chronic carriers are rarely found in households of index cases of acute typhoid fever and showed that secondary transmission and concomitant cases within the households are rare.

D. Typhoid Fever in Infants

The peak age incidence of typhoid fever in Santiago, as in other endemic areas is in school age children, 5-19 years of age. In contrast, the reported incidence of typhoid fever is very low in infants and
toddler. One of the possible explanations for this could be that young children less than two years of age do not consume the vehicles of transmission that are ingested by older individuals. However, it is also possible that contaminated vehicles are consumed by infants and toddlers but that these very young hosts do not clinically manifest an illness recognized as typhoid fever. A systematic collection of blood cultures was initiated in two primary health care clinics to answer this question. Results of this study are contained in APPENDIX D. It was found that bacteremia due to S. typhi and S. paratyphi occurred in approximately 4% of young children presenting to a primary health care facility with fever. In no instance was any child suspected clinically of having typhoid fever; therefore, this syndrome has been referred to as benign bacteremia due to S. typhi.

III. STUDIES OF CHRONIC TYPHOID CARRIERS

A. The Prevalence of Chronic Typhoid Carriers among Persons with
Chronic Gallbladder Disease in Santiago, Chile.

Gallbladder contents were cultured from 1000 individuals undergoing cholecystectomy in seven hospitals in Santiago, Chile. Results are shown in APPENDIX E. Overall, S. typhi was recovered from 3.8% of the gallbladders.

B. A Precise Estimation of the Prevalence of Chronic Typhoid Carriers
in Santiago

A precise estimate of the number of chronic biliary carriers of S. typhi was made using the detailed census of Santiago, data on the prevalence of gallbladder disease among individuals of various ages, and the measured prevalence of chronic carriage among persons with chronic gallbladder disease. Results are found in (APPENDIX F).
C. Serologic Screening to Detect Chronic Typhoid Carriers in an Endemic Area

A passive hemagglutination assay utilizing highly purified Vi antigen to measure Vi antibody was evaluated as a serological screening test to identify chronic typhoid carriers in a typhoid-endemic area, Santiago. The Vi serology proved to be very practical, sensitive and highly specific in identifying chronic carriers. The results are contained in Appendix G.

D. Development of an ELISA to Measure Vi Antibody and its Utility as a Serological Screening test for Chronic Typhoid Carriers

Based on the excellent results with the passive hemagglutination test for Vi antibody as a serological screening test to detect chronic typhoid carriers, an enzyme-linked immunosorbent assay (ELISA) was developed to measure Vi antibody. The ELISA utilized a tyraminated Vi polysaccharide as antigen. The advantages of the Vi ELISA include the ability to measure immunoglobulin class specific antibodies and the capacity to process very large numbers of sera. Results are shown in Appendix H.

E. Non-Surgical Domiciliary Treatment of Chronic Typhoid Carriers with a 28 Day Course of Amoxicillin and Probenecid

A 28 day oral regimen of amoxicillin and probenecid was evaluated as a non-surgical therapy to eradicate the chronic typhoid carrier state. A long-term cure was obtained in 15 of 26 carriers (56%). Those carriers who were successfully cured had a significantly higher serum antibiotic level than carriers in whom the treatment failed. Results are presented in detail in Appendix I.
F. Non-Surgical Antibiotic Therapy of the Chronic Typhoid Carrier State using Oral Ciprofloxacin

The new generation of quinolone antibiotics that has appeared in recent years includes ciprofloxacin, an agent with exceptionally good activity against *S. typhi* in vitro, with minimum inhibitory concentrations <0.06 mcg/ml. Pharmacokinetic studies in man indicate that the body fluid and tissue penetration of ciprofloxacin is excellent, including bile levels. For example, in a pilot study in which the bile levels of ciprofloxacin were measured after oral administration of 500 mg of ciprofloxacin, concentrations of drug of up to 10 mcg/ml were detected. Side effects of this antibiotic at either the 500 or 750 mg twice daily dosage schedule have been minimal. Based on these observations, we undertook a preliminary evaluation of ciprofloxacin in the treatment of chronic gallbladder carriers of *S. typhi*.

Twelve chronic carriers were enrolled into the study between June and December, 1985. Patients were treated with oral ciprofloxacin 750 mg twice daily, with careful monitoring for compliance and for possible adverse effects. Therapy was stopped in two cases after 10 days: one patient had an allergic reaction and one had a minimal drop in hematocrit of uncertain etiology. The remaining patients received the complete 28 day course of drug. Stool and bile-stained duodenal string cultures were obtained before therapy and at least monthly after discontinuation of therapy.

Of the total 12 carriers, one patient who completed the course of drug had a bacteriologic relapse within one week after completing therapy. A second patient whose stool and bile cultures were negative for six months following treatment became positive again for *S. typhi*. However, phage
typing of the isolates showed that the organism recovered after six months of negative cultures was distinct from the original infecting strain; thus this patient represents a re-infection. The other 10 patients have remained bacteriologically negative for at least six months, including the two individuals who had their courses of therapy interrupted before the full 28 days.

These preliminary results are extremely encouraging and suggest that ciprofloxacin is efficacious in treating chronic typhoid carriers and may achieve a higher cure rate than previous antibiotic regimens. Further, more comprehensive studies will undertaken to explore this possibility.

IV. ENVIRONMENTAL BACTERIOLOGY STUDIES

A. Recovery of S. typhi from Epidemiologically-Incriminated Surface Waters

Epidemiologic studies suggested that the lack of untreated sewage water for irrigation of salad vegetables during the dry summer months in Santiago represents a significant factor in the transmission of typhoid fever. Earlier environmental bacteriology studies, however, by Chilean bacteriologists had failed to recover S. typhi from the irrigation waters. Nevertheless, we proceeded to carry out environmental bacteriology studies using the same bacteriological methods as employed in the earlier studies but instituting the use of Moore swabs as the method of sampling the irrigation waters. By means of this new method of sampling, we were able to recover S. typhi repeatedly from surface waters used for irrigation. Details of these studies are contained in APPENDIX J.
B. Standardization of the Sensitivity of Moore Swabs for Isolating S. typhi from Environmental Sources

Moore swabs consist of large portions of gauze that are suspended for 48–72 hours in environmental sources to bacteriologically sample water; they act as filters to concentrate bacteria as the waters pass through the gauze. The sensitivity of Moore swabs in the recovery of S. typhi was evaluated by sampling sewers that drain the houses of known chronic typhoid carriers in Santiago. Results are presented in detail in APPENDIX K.

V. CLINICAL AND MOLECULAR BACTERIOLOGIC STUDIES OF S. TYPHI

A. Clinical Bacteriology Studies

The sensitivity of blood, bone marrow, and duodenal string cultures were compared in the isolation of S. typhi from 103 children with suspect typhoid fever. The combination of two blood and one duodenal string cultures equalled the sensitivity of a bone marrow in bacteriologically confirming the diagnosis of acute typhoid fever. These results are presented in detail in APPENDIX L.

B. Molecular Analyses of S. typhi

In the first study, 100 isolates of S. typhi from Santiago were examined for the presence of plasmids. Plasmids were found in only 8 isolates. None of the plasmids encoded antibiotic resistance. In fact, none of the 100 strains were found to be resistant to chloramphenicol, ampicillin, or trimethoprim, the clinically important antibiotics in the treatment of typhoid fever. These results are contained in APPENDIX M.

In a second study, a total of 141 S. typhi strains, including 70 from Santiago and 71 from Lima, Peru were examined for the presence of plasmids. Plasmids were present in only 12 of 70 (17%) of the Chilean and
5 of 71 (7%) of the Peruvian strains. Identical 21 kilobase plasmids (as defined by restriction endonuclease digest patterns) were found in 13 of the 17 plasmid-containing S. typhi from Santiago and Lima. These results and their significance for epidemiologic studies are found in APPENDIX N.

VI. FIELD TRIALS OF EFFICACY OF LIVE ORAL TYPHOID VACCINE TY21a

A series of four separate field trials of efficacy have been carried out in Santiago, involving more than 640,000 schoolchildren. In these trials three different formulations of vaccine and several different immunization schedules were compared in randomized, controlled trials. The enteric-coated formulation was found to be significantly superior to a formulation consisting of gelatin capsules containing NaBFeO₃ and lyophilized vaccine. Three doses of Ty21a in enteric-coated capsules given within one week has so far provided 67% efficacy for at least three years. Increasing the interval between doses to 21 days did not increase efficacy. Administering fewer doses (one or two) of vaccine in enteric-coated capsules provided lower levels of protection that endured for only two typhoid seasons. In contrast, administering four doses of enteric-coated vaccine conferred significantly higher protection than three doses. A fourth trial initiated in October, 1986, is comparing the relative efficacy of three doses of Ty21a given within one week in enteric-coated or liquid formulation. Results of these field trials of efficacy of Ty21a are contained in APPENDIX 0 and APPENDIX P.

VII. EVALUATION OF THE SAFETY AND IMMUNOGENICITY OF A PURIFIED VI POLYSACCHARIDE PARENTERAL VACCINE

The safety and immunogenicity of a purified Vi parenteral vaccine was carried out in healthy young adults in Maryland and in Chilean Air Force recruits of the same age. Meningococcal polysaccharide served as the
control vaccine. Results are summarized in APPENDIX Q.

VIII. STUDIES WITH TY21a ORAL VACCINE IN INFANTS AND TODDLERS

A. Background

Live oral typhoid vaccine Ty21a has proven to be an important advance for the prevention and possible control of typhoid fever in endemic areas because it provides significant protection without causing adverse reactions. Although typhoid fever in endemic areas is largely a disease of schoolage children, the main delivery system for pediatric vaccines in most developing countries occurs through the expanded program on immunization (EPI) which is heretofore usually targeted exclusively at infants. Thus it is intriguing to consider whether immunization of infants might protect these children later when they reach schoolage. To even consider such a proposition it will be necessary to show that Ty21a is immunogenic in infants and young children. Because of the innocuity of Ty21a and the propensity of Salmonella to avidly interact with M cells of gut lymphoid tissue, many investigators have introduced genes encoding putative protective antigens of other organisms to obtain expression in Ty21a, thereby using the attenuated S. typhi as a "carrier" bacteria. Among the combinations reported so far are Ty21a expressing Shigella sonnei O antigen (1), B subunit of Escherichia coli heat-labile enterotoxin (LT) (2), and an outer membrane protein of Vibrio cholerae (3). Important target age groups for these bivalent vaccines are also infants and young children. Heretofore, however, the youngest age group to have received Ty21a vaccine is six year olds. We therefore initiated studies to evaluate the clinical acceptability and immunogenicity of Ty21a in infants and young children (less than five years of age) in Santiago, Chile, an area endemic for typhoid fever.
B. Materials and Methods

Vaccine was administered in three separate, randomized, placebo-controlled, double-blind studies.

1. Study #1

Study #1 involved healthy children 6-24 months of age recruited from the well baby clinic of the Centro Diagnostico of the Universidad Catolica School of Medicine, Santiago. Following explanation of the study to the parents and obtaining written consent, infants were randomized to receive three doses of vaccine (10⁹ organisms per dose) or placebo which were given within eight days. Cups containing vaccine or placebo were prepared in a separate room by an unblinded nurse. She dissolved the contents of an enteric-coated capsule of Ty2la vaccine into 90 ml of cow's milk formula containing 0.5 gm of NaHCO₃. (A similar milk/bicarbonate "cocktail" method had been previously successfully used to vaccinate Chilean six year olds who demonstrated a good serologic response post-vaccination) (20). Milk containing NaHCO₃ alone served as the placebo. The coded cups containing vaccine or placebo were presented to a second nurse who administered the contents to the children in double blind fashion. The infants were examined 24 and 48 hrs after each dose of vaccine at which time the child's temperature was recorded; axillary temperatures were obtained because this is the accepted custom in Chile. The mother was interviewed to elicit evidence of adverse reactions in the previous 24 hrs.

A 4 ml specimen of blood was collected prior to and 21 days after vaccination. The blood was passed through a Piscoll-Hyphaque column to obtain mononuclear cells to carry out lymphocyte replication studies with selected S. typhi and appropriate control antigens to measure the
cell-mediated immune response to vaccination with Ty2la. Plasma was utilized to measure serum antibodies: IgG antibody to O antigen was measured by enzyme-linked immunosorbent assay (4); H antibody was measured by Widal tube agglutination as previously described (5) and Vi antibody was detected by passive hemagglutination using highly purified Vi polysaccharide (5).

2. Study #2

This study was carried out among children 2-5 years of age (three-fourths were three or four year olds) in a nursery school in the Pincoya district of Area Norte, Santiago. Children of consenting parents were randomized to receive three doses of Ty2la vaccine (10^9 viable organisms per dose) or placebo given within a period of eight days. Capsules of vaccine were opened by an unblinded individual in a separate room and the contents suspended in 50 ml of cow's milk containing 0.75 gm of NaHCO₃; placebo consisted of milk with bicarbonate only. The coded cups containing vaccine or placebo were presented to a nurse who distributed them to the children in double blind fashion. Children were examined 24 and 48 hrs after vaccination at which time axillary temperatures were taken and the parents were interviewed.

Before vaccination and 21 days thereafter 4 ml of blood were collected and the sera separated and frozen to be tested later for antibody as described above.

3. Study #3

The third study was carried out in 2-5 year old children in a second nursery school in Pincoya where children of consenting parents were randomized to receive four doses of Ty2la vaccine (10^9 viable organisms per dose) or placebo. Vaccine was administered identically as in Study #2 but a fourth dose was given within the eight day period in attempt to
increase vaccine immunogenicity. Blood was collected before and 21 days after vaccination for serologic tests as described above.

C. Results

1. Clinical Response to Vaccine

Table 1 shows the number of children in each study who received vaccine or placebo and the frequency of adverse reactions. Diarrhea, fever, vomiting and abdominal pain were uncommon in either group with no difference evident between vaccine and placebo recipients.

2. Immune Response to Ty21a

The serologic response to vaccination of infants and young children is summarized in Table 2. In Study #1, involving infants and toddlers less than two years of age, no significant rises in O antibody measured by IgG-ELISA were detected. Because these results contrast so notably from the serologic response of six year olds administered Ty21a vaccine by this method of administration in a previous study (4), we proceeded in the next study to vaccinate slightly older children, 2-5 years of age. These children in Study #2 showed some serologic reactions to both O and H antigens; in total 8 of 24 vaccinees showed a significant rise in one or another serologic test versus 0 of 25 pre-school children who received placebo (p<0.002).

In the third study, we attempted to increase the immunogenicity of the vaccine by administering an additional dose to preschool children. The addition of a fourth dose did not increase the serologic response to the vaccine.

Ty21a vaccine does not contain Vi antigen and therefore even in older individuals does not stimulate Vi antibodies. Thus the total lack of serologic response to Vi where measured in these studies in young children
is completely as expected (Table 2).

Lymphocyte cultures from the vaccinated and placebo infants responded to mitogens. However, the lymphocytes collected post-vaccination failed to show evidence of replication in the presence of S. typhi 0 polysaccharide or control (S. thompson or E. coli) 0 polysaccharides.
REFERENCES


### TABLE 1

**OCCURRENCE OF ADVERSE REACTIONS IN INFANTS AND PRE-SCHOOL CHILDREN FOLLOWING INGESTION OF LIVE ORAL TYPHOID VACCINE TY21A VACCINE OR PLACEBO**

<table>
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<th>Study</th>
<th>Age Group</th>
<th>No. Dose</th>
<th>Diarrhea Vaccine</th>
<th>Diarrhea Placebo</th>
<th>Fever Vaccine</th>
<th>Fever Placebo</th>
<th>Vomiting Vaccine</th>
<th>Vomiting Placebo</th>
<th>Abdominal Pain Vaccine</th>
<th>Abdominal Pain Placebo</th>
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<td>TyCh 6001</td>
<td>6-24 mos.</td>
<td>3</td>
<td>2/18</td>
<td>1/18</td>
<td>2/18</td>
<td>2/18</td>
<td>3/18</td>
<td>2/18</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TyCh 6003</td>
<td>2-5 yrs.</td>
<td>3</td>
<td>0/24</td>
<td>0/25</td>
<td>0/24</td>
<td>0/25</td>
<td>1/24</td>
<td>0/25</td>
<td>2/24</td>
<td>0/25</td>
</tr>
<tr>
<td>TyCh 6004</td>
<td>2-5 yrs.</td>
<td>4</td>
<td>1/17</td>
<td>2/24</td>
<td>1/17</td>
<td>0/24</td>
<td>0/17</td>
<td>1/24</td>
<td>1/17</td>
<td>2/24</td>
</tr>
</tbody>
</table>

*No. positive/No. vaccinated*
TABLE 2
SEROLOGIC RESPONSE FOLLOWING VACCINATION OF INFANTS AND YOUNG CHILDREN WITH LIVE ORAL VACCINE TY2IA OR PLACEBO

<table>
<thead>
<tr>
<th>Study</th>
<th>Age</th>
<th>No.</th>
<th>O Antibody by IgG-ELISA</th>
<th>H Antibody by Widal</th>
<th>VI Antibody by PHA</th>
<th>Rises by Any Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>TyCh 6001</td>
<td>6-24 mos.</td>
<td>3</td>
<td>0/18*</td>
<td>0/18</td>
<td>0/18</td>
<td>0/18</td>
</tr>
<tr>
<td>TyCh 6003</td>
<td>3-5 yrs.</td>
<td>3</td>
<td>5/24</td>
<td>3/24</td>
<td>0/24</td>
<td>5/24</td>
</tr>
<tr>
<td>TyCh 6004</td>
<td>3-5 yrs.</td>
<td>4</td>
<td>0/17</td>
<td>0/17</td>
<td>NT</td>
<td>0/17</td>
</tr>
<tr>
<td>Totals</td>
<td></td>
<td></td>
<td>5/59</td>
<td>3/59</td>
<td>0/42</td>
<td>5/59</td>
</tr>
</tbody>
</table>

* V = vaccine group, P = placebo group
+ No. positive/No. vaccinated
$ Positive hemagglutination using highly purified VI antigen
CONTRACT-RELATED PUBLICATIONS

Papers


Chapters


Presentations at National and International Meetings

Presentations at National and International Meetings (cont.)


INTERVENTIONS TO CONTROL ENDEMIC TYPHOID FEVER:
FIELD STUDIES IN SANTIAGO, CHILE

Myron M. Levine, Robert E. Black, Catterine Ferreccio, Mary Lou Clements, Claudio Lanata, Stephen Sears, J. Glenn Morris, Luis Cisneros, and Rene Germanier, and the Chilean Typhoid Commission

Introduction

Typhoid fever, the acute, often debilitating, febrile illness representing generalized infection of the reticuloendothelial system, intestinal lymphoid tissue and gall bladder, is endemic in many less-developed areas of the world. Man is the sole reservoir and host of the infection (Figure 1), in contrast with other Salmonella, which are typically zoonotic infections of domestic and herd animals (1). Approximately 3-5% of acute S. typhi infections result in chronic gall bladder infection, giving rise to long-term biliary carriers. The propensity to become a carrier following acute infection increases with age and is greater in females, thus paralleling the epidemiology of gall bladder disease (2-4). Asymptomatic chronic carriers comprise the reservoir that maintains the endemicity of the disease by contamination of food and water vehicles (Figure 1); direct contact spread of typhoid fever is relatively uncommon (7).

Recognition of the above-mentioned facts helps explain most of the observations regarding the global occurrence of typhoid fever. It is endemic in less-developed areas where sanitation and food hygiene are primitive. However, the highest incidences occur where piped water is available but the water is fe-
cally contaminated and untreated. a situation prevalent in many large cities of Europe and North America in the late 19th century (5-7). This phenomenon of piped transport of impure water can be regarded epidemiologically as an example of amplification of disease transmission.

The introduction of purification (including chlorination) of water supplies and treatment of sewage prior to discharge, interrupted the amplification step and caused a precipitous fall in the incidence of typhoid fever in the cities of Europe and North America in the first three decades of the 20th century (5-7). Figure 2 illustrates this drop in incidence of typhoid fever in the United States. This pattern is typical of virtually all countries as they industrialize and provide chlorinated water and sewerage systems for their urban populations (8).

One country, Chile, in the cone of South America, represents an exception to the above pattern. By most criteria of health and quality of life, Chile is advanced well beyond the
Figure 1. A schematic diagram of the cycle of endemic typhoid fever and intervention points.

A HIGH INCIDENCE

INTERVENTIONS:
- Environmental measures
- Identify vehicles
- Immunization

ENDEMIC TYPHOID FEVER

CONTAMINATION OF VEHICLES OF TRANSMISSION

INTERVENTIONS:
- Identify carriers

HIGH PREVALENCE OF CARRIERS

SANITARY AND HYGIENIC PRECAUTIONS

Chronic carriers comprising the reservoir contaminate food and water vehicles. These are consumed by susceptible hosts leading to a high incidence of typhoid fever. Approximately 3-5% of infected persons become chronic gall bladder carriers of Salmonella typhi and perpetuate the reservoir. The various interventions are discussed in the text.

ranks of the less-developed countries. Chile has a world-renowned health care delivery system, a low infant mortality rate (Table 1), most immunizable communicable diseases have been impressively controlled (Table 1), and the literacy rate is 94%. In the capital city, Santiago, 96% of households have bacteriologically monitored, chlorinated water and 75% have connections to the municipal sewerage system (9). Nevertheless, despite these manifestations of a high level of development and control of most other communicable diseases, typhoid fever is highly endemic in Chile, particularly in Santiago. The incidence of typhoid fever in Chile from 1960 to 1981 is shown in Table 2. During that period, the incidence rate has usually exceeded 50 cases per 10^5 population; since 1977 the rate has surpassed 90 per 10^5 in Chile and 150 per 10^5 in Santiago.

Santiago, Chile thus provided a unique opportunity to intensively study the epidemiology of endemic typhoid fever and its control in a country of relatively advanced technology and educational development. Accordingly, following some preliminary epidemiological
and seroepidemiological studies in late 1978, a multifaceted program "Studies to Control Endemic Typhoid Fever in Chile" was designed. The ultimate goal of the program is to reduce the incidence of typhoid fever in Chile to the level where it no longer represents a major public health burden. The specific objectives of the program have included (Figure 1):

1) Epidemiological investigations to identify high-risk groups, risk factors, protective factors, major modes of transmission and specific contaminated food and water vehicles.

2) A quantification of the magnitude of the reservoir of chronic S. typhi carriers.

3) Development of a simplified, sensitive, and specific serological assay to screen large numbers of food handlers and other epidemiologically important groups for the presence of chronic typhoid carriers.

4) Evaluations of possible nonsurgical domiciliary antibiotic treatments to eradicate the chronic carrier state.

5) Environmental bacteriology studies to confirm the presence of S. typhi in epidemiologically incriminated waters.

6) Large-scale controlled field trials to assess the efficacy of a live oral attenuated S. typhi vaccine (strain Ty21a) in the prevention of typhoid fever in Chilean schoolchildren and its use as a public health intervention.

Each of these components of the program will be reviewed below. The project represents a collaborative effort involving participants from several institutions in Chile and several abroad as well as international agencies. Included are the Chilean Ministry of Health, the Center for Vaccine Development of the University of Maryland School of Medicine, the Pan American Health Organization, the World Health Organization, the Walter Reed Army Institute of Research and the Swiss Serum and Vaccine Institute.

Epidemiological Investigations

Descriptive Epidemiology

Chile is a long narrow country stretching more than 3,000 miles from north to south. Approximately 4.5 million of Chile's 11.8 mil-

Table 1. Infant mortality rate and incidence of certain immunizable communicable diseases in Chile, 1964-1980.

<table>
<thead>
<tr>
<th>Year</th>
<th>Measles</th>
<th>Pertussis</th>
<th>Poliomyelitis</th>
<th>Infant Mortality Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Incidence</td>
<td>Cases</td>
<td>Incidence</td>
</tr>
<tr>
<td>1964</td>
<td>35,941</td>
<td>428.3*</td>
<td>5,279</td>
<td>62.9*</td>
</tr>
<tr>
<td>1969</td>
<td>9,538</td>
<td>99.7</td>
<td>2,905</td>
<td>30.4</td>
</tr>
<tr>
<td>1972</td>
<td>8,413</td>
<td>82.1</td>
<td>2,550</td>
<td>24.9</td>
</tr>
<tr>
<td>1975</td>
<td>3,544</td>
<td>34.0</td>
<td>2,795</td>
<td>23.2</td>
</tr>
</tbody>
</table>

* Rate per 100,000.

* Rate per 1,000 live births.
lion inhabitants live in metropolitan Santiago, which is located in the center of the country in a valley between the Andes mountains and the Pacific Ocean. Santiago has a temperate "Mediterranean" climate with wet winters and rainless summers.

In Table 2 are listed the population of Santiago and of Chile, the cases of typhoid fever, and incidence rates from 1960-1981. Approximately one-half of the cases of typhoid fever in Santiago are reported from Santiago. In 1977 the incidence of typhoid fever doubled and remained at elevated rates for several years. It is not clear what factors were responsible for the doubling of the notification rates for typhoid fever since 1977. It is apparently not due to an administrative change in notification, since there was not a similar rise in nonenteric infections.

Typhoid fever shows a striking seasonality in Santiago, where it is a warm-season disease. Approximately 65% of cases occur between December 1 and April 30 of each year (Table 3).

The incidence of typhoid fever drops as one goes further north or south from Santiago. One area of particular interest is the Los Lagos Region, where many persons from Santiago vacation in summer. The daytime temperatures in this region can be quite warm, but there is rainfall all year long. Typhoid is very uncommon here.

Table 4 shows the incidence of typhoid fever by administrative area of Santiago in years 1977 and 1978. Area Oriente, an area of high affluence, has high rates in addition to poorer areas of Santiago. Furthermore, the incidence of typhoid in Area Oriente is believed to be very underreported because in the Los Lagos Region, where many persons from Santiago vacation in summer. The daytime temperatures in this region can be quite warm, but there is rainfall all year long. Typhoid is very uncommon here.

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<table>
<thead>
<tr>
<th>Month</th>
<th>1970-76 % of Total</th>
<th>1977-81 % of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>421 14.1</td>
<td>948 13.3</td>
</tr>
<tr>
<td>February</td>
<td>403 13.5</td>
<td>917 12.9</td>
</tr>
<tr>
<td>March</td>
<td>415 13.9</td>
<td>1015 14.3</td>
</tr>
<tr>
<td>April</td>
<td>400 13.4</td>
<td>867 12.2</td>
</tr>
<tr>
<td>May</td>
<td>302 10.1</td>
<td>605 8.5</td>
</tr>
<tr>
<td>June</td>
<td>183 6.1</td>
<td>529 7.4</td>
</tr>
<tr>
<td>July</td>
<td>103 3.5</td>
<td>282 4.0</td>
</tr>
<tr>
<td>August</td>
<td>76 2.5</td>
<td>124 1.7</td>
</tr>
<tr>
<td>September</td>
<td>62 2.1</td>
<td>162 2.3</td>
</tr>
<tr>
<td>October</td>
<td>109 3.7</td>
<td>215 3.0</td>
</tr>
<tr>
<td>November</td>
<td>167 5.6</td>
<td>601 8.5</td>
</tr>
<tr>
<td>December</td>
<td>340 11.4</td>
<td>841 11.8</td>
</tr>
</tbody>
</table>

Table 4. Incidence rates of typhoid fever by administrative area, Santiago, Chile.

<table>
<thead>
<tr>
<th>Area</th>
<th>Mean socioeconomic level</th>
<th>Incidence rate 100000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sur</td>
<td>Low, middle</td>
<td>317.5</td>
</tr>
<tr>
<td>Sur Oriente</td>
<td>Low, middle</td>
<td>117.0</td>
</tr>
<tr>
<td>Occidente</td>
<td>Low, middle</td>
<td>140.9</td>
</tr>
<tr>
<td>Norte</td>
<td>Low, middle</td>
<td>125.5</td>
</tr>
<tr>
<td>Central</td>
<td>Middle</td>
<td>185.3</td>
</tr>
<tr>
<td>Oriente</td>
<td>Middle, upper</td>
<td>113.7</td>
</tr>
</tbody>
</table>

In summary, an explanation for the endemicity of typhoid in Santiago must explain:

- the high incidence in schoolchildren.
- a summer seasonality with the highest incidence (December to mid-March) occurring when children are out of school on summer recess.
- an apparent low incidence in children less than 2 years of age.
- amplified transmission despite high levels of sanitation.
- why the incidence of typhoid fever is very low in the Los Lagos Region, despite the influx into that region of many persons (including presumably chronic carriers) from Santiago.

Seroepidemiology

Antibodies to the flagellar (H) antigen of *S. typhi* (d) are IgG and long-lived (10). They may derive from immunization or clinical or subclinical infection (10). Where parenteral vaccine is not commonly used, as in Chile, measurement of the prevalence of *S. typhi* H antibodies can give helpful insights into the epidemiology of typhoid fever (10). In November 1978, sera were obtained from healthy 15-19 year olds, as well as other age groups, in three areas of Chile. These included: 1) Area Norte, Santiago, representing a low and low-middle socioeconomic population; 2) children from an exclusive private school in Providencia, Area Oriente, representing an affluent group; and 3) schoolchildren in the Los Lagos Region of southern Chile, where the reported incidence of typhoid fever is low. As shown in Figure 3, 25% of 15-19 year olds in Area Norte had *S. typhi* H agglutinins at a reciprocal titer of 40. The prevalence in Providencia 15-19 year olds was as high (in fact slightly higher, 34%). In contrast, the prevalence of antibodies in teenagers in the Los Lagos Region (12%) was significantly lower than in Santiago (p = 0.04). The seroepidemiological data confirm the notification data regarding the occurrence of typhoid, i.e., it is indeed common in high socioeconomic areas of Santiago but is rare in the Los Lagos Region.

Approximately 80-90% of children in the other areas of Santiago are cared for by physicians at National Health Service facilities where reporting is compulsory.

The highest incidence of typhoid fever is found in 10-14 year olds (Table 5); approximately 60% of cases occur in school-age children 6-19 years of age. In contrast, notification rates in children less than 2 years of age are very low. Notable sharp increases in the incidence in childhood occur in 3 year olds (versus 2 year olds) and in 6 year olds (versus 5 year olds).

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean % of Total</td>
<td>Mean incidence per 10^3</td>
</tr>
<tr>
<td>0–4</td>
<td>5.9</td>
<td>38.7</td>
</tr>
<tr>
<td>5–9</td>
<td>18.3</td>
<td>126.5</td>
</tr>
<tr>
<td>10–14</td>
<td>21.8</td>
<td>152.2</td>
</tr>
<tr>
<td>15–19</td>
<td>17.3</td>
<td>135.5</td>
</tr>
<tr>
<td>20–24</td>
<td>14.7</td>
<td>126.4</td>
</tr>
<tr>
<td>25–34</td>
<td>14.2</td>
<td>72.0</td>
</tr>
<tr>
<td>35–44</td>
<td>4.7</td>
<td>22.6</td>
</tr>
<tr>
<td>45–54</td>
<td>1.8</td>
<td>17.4</td>
</tr>
<tr>
<td>55–64</td>
<td>0.8</td>
<td>10.8</td>
</tr>
<tr>
<td>65-</td>
<td>0.3</td>
<td>5.2</td>
</tr>
</tbody>
</table>


Figure 3. The prevalence of *Salmonella typhi* M antibodies in various age groups from three population groups in Chile.

**AGE PREVALENCE OF SALMONELLA TYPHI M ANTIBODY IN SELECTED AREAS OF CHILE**

- **Area Norte**, a low and middle socioeconomic sector of Santiago with a high reported incidence of typhoid fever
- **children** from a private school in Providencia, an affluent area where, nonetheless, the incidence of typhoid fever is high
- **Llanquihue** and **Puerto Varas**, in the Los Lagos Region of Southern Chile where the reported incidence of typhoid fever is low.

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42 Control of Endemic Typhoid Fever
Case/Control Study

A case/control study attempted to identify specific vehicles of transmission as well as risk factors and protective factors (11). This study, which involved 81 cases age 3-14 years and 81 matched controls, incriminated only one possible vehicle, flavored ices sold by street vendors. One aspect of the study involved the collection of multiple coprocultures from the food handlers in both the case and control households. Chronic *S. typhi* carriers were identified in only 2 of the 81 (2.5%) cases and 1 of 81 (1.2%) control households. This observation was the first evidence to demonstrate that chronic typhoid carriers in the home are not responsible for most cases of typhoid fever in children in Santiago.

Family Studies

We sought to further examine factors involved in the transmission of *S. typhi* in Santiago by interviewing and culturing the household members of recently confirmed pediatric cases. This represents a more intensive study of the household as the possible site in which transmission of typhoid infection may be occurring. Two separate studies involving 24 and 39 households, respectively, were carried out: in which attempts were made to identify chronic typhoid carriers as well as possible concurrent (or secondary) cases by culturing household foodhandlers and contacts below 19 years of age (these represent high-risk individuals) (12, C. Ferreccio et al. unpublished data).

Ninety-six percent of the households had municipal water and 79% were connected to the city sewerage system. A chronic *S. typhi* carrier was identified in only one (1.6%) household. Most importantly, only two concomitant cases were identified among the scores of high-risk children less than 19 years of age who were cultured. Eighty-six percent of index cases gave a history of eating outside the household at least once each week.

In summary, the data from family-based studies confirm the earlier observation that it is uncommon to find a chronic carrier in the household of an index case. Furthermore, the low frequency of concomitant cases among high-risk siblings strongly suggests that the vehicle of transmission in children and teenagers is usually consumed outside the home, otherwise more concomitant cases would be expected.

*S. typhi* Infection in Infants

Few cases of typhoid fever are reported in children less than 2 years of age in Santiago. This could represent a lack of consumption of the vehicles that transmit *S. typhi* to older children or could imply that, following ingestion of the usual vehicles of transmission, infants manifest an atypical response to infection that is not readily recognized clinically. To help resolve this question we systematically performed blood cultures in children less than 2 years of age with fever who were seen at two health centers in Santiago during the three peak months of the typhoid fever season (13). Of 197 outpatients less than 2 years of age with fever who were cultured, *S. typhi* was isolated from the blood cultures of four infants (2%), *S. paratyphi* B from two (1%) and *S. paratyphi* A from one (0.5%). The clinical syndrome in these infants was very mild, consisting of 1-5 days of fever (38.3-38.8°C) and respiratory symptoms. In no instance was enteric fever considered in the differential diagnosis and, were it not for the study protocol, a blood culture would not have been taken from any infant.

These data demonstrate that during the typhoid fever season in Chile children less than 2 years of age are becoming infected at a much higher rate than previously appreciated. The mode of transmission and specific vehicles have yet to be identified.
Environmental Bacteriology Studies

The observation that most cases of typhoid fever are not associated with a chronic carrier in the home made us turn our attention to a closer inspection of the water and sewerage system. We learned that although three-fourths of Santiago households were connected to the sewerage system there is no treatment of sewage. Thus, raw, untreated sewage enters the Mapocho River (which traverses northern Santiago) or the Zanjón de la Aguada (a large open sewer that traverses southern Santiago from east to west before emptying into the Mapocho River southwest of the city) (Figure 4). As the Zanjón and the Mapocho River reach the westernmost portion of metropolitan Santiago, their fecally polluted, untreated waters are diverted for irrigation of crops during the rainless summer season. Prior to May 1983, 90% of the crops grown in this region were lettuce, cabbage and celery, vegetables that are difficult to wash and are eaten raw in salads in Chile.

The hypothesis that raw vegetables grown with untreated waste waters and fruit “freshened” with contaminated river water represent important vehicles of transmission successfully explains the following epidemiological observations:

- the striking seasonality of typhoid (irrigation is used in the summer when there are no rains);
- the low reported incidence in young children (raw vegetables are not an important food item for infants and toddlers);
- the high incidence of typhoid fever in high socioeconomic neighborhoods of Santiago (they eat salads in restaurants and at home);
- the low incidence of typhoid fever in the Los Lagos Region of Chile (because of year-round rains in this region, irrigation is not used).

Prior to 1983. Chilean microbiologists carried out a series of environmental microbiology studies in attempts to isolate S. typhi from River treatment plants. The hypothesis that raw vegetables grown with untreated waste waters and fruit “freshened” with contaminated river water represent important vehicles of transmission successfully explains the following epidemiological observations.

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from waters of the Zanjón and the Mapocho River and from vegetables irrigated with untreated wastewater (14-16). While heavy coliform counts and many nontyphoidal Salmonella were found, S. typhi was never isolated from vegetables or from the Zanjón and was isolated only once from the Mapocho River (17). The past failure to isolate S. typhi from the polluted waters was in conflict with our epidemiological incrimination of this wastewater. In review of the earlier Chilean studies, we concluded that the bacteriological methods were efficient, but the techniques of environmental sampling appeared suboptimal. Therefore we initiated new environmental bacteriological studies (18) using Moore swabs (19-21) (thick wads of cotton gauze which are left in the flowing wastewater for 2-3 days allowing the gauze to act as a filter) to collect samples. The Moore swab, originally described in England in 1948 (19), is a concentrating method that has been used successfully to locate the homes of chronic S. typhi carriers by isolating the organism from sewage effluents (19-24). Moore swabs have been extremely useful in the investigation of urban typhoid fever outbreaks in Europe, Japan, and the United States of America (19-24). However, the efficacy and reliability of Moore swabs in endemic areas had not previously been assessed. Nevertheless, based on its success in finding S. typhi in sewage contaminated by carriers in industrialized areas, we decided to employ modified Moore swabs to isolate S. typhi from environmental sources in Santiago.

Microbiological examination of rivers and irrigation canals of Santiago, Chile was carried out from January to March 1983. The two major waterways in Santiago that carry wastewater are the Mapocho River in the north and the Zanjón de la Aguada canal in the south (Figure 4). Untreated sewage flows directly into these waters, which are used for irrigation in the agricultural districts of Maipú and Pudahuel (on the perimeter of the city). The Zanjón de la Aguada, which is heavily contaminated with industrial waste from the central section of the city, receives untreated sewage and becomes fecally polluted as it flows westward. During the final few kilometers as it approaches the agricultural areas, no further sewage is discharged in an attempt to allow a degree of self-purification of the wastewater.

We placed 133 swabs into the Mapocho River and the Zanjón de la Aguada and recovered 93. None of the 17 swabs placed in industrial areas grew S. typhi. In contrast, 4 of the 31 swabs from the Zanjón de la Aguada without industrial discharge (13%) and 4 of 45 from the Mapocho River (8.3%) contained S. typhi. Of the 76 swabs placed in agricultural areas, 8 were culture-positive (11%). Of the 8 isolates, 5 were phage type E1 and 46, the two most common disease-causing types in Chile, were untypeable, and the other two were N and M1.

Thus, using Moore swabs, we were able to isolate S. typhi from irrigation water in Santiago, Chile. Since the sensitivity of the Moore swab is inversely related to the size of the waterway sampled (21), our isolation rate of 11% from these large waterways is probably an underestimate. S. typhi is fastidious, easily inhibited by coliforms, and usually present in relatively small numbers in environmental samples (22). The Moore swab, by acting as a filter, improves the chance of isolating rare S. typhi among millions of coliforms, and we have now shown it to be a practical, reliable tool to isolate S. typhi from irrigation water in endemic areas. Finding S. typhi with the same phage types as disease-causing isolates in irrigation water supports the hypothesis, based on epidemiological observations, that contaminated vegetables in Santiago serve as important vehicles of transmission.

Studies with Chronic Carriers

Quantitation of the Reservoir

Using epidemiological techniques, we estimated that in 1980 there existed 25,019 fe-
male and 4,575 male chronic S. typhi carriers among the 4,264,515 inhabitants of Santiago, giving a prevalence of 694 carriers per 10^6 population (24, 25).

**Simple Serological Screening Test for Carriers**

Simple, yet reliable, screening tests are required to allow rapid and effective identification of chronic carriers. Shortly after the original description of the Vi antigen by Felix and Pitt in the 1930s (26), they noted that chronic carriers had high titers of Vi antibody (measured by bacterial agglutination using Vi-rich bacteria) and suggested that this serology might serve as a screening test to detect carriers (27). Over the next 45 years, great debate occurred on this subject due to wide diversity of results of various investigators (28-31). Until recently, all assays were limited by the lack of purity of the antigen. However, a few years ago highly purified Vi antigen became available (32). Vi serology using this purified antigen in a passive hemagglutination (HA) test was successful in detecting chronic carriers in outbreak situations in non endemic areas (33). Therefore we undertook to evaluate the utility of this serological screening test to identify carriers in an endemic area, Santiago, Chile (34). Sera were tested from the following Chilean populations:

1) 36 bacteriologically-confirmed known chronic carriers.
2) 29 patients of both sexes, age 18 years and over, with acute typhoid fever.
3) 388 women who had confirmed typhoid fever 12-48 months earlier and who were apparently not carriers (based on three negative stool cultures).
4) 59 healthy individuals, age 16-46 years.

Of the 36 chronic carriers, 27 (75%) had Vi reciprocal titers of ≥160 (see Table 6), whereas only 8% of the 388 noncarrier women (p < 0.001) and 3% of 59 health subjects (who had no bacteriological screening) (p < 0.001) had titers ≥160. The frequency of titers ≥160 in patients with acute typhoid fever (38%) was also significantly lower than that in chronic carriers (p < 0.005). The geometric mean titer in the chronic carriers was significantly (p < 0.001) higher than that in any of the other groups. Using the 388 culture-negative women as negative controls, a Vi antibody titer of ≥160 was 75% sensitive and at least 92% specific in detecting chronic carriers.

In Santiago, the predictive value (35) of a

---

**Table 6. Prevalence of Vi antibody* in chronic Salmonella typhi carriers, acute typhoid fever and healthy populations in Santiago, Chile.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Characteristics</th>
<th>Reciprocal geometric mean titer</th>
<th>% with reciprocal titer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>≤ 40</td>
</tr>
<tr>
<td>Chronic S. typhi carriers</td>
<td>92% females</td>
<td>296</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>17-59 years</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Acute typhoid fever patients</td>
<td>Both sexes</td>
<td>296</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>18-30 years</td>
<td>29</td>
<td>14</td>
</tr>
<tr>
<td>Chronic carriers with typhoid fever 1-4 years earlier</td>
<td>100% females</td>
<td>24-62 years</td>
<td>388</td>
</tr>
<tr>
<td></td>
<td>100% females</td>
<td>24-62 years</td>
<td>388</td>
</tr>
<tr>
<td>Healthy Chileans</td>
<td>Both sexes</td>
<td>16-46 years</td>
<td>59</td>
</tr>
</tbody>
</table>

* Measured by passive hemagglutination using highly purified Vi antigen.
carrier ≥160 is at least 8% in the general adult population. 16% in women 40 years and older, and 3% in women 25 years and older with history of confirmed typhoid fever. The practical application of the simple passive hemagglutination assay with highly purified Vi antigen to detect chronic *S. typhi* carriers in an endemic area like Santiago, Chile depends greatly on its predictive value. Since the predictive value is high in populations with high chronic *S. typhi* carrier rates (such as older women), screening high-risk groups of the population is warranted as part of a program to control typhoid fever. For this reason, systematic serological screening of foodhandlers in Santiago schools (90% of whom are women over 30 years of age) has been initiated.

Treatment of Chronic Typhoid Carriers

When a chronic *S. typhi* carrier is identified, interventions must be initiated to minimize the chance for transmission of *S. typhi* by the carrier to susceptibles. Health education, including counseling on personal hygiene and food preparation techniques, is fundamental. Ideally, however, therapy to eradicate the chronic carrier state is desired. The currently recognized "gold standard" of therapy involves cholecystectomy followed by several weeks of antibiotic (usually ampicillin or amoxicillin) therapy. Obviously, such a therapeutic regimen involving major abdominal surgery is unsuitable as a routine public health intervention in endemic areas where the prevalence of carriers is high. Thus, for decades, an alternative, nonsurgical therapeutic regimen has been sought to successfully cure chronic *S. typhi* carriers.

Italian investigators (36) reported that two weeks of intravenous ampicillin (1.0 g q 8 h) successfully cured 19 chronic *S. typhi* biliary carriers. However, intravenous antibiotic therapy precludes self-administered domiciliary treatment and thus is also not practical for public health use. The advent of amoxicillin made available a superbly absorbed analog of ampicillin that provides serum levels following oral administration that were previously achievable only with parenteral administration of ampicillin. Furthermore, like ampicillin, amoxicillin is concentrated in bile. Nolan et al. (37) recognized that these features of amoxicillin made it worthy of evaluation as a nonsurgical treatment for the chronic *S. typhi* carrier state. Nolan et al. (37) treated 15 chronic *S. typhi* biliary carriers with oral amoxicillin (2.0 g three times daily) for 28 days. Long-term cures were observed in 9 of 10 carriers who were able to complete the month of therapy.

Encouraged by these preliminary results of Nolan et al. (37), we proceeded to evaluate a 28-day course of oral amoxicillin (2.0 g three times daily) plus probenecid (0.5 g three times daily) in treatment of chronic *S. typhi* carriers in Santiago, Chile. (C. Lanata et al., unpublished data). Twenty-eight confirmed chronic carriers (27 females) began the course of therapy. Antibiotic and probenecid for each day of therapy were provided in small vials. Medication was taken at home or at work and the times of dosing were recorded by the patient in a small diary. Patients were visited in their homes at least once weekly on a scheduled basis. In addition, random unscheduled visits were made at least once weekly. At both scheduled and surprise visits, urine specimens were collected for measurement of amoxicillin levels.

Two of the 28 patients were unable to complete the course of amoxicillin therapy because of severe allergic reactions which were manifested in the first or second day of therapy. Of the remaining 26 carriers who successfully completed the 28-day course of amoxicillin and probenecid, many complained at one time or another of mild diarrhea, rash, nausea, abdominal discomfort, or gastritis. In no instances were the symptoms sufficiently severe to cause discontinuation of therapy.

The success of therapy was monitored by means of stool cultures and bile cultures (obtained by string capsule device) as monthly in-
tervals following completion of therapy. This nonsurgical, ambulatory, domiciliary oral treatment regimen resulted in long-term (1 year) cure of 15 of the 26 carriers (58%). When failure occurred it was usually evident within the first 6 weeks following cessation of therapy. Thirteen of the 26 carriers have had radiological evaluation of their gall bladder function: cholelihiasis, failure of the gall bladder to fill during cholecystogram, or other pathology was present in 13 of 13 carriers examined so far.

A cure rate of 58% with a domiciliary oral antibiotic regimen, despite the presence of gall bladder dysfunction, is encouraging news for treating an individual patient, since there is a greater than ever chance of cure without surgery. However, such a cure rate is too low to advocate its use in public health programs. Therefore, we are continuing to seek an antibiotic regimen that will cure at least 80% of carriers, even with gallstones, without cholecystectomy.

Large-Scale Field Trials of the Ty2la Live Oral Typhoid Vaccine

The live oral typhoid vaccine, Ty2la, developed by Germanier and coworkers (39) represents a potentially major breakthrough for the control of typhoid fever by immunization. In the initial clinical studies with this live attenuated Salmonella typhi oral vaccine in North American volunteers, it was shown to cause no adverse reactions and to be genetically stable and highly protective (39).

The first field trial with Ty2la was carried out in Alexandria, Egypt where approximately 16,000 6 and 7 year old schoolchildren were given three doses (10^4 viable vaccine organisms per dose) within one week (40). Individual doses of lyophilized vaccine contained within small glass vials were reconstituted on the spot, and the children were vaccinated a few minutes after they chewed a tablet containing 1.0 g of NaHCO₃ to neutralize gastric acid. An equal number of children ingested placebo. In this trial, the vaccine provided 96% efficacy for at least three years in an area where the incidence of confirmed typhoid fever in the control group was 40 per 10^5 schoolchildren.

Stimulated by highly encouraging results of the Egyptian trial, a collaborative effort was undertaken to carry out field trials of Ty2la in Santiago, Chile to obtain new information and to evaluate the possible use of this vaccine as a public health intervention to control endemic typhoid fever in Chile.

Two separate field trials of efficacy of one Ty2la vaccine have been undertaken in Santiago, Chile in Area Norte (Trial 1) and Area Occidente (Trial 2). Results of these controlled field trials are summarized below.

Area Norte Trial

The goals of the first Chilean field trial in the Northern Administrative Area (Area Norte) included:

1) To evaluate the efficacy of a new formulation of Ty2la vaccine (enteric-coated capsules) that is more amenable to mass vaccination, since NaHCO₃ pretreatment is unnecessary.
2) To investigate the efficacy of fever (one or two) doses of vaccine than were used in the Alexandria, Egypt field trial.
3) To assess the efficacy in an area of particularly high endemicity and force of infection.

Parents of 91,354 of the 137,697 schoolchildren in Area Norte gave permission for their children to participate in the trial. These children were randomized so that in May and June, 1982, 31,762 received two doses of placebo, 32,707 received one dose of vaccine and one of placebo, and 27,485 received two doses of vaccine (one week apart). The remaining 45,743 unvaccinated children were considered as a separate "control" group.

A summary of 24 months of surveillance is contained in Table 7. Briefly, two doses of the vaccine stimulated a moderate degree (59%) of protection which continued over two typhoid seasons. In Table 8 the results are di-
Table 7. Area Norte field trial. Efficacy of one and two doses of Ty21a live oral typhoid vaccine given in enteric-coated capsules.

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>No. of children</th>
<th>No. of cases*</th>
<th>Incidence (per 10^2)</th>
<th>Percent vaccine efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 doses</td>
<td>27,485</td>
<td>40</td>
<td>145.5</td>
<td>59</td>
</tr>
<tr>
<td>1 dose</td>
<td>32,707</td>
<td>85</td>
<td>259.9</td>
<td>26</td>
</tr>
<tr>
<td>Placebo</td>
<td>31,762</td>
<td>112</td>
<td>352.6</td>
<td></td>
</tr>
<tr>
<td>Not vaccinated</td>
<td>45,743</td>
<td>147</td>
<td>3211.4</td>
<td></td>
</tr>
</tbody>
</table>

* Bacteriologically confirmed by blood or bone marrow culture.

vs p < 0.2.
vs p < 0.04.
vs p < 0.003.

Table 8. 24 Months of surveillance of the Area Norte field trial. Efficacy of two doses of enteric-coated formulation of Ty21a oral typhoid vaccine.

<table>
<thead>
<tr>
<th>Surveillance period</th>
<th>Placebo (31,762)</th>
<th>Two doses (27,485)</th>
<th>Percent vaccine efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cases*</td>
<td>Rate '10^2</td>
<td>No. of cases</td>
</tr>
<tr>
<td>1982 July-Sept.</td>
<td>1</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td>Oct.-Dec.</td>
<td>15</td>
<td>4.2</td>
<td>4</td>
</tr>
<tr>
<td>1983 Jan.-Mar.</td>
<td>37</td>
<td>116.5</td>
<td>10</td>
</tr>
<tr>
<td>Apr.-June</td>
<td>16</td>
<td>50.4</td>
<td>16</td>
</tr>
<tr>
<td>July-Sept.</td>
<td>4</td>
<td>12.6</td>
<td>1</td>
</tr>
<tr>
<td>Oct.-Dec.</td>
<td>14</td>
<td>44.1</td>
<td>1</td>
</tr>
<tr>
<td>1983 Jan.-Mar.</td>
<td>19</td>
<td>59.5</td>
<td>6</td>
</tr>
<tr>
<td>Apr.-June</td>
<td>8</td>
<td>25.2</td>
<td>3</td>
</tr>
</tbody>
</table>

* Bacteriologically confirmed by blood or bone marrow culture.

Provided into three-month periods of observation. In this analysis one notes that there was one three-month period (April-June, 1983) during the 24 months of surveillance when the protective effect of the vaccine appeared to have been overwhelmed (Table 8); in all other periods vaccine efficacy exceeded 57% (Table 8).

Other important observations from the Area Norte field trial include:

1) The vaccine caused no significant adverse reactions in 60,000 vaccinated children.
2) The enteric-coated formulation was found to be highly practical and well suited to mass vaccination.
3) The annual incidence of culture-confirmed typhoid fever in the placebo control group in the first year of surveillance in Area Norte was 214.10^3, a rate more than five times higher than the rate in the control group in the Egyptian trial.
4) One dose of vaccine gave much less protection (30%) than two doses of vaccine (59%).
5) As shown in Table 9, two doses of vaccine also provided moderate protection against S. paratyphi B infection. This makes sense since protection with Ty21a and other live Salmonella vaccines is known to be related to the lipopolysaccharide O antigen (41, 42). The O antigens of S. typhi and S. paratyphi B are related.
Table 9. Efficacy of Ty21a attenuated Salmonella typhi vaccine against S. paratyphi B disease.

<table>
<thead>
<tr>
<th>Vaccine group</th>
<th>No. of children</th>
<th>No. cases of paratyphi B*</th>
<th>Incidence per 10⁵</th>
<th>Percent vaccine efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 doses</td>
<td>27,465</td>
<td>6</td>
<td>21.8⁷</td>
<td>54</td>
</tr>
<tr>
<td>1 dose</td>
<td>32,707</td>
<td>10</td>
<td>30.6⁷</td>
<td>31</td>
</tr>
<tr>
<td>Placebo</td>
<td>31,762</td>
<td>14</td>
<td>44.1¹</td>
<td></td>
</tr>
<tr>
<td>Not vaccinated</td>
<td>45,743</td>
<td>23</td>
<td>50.3</td>
<td></td>
</tr>
</tbody>
</table>

* Bacteriologically confirmed by blood or bone marrow culture.
* vs ¹, p = 0.2.

Area Occidente Field Trial

Based on results of the field trial in Area Norte which showed only moderate efficacy with two doses of enteric-coated vaccine and an apparent overwhelming protection in vaccinees during one three-month period, a second field trial was initiated in Area Occidente of Santiago. In this trial 141,127 children of consenting parents (representing 95% of all schoolchildren in Area Occidente) were randomized to one of five groups to receive:

Group 1—Three doses of vaccine in enteric-coated capsules given within one week.

Group 2—Three doses of vaccine with NaHCO₃ given within one week. The commercial gelatin capsule/NaHCO₃ formulation was used, which consists of two gelatin capsules each containing 0.5 g of NaHCO₃ and one gelatin capsule containing lyophilized vaccine.

Group 3—Three doses of enteric-coated vaccine with an interval of three weeks between the doses.

Group 4—Three doses of the gelatin capsule/NaHCO₃ formulation with an interval of three weeks between the doses.

Group 5—Three doses of placebo.

The design of this trial was intended to allow a direct comparison of two different formulations of vaccine as well as two different immunization schedules. We would like to have included the Egyptian formulation as one cell in this trial; however, that formulation was not available (Table 10). The Egyptian formulation was prepared only for the Alexandria trial and then was replaced by the gelatin capsule/NaHCO₃ formulation, which became available commercially in many countries of the world.

Similarly, the more practical enteric-coated formulation was made in two special lots for the Area Norte and Area Occidente trials in Chile (Table 10): this enteric-coated formulation was not commercially available at that time.

Results of the Area Occidente field trial are shown in Table 11. The clear-cut results allow the following conclusions to be drawn:

1) The extraordinary safety of Ty21a oral vaccine was once again shown in 107,450 Area Occidente schoolchildren who ingested at least two doses of vaccine between July and September, 1983.

2) The enteric-coated formulation was found to be significantly more protective than the gelatin capsule/NaHCO₃ formulation.

3) The long-interval immunization schedule (21 days between doses) gave no greater protective

Table 10. Field trial formulation of Ty21a live oral typhoid vaccine.

<table>
<thead>
<tr>
<th>Vaccine formulation</th>
<th>NaHCO₃</th>
<th>Where used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyophilized vaccine</td>
<td>1.0 g</td>
<td>Alexandria.</td>
</tr>
<tr>
<td>in glass vials under vacuum</td>
<td></td>
<td>Egypt</td>
</tr>
<tr>
<td>Lyophilized vaccine</td>
<td>0.5 g</td>
<td>Santiago, Chile</td>
</tr>
<tr>
<td>capsules</td>
<td></td>
<td>(Area Norte and Area Occidente)</td>
</tr>
<tr>
<td>Lyophilized vaccine</td>
<td>0.5 g</td>
<td>Santiago, Chile</td>
</tr>
<tr>
<td>in gelatin capsules each with</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 11. Efficacy of Ty21a oral typhoid vaccine in Area Occidente after 10 months of surveillance: comparison of two different formulations and immunization schedules.

<table>
<thead>
<tr>
<th>Three doses within one week</th>
<th>No. of children</th>
<th>No. of cases</th>
<th>Rate 10^3/100</th>
<th>Percent vaccine efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteric-coated</td>
<td>22,170</td>
<td>7</td>
<td>31.6</td>
<td>74</td>
</tr>
<tr>
<td>gelatin/NaHCO₃</td>
<td>22,379</td>
<td>24</td>
<td>107.2</td>
<td>12</td>
</tr>
<tr>
<td>Three doses, 21 days between each dose</td>
<td>Enteric-coated</td>
<td>21,596</td>
<td>8</td>
<td>37.0</td>
</tr>
<tr>
<td>gelatin/NaHCO₃</td>
<td>21,541</td>
<td>19</td>
<td>74.2</td>
<td>39</td>
</tr>
<tr>
<td>Placebo</td>
<td>27,793</td>
<td>34</td>
<td>122.3</td>
<td>39</td>
</tr>
<tr>
<td>Not vaccinated</td>
<td>14,962</td>
<td>16</td>
<td>106.9</td>
<td>39</td>
</tr>
</tbody>
</table>

* Bacteriologically confirmed by blood or bone marrow culture.

* or * vs *; p 0.002.

* vs *; p 0.005.

than when all doses of vaccine were given within one week.

4) The level of protection achieved with three doses of enteric-coated vaccine was approximately 0.2%.

5) Some children were absent during one day of vaccination and consequently received only two doses of vaccine. The numbers of such children are small and these children may represent a skewed group at different risk from those who were not absent on any vaccination days. Nevertheless, it seemed worthwhile to compute the incidence rate in the children who received only two doses and compare it to the serum. It was found that the incidence rate was 3.0 cases per 1000 children for the group that received three doses of vaccine or placebo. In Table 12. the incidence of typhoid fever in all children who received three doses of enteric-coated vaccine (both short and long immunization schedules) is compared with the rate in all recipients of two doses of enteric-coated vaccine and with the incidence rate in placebo children. In this analysis, no evidence is found for lesser immunity in recipients of two doses of vaccine. Again one must stress that the groups of two- and three-dose recipients were not randomized and therefore comparison may be inappropriate.

A Third Santiago Field Trial

Based on results of the Area Occidente field trial, it is obvious that the enteric-coated formulation is the formulation of choice and that a short interval immunization schedule is satisfactory. However, it is still unclear whether there is a difference in the protection conferred by two versus three doses given within eight days. Nor is it clear whether a fourth

Table 12. Comparison of efficacy of three versus two doses of enteric-coated Ty21a vaccine in the Area Occidente field trial (10 months of surveillance).

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>No. of children</th>
<th>No. of cases</th>
<th>Incidence per 10^3</th>
<th>Percent vaccine efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteric-coated* 3 doses</td>
<td>43,766</td>
<td>15</td>
<td>34.3</td>
<td>72</td>
</tr>
<tr>
<td>Enteric-coated* 2 doses</td>
<td>9,920</td>
<td>2</td>
<td>20.2</td>
<td>83</td>
</tr>
<tr>
<td>Placebo</td>
<td>27,793</td>
<td>34</td>
<td>122.3</td>
<td>39</td>
</tr>
</tbody>
</table>

* Includes both short- and long-interval schedules.
dose might significantly enhance protection. The answers to these questions were to be sought in a third field trial that began with vaccinations in October 1984. In this trial 285,000 schoolchildren in Area Sur and Area Central of Santiago were randomized to receive two, three, or four doses of enteric-coated vaccine given within eight days. For ethical reasons, no placebo group was included; thus only relative efficacy will be determined.

Implementation Phase

During the past five years, the epidemiology of endemic typhoid fever has been intensively studied and various interventions evaluated. We are now at the point of definitive intervention. Considering severe age wastage: This representation in the settings for the mass Santiago and enteric-coated vaccine Results of the two, three or expected to deviation schedule. Since approx schoolchildren: cost-benefit of high efficiency costs in Santiago apparently all incidence in 40% from the

REFERENCES

Case-control study to identify risk factors for paediatric endemic typhoid fever in Santiago, Chile

ROBERT E. BLACK,1 LUIS CISNEROS,2 MYRON M. LEVINE,3 ANTONIO BANNI,4 HERNAN LOBOS,5 & HECTOR RODRIGUEZ 6

Typhoid fever is an important endemic health problem in Santiago, Chile. Its incidence has more than doubled in recent years, during which access to potable water and sewage disposal in the home became almost universal in the city. A matched case-control study was carried out to identify risk factors and vehicles of transmission of paediatric typhoid fever; 81 children in the 3-14-years age group with typhoid fever were compared with controls, matched with respect to age, sex, and neighbourhood. It was found that case children more frequently bought lunch at school and shared food with classmates. Also, case children more often consumed flavoured ices bought outside the home; none of 41 other food items considered in the study was associated with a higher risk of typhoid fever. Only two food handlers for cases and one for controls were positive for Salmonella typhi, indicating that persons preparing food solely for their own family were not the main source of S. typhi infection. Rather, the risk factors identified in this study are consistent with the hypothesis that paediatric endemic typhoid fever in Santiago is largely spread by consumption of food-stuffs that are prepared outside the individual’s home and are shared with or sold to children.

Typhoid fever is an endemic health problem in Chile, presenting some interesting and mostly unexplained epidemiological features. The illness has a marked seasonality with a peak during the summer months and the highest incidence is in children in the 8-13-years age group (1, 2). Furthermore, its incidence is high in children from both low and high socioeconomic groups, even those who live under apparently nearly optimum sanitary conditions (3).

Significant improvement has been achieved in reducing the mortality rate of typhoid fever in Chile from 12 per 10 000 inhabitants in the 1940s to less than 1 per 10000 in the late 1970s; however, over the same period the morbidity rate has increased from 50 per 10 000 inhabitants to 100 per 10 000 (4). Paradoxically, this increase in morbidity occurred during a period in which access to potable water and sewage disposal in the home increased and became almost universal in urban areas (1, 3). Furthermore, during this time there was a striking reduction in the frequency of most other communicable diseases in Chile (6).

Little is known about the routes of transmission for typhoid fever in Santiago. The two principal hypotheses proposed suggest contamination of food (a) by foodhandlers who are asymptomatic carriers of Salmonella typhi (2) or (b) by the irrigation of fruit and vegetables with sewage-contaminated water (7, 8). As far as the first hypothesis is concerned it should be noted that the prevalence of cholelithiasis in Chile is one of the highest in the world (9) and that this, together with the endemic presence of typhoid fever in the country (1-4), produces a high rate of chronic biliary carriage. It has been estimated that there are nearly 30 000 such carriers of S. typhi in Santiago (a prevalence rate of 694 per 10 000 (10)). With regard to the second hypothesis, sewage in Santiago is discharged untreated into the Mapocho river and a large canal; water drawn from these sources is used to irrigate crops, such as lettuce and celery, which are grown near the city (7). High faecal
coliiform counts have been measured in this water, and \textit{S. typhi} organisms have been isolated from it \textit{(8)}. The aim of the present study was to identify risk factors and vehicles of transmission of typhoid fever in the eastern part of Santiago, an area that accommodates families of all economic strata, but mostly middle- and high-income persons living in modern housing.

**MATERIALS AND METHODS**

A matched case-control study was conducted from December 1980 to June 1981. Cases selected were children of either sex in the age group 3-14 years who lived in the eastern area of Santiago and who were diagnosed as having typhoid fever as confirmed by blood and/or bone marrow cultures that were positive for \textit{S. typhi}. Children were diagnosed and treated at the Calvo McKenna Hospital (serving mainly low and middle socioeconomic groups) or by 20 paediatricians who care more for middle and upper socioeconomic groups in their private practices. A surveillance system was established to ensure that two blood cultures were obtained from each child with suspected typhoid fever at either the hospital or the private practices. Blood was cultured in a medium of supplemented peptone broth,\textsuperscript{a} and processed by standard methods \textit{(11, 12)}.

Controls were children of the same sex and age (= one year of age) as the cases, and lived in the same neighbourhood. They were identified by following a standardized routine which started at the home of the case. Controls who had had a febrile illness suggestive of typhoid fever during the four weeks prior to their participation in the study were excluded and a new control was selected.\textsuperscript{a} Once an appropriate control was identified, return visits to the neighbourhood were made until complete information was obtained.

Two public health nurses filled in questionnaires concerning cases and their matched controls, and the answers given by the children were verified by interviewing their mothers. For young children, the questions were answered by their mothers. Cases and matched controls were interviewed by the same nurse.

The questionnaire explored the following areas: socioeconomic level (type of house construction and ownership: number of rooms, bed(s), and persons in house, and ownership of car); sanitary conditions at home (existence of water source and bathroom facilities), food and drink consumption (42 items), both at home and out of home (for the two weeks prior to the onset of illness in cases and over the same period for the matched controls); existence of cooks and/or maids at home and their role in food preparation; frequency of eating food from street vendors, in restaurants, or at school; history of gall bladder disease among the food handlers at home; contact with known cases of typhoid fever (in the two months preceding the study); and travel and swimming activities (in the month prior to onset of illness for cases and in the same period for the matched control).

For both cases and controls, two stool samples were obtained from the primary food handler in the household. The initial sample was obtained by rectal swab at the time of the interview and placed in Cary-Blair transport medium and cultured the same day. The second sample was a stool obtained during the morning of the following day which was kept refrigerated and was cultured within six hours. Faecal samples were cultured on \textit{Salmonella-Shigella}, MacConkey's, or bismuth sulfit agar, either directly or after selective enrichment for 24 hours; colonies harbouring \textit{Salmonella} or \textit{Shigella} bacteria were identified using standard techniques \textit{(12)}.

Statistical analysis of the results for matched pairs was carried out using the McNemar test \textit{(13)} if the outcome was dichotomous, and a similar test derived by Fleiss \textit{(13)} if triphotonous.

**RESULTS**

Eighty-one cases met the criteria for inclusion in the study; 67 were from Calvo McKenna Hospital and 14 were from the practices of private paediatricians in Santiago. The largest number of cases were 6-year olds, with relatively few below this age and fairly uniform frequencies above it. There were equal numbers of male and female children (Fig. 1).

\textsuperscript{a} From Becton Dickinson and Co., Oxnard, CA 93030, USA.
RISK FACTORS FOR PAEDIATRIC ENDEMIC TYPHOID FEVER

Table 1. Association of selected risk factors with paediatric typhoid fever in Santiago, Chile

<table>
<thead>
<tr>
<th></th>
<th>Case:Control pairs</th>
<th></th>
<th>Relative risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes:Yes</td>
<td>Yes:No</td>
<td>No:Yes</td>
</tr>
<tr>
<td>History of typhoid fever in a relative</td>
<td>0 16</td>
<td>5 58</td>
<td>5 58</td>
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<tr>
<td>Bought lunch at school*</td>
<td>2 12</td>
<td>3 35</td>
<td>3 35</td>
</tr>
<tr>
<td>Travel outside Santiago</td>
<td>12 8</td>
<td>21 40</td>
<td>21 40</td>
</tr>
<tr>
<td>Swimming in a lake</td>
<td>9 0</td>
<td>8 72</td>
<td>8 72</td>
</tr>
<tr>
<td>Swimming in a pool</td>
<td>10 7</td>
<td>18 48</td>
<td>18 48</td>
</tr>
</tbody>
</table>

* Analysis confined to cases that occurred while school was in session.

Rectal swabs were obtained from 78, and stool samples from 77, of the 81 domestic food handlers for cases; 2 food handlers were positive for *S. typhi*, including the mother of one case and the cook (female) of another. Swabs were also obtained from 61, and stool from 71 food handlers of controls. One of these food handlers was positive for *S. typhi*; this was the mother of a control child whose own children were negative for *S. typhi* but who prepared flavoured ice cream for the sick child to eat. Food handlers who prepared ice cream for the sick child were more likely to have their own children with typhoid fever than were food handlers who did not prepare ice cream for sick children. Four of the food handlers had a family member with typhoid fever prior to the onset of illness in the matched case (Table 1).

Controls travelled away from Santiago more frequently than did cases in the month before the onset of illness in the matched case (Table 1). This lower risk for controls who travelled seemed to hold during both summer (relative risk 5.10) and non-summer (relative risk 3.10) months. Controls also swam more frequently in a lake or pool than cases (Table 1).

Seven additional food handlers had stool cultures positive for other enteropathogens, including *S. paratyphi* B (3), other salmonellae (3) and *Shigella* (1).

The families of cases and matched controls had the same number of persons in the home, and all had household electricity, sewage disposal facilities, sinks in bathroom and kitchen, and a source of water, or owned a refrigerator or an automobile. The relatives of cases (usually cousins), but not friends, were more frequently (relative risk 3.3) reported ill with typhoid fever during the two months preceding the study (Table 1). Cases more frequently (relative risk 4.0) ate lunch bought at school than controls (Table 1), but both groups ate food from street vendors, school kiosks, and restaurants. In addition, cases more frequently shared food at school with friends (Table 2). This increased risk was particularly apparent for children who shared food in this way three or more times per week (relative risk 6.0) (Table 2), and seemed to be more important for children who brought food to school (relative risk 10.2, $\chi^2 = 5.4$, $P < 0.05$) than for children who purchased school food (relative risk 2.0, not significant).

Controls travelled away from Santiago more frequently than did cases in the month before the onset of illness in the matched case (Table 1). This lower risk for controls who travelled seemed to hold during both summer (relative risk 5.10) and non-summer (relative risk 3.10) months. Controls also swam more frequently in a lake or pool than cases (Table 1).

The consumption patterns of cases for fruits and vegetables and other food products were obtained for two weeks prior to onset of their illness and for the same period in matched controls. Consumption of

<table>
<thead>
<tr>
<th>No. of cases sharing food</th>
<th>No. of matched controls sharing food</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-2 times/week</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>No. of cases sharing food</th>
<th>No. of times/week</th>
<th>No. of mat. controls sharing food</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\geq 3$ times/week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(121)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\geq 1$-2 times/week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(34)</td>
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</table>

* $\chi^2 = 7.3$, $P < 0.01$ (4 degrees of freedom).

** Figures in parentheses are cases.
Table 3. Frequency of consuming purchased flavoured ices by typhoid fever cases and matched controls

<table>
<thead>
<tr>
<th>Case consumption</th>
<th>0-2 times/week</th>
<th>1-2 times/week</th>
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<tr>
<td>≥3 times/week</td>
<td>15</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>1-2 times/week</td>
<td>4</td>
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<td>2</td>
</tr>
<tr>
<td>Never</td>
<td>3</td>
<td>5</td>
<td>28</td>
</tr>
</tbody>
</table>

*p = 7.3, P < 0.05-2 degrees of freedom.
* Figures in parentheses are totals.

purchased flavoured ices was associated with a higher risk of typhoid fever, particularly among children consuming such ices three or more times per week (relative risk 3.0) (Table 3). Consumption of flavoured ices made in the home was not associated with typhoid fever (relative risk 5/9). No association was found between consumption of the other food items, including suspected vehicles or groups of these items, and development of typhoid fever. For example, the relative risk associated with consumption of lettuce was 14/19 (0.74) and strawberries 22/19 (1.2). One food item, more con huesillos (a local drink made from corn and apricots), was associated with a significantly lower risk of typhoid fever (relative risk 4/16, $\chi^2 = 9.9, P < 0.01$).

**DISCUSSION**

The epidemiology of endemic diseases is frequently complex. Unlike common-source outbreaks, many vehicles, each responsible for a few cases, may be involved, and this might be the situation regarding typhoid fever in Santiago, Chile. The present study incriminated only one food item but identified a number of factors associated with a higher or lower risk of developing typhoid fever.

Since more than 70 variables were investigated, it can be expected that a few of them are statistically significant by chance alone at a $P$ level of less than 0.05. In some instances, however, the statistical significance of the association was greater than 0.05. Furthermore, some risk factors were corroborated by statistical significance of two or more related variables, e.g., travel outside Santiago and swimming in lakes or pools, usually outside the city. Since these are related variables, the finding of all three to be important factors suggests that this was not due to chance. Cases were probably matched so closely with controls from the same neighbourhood for socioeconomic status that potential risk factors associated with wealth, education, or place of residence may have been overlooked. On the other hand, this study design allowed examination of important risk factors without the potentially confounding effects that may have arisen had cases and controls differed in socioeconomic status.

The study identified flavoured ices as a vehicle of transmission of typhoid fever among children in Santiago; however, the precise means of contamination of the ices has not yet been established. One possibility is that the water used to prepare them was contaminated, but the almost universal household access to water of good quality in study families and in Santiago in general (3) and the failure of this study to implicate the water source as an important risk factor suggest that this is unlikely. Another possible explanation is that S. typhi carriers contaminated the containers, the water, or the ice in their homes while preparing the flavoured ices for sale. In this respect, it is pertinent that we identified an S. typhi carrier who had prepared and sold flavoured ices to a child who subsequently developed typhoid fever. The lack of significance, as a risk factor, of flavoured ices made and consumed at home further emphasizes that it is the preparation of the food item by carriers outside the home that is important.

Consuming lunch bought at school cafeterias and sharing food with classmates who did not buy lunch at school were both associated with a higher risk of typhoid fever. Although we did not detect any clustering of cases in particular schools, these data suggest that one transmission route of S. typhi is the consumption of school food that was prepared on the school premises, brought by their children from home, or bought from food vendors. It is also possible that certain children have a tendency to eat food prepared outside their own homes, thus exposing themselves to food prepared by a wide variety of persons, some of whom could be chronic carriers of S. typhi. The higher frequency of reported (but undocumented) typhoid fever among relatives of cases compared to those of controls could be due to reporting bias; however, it may indicate that the case and the relative had shared a common food exposure.

The reduced risk of typhoid fever among children who travelled out of Santiago may be a marker of socioeconomic status but could also indicate that they were removed from the source of infection, being safer away from the city. The incidence rates of typhoid fever in the popular holiday resorts are much lower than those in Santiago (2).

This study indicates that food handlers who prepared food solely for their own families were not
the main source of \textit{S. typhi} infection. Cultures of two stool samples should identify most asymptomatic carriers, and only two carriers were found in the homes of the 81 cases. Furthermore, this finding was corroborated by a later study of family members of typhoid fever patients in Santiago (15). Using three stool cultures and measurement of Vi antibodies (16), only one chronic carrier was found among the family members of 24 patients with typhoid fever. Although chronic carriers are undoubtedly important in the transmission of \textit{S. typhi}, all these studies indicate that such carriers within the household could account for only a small fraction of typhoid fever cases. The risk factors identified in the present study are consistent with the hypothesis that endemic typhoid fever in Santiago is largely spread by exposure to food items that are prepared in schools, private homes, or by food vendors and that are shared with or sold to children. From this study, it cannot be determined whether contamination of these items with \textit{S. typhi} was a result of their preparation by chronic \textit{S. typhi} carriers or because the raw foodstuffs were contaminated by \textit{S. typhi} from Santiago sewage. Further epidemiological and bacteriological studies are planned to resolve this issue.

ACKNOWLEDGEMENTS

This study was funded by grants from the World Health Organization, the Pan American Health Organization, and by research contract No. DAMD 17-0-015 from the US Army Medical Research and Development Command. The authors would like to thank the paediatricians of the eastern area of Santiago who helped in identifying cases of typhoid fever, Margarita Canales and Viviana Sotomayor for field data collection, Aurora Maldonado for laboratory assistance, and Dr. Caterine Ferreccio for helpful comments on the manuscript.

RÉSUMÉ

\textbf{ETUDE CAS-TÉMOINS EN VUE DE DÉTERMINER LES FACTEURS DE RISQUE DE LA FIÈVRE TYPHOIDE EN DÉMOCRATIE DE L'ENFANT A SANTIAGO DU CHILI.}

La fièvre typhoïde est un problème de santé endémique au Chili, qui atteint un paroxysme pendant l'été et se répand surtout chez les enfants de 8 à 13 ans. À Santiago, l'incidence de la fièvre typhoïde a doublé ces dernières années, bien que la quasi-totalité des ménages aient accès à l'eau potable et à l'évacuation des eaux usées; l'incidence est élevée, tant dans les quartiers pauvres que dans les quartiers riches de la ville. Une étude de cas et de témoins apparaît à être entreprise en vue de déterminer les facteurs de risques et les véhicules de transmission de la maladie. Quatre-vingt-un enfants de trois à quatorze ans, souffrant de fièvre typhoïde confirmée par analyse bactériologique ont été comparés à des sujets témoins appariés par âge, sexe et voisinage. L'étude a révélé des facteurs de risques qui correspondent à l'hypothèse selon laquelle, à Santiago, la contamination de produits alimentaires par les enfants vendus à ceux-ci hors de la maison est une large mesure responsable de la propagation de la maladie. La contamination de ces produits par \textit{Salmonella typhi} peut être due à leur préparation par des porteurs chroniques de \textit{S. typhi} ou au fait que les derniers entrant dans leur composition contiennent \textit{S. typhi}, par suite d'une pollution par les eaux usées.

REFERENCES


TYPHOID FEVER IN SANTIAGO, CHILE: A STUDY OF HOUSEHOLD CONTACTS OF PEDIATRIC PATIENTS*

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Abstract. We obtained clinical, epidemiological, and laboratory data (including three stool cultures) from 155 (96%) of 161 household contacts of 24 patients <16 years old with culture-confirmed typhoid fever; these 24 patients represented approximately 40% of such patients seen in three hospitals in Santiago during a 12-week period. A chronic typhoid carrier was identified in only one household, with concurrent or secondary cases seen in two other households. When index cases were matched with household members nearest in age, no specific risk factors for illness could be identified. There was evidence of generalized exposure to enteric pathogens within these households, with nine persons from seven different households culture-positive for non-typhoidal Salmonella, and nine, from eight different households, culture-positive for Shigella; transmission of these pathogens within households did not appear to be common since no household had more than one family member with the same serotype or species of either pathogen.

Typhoid fever is endemic in Santiago, Chile, where an average of 170 cases per 100,000 population per year was reported for the years 1977 - 1981. The incidence of typhoid fever is highest among children 3 - 13 years of age and relatively low among children ≤ 4 and adults aged ≥ 25 years. Over 75% of cases occur during the summer and early fall (December-May). Despite generally high standards of sanitation and medical care, the incidence rate for typhoid in Santiago, rather than declining, has almost doubled during the past decade. The reasons for the large number of cases in the city for the recent increase in incidence are still not well understood; contributing factors may include a high chronic carrier rate (estimated at 694 carriers per 10⁶ population), problems with food sanitation exacerbated by recent deregulation of the local food service industry, and use of untreated waste water for irrigation of crops in the summer.

We sought to examine these and other factors by studying households in Santiago in which a child had recently been diagnosed as having typhoid fever. Within households we attempted to identify chronic typhoid carriers and possible concurrent cases; we also attempted to identify risk factors for infection, including patterns of food consumption away from the household and preferences for specific "high risk" foods such as raw vegetables. Although our study was designed to evaluate infections with Salmonella typhi, the culture techniques used allowed us to identify other Salmonella and Shigella species in stool samples; as we found a relatively high rate of stool carriage of both non-typhoidal Salmonella and Shigella, clinical and epidemiological data related to these pathogens have been included in our analysis.

METHODS

Laboratory records in three Santiago hospitals (Hospiral Felix Bulnes, Hospital San Juan de Dios, and the Infectious Diseases Hospital) were reviewed daily or every other day during a 12-week period in March, April, and May 1983. Efforts were made to visit the households of all patients <16 years of age who had culture-confirmed typhoid fever; if more than one case was identified on a single day, the household of the youngest patient identified was visited. A standardized questionnaire was administered to each index case and to each household member; data

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requested included the occurrence of symptoms such as fever or diarrhea, frequency of eating food away from the household (at school, at the homes of relatives or friends, or purchased from street vendors), recent travel history, history of exposure to persons with typhoid outside of the family, and a limited list of food preferences (ice cubes purchased outside of the home; raw vegetables, including lettuce, cabbage, celery, and tomatoes; fresh fruit; and fresh seafood). A second questionnaire related to the physical characteristics of the household was administered to the head of each household.

Three stool cultures were collected from each household member, with efforts made to obtain cultures on days 1, 2, and 7 after the initial household visit. Persons were instructed to sample a fresh stool with a sterile swab and place the swab in a vial containing Cary-Blair transport medium. At the Enteric Laboratory of the Institute of Public Health swabs were directly plated on SS, MacConkey's and bismuth sulfate agar, and then placed in selenite enrichment for 24 hours. Colonies suspicious for Salmonella or Shigella were identified using standard techniques. All isolates were serotyped or speciated, or both, by the Reference Laboratory of the Institute of Public Health. Plasmids were identified in all Shigella isolates using an alkaline plasmid extraction technique. At the time of the first household visit a blood sample was collected from each household member over 10 years of age. Serum was frozen at -20°C until the conclusion of the study, at which time samples were assayed for Vi antibodies by passive hemagglutination, using purified S. typhi Vi antigen (kindly provided by John Robbins, Bureau of Biologics, Bethesda, MD); titers \( \geq 1:160 \) were regarded as positive. Four water samples for fecal coliform and chlorine analysis were collected on successive visits from the kitchen water supply in each household.

RESULTS

Families of 24 patients (representing approximately 40% of eligible families) were contacted and agreed to participate in the study. The average age of these patients was 10 years (standard deviation ± 4 years), compared to an average age of 8.9 years for all reported typhoid cases in Santiago among patients <16 years old. Excluding the index cases, a total of 161 persons lived in the 24 households: complete clinical, epidemiologic, and laboratory data were obtained for 138 (96%). Thirty persons in 18 households, were <10 years of age; 14 in 10 households, were ≥4 years of age.

Households included in the study were concentrated in the western part of the city near the three hospitals surveyed (Fig. 1). Households had an average of 2.4 persons per room (range 0.9–4.6). Nineteen (79%) of the households were connected to the city sewerage system, all had electricity, and all but one had municipal water. Because of delays in obtaining initial cultures and in confirming the identification of isolates, the average time between a patient’s onset of symptoms of our initial visit to the household was 21.5 days (range 11–37 days).

Salmonella typhi

We identified a chronic S. typhi carrier on only one household. The carrier, the 38-year-old stepfather of the index case, gave a history of having had typhoid fever in 1960. He was asymptomatic at the time of the study, with multiple positive stool cultures for S. typhi; he had a strongly positive Vi antibody titer (1:640).
Apparent concurrent or secondary cases were identified in two households. In the first, all six household members were culture positive for *S. typhi*, four were symptomatic and two were hospitalized. No household member had a history of *typhoid* and none had an elevated Vi antibody titer. The water supply for the household came from a well which was not chlorinated, and water samples had consistently high fecal coliform counts. In a second household, the index case's 28-year-old sister developed fever and gastrointestinal complaints, with positive stool cultures for *S. typhi* 5 weeks after onset of symptoms in the index case; she had no previous history of *typhoid* and did not have an elevated Vi antibody titer.

Thirty-six (60%) of 60 culture-negative household members <16 years of age ate food outside of the household at least once during an average week, compared to 19 (35%) of 22 index cases (excluding 2 index cases for whom no matched household controls were available) (*P* = 0.01, by the method of Pike and Morrow for matched cases and variable numbers of controls). However, index cases were significantly older than household controls (mean age 10 years vs. 8.5 years; *P* = 0.03, Mann-Whitney U test), and it was possible to show within the control group that older children were more likely than younger children to eat away from home (*P* = 0.01, Mann-Whitney U test). When index cases were matched with the culture-negative household member closest in age (but <16 years old) the difference in food consumption outside of the household was not significant (*P* > 0.05, McNemar test for matched cases and controls). No other risk factors for illness could be identified, using both age-matched and non-aged-matched controls.

Serum samples were obtained from 122 household members. Two persons had Vi antibody titers of ≥1:160: one was a presumed chronic carrier, as noted above, the other, with a titer of 1:160, was a 32-year-old female with no previous history of *typhoid* who had three negative stool cultures. In two households municipal water was collected in tanks which, when tested, contained no chlorine; all other municipal water samples had adequate levels of chlorine, and none had significant numbers of fecal coliforms.

**Non-typhoidal Salmonella and Shigella**

Nine (5.8%) of the 155 household members had positive stool cultures for non-typhoidal *Salmonella* (Table 1a), with culture-positive persons identified in seven households. Ages of persons culture-positive for *Salmonella* did not differ significantly from ages of culture-negative persons (*P* > 0.05, Mann-Whitney U test) (Fig. 2a). No significant risk factors for infection were identified when culture-positive household members were matched with culture-negative household members, nor was it possible to show an association between household characteristics (number of persons in household, number of persons per room, water and sanitation facilities) and the presence of culture-positive persons in the household. In the two households with more than one culture-positive person, household members had different species or serotypes of *Salmonella*.

Nine household members (5.8%) had positive cultures for *Shigella* (Table 1b), with culture-positive persons identified in eight households (including 2 households in which persons culture-positive for *Salmonella* were also present). The ages of persons culture-positive for *Shigella* did not differ significantly from ages of culture-negative persons (Fig. 2b). None of the 30 household members <10 years of age were culture-positive. No significant risk factors for infection were identified, either between matched culture-positive and culture-negative household members, or between households with and without culture-positive persons. In the one household that had more than one person culture-positive for *Shigella*, household members were colonized with different species of *Shigella*. All isolates contained the 146-md plasmid associated with invasiveness.

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. symptomatic</th>
<th>No. asymptomatic</th>
</tr>
</thead>
<tbody>
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<td><em>Salmonella</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>typhi</em></td>
<td>0</td>
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</tr>
<tr>
<td><em>typhimurium</em></td>
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<td>1</td>
</tr>
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<td><em>typhi</em></td>
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<td><em>typhi</em> B</td>
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</tr>
<tr>
<td>Total</td>
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<td>4</td>
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<td>3</td>
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<tr>
<td>Total</td>
<td>4</td>
<td>5</td>
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</table>
TYPHOID FEVER IN SANTIAGO, CHILE

We identified only a small number of the observed cases. We did identify a second person with a borderline elevated Vi antibody titer; the significance of this result is unclear; as the person had three negative stool cultures, this may represent a false-positive test.

We identified possible concurrent or secondary cases in two households. As we did not visit homes until an average of 3 weeks after onset of symptoms in the index case, we may have missed some early cases among household members; in untreated infections, however, stool cultures are most likely to be positive at 3 weeks, with stool carriage continuing until the 7th or 8th week in over 50% of patients. Given the number of young (and potentially susceptible) children in the households studied, our inability to identify additional cases suggests that transmission of S. typhi within households (including acquisition by consumption of a common vehicle within a household) was not a frequent occurrence.

We were unable to implicate any one specific vehicle (such as raw vegetables) in the transmission of typhoid. There was an association between age and eating food outside of the household, and a possible interrelationship between these factors and illness: index cases were more likely to eat food outside of the household than were household controls (culture-negative household members < 16 years of age), but cases were also older than household controls, and it was possible to show that older children were more likely than younger children to eat away from home. Typhoid cases in Santiago occur more frequently in older children, with very few cases in children ≤ 4; if eating food outside of the household is a risk factor for illness, it may partially explain the observed age distribution of cases in the city.

We found a relatively high rate of carriage of non-typhoidal Salmonella and Shigella, with 11% of household members, in 54% of the households studied, infected with at least one pathogen other than S. typhi. In the absence of data from control families it is difficult to know how accurately this reflects the carriage rate of such pathogens in the general population; within the study population, however, the rates are comparable to those seen in countries such as Bangladesh. No household in the study had more than one person culture-positive for the same species of either Salmonella or Shigella, suggesting, as with S. typhi, that these pathogens were infrequently transmitted within households; one might also have
expected to see more cases among young children.

With the relatively small size of our study we were not able to identify any specific risk factors for transmission of S. typhi non-typhoidal Shigella. However, the data did show a relatively high level of exposure to bacterial enteric pathogens within the study population. Future studies should focus on events of transmission outside the household.

REFERENCES


Benign bacteremia caused by Salmonella typhi and paratyphi in children younger than 2 years

Catterine Ferreccio, M.D., Myron M. Levine, M.D., D.T.P.H., Alejandro Manterola, M.D., German Rodriguez, M.D., Isabel Rivara, M.D., Ingeborg Prenzel, M.D., Robert E. Black, M.D., M.P.H., Thomas Mancuso, M.D., and Dorothy Bulas, M.D.
Baltimore, Md., and Santiago, Chile

Typhoid fever has remained endemic in Santiago, Chile, for decades; since 1977 the incidence has exceeded 150 cases per 100,000 population. Typhoid fever occurs mainly in persons 5 to 25 years of age (Table 1), and is generally manifested as a classic clinical syndrome including fever, abdominal discomfort and distention, headache, malaise, constipation, and hepatosplenomegaly.

Few cases of typhoid fever are reported in children younger than 2 years. Thus it was necessary to determine whether the very low reported incidence of typhoid fever in young children represents a lack of consumption of the vehicles that transmit Salmonella typhi to older children or whether infection occurs but the infant host manifests an atypical response that is not readily recognized clinically. To help resolve this question, we systematically performed blood cultures in children younger than 2 years with fever who were seen at two health centers in Santiago during the 3 peak months of the typhoid fever season.

METHODS

Rectal temperatures were recorded in all children younger than 2 years who were seen at Pincoya and Consultorio Dos health centers in the northern administrative area (Area Norte) of Santiago from January through March 1983. In all children with a temperature ≥38°C, 2 ml blood was drawn for culture and inoculated into a flask containing 35 ml brain-heart infusion with 0.017 sodium polyanethol sulfonate. The reason for the blood culture was explained to the parents, and verbal informed consent was obtained, according to local custom. The study was discontinued in the last week of March, by which time blood from 197 consecutive children had been cultured. Cultures were
Table I. Age-specific incidence and cases of typhoid and paratyphoid fever, Santiago, Chile, 1977-1931

<table>
<thead>
<tr>
<th>Age</th>
<th>Metropolitan Santiago*</th>
<th>Northern Administrative Area of Santiago - Area Norte†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean annual cases</td>
<td>Mean annual incidence per 100,000</td>
</tr>
<tr>
<td></td>
<td>Per 100,000</td>
<td></td>
</tr>
<tr>
<td>0 to 4</td>
<td>222</td>
<td>89.2</td>
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<tr>
<td>5 to 9</td>
<td>239</td>
<td>272.2</td>
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<tr>
<td>10 to 14</td>
<td>143</td>
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<td>15 to 19</td>
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*Mass population 144,421
†Mass population 142,451
‡Approximately 95% of cases in all age groups are typhoid fever, 10% paratyphoid fever

Table II. Clinical findings in infants* with S. typhi and S. paratyphi bacteremia

<table>
<thead>
<tr>
<th>Age</th>
<th>Temperature of fever (°C)</th>
<th>Duration of fever (days)</th>
<th>Anorexia</th>
<th>Vomiting</th>
<th>Constipation</th>
<th>Diarrhea</th>
<th>Cough</th>
<th>Hepatosplenomegaly</th>
<th>Clinical diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 4</td>
<td>38.3</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5 to 9</td>
<td>38.3</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10 to 14</td>
<td>38.3</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15 to 19</td>
<td>38.3</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>20 to 24</td>
<td>38.3</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>25 to 29</td>
<td>38.3</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>30 to 34</td>
<td>38.3</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>35 to 44</td>
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<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>45 to 64</td>
<td>38.3</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>65+</td>
<td>38.3</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Includes only the seven infants detected by active surveillance in this prospective study.

Isolated at 37°C for 7 days, and suspicious colonies were confirmed as S. typhi by standard biochemical and serologic techniques. S. typhi were phage typed at the Institute of Public Health, Santiago. A standardized medical history and physical examination were recorded for all infants. Infants with positive cultures were recalled, reexamined, and given chloramphenicol (50 mg/kg/day PO).

RESULTS

Of the 197 children, 50 were younger than 6 months (no newborn infants). 68 were 6 to 11 months of age, 57 were 12 to 17 months, and 22 were 18 to 23 months; 93% of the fevers recorded were between 38°C and 39°C. Acute respiratory infections (44%), diarrhea (20%), and viral syndrome (17%) were the most common clinical diagnoses at the time of examination. None of the infants appeared severely ill, and in no instance was enteric fever considered in the differential diagnosis. Consequently, were it not for the study protocol, a blood culture would not have been taken from any infant.

S. typhi was isolated from four children (2%), S. paratyphi B from 2 (1%), and S. paratyphi A from 1 (0.5%). All other blood cultures were negative. Four isolations occurred in January, one in February, and two in March. Two S. typhi strains were nontypable. However, the remaining two were phage type E1 and 46, the two most common types in Santiago.

The clinical syndrome in these infants prior to examination was mild, consisting of 1 to 5 days of fever between 38.3°C and 38.8°C (Table II). Six of the seven infants, including all four with S. typhi, had cough, and one had clinical and radiographic evidence of pneumonia. None had splenomegaly, but one had minimal hepatomegaly. On follow-up it was found that none of the infants had completed the course of chloramphenicol therapy; the mothers had spontaneously discontinued the medication.
Discussion

Most information on the age distribution of typhoid fever stems from hospital-based studies. Three major points recur in these reports: (1) S. typhi infection is notably less common in children younger than 2 years (usually <10% of the cases). (2) The clinical syndrome is often distinct from that encountered in older children, and commonly includes vomiting, diarrhea, convulsions and meningismus, and respiratory signs, in addition to fever. (3) Most reports state that hospitalized infants with typhoid fever are quite ill and that a bacteremic infectious process (e.g., sepsis, meningitis) is usually suspected.

Two main hypotheses have been put forth to explain the low reported incidence of typhoid fever in children younger than 2 years: (1) Infants and young children do not ingest the vehicles of transmission of S. typhi that are consumed by older children. (2) Infants and young children consume contaminated vehicles of transmission but do not readily develop recognizable clinical illness because of host factors peculiar to the age group. If the latter is correct, and infants are becoming infected but are manifesting only mild illness, evidence of such infections would have to be sought by systematic investigation of nonhospitalized, mildly ill infants. This pilot study in Santiago, an area where typhoid fever is endemic, represents the first systematic attempt to decipher this problem. The isolation of S. typhi and S. paratyphi from blood cultures of 3.6% of 197 febrile but mildly ill infants seen at health centers during the summer months demonstrates that during the peak typhoid fever season, children younger than 2 years are becoming infected at a much higher rate than previously appreciated. During this same 3-month period, two infants from the registered population served by these two National Health Service community health centers were admitted directly to the hospital with severe illness confirmed by blood culture to be typhoid fever. Thus at least two mild, unrecognized, bacteremic S. typhi infections in young children may exist for every clinically overt, confirmed case.

In the pathogenesis of typhoid fever, two bacteremias occur at distinct stages. The primary bacteremia appears within hours after ingestion of the pathogen. On reaching the small intestine, the S. typhi rapidly pass through the mucosa to reach the lamina propria, where they elicit a chemotactic response resulting in an influx of macrophages. Primary access to the bloodstream occurs either during mucosal invasion or after drainage to mesenteric lymph nodes and entrance into the blood by way of the thoracic duct. This primary bacteremia is short-lived and clinically inapparent. Viable S. typhi persist in the reticuloendothelial system after being cleared from the blood. After incubation of 10 to 14 days, and concomitant with the onset of clinical illness, the secondary bacteremia characteristic of typhoid fever occurs. It is not clear whether the enteric fever organisms in the blood of these infants represent the fortuitous detection of primary bacteremia or whether it denotes secondary bacteremia in infants with a particularly benign form of the disease. Earlier reports noted the mildness of pathologic alterations caused by S. typhi in the intestines of infants compared with those in older children, as well as the frequency of respiratory signs and symptoms.

Ashcroft pondered why some less developed areas with appalling sanitation have little typhoid fever, whereas other somewhat more developed countries have endemic disease with high incidences in schoolchildren and young adults. He hypothesized that in areas with the most primitive sanitation and hygiene, widespread asymptomatic or mild infection of infants and young children occurs, leading to immunity and exhaustion of susceptible individuals after the first few years of life. According to Ashcroft's hypothesis, frequent infection of infants and young children would not be expected in a more developed country such as Chile, where the epidemiologic pattern of typhoid fever reveals the peak reported incidence in schoolchildren and young adults. Nevertheless, our preliminary data support the concept that infants become infected at a higher rate than is commonly appreciated and manifest a very mild clinical illness (not recognized as enteric fever), albeit accompanied by demonstrable bacteremia.

References

INVESTIGACION SOBRE EL ESTADO DE PORTADOR DE SALMONELLA TYPHI-PARATYPHI EN PACIENTES INTERVENIDOS POR PATOLOGIA VESICULAR

Conrado Ristori, Héctor Rodríguez, Patricia Vicent, Hernán Lobos, Karen D’Ottone, Julio García, María Eugenia Pinto, Patricio Nercedes y Luis Cisneros

La elevada morbilidad de la fiebre tifoidea en Chile, junto a las datos demostrativos de que existe una correlación entre el estado de portador de S. typhi y las colecistopatías encontradas en otras regiones, condujo a analizar 1 000 muestras de bilis de pacientes con colecistopatía. Los resultados indican que la prevalencia excepcionalmente elevada de colecistopatías en Chile constituye un factor importante en la transmisión de la fiebre tifoidea.

Introducción

En Chile, la morbilidad por fiebre tifoidea presenta una tendencia ascendente, intensificada en forma notable a partir del último quinquenio, con tasas superiores a 120 por 100 000 habitantes (7). Esta situación resulta sorprendente, ya que la mayoría de los casos se asocian con enfermedades crónicas u otras condiciones sanidad, por lo que el fenómeno se atribuye al hecho de que la mayoría de los enfermos se encuentran en Santiago que, si bien dispone de la más alta cobertura del país en agua potable y sistemas para eliminación de excretas, contribuye con dos tercios al total de casos (1-7).

Es bien conocido que el papel más importante en la transmisión de la enfermedad lo desempeñan los portadores (6-7), cuyo número se incrementa por la existencia de por lo menos tres casos subclínicos o inaparentes por cada caso diagnosticado. Por este motivo, la elevada prevalencia de las colecistopatías en el país (7-11) nos indica que la relación entre la enfermedad y la transmisión de la fiebre tifoidea, ya mencionada en trabajos realizados en otros países, (Estudios epidemiológicos y anatomicopatológicos en Chile han revelado que entre los adultos la linhias vesicular se observa en una proporción del 50% en el sexo femenino y del 20,5% en el masculino (7,8).

Propósito del estudio

El objetivo del estudio fue analizar la flora microbianas que se encuentra en la bilis de enfermos sometidos a colecistectomía en la ciudad de Santiago, y en especial las salmonellas del grupo tifico-paratípico. En trabajos similares realizados en países con baja inci-

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3 Ministerio de Salud Pública de Chile.
4 Hospitales San Juan de Dios, Santiago.
5 Cátedra de Medicina Preventiva, Universidad de Maryland, EEUU.
dencia de infecciones entéricas, se ha señalado la presencia en la bilis de una gran variedad de bacterias en un tercio de los casos de colecistopatías intervenidas quirúrgicamente, pero sin participación de las salmonelas del grupo tífico-paratífico (12-18). Al proyectarse sobre el total de colecistopatías estimado para Santiago, el resultado de esta investigación permitiría disponer de una orientación aproximada con respecto al número de portadores de fiebre tifoidea en la capital.

Material y método

La investigación se realizó con exclusividad en el área urbana de Santiago con la colaboración de siete servicios quirúrgicos de los principales hospitales de adultos. Se tomaron muestras de bilis durante tres meses, a partir de julio de 1980, de pacientes seleccionados al azar, hasta lograr el total preestablecido como meta de 1 000 muestras.

Durante las colecistectomías los cirujanos extrajeron bilis por punción vesicular de los pacientes y todas las muestras se remitieron al Instituto de Salud Pública de Chile, junto con suero sanguíneo de los enfermos. En el Instituto se investigó la presencia de microorganismos aerobios en las muestras mediante cultivos en agar sangre y en medios selectivos salmonella-shigella y desoxicolato xilosa-lactosa para salmonelas, y las muestras de suero sanguíneo se sometieron a la prueba de Widal para detectar la presencia de S. typhi. Al mismo tiempo, uno de los hospitales colaboradores (el Hospital San Juan de Dios) realizó una investigación de microorganismos anaerobios en la bilis de sus propios pacientes, cuyos resultados se publicarán por separado.

Resultados

En el cuadro 1 se presenta la distribución por sexo y edad de los 1 000 pacientes con colecistectomía de los cuales se obtuvieron muestras de bilis. La frecuencia de colecistectomías fue mayor en el sexo femenino, con una relación de 4:1 ó 5:1 mujeres por hombre en todos los hospitales, excepto en la Clínica Central que sólo atiende emergencias, donde la relación fue de 2:1 aproximadamente. Este resultado podría explicarse por el número más elevado de intervenciones en hombres, a causa de procesos agudos (empiemas, colangitis y cuadros obstructivos).

Como se indica también en el cuadro 1, la proporción de hombres sometidos a esta operación aumentó con la edad: fue mínima en los menores de 25 años (6,4%) y máxima (32,8%) en los mayores de 35 años. En las mujeres la distribución fue más uniforme, 18,1% en el grupo de menores de 25 años y 19,3% en las que tenían más de 54 años.

Entre las causas de la intervención predominó la litiasis vesicular, en 45,7% de los hombres y 51,9% de las mujeres, seguida por la colecistitis crónica, en 28,6 y 31,2% respectivamente, y por la colecistitis aguda en 23,0 y 13,5%.

El empiema vesicular, el cáncer y otros diagnósticos sólo participaron en una proporción muy pequeña de las intervenciones (menos de 4%). Sin embargo, el porcentaje de casos de litiasis vesicular fue algo mayor que el indicado ya que un número importante de pacientes operados por colecistitis presentaba litiasis vesicular.

En el cuadro 2 se indica la proporción de bilícultivos que dieron resultados bacteriológicos positivos, según el lugar de intervención y el sexo; uno de los porcentajes más altos de positividad correspondió al Hospital San Juan de Dios, lo que podría deberse a circunstancias especiales en relación con algunas de las muestras. En dicho hospital, además de hacer una investigación de microbios anaeróbios, se investigaron también las bacterias aerobias. Estos exámenes no fueron exactamente los mismos que los que se realizaron en el Instituto
CUADRO 1 — Pacientes intervenidos que proporcionaron muestras de bilis, según sexo y edad, y hospitales e clínicas de Santiago donde se realizó la operación (julio-agosto 1980).

<table>
<thead>
<tr>
<th>Hospital o clínica que proporcionó muestras de bila</th>
<th>Hombres, según grupos de edad (én anos)</th>
<th>Mujeres, según grupos de edad (én anos)</th>
<th>Total hombres/mujeres</th>
<th>Total ambos sexos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C25-34 35-44 45-54 55-64</td>
<td>C25-34 35-44 45-54 55-64</td>
<td>C25-34 35-44 45-54 55-64</td>
<td>C25-34 35-44 45-54 55-64</td>
</tr>
<tr>
<td>San Juan de Dios</td>
<td>1 8 1 6 7 10 37</td>
<td>29 34 43 17 50 153</td>
<td>198</td>
<td>108</td>
</tr>
<tr>
<td>Barros Luco-Truco</td>
<td>2 9 6 9 11 37</td>
<td>26 33 35 21 29 144</td>
<td>181</td>
<td>141</td>
</tr>
<tr>
<td>Salvador</td>
<td>4 9 9 7 6 35</td>
<td>22 40 32 22 36 152</td>
<td>187</td>
<td>157</td>
</tr>
<tr>
<td>San José</td>
<td>1 2 2 3 16 78</td>
<td>17 17 26 11 20 91</td>
<td>117</td>
<td>117</td>
</tr>
<tr>
<td>J. J. Aguirre</td>
<td>3 0 2 8 13</td>
<td>13 14 11 8 13 59</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Asistencia Pública</td>
<td>0 5 9 3 9 28</td>
<td>28 56 36 20 16 156</td>
<td>164</td>
<td>164</td>
</tr>
<tr>
<td>Total</td>
<td>15 38 44 42 67 204</td>
<td>115 185 195 116 155 76</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

Porcentaje de hombres y mujeres que proporcionaron muestras de bila

<table>
<thead>
<tr>
<th></th>
<th>Hombres</th>
<th>Mujeres</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Próporció de hombres y mujeres</td>
<td>6.4</td>
<td>10.6</td>
<td>8.0</td>
</tr>
</tbody>
</table>

de Salud Pública, ya que en éste se utilizaron medios selectivos para S. typhi, los cuales tal vez limitaron el desarrollo de otras especies. Por lo tanto los resultados de los 51 exámenes realizados en el laboratorio del Hospital San Juan de Dios, que se incluyen en este estudio, quizás contribuyeran al elevado índice de positividad que se encontró en las muestras tomadas en esa institución.

En la Clínica Central de la Asistencia Pública, donde sólo se practican intervenciones de emergencia, con una proporción alta de procesos agudos, se observó el mayor porcentaje de resultados positivos.

Al considerar en conjunto las muestras que se tomaron en los diez establecimientos, la proporción de bilicultivos positivos fue superior en hombres (35.8%) que en mujeres (26.6%), lo que puede relacionarse con la mayor frecuencia de cuadros agudos entre los primeros.

En el cuadro 3 se comparan los resulta-
dos obtenidos en hombres y mujeres de diferentes grupos de edad. En los hombres, si no se toma en consideración el pequeño número de muestras de los menores de 25 años, se observa un aumento de positividad en los grupos de mayor edad. Este aumento no fue tan pronunciado en las mujeres, con excepción del grupo mayor de 55 años en donde el porcentaje de positividad fue 49,4.

Como se indica en el cuadro 4, de los 1 000 bilicultivos examinados se aislaron 340 bacterias, lo que coincide con los resultados de investigaciones realizadas en otros países. *Escherichia coli* fue la bacteria que se encontró con mayor frecuencia (con 33,5% del total de muestras positivas) seguida por las del grupo uísco-paraísico (21,9%), *Klebsiella pneurnonieae* (11,8%) y *Streptococcus viridans* (6,5%). Las demás bacterias aerobias se encontraron en proporciones menores. En total se aislaron 340 bacterias de 285 bilicultivos, ya que en algunos de ellos coexistían varias bacterias.

En el cuadro 5 se muestra la distribución de salmonelas, *E. coli* y otras bacterias, según el sexo y la edad de los pacientes. En general, se halló un mayor porcentaje de *E. coli* en bilicultivos positivos de hombres (18,1%) que de mujeres (9,7%). Su proporción aumentó con la edad en ambos sexos y resultó máxima en el grupo mayor de 54 años. A menudo se aisló *E. coli* en bilicultivos que contenían también otras bacterias. La proporción de muestras de bilis positivas para *S. typhi* y *S. paratyphi* fue muy similar. *S. typhi* se encontró en el 2,9% de los bilicultivos del sexo femenino y 4% de los del sexo masculino, y *S. paratyphi* en el 2,3 y 3,1% respectivamente. En cuanto a la distribución de estas variedades según la edad de los pacientes, no pudo analizarse eficazmente en los hombres debido al reducido número de muestras. En las mujeres se observó una leve disminución de los porcentajes de cultivos positivos al aumentar la edad, pero no fue estadísticamente significativo. Como *S. typhi* es responsable de la mayoría de las infecciones entéricas causadas también por *S. paratyphi* es evidente que los resultados (que muestran porcentajes aproximadamente iguales de los dos microorganismos) no reflejan lo observado en la clínica.

CUADRO 5—Comparación de los resultados obtenidos en bilicultivos de pacientes de ambos sexos, según la edad.

<table>
<thead>
<tr>
<th>Edad de los pacientes que proporcionaron muestras de bilis y resultados de los bilicultivos</th>
<th>&lt;25</th>
<th>25-34</th>
<th>35-44</th>
<th>45-54</th>
<th>&gt;55</th>
<th>Todos los grupos de edad</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sexo de los pacientes que proporcionaron muestras de bilis y resultados de los bilicultivos</strong></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td><strong>Mujeres de hombres:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positivo</td>
<td>6</td>
<td>16,7</td>
<td>6</td>
<td>16,7</td>
<td>17</td>
<td>40,5</td>
</tr>
<tr>
<td>Negativo</td>
<td>8</td>
<td>57,1</td>
<td>31</td>
<td>83,3</td>
<td>20</td>
<td>52,6</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>100,0</td>
<td>37</td>
<td>100,0</td>
<td>37</td>
<td>100,0</td>
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<td><strong>Mujeres de mujeres:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positivo</td>
<td>1</td>
<td>14,3</td>
<td>2</td>
<td>17,6</td>
<td>10</td>
<td>20,8</td>
</tr>
<tr>
<td>Negativo</td>
<td>6</td>
<td>85,7</td>
<td>11</td>
<td>82,4</td>
<td>38</td>
<td>79,2</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>100,0</td>
<td>13</td>
<td>100,0</td>
<td>48</td>
<td>100,0</td>
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<td><strong>Todos los muestra:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positivo</td>
<td>7</td>
<td>23,4</td>
<td>8</td>
<td>25,8</td>
<td>37</td>
<td>30,4</td>
</tr>
<tr>
<td>Negativo</td>
<td>26</td>
<td>76,6</td>
<td>25</td>
<td>74,2</td>
<td>113</td>
<td>69,6</td>
</tr>
<tr>
<td>Total</td>
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<td>100,0</td>
<td>33</td>
<td>100,0</td>
<td>150</td>
<td>100,0</td>
</tr>
<tr>
<td>Bacterias aisladas</td>
<td>Hospital Central de Asistencia Pública</td>
<td>Hospital槿</td>
<td>Hospital San José</td>
<td>Hospital de del Río</td>
<td>Total (todos los hospitales)</td>
<td>%</td>
</tr>
<tr>
<td>--------------------</td>
<td>--------------------------------------</td>
<td>-----------</td>
<td>------------------</td>
<td>-------------------</td>
<td>-----------------------------</td>
<td>---</td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td><em>Salmonella paratyphi A</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Salmonella paratyphi B</em></td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>14</td>
<td>23</td>
<td>3</td>
<td>15</td>
<td>9</td>
<td>37</td>
</tr>
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<td><em>E. coli</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Escherichia ludwigii</em></td>
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<td>6</td>
<td>6</td>
<td>2</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td><em>Enterobacter agglomerans</em></td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>3</td>
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<td>-</td>
</tr>
<tr>
<td><em>Enterobacter hafniae</em></td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Cetriobacter freundii</em></td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Cetriobacter diversus</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>Alcaligenes faecalis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Prevotella melaninogenica</em></td>
<td>3</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td><em>Prevotella alcaligenes</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Bacteroides anaerobius</em></td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas stutzeri</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus epidemidies</em></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>Staphylococcus viridans</em></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td><em>Enterococcus</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Anaerobios</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| Total de bacterias | 42 | 33 | 21 | 40 | 26 | 106 | 66 | 540 | 100,0 |
| Total de muestras | 89 | 179 | 72 | 189 | 117 | 190 | 164 | 1000 | 100,0 |

| % de bacterias aisladas | 47,5 | 29,6 | 29,2 | 23,4 | 20,5 | 55,8 | 28,0 | 34,0 |

En cuanto al tiempo transcurrdo entre la obtención y el análisis de las muestras de bilis, el cuadro 6 indica que las muestras procesadas 72 horas después de la colecistectomía resultaron positivas en 50,4%, y disminuyeron a 24% cuando el plazo fue mayor. (Todas las muestras se procesaron dentro de los siete días después de su recolección.) En general, en las bacterias del grupo sérico-paratífico se halló una positividad de 7,7% cuando el tiempo transcurrido antes de la siembra fue menor de 72 horas, con una disminución a 6,6% en un lapso mayor. Los otros tipos de bacterias resultaron aún más afectados al aumentar el tiempo transcurrido, con 22,7% de positividad en lapsos menores de 72 horas y 17,4% en los mayores de ese límite. En el cuadro 7 se comparan los resultados de los biliuritos y las reacciones de aglutinación (Widal), obtenidas con las muestras de sangre de los pacientes. Con este estudio se observó que hubo un aumento de positividad del cultivo a medida que se elevaban los títulos de anticuerpos H y O, detectados por la reacción de Widal. Sólo 1,6% de los enfermos con reacción negativa para el antígeno H (con títulos menores de 1:4...
No se proporcionó un texto en formato natural para esta página. Sin embargo, se adjunta una imagen con un cuadro de datos y un párrafo final que se transcribe como sigue:

**Discusión y conclusiones**

En Chile, la incidencia anual de la fiebre tifoiide supera a la de países con menor desarrollo económico y condición climática más favorable para la transmisión de enfermedades. El hecho es aún más notorio en la ciudad de Santiago, donde se registran dos tercios del total de casos con sólo un tercio de la población total del país.

Se ha comprobado que más que los enfermos, los portadores de S. typhi juegan un papel preponderante en la transmisión de la enfermedad, pero los estudios destinados a demostrar este hecho se han basado siempre en el coprocultivo, método impreciso y resistido por los pacientes si se repite en forma seriada.
CUADRO 6— Efecto del tiempo transcurrido entre la obtención de la muestra y la alimentación, sobre el aislamiento de bacterias.

<table>
<thead>
<tr>
<th>Resultados bacteriológicos</th>
<th>Tiempo transcurrido entre la obtención de la muestra y la alimentación</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>672 horas</td>
</tr>
<tr>
<td>Resultados negativos</td>
<td>467</td>
</tr>
<tr>
<td>Resultados positivos para:</td>
<td></td>
</tr>
<tr>
<td>S. typhi-paratyphi</td>
<td>55</td>
</tr>
<tr>
<td>Otras bacterias</td>
<td>162</td>
</tr>
<tr>
<td>Total de muestras</td>
<td>714</td>
</tr>
</tbody>
</table>

Además, existen datos de que las bacterias del grupo tifoico-paraóptico son causa de colecistopatías y que si infectan a personas que ya padecen de procesos vesiculares, la persistencia del estado de portador es más frecuente y prolongada que en las personas que no padecen colecistopatía. A lo anterior debe añadirse que la prevalencia de colecistopatías en Chile es una de las más elevadas del mundo.

Otro dato significativo es que durante el estudio se practicaron cuatro veces más colecistopatías en mujeres que en hombres. En general, estos tuvieron intervenciones por procesos agudos, mientras que en la mayoría de las mujeres predominó el diagnóstico de colecistitis crónica o litiasis vesicular.

En lo que se refiere a los resultados de los bilicúltivos, sólo E. coli, presente en 33,5% de las muestras positivas, superó en frecuencia al grupo S. typhi-paratyphi, que se halló en 21,5%, así como a cualquier otra magnitud registrada en la bibliografía. Al respecto, conviene notar que los informes anteriores sobre este tema corresponden a países con mayor desarrollo económico y baja incidencia de infecciones entericas. La repetición de este tipo de investigaciones en

CUADRO 7— Relación entre los pacientes con reacciones de aglutinación positiva (Widal) para antisuero H y O (titulos 1:40 o más altos) y los pacientes con muestras de bilis positivas para S. typhi.

<table>
<thead>
<tr>
<th>Resultados bacteriológicos de bilicúltivos</th>
<th>Positivo para S. typhi</th>
<th>Negativo para S. typhi</th>
<th>Total de bilicúltivos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titulos obtenidos con antígeno H:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61:20</td>
<td>12</td>
<td>1,6</td>
<td>728 98,4</td>
</tr>
<tr>
<td>1:40 6 1:20</td>
<td>15</td>
<td>8,3</td>
<td>166 91,7</td>
</tr>
<tr>
<td>1:160</td>
<td>8</td>
<td>34,8</td>
<td>15 65,2</td>
</tr>
<tr>
<td>Titulos obtenidos con antígeno O:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>61:20</td>
<td>16</td>
<td>1,7</td>
<td>787 98,3</td>
</tr>
<tr>
<td>1:40 6 1:20</td>
<td>15</td>
<td>11,6</td>
<td>117 88,6</td>
</tr>
<tr>
<td>1:160</td>
<td>6</td>
<td>54,5</td>
<td>5 45,5</td>
</tr>
<tr>
<td>No. de pacientes examinados</td>
<td>33</td>
<td>909</td>
<td>944</td>
</tr>
<tr>
<td>No. de pacientes no examinados</td>
<td>3</td>
<td>53</td>
<td>36</td>
</tr>
<tr>
<td>Total de pacientes</td>
<td>36</td>
<td>962</td>
<td>1000</td>
</tr>
</tbody>
</table>
países donde la fiebre tifoidea aún constituye un problema grave, otorgaría mayor validez a las conclusiones de este estudio.

No se observó una relación clara entre el aislamiento de bacterias específicas en los biliculíticos y los antecedentes de enfermedad, salvo en los casos de infección muy reciente. Esto puede atribuirse sobre todo a la frecuencia de formas ambulatorias, no advertidas, en especial en el caso de S. paratyphi B, cuyo aislamiento de los biliculíticos, casi tan alto como el de S. typhi, no guarda relación con la frecuencia mínima de su diagnóstico clínico.

En cambio, hubo cierta concordancia entre la positividad de los cultivos y los títulos de anticuerpos H y O detectados mediante las reacciones de aglutinación de Widal.

La positividad de S. typhi-paratyphi (7,3 %) obtenida en los biliculíticos examinados, al proyectarse sobre el total de colecistopatías en el área metropolitana de Santiago (500 000), permite deducir la enorme cantidad de portadores circulantes, sobre todo, del sexo femenino, quienes en su mayoría suelen ocuparse de la manipulación de alimentos.

El riesgo de que los casos diagnosticados y notificados de infecciones por S. typhi y S. paratyphi, junto con el mayor número de casos subclínicos o inaparentes, se transformen en portadores crónicos se magnifica por las elevadas tasas de colecistopatías y litiasis vesiculares. Se explica así la inusitada incidencia de estas infecciones en un país cuyo nivel socioeconómico y condiciones sanitarias como clínicas no se encuentran entre los más desfavorables del mundo para la transmisión de esos organismos. Las esperanzas de reducir al máximo este problema dependen en gran parte del éxito que se logre en los ensayos de nuevas vacunas vivas y atenuadas de administración oral que, además de proporcionar protección contra las manifestaciones clínicas, sean capaces de producir inmunidad intestinal, con la consecuente reducción del número de portadores.

Resumen

En años recientes la morbilidad de la fiebre tifoidea en Chile ha sido relativamente alta y la incidencia de la enfermedad se ha elevado hasta 120 casos por 100 000 habitantes. Como en estudios realizados en otros países se ha encontrado una relación entre la colecistopatía y el estado de portador, al que puede atribuirse gran parte de la transmisión de la fiebre tifoidea, se realizó un análisis de muestras de bilis y de sangre de
1,000 pacientes intervenidos por colecistopatía, durante el período de julio a octubre de 1980. Los siete hospitales que proporcionaron las muestras se encontraban ubicados en el área metropolitana de Santiago, en donde la incidencia de la fiebre tifoidea era considerablemente más alta que en el resto del país.

Las colecistectomías fueron aproximadamente cuatro veces más frecuentes en las mujeres que en los hombres, lo que confirma el hecho de que la incidencia de colecistopatías es mayor en el sexo femenino. Sin embargo, un porcentaje más alto de hombres ingresaron en los hospitales por colecistitis aguda.

Se encontraron bacterias en el 35,8% de los bilicicativos de pacientes del sexo masculino y en el 28,5% de los del sexo femenino. En los 285 bilicicativos positivos se encontraron 38 Salmonella typhi y 35 S. paratyphi. En conjunto, sólo se aisló S. typhi en el 11,2% de los bilicicativos positivos y en el 3,8% de las 1,000 muestras examinadas. Estos resultados concuerdan bastante bien con los obtenidos mediante las reacciones de aglutinación de Widal que se efectuaron con muestras de sangre de los mismos pacientes.

Las colecistopatías son bastante frecuentes en Chile; sólo en el área de Santiago se ha estimado que existen 500,000 casos. Este hecho, unido a la frecuencia de estados portadores en los casos de colecistopatía, como se deduce de los resultados de este estudio, permite explicar la gran incidencia de la fiebre tifoidea.

Agradecimiento

Los autores de este trabajo agradecen la colaboración prestada por los servicios de cirugía de los hospitales San Juan de Dios, Salvador Barros Luco-Trudeau, Síberor del Río, San José, J.J. Aguirre y Clínica Central de la Asistencia Pública, como también de los epidemiólogos de los servicios de salud de Santiago. Asimismo expresan su reconocimiento a los Dres. Jorge Toro, María Reyes y Carmen Ferrocco, y a la técnica Luis Zapata y Aurora Maldonado, del Instituto de Salud Pública de Chile.

REFERENCIAS

9. Medina, E., Kezampiter, A.M., Croiset, V., Larrazabal, M. y Topozañevicz, M. Epidemiología de las colecistopatías en Chile: I. Volumen
Investigation of the Salmonella typhi-paratyphi carrier state in cases of surgical intervention for gallbladder disease (Summary)

Chile has experienced relatively high typhoid morbidity in recent years, the annual incidence going as high as 120 cases per 100,000 inhabitants. Because correlations had been found elsewhere between gallbladder disease and the carrier state responsible for much typhoid transmission, a study was made of bile and blood specimens from 1,000 patients whose gallbladders were surgically removed in July-October 1980. The seven health facilities providing these specimens were located in metropolitan Santiago, which had been experiencing a considerably higher typhoid incidence than the rest of the country.

About four times as many surgical interventions were performed on women than on men, confirming that there was a generally higher incidence of gallbladder disease among the former. However, a higher proportion of the male patients were admitted for acute vesicular disease.

Bile specimens yielding bacterial isolates were obtained from 35.8% of the male patients and 28.5% of the female patients. These 285 positive specimens yielded 38 Salmonella typhi and 35 S. paratyphi isolates. Overall, S. typhi was isolated from 11.2% of the positive bile specimens and 3.8% of the 1,000 specimens examined. These results correlated fairly well with the results of Widal agglutination tests performed with blood specimens from the same patients.

Gallbladder pathologies are quite common in Chile, there being an estimated 500,000 cases in Santiago alone. This fact, together with the frequent occurrence of the carrier state in gallbladder disease cases — as shown by the findings of this study — helps to explain the high observed incidence of typhoid fever.

Pesquisa sobre o estado de portador da Salmonella typhi-paratyphi em doentes operados devido à patologia vesicular (Resumo)

Nestes últimos anos a morbidade da febre tifoide no Chile tem sido relativamente alta e a incidência da doença atingiu até 120 casos por 100 000 habitantes. Da mesma maneira em que estudos feitos em outros países o demonstraram, encontrou-se uma relação entre a colecistopatia e o estado de portador, ao qual se pode atribuir em grande parte a
transmissão da febre tifoíde. Foi-se analisada amostras de bila e de sangue de 1-1 000 doentes operados de coleciostomias durante o período de julho a outubro de 1980. Os 285 biliculturas foram feitas na área metropolitana de Santiago, onde a incidência da febre tifoíde era notavelmente mais alta do que no resto do país.

As coleciostomias foram aproximadamente quatro vezes mais frequentes entre as mulheres que entre os homens, o que confirma o fato de que a incidência de colecistopatias é mais alta no sexo feminino. No entanto, uma percentagem maior de homens baixou aos hospitais devido à colecistite aguda.

Acharam-se bactérias em 35,4% das biliculturas de doentes do sexo masculino e em 28,3% das do sexo feminino. Nas 285 biliculturas positivas, acharam-se 38 Salmonella typhi e 35 S. paratyphi. Em conjunto, somente se isolou S. typhi em 11,3% das biliculturas positivas e em 3,8% das 1 000 amostras examinadas. Esses resultados concordam bastante bem com os que foram obtidos mediante as reações de aglutinação de Widal feitas com amostras de sangue dos próprios doentes.

As colecistopatias são bem frequentes no Chile. Foi-se uma estimativa que se na área de Santiago há 500 000 casos. Todo isso acrescentado à frequência de estudos portadores nos casos de colecistopatia, como se pode deduzir dos achados deste estudo, permite explicar a grande incidência da febre tifoíde.

Recherche sur l'état de portateur de Salmonella typhi-paratyphi chez des patients opérés pour pathologie vésiculaire (Résumé)

Au cours des dernières années, la mobilité causée par la fièvre typhoïde au Chili a été relativement importante et l'incidence de cette maladie s'est élevée jusqu'à 120 cas par 100 000 habitants. Étant donné que, lors d'études réalisées dans d'autres pays, on a observé entre la colecistopathie et le fait d'être porteur, auquel on peut attribuer une grande partie de la transmission de la fièvre typhoïde, une analyse d'échantillons de bile et de sang de 1 000 patients ayant subi une colecystostomie fut effectuée, pendant la période de juillet à octobre 1980. Les sept hôpitaux où furent réalisées les échantillons se trouvaient situés dans la zone métropolitaine de Santiago, où l'incidence de fièvre typhoïde était considérablement plus élevée que dans le reste du pays.

Les colecystotomies furent : 2x approximativement quatre fois plus fréquentes chez les femmes que chez les hommes, ce qui confirme que l'incidence de colecystopathie est plus courante dans le sexe féminin. Cependant, un pourcentage plus élevé d'hommes entrèrent à l'hôpital pour colecystite aiguë.

On observa des bactérias dans 35,4% des bilicultures de patients do sexe masculin et dans 28,5% das bilicultures de malades do sexe feminin. Dans les 285 bilicultures positivas on de-
couvrit 38 Salmonella typhi e 35 S. paratyphi. Pour l'ensemble, on isola S. typhi dans 11,2% das bilicultures positivas e dans 3,8% das 1 000 échantillons examinados. Cez resultados concordam assez bien avec ceux obtenus par les réactions d'agglutinação de Widal que furem effectuées avec des échantillons de sang de mêmes patientas.

Les colecystopathies sont assez fréquentes au Chili; on a estimé leur nombre à 500 000 cas pour la seule région de Santiago. Ce fait, uni au nombre de porteurs dans les cas de colecystopathie, comme on peut le déduire des résultats de cette étude, permet d'expliquer la gran-
de incidence de la fièvre typhoïde.
Precise Estimation of the Numbers of Chronic Carriers of Salmonella typhi in Santiago, Chile, an Endemic Area

Myron M. Levine, Robert E. Black, Claudio Lanata, and the Chilean Typhoid Committee

As part of a program to control endemic typhoid fever in Santiago, Chile, an assessment was made of the magnitude of the reservoir of chronic carriers of Salmonella typhi. The availability of an accurate census and reliable data on the prevalence of biliary disease and of S. typhi carriage among persons with cholecystitis allowed an unusually precise estimate of the number of carriers. In 1930 there existed 25,019 female and 4,573 male carriers in a population of 4,264,514, yielding a crude prevalence of 694 carriers per 100,000 population. Because of the magnitude of this human reservoir, which includes many females of <40 years of age, it is recommended that a typhoid control program include the identification of carriers followed by health education and therapeutic interventions.

The human population is the reservoir as well as the natural host for Salmonella typhi. In general, ~2%--5% of all individuals who develop clinical or subclinical infection with S. typhi become chronic gallbladder carriers and thereby serve to maintain endemicity of the disease [1-6]. The propensity to become a chronic carrier after acute infection increases with age and is greater in women [1, 2, 5, 7], observations which are in keeping with the epidemiology of cholecystis[5-11].

Typhoid fever is highly endemic in Santiago, Chile, despite the widespread availability of potable water, the sewer sanitation, and the effective control of most other communicable diseases [12, 13]. Chile also has one of the highest prevalences of cholecystis[10, 11, 14]. This combination of a high incidence of typhoid fever and a high prevalence of gallbladder disease probably results in a high prevalence of chronic carriers. Continued contamination of vehicles of transmission by these carriers maintains the endemic cycle and interferes with effective control of typhoid fever.

As part of a program to control endemic typhoid fever in Santiago, we estimated the number of chronic S. typhi carriers. The availability in Santiago of a reliable census, coupled with a large necropsy survey of the prevalence of cholecystitis and quantitative data on the frequency of S. typhi carriage among persons with cholecystic disease, provided an opportunity to assess the magnitude of the human reservoir of infection with a precision heretofore not possible.

Materials and Methods

The sizes of the male and female populations of Santiago were obtained from official census data [13]. The prevalence of persons with gallbladder disease in each decennial age group over 10 years of age was obtained from 1,967 autopsies performed at the Medico-Legal Institute, Santiago [10]; in the vast majority of instances, these autopsies were performed on persons who died as a result of motor vehicle accidents or other trauma [10]. The percentage of persons in each age group who had gallbladder disease was multiplied by the number of persons of that age in the general population to estimate the number with gallbladder disease.

The prevalence of chronic infection of the gallbladder with S. typhi among persons with biliary disease in 1980 is known from a recent study of persons undergoing cholecystectomy in seven ma...
Table 1. Estimate of the number of chronic carriers of Salmonella typhi in Santiago, Chile, in 1930 based on the prevalence of gallbladder disease in the population and the prevalence of chronic infection with S. typhi in persons with cholelithiasis.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>10-19</th>
<th>20-29</th>
<th>30-39</th>
<th>40-49</th>
<th>50-59</th>
<th>60-59</th>
<th>70-79</th>
<th>80+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>443,408</td>
<td>407,190</td>
<td>323,987</td>
<td>221,209</td>
<td>169,221</td>
<td>113,551</td>
<td>59,233</td>
<td>21,714</td>
<td>1,759,548</td>
</tr>
<tr>
<td>Cholelithiasis (%)*</td>
<td>9.7</td>
<td>23.4</td>
<td>43.1</td>
<td>51.7</td>
<td>60.0</td>
<td>69.2</td>
<td>69.2</td>
<td>55.5</td>
<td>...</td>
</tr>
<tr>
<td>Cholelithiasis†</td>
<td>43,010</td>
<td>93,282</td>
<td>139,638</td>
<td>114,365</td>
<td>101,332</td>
<td>83,593</td>
<td>40,993</td>
<td>12,051</td>
<td>620,673</td>
</tr>
<tr>
<td>S. typhi carriers‡</td>
<td>1,720</td>
<td>3,811</td>
<td>5,586</td>
<td>4,575</td>
<td>4,041</td>
<td>3,144</td>
<td>1,010</td>
<td>452</td>
<td>25,019</td>
</tr>
<tr>
<td>Carriers per 10⁶ population</td>
<td>388</td>
<td>940</td>
<td>1,724</td>
<td>2,068</td>
<td>2,400</td>
<td>2,768</td>
<td>2,768</td>
<td>2,220</td>
<td>...</td>
</tr>
<tr>
<td>Male</td>
<td>438,865</td>
<td>373,895</td>
<td>294,395</td>
<td>192,984</td>
<td>139,793</td>
<td>122,533</td>
<td>80,211</td>
<td>9,516</td>
<td>1,572,551</td>
</tr>
<tr>
<td>Cholelithiasis (%)*</td>
<td>0.3</td>
<td>4.5</td>
<td>13.4</td>
<td>16.3</td>
<td>19.3</td>
<td>24.7</td>
<td>43.5</td>
<td>40.0</td>
<td>...</td>
</tr>
<tr>
<td>Cholelithiasis†</td>
<td>0</td>
<td>16,825</td>
<td>39,476</td>
<td>32,395</td>
<td>27,655</td>
<td>20,472</td>
<td>17,061</td>
<td>3,814</td>
<td>157,718</td>
</tr>
<tr>
<td>S. typhi carriers‡</td>
<td>0</td>
<td>488</td>
<td>1,145</td>
<td>939</td>
<td>805</td>
<td>594</td>
<td>495</td>
<td>111</td>
<td>4,575</td>
</tr>
<tr>
<td>Carriers per 10⁶ population</td>
<td>0</td>
<td>131</td>
<td>389</td>
<td>484</td>
<td>575</td>
<td>17</td>
<td>1,262</td>
<td>1,164</td>
<td>...</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of persons except where percentages are indicated.

* Percentages based on 1,267 persons studied at autopsy at the Medical Institute, Santiago.
† Estimate computed by multiplying the no. of persons in each age group of the total population by the percentage of persons in that age group who were found to have cholelithiasis at autopsy.
‡ Estimate based on the observation that 4% of females and 2.9% of males with gallbladder disease in Santiago have chronic S. typhi bile infection [16].

Results

A summary of the population of Santiago in 1980 by age and sex, the number of persons with gallbladder disease, and the calculated number of S. typhi carriers is shown in table 1. In total, 25,019 female and 4,575 male chronic carriers of S. typhi over 10 years of age were calculated to exist among the population of 4,264,514 in greater Santiago; the overall prevalence was 694 carriers per 10⁶ population. The prevalence of chronic carriers increased with age; among women over 40 years of age, 2.1%-2.8% (that is, 2,068-2,768 per 10⁶ women) were computed to be chronic S. typhi carriers.

Discussion

Typhoid fever is highly endemic in Chile, (the annual incidence since 1975 has ranged from 59 to 121 cases per 10⁶ population), particularly in Santiago where peak incidence rates occur in older schoolchildren and young adults. This has been somewhat enigmatic to epidemiologists, since based on other demographic, socioeconomic, and health indicators, Chile is a fairly developed country. The relationship between biliary disease and chronic S. typhi carriage has been recognized for many decades [1-7]. Chile also has one of the highest prevalences of gallbladder disease in the world [10, 11, 14], and gallbladder disease appears among young female Chileans [10, 11]. We therefore surmised that there must exist a particularly high prevalence of chronic carriers in Santiago who serve as reservoirs and disseminators of S. typhi and who help maintain a high level of endemicity of typhoid fever.

The present report provides the most precise estimation ever made of the number and prevalence of chronic carriers of S. typhi in an endemic area. This precision was possible because of the existence of accurate data providing the age-specific prevalence of cholelithiasis [10, 11] and the frequency of chronic biliary infection with S. typhi.
among persons with gallbladder disease [16]. The few
previous attempts at estimating the number of
chronic carriers of S. typhi in other geographic
areas were rough estimates which, with one excep-
tion, did not take into account the relationship be-
tween the age of the patient at the time of acute in-
fec-tion and the development of the chronic carrier
state [7, 17, 18].

The large number of chronic carriers of S. typhi
(29,594 persons) and the high crude prevalence
rate (694 carriers per 10^4 population) calculated
for Santiago demonstrate the notable magnitude
of the human reservoir of S. typhi. Furthermore,
the existence of many carriers younger than 30
years of age implies that a significant reservoir will
be present for many decades to come.

The outstanding efficacy of Ty 21a attenuated
S. typhi oral vaccine in preventing acute typhoid
fever in Alexandria, Egypt, has generated con-
siderable hope that mass application of this vac-
cine in endemic areas may greatly diminish the in-
sidious spread of the human reservoir of
S. typhi. The possibility of using this vaccine in
large numbers of individuals in endemic areas
is strongly indicated by recent findings in the
Alexandria, Egypt, typhoid fever control pro-
gram.

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gram.

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Public Health

VI SEROLOGY IN DETECTION OF CHRONIC SALMONELLA TYPHII CARRIERS IN AN ENDEMIC AREA

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SUMMARY

A passive haemagglutination assay measuring antibody to highly purified Vi antigen, known to be sensitive and specific for the detection of chronic Salmonella typhi carriers in an endemic area, was evaluated in an endemic area. A control serum Vi antibody titre of 160 was found to have a sensitivity of 75% and specificity of 93%, and a high predictive value of seroconversion for chronic S. typhi carriers in high-risk populations groups (e.g., women over 40 years). The simple assay can screen for chronic S. typhi carriers even in areas where typhoid fever is highly endemic.

INTRODUCTION

Since the description in the 1930s of a Vi antigen of Salmonella typhi and of the relation between high serum titres of Vi antibody and the chronic S. typhi carrier state, there have been many conflicting reports about the usefulness of Vi serology in the detection of chronic S. typhi carriers. The disagreements stem from both the methods used and the interpretation of results. Felix et al. used a Vi-rich S. typhi strain as the antigen in a direct bacterial agglutination test, which they considered helpful in identifying chronic S. typhi carriers. Some authorities placed much confidence in this screening test that they required a negative Vi serology to be documented in all individuals surveyed in certain industries, such as the water and food industries. However, the usefulness of this test, especially in areas where typhoid fever was endemic, was challenged by others; they pointed out that in such areas up to 20% of bacteriologically confirmed chronic S. typhi carriers lacked Vi antibody and up to 20% of normal individuals with negative serology for S. typhi had positive direct bacterial agglutination tests.

When the direct bacterial agglutination test was used in mass screening of unselected populations, less than 2% of people with positive Vi serology were found to be by intensive bacteriological culturing to be chronic S. typhi carriers.

Immunologically identical Vi antigen was discovered in other Enterobacteriaceae, including Citrobacter coli, Citrobacter freundii, and S. paratyphi C. Crude or partially purified Vi antigen prepared from S. typhi Vi 1, 2, and Citrobacter species was adsorbed to human group O or sheep erythrocytes for a passive haemagglutination assay. This assay had greater sensitivity than the direct bacterial agglutination test but the high false-positive rate persisted.

In 1972 Wong and Felley described a method to prepare highly purified Vi antigen. They and their colleagues used the highly purified antigen in a passive haemagglutination assay to detect a small number of chronic S. typhi carriers in a non-endemic area (Arkansas, USA). Vi serology was positive in all 7 chronic S. typhi carriers tested but in only 1 of 37 (3%) of the no-culture-negative contacts of these carriers. To determine whether this test might be useful in an endemic area, we have evaluated the sensitivity and specificity of the passive haemagglutination assay with highly purified Vi antigen to detect chronic S. typhi carriers in Santiago, Chile, where an estimated 28,000 or more chronic S. typhi carriers and where typhoid fever is still an important public health problem.

SUBJECTS AND METHODS

We defined a chronic S. typhi carrier as an individual from whom S. typhi was isolated 1 or more years after a bacteriologically confirmed episode of typhoid fever. Since there is a high prevalence of carrier carriage of S. typhi among Chilean women, we identified from medical records of the Infectious Disease Hospital, Santiago, women 25 years and older who had had bacteriologically confirmed typhoid fever 1–4 years previously. We were interested in the possibility of being chronic S. typhi carriers and avoiding them to come to the Public Health Laboratory for screening. Known male carriers were also included.

Bacteriological evaluation consisted of a normal culture on each of 3 consecutive days and one double-dose fluid culture. The women were instructed to sample a fresh stool with a sterile swab, inoculate it into Cary-Blair transport medium and to bring the sample to the bacteriology laboratory within 24 h. Samples of doubly-dose fluid containing bile were obtained by means of a sterile-enema apparatus (EnteroTest, Hudson) ingested by the subject under supervision. stools and bile-stained double-dose fluids were inoculated onto MacConkey, Wilkins-Chalgren, and Salmonella-Shigella agar. and immediate F broth. S. typhi was identified by standard biochemical and serological reactions. A serum sample was obtained from each subject.

We also obtained serum samples from 29 patients of both sexes, aged 18 or over, admitted to the Infectious Disease Hospital, with bacteriologically confirmed typhoid fever and from 59 healthy subjects of both sexes, aged 16–64, who had no bacteriological investigation.

Serum antibodies were measured by the passive haemagglutination assay method of Naja et al. with highly purified Vi antigen prepared from Citrobacter freundii by the modification of Wong and Felley's technique (provided by J. E. Rubins, Division of Bacterial Products, National Center for Drugs and Biologics, FDA). Serum samples were first allowed to react with unasorbed sheep erythrocytes to absorb anti-sheep-cell antibodies. Gentamicin-sensitive sheep erythrocytes were sensitized with highly purified Vi antigen (10 μg/ml). Serial two-fold dilutions of serum samples, from 1/20 to 1/5120, were added to equal volumes of affected and non-sensitized cells. The agglutination patterns were read after 2 h incubation at room temperature and again after incubation overnight at 4°C. Titres were recorded as the reciprocal final dilution showing a positive haemagglutination result. Known positive and negative control sera were assayed with each test. Student's t and chi-squared tests were used for statistical analysis.

RESULTS

Of the 36 chronic carriers (3 known and 33 detected by bacteriological screening), 27 (75%) had Vi titres of ≥ 160 (see Table), whereas only 3% of the 358 non-carrier women (p<0.001) and 3% of 59 healthy subjects who had no bacteriological screening (p<0.001) had titres ≥ 160. The
PREVALENCE OF V. ANTIODY IN CHRONIC S TYPHII CARRIERS.
ACUTE TYPHOID FEVER PATIENTS, AND HEALTHY SUBJECTS
IN SANTIAGO, CHILE.

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Group</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Percentage with sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic S typhi carriers</td>
<td>n = 36</td>
<td>17-50</td>
<td>25-63</td>
<td>All women</td>
<td>25-63</td>
<td>10-14</td>
</tr>
<tr>
<td>Active typhoid fever patients</td>
<td>n = 29</td>
<td>20-30</td>
<td>53</td>
<td>Both men</td>
<td>14-46</td>
<td>6-12</td>
</tr>
<tr>
<td>Non-typoidal LT cases</td>
<td>n = 30</td>
<td>20-30</td>
<td>53</td>
<td>Both men</td>
<td>14-46</td>
<td>6-12</td>
</tr>
</tbody>
</table>

The sensitivity and specificity of each V. antibody titre as a cut-off point in screening for chronic S. typhi carriers was determined with the 388 culture-negative women as negative controls (Fig. 1). With a V. antibody titre ≥160 as positive, the passive haemagglutination assay with highly purified V. antigen had 75% sensitivity and at least 92% specificity.

The predictive value of each V. antibody titre as cut-off point in screening for chronic carriers (defined as the percentage of subjects with positive V. serology who will be confirmed as chronic S. typhi carriers) was determined in populations with different carrier prevalence rates (Fig. 2). When we used the 388 culture-negative women with history of confirmed typhoid fever as the negative controls, the specificity of a V. antibody titre ≥160 was 92%. Thus, in Santiago the predictive value of this titre is 8% in the general adult population, 16% in women 40 years and older, and 31% in women 25 years and older with history of confirmed typhoid fever (Fig. 2A). However, the 59 healthy Chileans (who were not studied bacteriologically) may be more representative of the general population in Santiago; when they were used as the negative controls, the specificity of a V. antibody titre ≥160 rose to 97% and the predictive value rose from 8% to 17% in the general adult population and from 16% to 31% in women 40 years and older (Fig 2B).

DISCUSSION

3-5% of patients with typhoid fever become chronic carriers and the carrier state persists throughout life. Since the only natural host and reservoir of S. typhi is man, the detection of chronic carriers is essential for the control of typhoid fever. The use of bacteriological cultures for the detection of chronic S. typhi carriers is limited by expense, logistical considerations, and the fact that carriers typically have intermittent excretion of S. typhi so repeated cultures are necessary. Some reports of the non-surgical treatment of chronic S. typhi carriers have been encouraging, as the use of V. serology to screen for carriers in selected high-risk groups in an endemic area may be justified as part of a programme to control typhoid fever.

The main reason for the conflicting reports of the usefulness of V. serology in the detection of chronic S. typhi carriers is the differing purity of the V. antigen. The original studies with direct bacterial agglutination used an S. typhi strain rich in V. antigen. This strain, however, also contained somatic O and flagellar H antigens so the sera had to be preabsorbed with a V. negative S. typhi strain. The isolation of S. typhi strain Vi 1, which was rich in V. antigen but almost without O and H antigens, simplified direct bacterial agglutination. However, both the direct bacterial agglutination test and the passive haemagglutination assay with crude or partially purified V. antigen have a high false-positive rate in the general population, especially where typhoid fever is endemic, apparently because of cross-reactivity with other antigens in these antigen preparations. The lack of specificity has been the reason for the loss of confidence in V. serology since the late 1950s.

The development of a method to produce highly purified V. antigen was important, since it provided an antigen that improved the specificity of the medically simple passive haemagglutination assay. A preliminary report...
suggested that the passive haemagglutination assay with highly purified Vi antigen had no greater sensitivity or specificity than direct bacterial agglutination and more elaborate techniques, such as fluorescent Vi antibody test, counter-immunoelectrophoresis, or solid-phase radioimmunoassay, have been advocated. However, these more sophisticated techniques require expensive equipment and their use in less-developed countries where typhoid fever is endemic is limited.

The practical application of the simple passive haemagglutination assay with highly purified Vi antigen in detecting chronic S. typhi carriers in an endemic area depends not only on the sensitivity and specificity of the titre used as cut-off but also on its predictive value. Since the predictive value of such titre cut-offs is greater in populations with higher chronic S. typhi carrier rates, screening high-risk groups of the population will be justifiable.

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REFERENCES

Development and Evaluation of an
Enzyme Linked Immunoassay Assay for Serum Vi Antibodies
for Detection of Chronic Salmonella typhi Carriers

Running Title: ELISA Vi Antibodies to Detect S. typhi Carriers

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Abstract

An enzyme linked immunosorbent assay (ELISA) measuring serum IgG, IgM, and IgA antibodies to Vi capsular polysaccharide antigen that had been tyraminates to increase its binding efficiency to microtiter plates (Vi-Tyr) was compared to the standard passive haemagglutination assay (PHA) as a screening test for chronic Salmonella typhi carriers. Initially, three populations were evaluated: 22 healthy U.S. adults, 17 young Chilean adults with acute typhoid fever, and 51 Chileans who had bacteriologically confirmed S. typhi chronic carriage. IgG specific Vi-Tyr antibodies were preferentially present in the S. typhi chronic carrier state. 44/51 (81%) chronic carriers, 0/22 (0%) healthy U.S. adults, and 2/17 (12%) Chileans with acute typhoid fever had reciprocal serum IgG Vi-Tyr ELISA antibody titers > 200. The IgG Vi-Tyr ELISA was then compared to the PHA as a screening test for chronic carriers in 141 Chilean female foodhandlers. One woman was serologically incriminated as a carrier by both the IgG ELISA and PHA; her coprocultures were positive for S. typhi. One other woman, identified as a carrier by PHA was negative by culture and IgG ELISA. The IgG Vi-Tyr ELISA is as sensitive as the PHA (86% vs 76%) and as specific (95% vs 95%) in screening for chronic carriers.
Asymptomatic excretion of *Salmonella typhi* in stools for greater than one year following an episode of acute typhoid fever occurs in approximately three percent of adults (9). These asymptomatic chronic biliary carriers represent an important reservoir of *S. typhi* and have been responsible for outbreaks of acute typhoid fever (13). Detection of carriers, therefore, becomes an important aspect of typhoid fever control. Bacteriological confirmation of the chronic carrier state requires either multiple stool cultures or cultures of bile or bile-stained duodenal fluid. These procedures are not amenable to large scale screening (10,7,6). In addition, because chronic biliary carriers are often intermittent or light fecal *S. typhi* excreters, multiple bacteriological examinations are usually required to reliably make the diagnosis (10,6,3). For these reasons, serologic screening for the carrier state of *Salmonella typhi* in areas of typhoid endemcity is preferable.

The passive hemagglutination assay (PHA), using Vi antigen from *Citrobacter freundii* or *S. typhi*, has been found to be both sensitive and specific for the screening of the chronic carrier state of *S. typhi* in endemic and non-endemic areas (8,11). However, this assay requires test sera to be pre-absorbed with sheep erythrocytes, which is inconvenient in screening large populations. Attempts at using an enzyme linked immunosorbent assay (ELISA) for the detection of the carrier state have been hampered by the poor binding of the Vi antigen to microtiter plates. Some researchers, using immune sera as the capture reagent for the Vi antigen, have had some success in detecting specific IgG antibodies (8,11). However, large amounts of a standard immune sera are needed and may not be readily available.

Highly purified Vi polysaccharide from *Citrobacter freundii* was tyraminated (Vi-Tyr) in an attempt to enhance binding of the polysaccharide to plastic microtiter plates (12). This Vi-Tyr was then used in the development
of an ELISA which was compared to the passive hemagglutination assay (PHA) as a screening test for typhoid carriers in a typhoid endemic area. The ELISA was adapted to assess the relative occurrence of IgG, IgM, and IgA Vi specific antibodies in the carrier state.

Materials and Methods

Subjects. To standardize the Vi-Tyr ELISA, 3 groups of subjects were examined: 22 healthy young adults from the United States, 17 young Chilean adults admitted to the Infectious Diseases Hospital in Santiago with bacteriologically confirmed acute typhoid fever, and 51 asymptomatic S. typhi carriers from Chile. These chronic carriers had bacteriologically confirmed typhoid fever 1-4 years previously and, at the time of the study, had S. typhi isolated bacteriologically.

Once a serum dilution for screening carriers was determined using these known populations, 141 Chilean female foodhandlers, age 25-65 years, were screened blindly for S. typhi chronic carriage using the Vi-Tyr ELISA and PHA. S. typhi carriage in this group was confirmed by coprocultures.

Specimens. One serum sample was obtained from each subject in all groups except for the subjects with acute typhoid fever who had sera obtained upon hospital admission and 21 days later. Those subjects with acute typhoid fever and those with chronic S. typhi carriage had bacteriologic evaluation consisting of three stool cultures obtained on consecutive days and one duodenal fluid culture obtained by a gelatin-encapsulated string device (1). Two coprocultures were obtained on successive days from the healthy female foodhandlers to confirm S. typhi carriage. All samples were inoculated onto
MacConkey, Wilson-Blair, and Salmonella-Shigella agar, and into Selenite broth. *S. typhi* was recovered and identified by standard biochemical and serological reactions (4).

**Tyramination of the Vi antigen.** The tyramination of the Vi polysaccharide has been previously described (12). Briefly, tyramine (30 mg/ml) was added to 10 mg Vi in the presence of carbodiimide and incubated at pH 4.9 -5.1 for 3 hours. The resultant reaction mixture was dialyzed and purified by gel exclusion through a C-100 Sephadex column (Pharmacia, Piscataway, NJ).

**Standardization of the Vi-Tyr ELISA.** Sera from 16 known chronic typhoid carriers and from 6 healthy U.S. volunteers were used as the positive and negative reference sera, respectively, to establish a standard curve for each isotype-specific Vi-Tyr ELISA. These samples were assayed twelve different times at two-fold dilutions starting at 1:25 and ending at 1:3200 by the following method:

The wells of Immulon I (Dynatech, Alexandria, Va) plates were incubated at 4°C overnight with 0.1 ml aliquots of Vi-Tyr antigen in phosphate buffered saline (PBS), pH 7.3. The wells were washed 5 times with PBS containing 0.05% Tween 20 (PBS-Tween) and then incubated at 37°C for 1 hour with 0.1 ml of human serum diluted in PBS-Tween containing 1% non-immune goat serum and 1% fetal bovine serum. The wells were then washed 5 times with PBS-Tween and incubated for 1 hour at 37°C with heavy chain specific antibody to human immunoglobulin G, M, and A conjugated to alkaline phosphatase (Kirkegaard and Perry, Gaithersberg, Md.) diluted in PBS-Tween. After washing, the wells were incubated at room temperature with 0.1 ml of *p*-nitrophenyl phosphate (1mg/ml) in 10% diethanolamine buffer (pH 9.8). Absorbance was monitored at 405nm.
Saturation kinetics using several high FFA titered serum samples were determined using Vi-Tyr coating concentrations of 0.5, 1.0, and 2.0 ug/ml. The specificity of the goat antibody conjugates were examined with purified IgG, IgM serum fractions and milk IgA obtained by filtration through a DEAE Biogel-A (Pharmacia) column.

The FFA was performed for each subject in each group by methods previously described (6). A titer of ≥ 160 was considered to be indicative of the S. typhi carrier state.

Results

Assay Standardization. The absorbance (A) of the pooled positive serum was determined as a function of the tyraminated Vi antigen coating concentration. Saturation kinetics were observed and a coating concentration of 1 ug/ml was chosen. The pooled IgG fraction contained 1450 mg/dl IgG and less than 1 mg/dl IgM. The pooled IgM fraction contained 140 mg/dl IgM and less than 1 mg/dl IgG. The IgA sample contained 35.5 mg/dl IgA with less than 1 mg/ml IgG and IgM. The conjugates were shown to be isotype specific.

A standard curve for IgG specific Vi antibody using positive sera was linear for absorbance (A) values ranging from 1.2 to 0.1 using serum dilutions ranging from 1:50 to 1:800 (figure 1). The negative serum pool gave optical densities (O.D.) below 0.1 for dilutions as low as 1:25. A dilution of 1:50 of the negative serum pool produced a mean O.D. of 0.04 with a standard deviation (S.D.) of 0.01. The cut-off absorbance value signifying significant IgG specific Vi antibody was set at an O.D. reading of 0.2 since this value is on the linear portion of the curve and well above background.

The IgM standard curve for Vi antibody was linear for A values ranging
from 1.4 to 0.15 using serum dilutions ranging from 1:25 to 1:400 (figure not shown). Because of high background values in the negative serum pool for dilutions less than 1:1000, starting dilutions of 1:100 were used in all samples. A cut off of 0.3 was used to determine a positive antibody titer since the absorbance of the negative serum pool, at a 1:100 dilution was 0.07 ± 0.05. Similarly, an IgA standard curve was determined as being linear for A values ranging from 0.9 ± 0.14 using serum dilutions ranging from 1:50 to 1:400 (figure not shown). An absorbance of 0.15 was conservatively chosen as the cut-off for a positive antibody titer since the pooled negative sera at a 1:50 dilution gave an O.D. of 0.02 ± 0.01.

Evaluation of the Vi-Tyr ELISA to detect S. typhi carriers. Table 1 shows the results of the IgG Vi-Tyr specific antibody titers in individuals with acute typhoid fever and S. typhi carriage, and a healthy population. Of the 51 chronic carriers tested, 444 (86%) had an IgG Vi-Tyr ELISA titer greater than or equal to 1:200. In contrast, only 12% of the acute typhoid patients and none of the healthy U.S. volunteers had similar titers (p < 0.00000001). An IgM specific Vi antibody titer >100 was detected in 19 (37%) of chronic carriers and in 3 (18%) of patients with acute typhoid fever (Table 2). The IgM Vi-Tyr ELISA was unable to discriminate acute typhoid fever patients from chronic carriers (p = 0.2), and did not increase the detection sensitivity of carriers of the IgG Vi-Tyr ELISA. Although Vi-Tyr specific IgA antibodies were present in 37 (72%) of the chronic carriers, they also were detected in patients who had acute typhoid fever (Table 3) (p = 0.2). ELISA Vi-Tyr antibodies of all three immunoglobulin classes were seen with equal but low frequency in the admission and follow-up serum samples obtained from patients with acute typhoid fever.
To assess the applicability of the IgG Vi-Tyr ELISA in a typhoid endemic area, 141 Chilean female food handlers were screened by the ELISA, PHA, and two coprocultures. Of these 141 women tested, one had an IgG ELISA titer $> 200$ and two women, one of whom also had the positive ELISA titer, had a PHA titer $> 160$ (Table 1). Of these two women serologically identified as possible carriers, only the one woman who was positive by the Vi-Tyr IgG ELISA was confirmed to be a carrier by culture.

The sensitivity of the IgG Vi ELISA titer of $> 200$ in screening for chronic S. typhi carriage as determined by analyzing the results obtained with the 51 known chronic carriers is 86% compared to 76% with the PHA using a titer of $> 160$. The specificity of the IgG specific Vi-Tyr ELISA in screening for chronic carriers using healthy U.S. volunteers and acute typhoid fever patients is 95%, which is equal to that obtained by using the PHA.

**Discussion**

Since man is the only reservoir of S. typhi, the detection of carriers is necessary for control of typhoid fever. In areas of typhoid endemicity, screening for chronic typhoid carriers by serological means is of practical importance since bacteriologic screening is expensive and logistically difficult to perform. The tyramine derivative of Vi provides sufficient binding of the antigen for detection of Vi specific antibodies by ELISA. In terms of rapidity and ease of performance, we find the IgG specific Vi-Tyr ELISA to be superior to our previously reported PHA for the detection of S. typhi carriers (1,12).

Analysis of the different classes of antibody involved in the chronic carrier state has shown that Vi antibody of the IgG class is present most
frequently. IgM and IgA Vi antibodies, although seen in chronic carriers, cannot be used to differentiate persons with acute or chronic S. typhi infection. It is probable that the IgG antibody response to Vi present in carriers reflects prolonged immunologic stimulation. It is interesting that a serum IgA Vi response is present in both the acute and chronic forms of S. typhi infection. This seems reasonable since S. typhi participates in an enterobacterial circuit in the pathogenesis of acute typhoid fever and also is a primary occupant of the biliary system in chronic infection. Further work on subclass specificities in both the IgG and IgA responses to Vi antigen in the acute and chronic forms of S. typhi infections may help elucidate other possible immunologic differences in these two disease states.
References


Figure 1. IgG specific antibody response to Vi-Tyr antigen in pooled sera from 16 asymptomatic *Salmonella typhi* carriers as measured by ELISA. Antibody results are represented as the mean (dots) and two standard deviations (bars) compiled from 12 separate runs.
<table>
<thead>
<tr>
<th>Group Description</th>
<th>T&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ELISA Titre&lt;sup&gt;b&lt;/sup&gt;</th>
<th>FFA Titer&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S. volunteer (22)</td>
<td>6</td>
<td>25</td>
<td>40</td>
</tr>
<tr>
<td>Acute typhoid patients</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Chronic carriers (51)</td>
<td>6</td>
<td>44</td>
<td>7</td>
</tr>
<tr>
<td>Food handlers (141)</td>
<td>140</td>
<td>1</td>
<td>139</td>
</tr>
</tbody>
</table>

a = reciprocal
b = number of

c = reciprocal

d = admission

e = the or

NA = not applicable
TABLE 2

Prevalence of IgM Specific VI Antibody

<table>
<thead>
<tr>
<th>Group Description</th>
<th>GMT</th>
<th>ELISA titer&lt;sup&gt;b&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>(no.)</td>
<td></td>
<td>&lt;100</td>
</tr>
<tr>
<td>U.S. volunteers</td>
<td></td>
<td>&gt;100</td>
</tr>
<tr>
<td>(22)</td>
<td>43</td>
<td>21</td>
</tr>
<tr>
<td>Acute typhoid patients</td>
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<td>1</td>
</tr>
<tr>
<td>(17)</td>
<td>34/40&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14</td>
</tr>
<tr>
<td>Chronic carriers</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>(51)</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
</tr>
</tbody>
</table>

<sup>a</sup> = reciprocal geometric mean VI titer
<sup>b</sup> = No. subjects with a given reciprocal titer
<sup>c</sup> = admission titer/ follow-up titer
<table>
<thead>
<tr>
<th>Group Description</th>
<th>GM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>ELISA Titer&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S. volunteers</td>
<td>n/a</td>
<td>&lt;50 &lt;50</td>
</tr>
<tr>
<td>(22)</td>
<td></td>
<td>18 20 2</td>
</tr>
<tr>
<td>Acute typhoid patients</td>
<td>n/a</td>
<td>50/51&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>(17)</td>
<td></td>
<td>6 11</td>
</tr>
<tr>
<td>Chronic carriers</td>
<td>n/a</td>
<td>52 14 37</td>
</tr>
<tr>
<td>(51)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> number of subjects with a given reciprocal titer
<sup>b</sup> admission titer/ follow-up titer
NON-SURGICAL TREATMENT OF CHRONIC SALMONELLA TYPHI CARRIERS WITH AMoxicillin AND PRObenicID

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Running Head: Antimicrobial Therapy of Chronic Typhoid Carriers

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10 S. Pine Street, Baltimore, MD 21201, U.S.A.
SUMMARY

An oral regimen of amoxicillin 2.0 gm combined with probenecid 0.5 gm three times a day for 28 consecutive days was evaluated in the treatment of chronic *Salmonella typhi* carriers in Santiago, Chile. Mild (mostly gastrointestinal) but tolerable side effects commonly occurred early in the therapy. Fifteen of 26 (58%) treated carriers (including three with gallstones) remained cured after 12 months of bacteriological follow-up; all failures became evident within the first four months after completing therapy. The serum amoxicillin blood level at four hours post-dose was significantly higher (19.50 ± 2.90 mcg/ml) in the cured carriers compared with the carriers who failed (14.69 ± 2.77 mcg/ml) (p<0.01). This regimen provides a reasonable alternative to cholecystectomy in selected carriers. However, the cure rate of 58% is arguably too low to justify systematic use in typhoid fever control programs.
INTRODUCTION

Approximately 3% of patients with typhoid fever become chronic gallbladder carriers and thereby serve as a reservoir for the transmission of Salmonella typhi (Ames & Robins 1943, Anderson et al., 1936). The combination of cholecystectomy and antibiotics is largely successful in eradicating the carrier-state (Munnich & Bakshi 1979, Perkins et al., 1966, Whitby 1964). However, because this regimen is invasive and expensive, it is not a practical public health tool. Based on these drawbacks, investigators have studied the effectiveness of antibiotics alone in eradicating the carrier state, using drugs to which S. typhi are susceptible in vivo. Overall, medical therapy alone has been disappointing and success has generally been correlated with the presence or absence of gallstones (Bullock 1963, Dinbar et al., 1969, Kaye et al., 1967, Munnich et al., 1974, Nolan & White. 1978, Sciolli et al., 1972, Simon & Miller 1966, Tynes & Utz 1962). Nevertheless, two reports have generated optimism. Sciolli et al (1972) successfully treated all of 19 chronic S. typhi carriers with a 15 day course of intravenous ampicillin, suggesting that high cure rates are achievable if sufficiently high serum and biliary levels of a bactericidal antibiotic can be maintained. Because of the impracticality of parenteral therapy, regimens are being sought to achieve this with an oral antibiotic. Amoxicillin, a congener of ampicillin which gives two to three-fold greater blood levels after oral dosing and is concentrated in bile, was considered an attractive drug to be evaluated in treatment of the S. typhi carrier state (Kosmidis et al., 1972, Waki et al., 1977). Nolan & White (1978) treated 15 carriers with amoxicillin, 6.0 gm/day. Of 10 carriers who completed this 28 day regimen, nine were cured, including three with gall bladder disease. The remaining five carriers had their amoxicillin dose reduced to 3.0 gm/day because of gastrointestinal
intolerance; of these, only two were cured.

Based on this background, we conducted a clinical trial in Santiago, Chile, where typhoid fever is highly endemic and the prevalence of chronic carriers and cholelithiasis is high (Levine et al., 1982), to evaluate an oral antibiotic regimen that might cure S. typhi carriers without surgery, regardless of the presence of gallstones. In this trial, amoxicillin was combined with probenecid to increase and prolong the amoxicillin blood levels.

PATIENTS AND METHODS

Subjects

Twenty-six bacteriologically-confirmed chronic S. typhi carriers who were free of any debilitating disease, did not have history of penicillin allergy, gastrointestinal or renal disease and were not pregnant or lactating women were enrolled into the study (Lanata et al., 1983). These included 22 persons who were known carriers for at least 12 months, two for nine months, and two who were carriers for six months. Written informed consent was obtained.

Bacteriology

All participating carriers had a baseline medical evaluation. Prior to treatment a stool culture on each of three consecutive days and one duodenal-fluid culture were obtained. The carriers were instructed to inoculate a sample of fresh stool with a sterile swab into Cary-Blair transport medium (Fingold & Martin 1982) and to bring the sample into the bacteriology laboratory of the Institute of Public Health within 24 hours. Samples of duodenal fluid containing bile were obtained by means of a gelatin-encapsulated string device ("Entero-Test", R.D.C. Corporation, Mountain View, Ca.) ingested by the subject under supervision (Gilman et al., 1979, Avendano et al., 1986). Stools and bile-stained duodenal fluids were inoculated onto MacConkey, Wilson-Blair, and Salmonella-Shigella agar
directly, as well as after 18 hours enrichment in selenite F broth. *S. typhi*
was identified by standard techniques (Edwards & Dwing 1972).

After treatment, three consecutive stool cultures and one duodenal-fluid
culture were obtained during the first week post-treatment and at the 1st,
3rd, 6th, 9th and 12th month post-treatment, whenever possible. Carriers with
all cultures free of *S. typhi* for 12 or more months after completion of
therapy were considered cured.

**Treatment Regimen**

The oral treatment regimen consisted of amoxicillin trihydrate, 2.0 gm
and probenecid 0.5 gm taken three times each day for 28 days by the patient at
home. To detect side effects and evaluate compliance, each carrier was
contacted at least twice a week by telephone or by home visits. A diary was
provided for the carriers to record the precise times when they took their
medication and the appearance of any side effects. The medication was
provided in single-dose vials, to assure ingestion of the correct number of
capsules. A spot urine sample was obtained during the home visits to be
subsequently tested for amoxicillin levels as an indicator of compliance.
Finally, during the second and last week of treatment, a serum sample was
obtained before the morning dose, and two, three and four hours thereafter for
amoxicillin blood levels.

**Amoxicillin Levels**

Sera and urine samples were frozen at -70°C and transported on dry ice to
Baltimore. Because of the loss of one box of specimens during transport,
samples were available from only 20 carriers. The serum and urine amoxicillin
levels were determined in quadruplicate on a gel-diffusion system using five
known standard dilutions in each plate (Bennett et al., 1966).
RESULTS

Twenty-six carriers (92% women) infected with amoxicillin-sensitive \textit{S. typhi} were enrolled; their mean age was 34.6 years (Table 1). The gallbladder status was known in 15 carriers (13 consented to have cholecystograms); none had a normal gallbladder; eight had gallstones; five had a non-functioning gallbladder; two others were hepatic biliary tree carriers who had had cholecystectomies four and 12 months earlier.

\textbf{Side Effects}

Most of the carriers (86\%) had mild and transient adverse reactions during the first week of therapy that disappeared without interrupting the antibiotic treatment. In total, 13 (50\%) carriers complained of mild epigastric pain, lasting a mean of 2 days; 11 (42\%) experienced nausea, enduring a mean of 2.5 days; seven (27\%) had diarrhoea, persisting for a mean of 5.4 days; six (23\%) had a diffuse pruritic rash, lasting a mean of 7.7 days; and six (23\%) had other symptoms. Two carriers developed intense epigastric pain that led to interruption of treatment. When the same treatment schedule was re-initiated five to seven days afterwards combined with antacids, mild epigastric discomfort recurred in one woman which lasted four days.

The unscheduled home visits demonstrated a high degree of compliance. On each visit each carrier had the correct number of unused individual doses. Of the urine samples available for testing from 20 carriers, multiple samples tested from 19 carriers had a high urinary level of amoxicillin; the remaining carrier had one of five urines negative for the drug.

\textbf{Bacteriological Response to Therapy}

After a period of 12 or more months of follow-up, 15 of the 26 carriers (58\%) persisted with negative cultures for \textit{S. typhi} and were considered cured.
(Table 1). Notably, this group included the two intrahepatic biliary S. typhi carriers (McFaddin 1966). Three (38%) of the eight carriers known to have gallstones and one (20%) of the five who had a non-functioning gallbladder were cured. When the probability of cure was observed by month post-treatment, it became stable after the 4th month (Figure 1). No failures occurred after that period.

Various parameters were analyzed to compare the carriers who failed with those who were cured. No differences were found with respect to age or duration of carriage prior to therapy (Table 1). Furthermore, the proportion of carriers who developed side effects was similar between the two groups.

One objective difference between the groups was found in the serum amoxicillin levels of the 20 carriers whose specimens were available for testing. There were no differences in the serum amoxicillin levels between the 12 cured and eight failed carriers at baseline (overnight level), or two and three hours after the morning dose (Figure 2). However, the mean serum amoxicillin level at four hours after the morning dose in the 12 tested carriers who were cured (19.50 ± 2.9 mcg/ml) was significantly higher than the mean level among the eight tested carriers who failed (14.69 ± 2.77) (p<0.01, Student's t test) (Figure 2).

**DISCUSSION**

After 28 days of 6.0 gm of amoxicillin combined with 1.5 gm of probenecid daily, we successfully eradicated S. typhi in 15 (58%) of 26 chronic carriers who were highly compliant in taking their medication. Our results are somewhat in contrast with those of Nolan & White (1978) who obtained a 90% cure rate among 10 carriers treated for the same period of time with the same amoxicillin dosage but without probenecid. Because of the small numbers involved, the difference in cure rates (nine of 10 versus 15 of 26) may very
well be due to chance (p=0.11, two-tailed Fisher's Exact test). However, the
most likely explanation of the difference in results is the high prevalence of
gall bladder disease among our carriers. All 13 carriers in our series who
had oral cholecystograms exhibited cholelithiasis or a non-functioning gall
bladder, in contrast with only 3 of 10 carriers in the report by Nolan & White
(1978) (p<0.005) who were treated with the same amoxicillin regimen but
without probenecid. Others have noted the relationship between gallstones and
failure rate in chronic carriers treated with antibiotics (Bullock 1963,

Nolan & White (1978) reported a significantly lower serum level of
amoxicillin six hours after the last treatment dose in the carriers who failed
treatment. Our results corroborate the importance of high and prolonged
antibiotic levels in achievement of cures: a significantly lower mean serum
amoxicillin level was observed at the 4th hour post-dose among the carriers
who failed, in comparison with the carriers who were cured (Figure 2).

We conclude that 2.0 gm of amoxicillin combined with 0.5 gm of probenecid
given three times a day for 28 days has a definite but limited role in the
ambulatory, non-surgical treatment of chronic S. typhi carriers. In providing
a >50% cure rate, this therapeutic regimen offers to the sporadic chronic
carrier a moderate chance to eradicate the carrier state without resort to
surgery. This is particularly relevant for carriers who, because of other
conditions, are deemed unacceptable surgical risks. In contrast, the cure
rate of 58% with this regimen is probably insufficiently high to justify its
use as a major component of typhoid fever control programs. Now that a
practical screening test is available to identify chronic typhoid carriers in
endemic areas (Lanata et al., 1983), an important intervention in control
programs must be the identification and treatment of carriers in
epidemiologically relevant groups such as foodhandlers. Therefore, the search must continue to identify a practical, non-surgical, ambulatory treatment for chronic *S. typhi* carriers that achieves >80% cure rate without causing notable adverse reactions.
Acknowledgment: We thank Ms. Irma Canepa from the Ministry of Health, the personnel of the outpatient department of the Infectious Diseases Hospital and of the laboratory of the Instituto de Salud Publica for their help in conducting this study; Beecham Laboratories for providing amoxicillin; and Ms. Fiorella L. Piazzon for reviewing the manuscript. This work was partly supported by grants from the World Health Organization, the Pan American Health Organization, and the HDC Corporation, Mountain View, California, and by research contract DAMD-17-C-1115 from the U.S. Army Medical Research and Development Command.
REFERENCES


**LEGENDS**

**Figure 1** - The probability of cure (±95% confidence interval) by month post-treatment among 26 chronic *Salmonella typhi* carriers in Santiago, Chile treated with amoxicillin and probenecid. Data shown as a Kaplan-Meier curve.

**Figure 2** - Mean serum amoxicillin levels (±95% confidence interval) in 20 chronic *Salmonella typhi* carriers after oral 2.0 gm oral dose of probenecid. Data arranged according to carriers who were cured (n=12) or who failed (n=8) on this antibiotic regimen.
SERUM AMOXICILLIN LEVELS (95% CONFIDENCE INTERVAL) OF 20 CHRONIC B. TYPHİM CARRIERS AFTER ONE ORAL DOSE OF 2 g OF AMOXICILLIN AND 0.5 g OF PROBENECID ACCORDING TO TREATMENT OUTCOME.


- CURED (●●●●)
- FAILED (●●●●)

* MEANS DIFFERENCE AT 4 HOURS SIGNIFICANT AT P<.05, UTMN U2 T TEST.
PROBABILITY OF CURE (95% CONFIDENCE INTERVAL) BY MONTH POST-TREATMENT OF 3 CHRONIC SALMONELLA TYPHI CARRIERS TREATED WITH AMOXICILLIN AND PROBENECID. KAPLAN-MEIER CURVE. SANTIAGO, CHILE 1981-1983
PROBABILITY OF CURE (95% CONFIDENCE INTERVAL) BY MONTH POST-TREATMENT OF 26 CHRONIC SALMONELLA TYPHI CARRIERS TREATED WITH AMOXICILLIN AND PROBENECID. KAPLAN-MEIER CURVE.
SERUM AMOXICILLIN LEVEL (95% CONFIENCE INTERVAL) OF 20 CHRONIC TYPHUS CARRIERS AFTER ONE ORAL DOSE OF 2 GM OF AMOXICILLIN AND 0.3 mg OF PROBENECID ACCORDING TO TREATMENT OUTCOME
SANTIAGO, CHILE 1981-1983

- CURVED (n=12)
- FAILED (n=8)

* MEANS DIFFERENCE AT 4 HOURS SIGNIFICANT AT P<.001, STUDENT'S T TEST
The Use of Moore Swabs for Isolation of *Salmonella typhi* from Irrigation Water in Santiago, Chile

S. D. Sears, C. Ferreccio, M. M. Levine, J. M. Cordano, J. Monreal, R. E. Black, K. D'Ottone, B. Rowe, and the Chilean Typhoid Committee*

In Chile, a country with an exceedingly high incidence of typhoid, untreated sewage is applied directly to fields where salad vegetables are cultivated. Water used for irrigation was examined for the presence of *Salmonella typhi* by making use of the sewer-swab technique. *S. typhi* was isolated in 8 (11%) of 76 irrigation samples examined from nonindustrial, polluted water. This supports the hypothesis that crops grown with water contaminated with feces are important vehicles in the transmission of *S. typhi* in this endemic area. Since sewage treatment plants will not be available in Santiago in the near future, emphasis is being placed on devising alternative methods of irrigation and on growing vegetables that are cooked before being eaten.

Typhoid fever is a major health problem in Santiago, Chile where the annual incidence has exceeded 150 cases per 100,000 population since 1977, with most cases occurring in summer [1]. This is unexpected because Chile has demographic features and health statistics consistent with a technologically advanced society: 94% of homes have bacteriologically monitored, chlorinated water, and 75% have flush toilets [2]. However, human waste is discharged without treatment into the local river, water from which is used to irrigate farmland during the dry summer months. Crops such as lettuce, cabbage, and celery grown with sewage-contaminated water may play an important role as vehicles of *S. typhi* when they are consumed raw by residents of Santiago.

Multiple bacteriologic examinations of irrigation water in Santiago have demonstrated high fecal coliform counts and many other *Salmonella*, but no *S. typhi* [3, 4]. Although the microbiological methods used in previous attempts were appropriate, optimal sampling and concentrating techniques were not used. The Moore swab, described in England in 1948 [5], is a concentrating method that has been used successfully to locate chronic *S. typhi* carriers by isolating the organism from sewage effluents [6, 7]. Its main use has been in the investigation of urban typhoid fever outbreaks [8, 9], and its efficacy and reliability in endemic areas are unknown. We used a modified Moore swab to isolate *S. typhi* from environmental sources in Santiago.

Materials and Methods

Microbiological examination of rivers and irrigation canals of Santiago, Chile was carried out from January to March, 1983. The two major waterways in Santiago that carry wastewater are the Mapocho River in the north and Zanjón de la Aguada canal in the south (figure 1). Untreated sewage flows directly into these waters, which are used for irrigation in the agricultural districts of Maipú and Pudahuel (on the perimeter of the city). The Zanjón de la Aguada, which is heavily contaminated with industrial waste from the central section of the city, receives untreated sewage and becomes polluted with feces as it flows westward. In the final few kilometers before it reaches the...
agricultural areas, no further sewage is discharged, in an attempt to allow a degree of self-purification of the wastewater. Moore swabs were prepared by wrapping cotton gauze, 15 cm wide by 120 cm long, around wire. The swabs were tied to nylon cord, and suspended in the flowing water of the Zanjón de la Aguada, Mapocho River, and their tributaries. Swabs were also placed directly into irrigation canals of selected farms.

After 48-72 hr, the swabs were removed and immediately placed into 500 ml of Selenite-F broth. The selenite broth was incubated at 41 C and sub-cultured at 24 hr and 48 hr on to salmonella-shigella, bismuth-sulfite, and deoxycholate-citrate agars. (All broth and media were from BBL Microbiology Systems, Cockeysville, Md.) Suspicious colonies were placed on triple-sugar-iron agar slants and confirmed as S *typhi* by standard methods [7]. *S* *typhi* isolates were sent to the Central Public Health Laboratory, Colindale, U. K. for phage typing.

**Results**

We placed 56 swabs into the Mapocho River and 77 swabs into the Zanjón de la Aguada, but recovered only 93. Most lost swabs were due to interference by passersby, who removed or cut the swab. After the first month, by camouflaging the swabs, we were able to decrease losses. Of the 48 swabs recovered from the Zanjón de la Aguada, 17 came from central city industrial areas where there is heavy chemical pollution, and 31 were from agricultural areas where there is a predominance of fecal contamination. None of the 17 swabs from industrial areas and 4 (8.3%) of 45 swabs from the Mapocho River contained *S* *typhi*. Of the 76 swabs placed in agricultural areas, 8 (11%) were culture positive. Five of the eight isolates were phage-type E1 and 46, the two most common disease-causing types in Chile, one strain was untypeable, and the other two were N and M1.

**Discussion**

Using Moore swabs, we were able to isolate *S* *typhi* from irrigation water in Santiago, Chile. To our knowledge, this is the first time Moore swabs have been used for this purpose. The sensitivity of the Moore swab is thought to have an inverse relationship to the flow volume of the waterway sampled [7]. Thus, our isolation rate of 11% from these large waterways is probably an underestimate. *S* *typhi* is fastidious, easily inhibited by coliforms, and usually present in relatively small numbers in environmental samples [8]. The Moore swab, by acting as a filter, improves the chance of isolating rare *S* *typhi* among millions of coliforms and has been useful in isolating *S* *typhi* from sewers during outbreaks of infection in industrialized nations. We have now shown that it is both a practical and reliable epidemiological tool with which to isolate *S* *typhi* from irrigation water in endemic areas.

Finding *S* *typhi* with the same phage types as disease-causing isolates in irrigation water supports the hypothesis, based on epidemiological observations, that contaminated vegetables in Santiago serve as important vehicles of transmis-
sion (M. Levine, unpublished data). These observations are as follows: (1) typhoid fever peaks during summer when rainfall is lowest and irrigation is used most heavily; (2) in the agricultural lake-region of southern Chile where rain water is available all year, there is little irrigation and typhoid fever has a low incidence; (3) persons from all socioeconomic groups in Santiago have a high incidence of typhoid fever, a finding suggesting vehicles for infection that are consumed in all areas of the city; and (4) bacteriologically monitored, chlorinated water is available in 94% of all households and is thus an unlikely vehicle for S. typhi.

Enteric diseases can be transmitted by vegetables contaminated by polluted water [11], but a cause and effect relationship is difficult to prove. A study of kibbutzim in Israel showed that communities that practiced wastewater irrigation had a two-to-four times higher incidence of enteric infections [12]. Although our study does not prove that S. typhi cultured from irrigation water is directly responsible for typhoid fever, its presence implies that an association likely exists between S. typhi-contaminated vegetables and infection. Recently the government of Chile has intervened to change the farming patterns and usage of contaminated irrigation water. In Maipu and Pudahuel, water from the Mapocho River and the Zanjón will no longer be used to irrigate salad vegetables that can become contaminated and serve as vehicles of transmission of S. typhi.

References
Sensitivity of Moore Sewer Swabs for Isolating Salmonella typhi

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Moore swabs (sewer swabs) have been used successfully to culture pathogenic organisms from wastewater. Sensitivity seems to depend on the size of the waterway sampled as well as the number of organisms present. In Santiago, Chile, we placed 24 swabs into the sewers draining the homes of 10 known chronic carriers of typhoid. Swabs were positive for Salmonella typhi in 5 of the 10 households (50%) and 6 of the 24 swabs placed (25%).

In 1948, Moore used large gauze pads (sewer swabs) to isolate Salmonella parararby B from sewage outflow of a coastal English village. Two years later, using the swab technique, he was able to isolate Salmonella typhi and locate the home of a chronic typhoid carrier (7). The swabs were collected 48 h after placement and cultured in Selenite enrichment broth, with subculturing on Wilson and Blair solid medium (WB). By placing swabs in various sizes of sewers, Moore was able to trace back the source of contamination. He suggested that this technique was most successful when sewer swabs were placed in medium-sized sewers since the sensitivity seemed to be inversely related to the diameter of the sewer sampled (8).

The Moore swab, placed into flowing sewer water, apparently acts as a filter to trap and concentrate pathogenic organisms. The swab shows a more accurate microbiologic composition of the wastewater than water samples since the swab reflects the sum of organisms which have passed through it over time. The Moore swab has been used successfully to isolate viruses, mycobacteria, salmonellae, and vibrios from sewage (1, 4) and has proven useful for investigating the epidemiology of typhoid fever, including typhoid epidemics in industrialized nations (10) and studies of endemic typhoid in Chile (9).

The observation of Moore regarding the relationship of the effectiveness of his swab to sewer size was reconfirmed in the 1964 typhoid outbreak in Aberdeen, Scotland, in which Callahan and Brodie (2) found the sewer swab to be an insensitive tool for random sampling of large sewers. More recently, Barrett et al. (1) found the Moore swab to be both a practical and a sensitive tool for the isolation of Vibrio cholerae 01 from relatively small sewers. In a previous study, Sears et al. reported that Moore swabs can be used successfully to isolate S. typhi from polluted irrigation water in areas with endemic typhoid fever (9). To evaluate further the sensitivity and reliability of the Moore swab, we placed swabs in the small sewers draining the homes of known, chronic carriers of S. typhi.

As part of the projects designed to control typhoid fever in Chile, studies have been performed to locate chronic carriers of S. typhi. Through one of these previous studies, which evaluated the efficacy of amoxicillin therapy for treatment of the carrier state, a registry of chronic carriers was compiled. Ten carriers, who were unable to participate in this drug trial, were identified. Permission was obtained to place Moore swabs in the outflow of the sewers draining their homes. The houses of these carriers had flush toilets connected to terra cotta or open pipe drainage. In the front yard of each home was an access panel to the sewer. Most of the houses shared a common sewer with at least one and often two other houses. Swabs were placed directly in the sewers of the homes of the 10 carriers and left for 48 or 72 h. Each swab was sampled at least two (and usually three) separate times, and an effort was made to assure that the carriers remained home during the time the swabs were in place.

Moore swabs were prepared by wrapping sterile cotton gauze, six inches wide by four feet long (115 cm by 120 cm), around a stiff wire. This was attached to a nylon cord and placed directly into the draining sewage. Most swabs were placed on Friday and collected on Monday to help ensure use of the facilities by the carriers in the households. After 48 to 72 h, each swab was removed from the sewage and placed directly into a wide-mouth jar containing 500 ml of Selenite-F broth (BBL Microbiology Systems, Cockeysville, Md.).

The swabs in the Selenite were incubated at 41°C and subcultured between 18 and 24 h onto Salmonella Shigella, bismuth sulfite (WB), and DCL3 (deoxycholate citrate lactose sucrose) agar. (All broth and media were from BBL.) Subculturing was done directly from the broth as well as with a 10-fold dilution of the broth. At 24 h after the swab was removed, the Selenite broth was again subcultured directly and with a 10-fold dilution on the same solid media. Suspicious colonies from the solid media were placed in TSI agar slants. Those giving TSI reactions typical of S. typhi were confirmed with standard biochemical tests and by agglutination with appropriate antisera (3). All isolates were then phage typed.

The homes of 10 asymptomatic, chronic carriers of S. typhi were visited. No carrier was taking antibiotics. At least two swabs were placed at different times in each of the sewers draining the homes of these carriers. Table 1 lists the households, the number of swabs placed in each sewer, and the number of times the cultures were positive for S. typhi. A total of 24 swabs were placed, and of these, 6 were positive (25%). Although only 25% of the swabs were positive, 5 of the 10 carrier households (50%) were found to have culture-positive swabs.

WB was the most effective medium for isolation of S. typhi from the swabs. In four of the six isolates, WB was the only medium on which S. typhi could be identified. In one case, an isolate was recovered from both Salmonella Shigella and S. typhi.
medium and WB, and in only one instance was S. typhi isolated from Salmonella Shigella and DCLS media without recovery on WB. Of the six isolates, four were recovered on the first subculture (18 to 24 h), both directly and in the 1:10 dilution. Only one isolate that was not recovered by direct isolation was recovered at the 1:10 dilution.

In this study, we placed Moore swabs into the small-diameter sewers draining the homes of known chronic typhoid carriers in Santiago, Chile. When two or three swabs were placed over time in each sewer, we were able to successfully recover S. typhi from one-quarter of the swabs and one-half of the carriers. The ability to isolate typhoid bacilli from these sewers seems to increase with increasing numbers of swabs. We suspect that as more swabs are placed, the ability to find a positive one for each carrier increases. Since we had no way of confirming that the carrier in the household was shedding typhoid bacilli during the time the swab was in place, this isolation rate probably represents a lower estimate of the true sensitivity of the swab.

In studies in England in 1954, Kwantes and Speedy (5), while investigating a paratyphoid outbreak with Moore swabs, found that carriers tried to avoid using the toilet facilities to escape detection. We do not know if the typhoid carriers in the households we sampled avoided using the toilet during our swabbing. Ideally, we would have preferred to have simultaneous stool cultures with swab cultures to correlate sensitivity, but due to the study design that was not possible. Even so, our finding that the Moore swab was successful in identifying S. typhi carriers 50% of the time suggests that in field epidemiologic situations, it is a useful and practical tool.

In a previous study of Moore swabs in Chile, Sears et al. were able to isolate S. typhi 11% of the time from fecally polluted irrigation canals (9). Moore swabs proved to be reliable, inexpensive epidemiologic tools for the isolation of S. typhi in Chile, an endemic area. In this study, we have sought to refine our previous observations and have attempted to determine the crude sensitivity of the Moore swab in a field situation. Moore swabs will detect a known carrier at least 50% of the time if small sewers are sampled at least two separate times. Thus, the Moore swab is a reasonably sensitive method to isolate S. typhi and may have practical applications such as sampling small sewers draining restaurants, food-processing plants, markets, or other institutions in which it could be important to detect carriers.

Moore, in his original studies, suggested that the sewer draining a block of homes was the ideal size for isolating S. typhi (8). We have taken this observation one step further and have shown that small sewers direc... draining the homes of carriers can be sampled effectively for S. typhi. Our observations also ref... the utility of WB, as well as Selenite broth enrichment for isolating S. typhi and suggest that the use of WB alone may be sufficient since we would have missed only one isolate with such solitary use.

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LITERATURE CITED


Duodenal String Cultures: Practicality and Sensitivity for Diagnosing Enteric Fever in Children

The diagnosis of enteric (typhoid or paratyphoid) fever must be confirmed by isolation of the causative organism from a suitable clinical culture. This verifies the appropriateness of antibiotic therapy, allows differentiation between Salmonella typhi and Salmonella paratyphi A, B, and C infections and provides isolates for phage typing if epidemiological investigations are indicated. Bone marrow culture, the most sensitive method to recover organisms associated with enteric fever [1-7], requires special instruments and technical expertise and is uncomfortable and invasive; it is therefore not amenable to routine use in children with suspected enteric fever. Blood culture, in contrast, is widely practiced wherever bacteriology is available, because of its relative simplicity, safety, and noninvasiveness. Unfortunately, the sensitivity of blood culture is significantly less than that of bone marrow culture [1-7]. Since Salmonella are present in the bile of patients with acute enteric fever [8-10], some investigators have cultured bile-containing duodenal fluid by means of string capsule devices [7, 11-13] and have reported significantly higher rates of isolation of Salmonella than when cultures of blood are used alone. With one exception [14], these studies have been largely confined to adults. However, in endemic areas such as Santiago, Chile, typhoid fever is predominantly a disease of school-age children, five to 14 years of age [15]. Thus we undertook this study to evaluate the practicality and clinical acceptability of cultures of duodenal string capsule in children <15 years of age with a clinical diagnosis of enteric fever and to compare the sensitivity of this culture method with that of cultures of blood and bone marrow.

Patients and Methods

Patients with a clinical diagnosis of acute enteric fever who were admitted to the Roberto del Río Children’s Hospital between January 1983 and February 1985 entered the study. From each child, an attempt was made to obtain blood, bone marrow, and bile-stained duodenal fluid for culture. Two 1-ml samples of blood, drawn 30 min apart by sterile technique, were inoculated into flasks containing 50 ml of brain-heart infusion broth (BBL Microbiology Systems, Cockeysville, Md) with 0.025% sodium polychlorohydrate. Aspirates of bone marrow from the iliac crest were inoculated into identical flasks.

To obtain samples of bile-containing duodenal fluid for culture, we instructed the children to swallow a string capsule device (Pediatric Entoretest, HEDECO, Mountain View, Calif) with a glass of water or flavored gelatin. The device consists of a nylon string coiled within a gelatin capsule. The proximal end of the string was taped to the cheek, and the string was left in place for 6 hr. (During this time the gelatin capsule digests in the stomach and allows the nylon string to uncoil and pass through the pylorus into the duodenum, where the distal end is impregnated with bile and duodenal fluid.)

When the string was removed, the distal portion was examined for bile staining, and the pH was measured to determine whether the string had reached the duodenum. The distal 20 cm were severed and divided into two equal portions, one of which was inoculated into 20 ml of selenite F enrichment broth and the other into 50 ml of brain-heart infusion broth. Flasks were incubated at 35 C, and positive broths were subcultured onto salmonella-shigella and bismuth-sulfite agar (BBL). Suspicious colonies were transferred to Kligler’s triple-sugar iron agar and characterized by standard biochemical and serological techniques. Chloramphenicol treatment was initiated (30 mg/kg per day) after all cultures were obtained.

Results

A complete set of cultures (two blood, one bone marrow, and one duodenal string) was obtained from 103 children,
three to 14 years of age, with a clinical syndrome compatible with typhoid fever. From the last 23 patients a second duodenal string culture was routinely obtained on the day following the initial cultures. Only three additional children attempted to swallow the string capsule device but were unsuccessful. Four children had received an antibiotic before admission.

Sensitivity of culture combinations. One of the three cultures was positive in all 103 clinically suspected cases, of whom 88 had S. typhi and 15 had S. paratyphi A, B, or C infection. The sensitivity of a single culture in bacteriologically confirming cases ranged from 61% for blood to 76% for bone marrow, with a single duodenal string culture as the intermediate (71%; table 1). Combinations of cultures greatly increased the sensitivity (table 1). A second culture of blood resulted in isolation of Salmonella from 69% of the cases, whereas adding a bone marrow culture to two cultures of blood increased the sensitivity to 84%. A single duodenal string culture in conjunction with two cultures of blood also notably increased sensitivity, a combination resulting in bacteriologic confirmation of 95 (92%) of 103 cases. Thus either a single bone marrow or a single duodenal string culture sufficiently increased the rate of bacteriologic confirmation over two cultures of blood alone. By means of two cultures of blood, one bone marrow culture, and one duodenal string culture, 101 of 103 patients were confirmed bacteriologically as having enteric fever (the remaining two patients were confirmed by means of a second duodenal string culture). The last 23 patients in this study had two duodenal string cultures routinely performed with isolation of Salmonella from 21 (91%) of 23 children by means of these two cultures.

Practicability, acceptability, and reliability of duodenal fluid cultures by the string-capsule device. Among the 103 children from whom duodenal cultures were obtained, eight children (8%) had notable difficulty in swallowing the capsule; a few had to be given a second capsule. No adverse effects were noted from the use of the string-capsule devices.

The pH of the distal tip of the string was recorded in 99 of the 103 children who had duodenal string cultures. The recovery of Salmonella from duodenal string cultures was clearly related to whether the string had passed through the pylorus into the duodenum (based on the pH of the tip of the string). Of 76 children whose strings had a pH >6.0, 59 (78%) had positive cultures. In contrast, when the pH was <6.0, only 10 (45%) of 23 yielded Salmonella (P = .0042); the pH in the 10 with positive cultures was 5.0 and was <4.0 in those with negative cultures. The rate of positive duodenal string cultures did not differ significantly in relation to age; 27 (79%) of 34 children three to nine years of age had positive cultures vs. 46 (67%) of 69 children 10 to 14 years of age (P = .70). The rate of positivity of duodenal string cultures in relation to duration of illness before entering the study is shown in table 2. The duration of illness was not significantly different for the 73 children with positive cultures vs. the 30 children with negative cultures (table 2).

Table 2. Positivity of duodenal string cultures in children with enteric fever in relation to the duration of illness.

<table>
<thead>
<tr>
<th>Days of illness before culture</th>
<th>No. of children</th>
<th>No. positive/ negative (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-7</td>
<td>26</td>
<td>17/9 (65.4)</td>
</tr>
<tr>
<td>8-14</td>
<td>48</td>
<td>38/10 (79.1)</td>
</tr>
<tr>
<td>15-21</td>
<td>26</td>
<td>16/10 (61.5)</td>
</tr>
<tr>
<td>22-31</td>
<td>3</td>
<td>2/1 (67)</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>73/30</td>
</tr>
<tr>
<td>Median*</td>
<td>10/10</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD*</td>
<td></td>
<td>12.0 ± 4.6/10.0 ± 4.3</td>
</tr>
</tbody>
</table>

NOTE. The duration of illness was not significantly different in those with positive vs. those with negative cultures (Student's t and Wilcoxon rank sum tests).

* Data are days of illness before culture in positive days of illness before culture in negatives.

Discussion

Other investigators have previously documented the usefulness and sensitivity of duodenal string cultures in typhoid fever in studies largely involving adults [7, 13]. Benavente et al. [13] found duodenal string cultures positive in 86% and positive cultures of bone marrow in 75% of 36 Peruvians with typhoid fever. Hoffman et al. [7] reported that the combination of one culture of blood, one rectal swab, and one duodenal string culture had 86% sensitivity vs. 92% sensitivity for the combination of one...
culture of blood, one rectal swab, and one bone marrow culture in isolating S. typhi or S. paratyphi from 118 Indonesians with enteric fever. The only previous pediatric study involved 118 Peruvian patients two to 13 years of age with suspected enteric fever [14] who swallowed “bone-made” string-capsule devices prepared locally at the hospital. Only 47% of 36 young Peruvian children two to six years of age tolerated the duodenal string cultures; furthermore, the sensitivity of the duodenal string cultures was much lower than that previously reported for adult patients at that hospital in Peru [12, 13].

Herein we report a systematic study of duodenal string cultures in comparison with cultures of bone marrow and blood in 103 Chilean children (14 years of age or younger) with a clinical diagnosis of enteric fever. A single duodenal string culture, in conjunction with two cultures of blood, allowed isolation of S. typhi or S. paratyphi from 92% of patients, a comparable rate occurred with two cultures of blood and a bone marrow culture (84%). The string capsule device was practical and surprisingly well tolerated by the children: 103 (97%) of 106 children who attempted succeeded in swallowing the string capsule, with 98 (92%) having no notable difficulty whatsoever. Furthermore, Salmonella were as readily isolated from duodenal string cultures in young children three to nine years of age (27 [79%] of 34) as older children >10 years of age (46 [67%] of 69; P = .70). The recovery of Salmonella from string cultures correlated highly with evidence (by measurement of the pH of the distal end) that the string had reached the duodenum; when the string had a pH >6.0, Salmonella was recovered from 78% of the cultures vs. only 43% when the string pH was <6.0 (P = .0042). Our results in Chilean children contrast sharply with those in Peruvian children [14], with both clinical acceptability and sensitivity being significantly greater in our study. The two studies differ so markedly in methods, however, that caution must be exercised in making comparisons. The Peruvian study utilized homemade rather than commercial string devices, and it was not stated if these were modified for pediatric patients. Furthermore, the Peruvian investigators removed the strings after 3 h and most importantly, did not verify the pH of the tip of the string. The high sensitivity in the Chilean study may be due, in part, to the strings being left in place for an average of 6 h; future comparative studies will assess if 3 or 4 h will suffice, thereby making duodenal string cultures more practical for outpatients.

In the last 23 patients a second duodenal string culture was routinely obtained, thereby allowing us to make a preliminary statement of the value of two duodenal string cultures. Among these 23 patients, at least one of the duodenal string cultures was positive in 21 patients (91%), vs. two cultures of blood yielding a Salmonella in only 14 (61%), cases and two cultures of blood plus a bone marrow culture confirming 18 (78%) cases of enteric fever. If two duodenal string cultures are desired without un-}


duly delaying the initiation of antibiotics, it may be prudent to obtain the cultures one immediately after the other.

In children, culture methods to confirm the diagnosis of enteric fever must compromise between sensitivity and practicality. Two cultures of blood represent the minimum effort to be expected wherever bacteriologic capability is available, since they are simple to obtain and noninvasive. Unfortunately, cultures of blood offer only moderate sensitivity. Sensitivity can be notably increased if clinical material can be obtained for culture from the reticuloendothelial system where Salmonella reside in specific macrophages. Heretofore, this has been accomplished by means of bone marrow cultures. This procedure, however, is invasive for children and requires skilled operators and special needles that are not always available.

Our systematic study in Chilean children with enteric fever demonstrates that the combination of two cultures of blood and a duodenal string culture offers excellent sensitivity (equal to two cultures of blood and a bone marrow culture) and noninvasive practicality and is effective in children from three to 14 years of age.

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cases of meningitis

The patient was neither pregnant nor menstruating. A gram stain of the smear of the CSF revealed no organisms, and bacterial cultures were sterile. An 11CSF neutrophilic leukocytosis was noted, however, consisting of depressed levels of glucose and elevated levels of protein. Cultures of blood and cervix were positive for Neisseria gonorrhoeae. Despite aggressive management, including parenteral antimicrobial therapy using penicillin and chloramphenicol, the patient died of overwhelming sepsis [9].

Case 2. A 15-year-old black woman was admitted to a second hospital with a three-day history of migratory pain in her joints. Rabinowitz and his colleagues have pointed out that such cases are not uncommon in persons with meningococcemia [10], and have called attention to the possibility of meningococcal arthritis [11].

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References


Survey of Plasmids in Salmonella typhi from Chile and Thailand

Salmonella typhi remains an important enteric pathogen in many parts of the world. Although a number of outbreaks of typhoid fever have been caused by antibiotic-resistant S. typhi, such as in Mexico in the early 1970s [1], and more recently in Peru, these organisms have in general remained surprisingly susceptible to antibiotics, particularly when one compares their resistance with that of other enteric pathogens, like the shigellae and non-typhoidal salmonellae. The current study was originally undertaken to investigate antibiotic resistance in S. typhi in Santiago, Chile and to examine total plasmid content of clinical isolates. This study also explored the possibility of a common "virulence" plasmid(s) and the potential utility of plasmid electropherotyping for epidemiological studies. When no resistance and few plasmids were found, further studies were undertaken to investigate possible reasons for these findings and to determine if similar results could be found in other geographic locations.

Materials and Methods

Bacterial strains and susceptibility testing. Clinical isolates of S. typhi from local hospitals in Chile were identified and phage typed at the Instituto de Salud Publica in Santiago: strains from Thailand were sent to the Department of Medical Science, Bangkok. Recipient strains included Escherichia coli 153 (pro met) and nalidixic acid-resistant mutants of three plasmid-free S. typhi clinical isolates from Chile. Donor strains were E. coli isolated either from urinary tract infections in Santiago, or from feces of a U.S. student in Mexico [2]. Antimicrobial susceptibilities were determined by the disk-diffusion method by using Mueller-Hinton agar (Difco Laboratories, Detroit) and disks purchased from BBL Microbiology Systems (Cockeysville, Md).

Conjugation studies. Total plasmid contents of all strains were examined by the method of Kado and Liu [3]; a subset of 30 strains from Chile were also cross-examined by other methods [4, 5]. Conjugations were performed in broth as previously described [2, 5]. The frequency of transfer was determined by dividing the number of transconjugants by the number of recipients.

Growth curves and stability studies. For growth rate determinations, single colonies were inoculated in duplicate into brain-heart infusion broth, grown overnight at 37°C, diluted 10-4 into brain-heart infusion broth and incubated in a rotary incubator at 200 rpm at 37°C. Growth was followed at 580 nm with a Spectronic 21 (Beckman Instruments, Palo Alto, Calif). The stability of plasmids was determined by inoculating 10 single colonies of each strain from trimethoprim-containing agar plates onto peptone agar slants; after incubation at 25°C for 3 months, each slant was subcultured and 8-12 single colonies from each slant (80-100 total per strain) were tested for resistance.

Results

Chilean strains. One hundred strains of S. typhi isolated within the preceding year in Chile were examined; 19 were examined within one week of isolation. Phage typing of 74 isolates revealed that the majority of strains were either type E1 (23 isolates), type 46 (17 isolates), or F8 (8 isolates); other types included F1 and M1 (4 strains each), A and 34 (3 strains each), 38 (2), D4 (1), nontypable (2), and Vi(-) (7). None of these isolates were resistant to any of seven antimicrobial agents tested; this corroborates the results of D'Onofrio et al. [6] in Chile in 1980 that showed only two of 661 isolates were resistant to chloramphenicol and the results of Rodriguez et al. [7] in 1977 that showed only 1.8% of 1,622 isolates were resistant to any of the six agents.

Of 100 Chilean isolates that were examined for the presence of extrachromosomal DNA, only eight were found to have plasmids; all eight were detected by the method of Kado and Liu. Five phage type F8 isolates and one Vi(-) isolate had a plasmid of 65 Mdal; one type 38 strain had a plasmid of 32 Mdal, and one nontypable strain had a plasmid of 3 Mdal.

Thai strains. Since Chile is somewhat isolated geographically, strains of S. typhi from another location were examined. Fifty strains from Thailand were screened by phase typing, and 38 revealed the following: type 46 (10 strains), type M1 (7), type E1 (6), type D1 (3), type 53 (2), types E9, J5, D6, and D5 (1 strain each), Vi(-) (2), and 3 strains were untypable. Three were found to have plasmids. One of these three strains (phage type D1) was resistant to ampicillin, chloramphenicol, streptomycin, and tetracycline. Another isolate was resistant by disk to streptomycin (9 mm zone of inhibition), and ten were intermediate in susceptibility to streptomycin; none of these had a plasmid.

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trimethoprim-sulfamethoxazole) are available over-the-counter in these as in most developing countries. Chloramphenicol, for example, considered the primary agent for typhoid fever, has been widely used for various illnesses since the 1950s. Studies of other enteric organisms that cause diseases and are treated with similar antibiotics, such as Salmonella newport and S. typhimurium in Chile and Salmonella kirschfels in Bangkok, reveal multiple antibiotic resistances [12, 13; P. J., unpublished data]. Surveillance of E. coli in Santiago and Bangkok has revealed increased resistance in this species [14].

In order to investigate the interaction of S. typhi and several R factors found in nature, we performed conjugation studies between clinical isolates of S. typhi and E. coli. E. coli seems a likely donor species in nature for the following reasons: (1) it is normally the most numerous coli-form in the human intestinal tract and therefore should come into contact with S. typhi in developing countries it is often multiply resistant; and (2) since both E. coli and S. typhi are Enterobacteriaceae and considerably homologous by DNA studies, they would be expected to exchange genetic information in vivo. The transfer frequencies of the R factors originating in E. coli into S. typhi were, at most, slightly decreased relative to an E. coli recipient (table 1). This implies that neither a cell exclusion barrier nor a restriction endonuclease impedes the entry or establishment of E. coli plasmid DNA in S. typhi.

As expected, a comparison of growth rates revealed that the clinical E. coli isolates grew more rapidly than did the laboratory K12 strain and much more rapidly than did the S. typhi. The presence of some but not all R factors further slowed the growth rates of both E. coli 353 and of S. typhi, but a consistent effect of a given plasmid upon all host strains was not seen. A slowing effect has been well documented for some plasmids, although some have no effect and some even enhance growth of the organism [15, 16]. Whether the slowing effect on S. typhi growth seen with some of these R factors would impart a selective disadvantage in nature is unknown.

Another, perhaps more important, difference between the E. coli and S. typhi hosts was the degree of stability of the R factors. Four of the R factors were unstable in S. typhi but stable in E. coli (table 1). Such instability suggests that a number of accessible R factors in nature do

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Figure 1. Experiments to determine growth curves of parental strains and their plasmid-containing derivatives were performed in brain-heart infusion broth. Experiments were performed with STP12, STP13, STP18, and STP20 Escherichia coli isolates from urinary tract infections in Chile: pSTP12, pSTP13, pSTP18, and pSTP20 are the resistance plasmids derived from the corresponding strains. S13, S25, and S82 are Salmonella typhi isolates from Chile. The upper-left figure shows the growth curves of all strains before transferring any R factors. The next four figures show S. typhi strains without (solid lines) and with (broken lines) various R factors. The lower-right figure shows STP20 and a spontaneously arising derivative (STP20-C), which has lost resistance.

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Disproportionate Expansion of a Minor T Cell Subset in Patients with Lymphadenopathy Syndrome and Acquired Immunodeficiency Syndrome

The evolution of acquired immunodeficiency syndrome (AIDS) involves alterations in lymphocyte subpopulations that may be a significant part of the underlying disease process. These alterations in lymphocyte subsets include an inversion of the T4:T8 ratio that is due to a reduction in the absolute numbers of T4 positive cells and either normal numbers or slightly elevated numbers of T8 positive cells [1]. As the disease progresses, lymphopenia results in lower absolute numbers of both T4* and T8* cells and the T4:T8 ratio becomes even more reduced.

In viral infections with such viruses as cytomegalovirus (CMV), herpesvirus, or Epstein-Barr virus (EBV) a reversal in the ratio of T4* to T8* cells also occurs, and the effect may persist for months after recovery [2]. The reversal in these viral infections is primarily due to a dramatic expansion of the T8* population, although a reduction in the numbers of T4* cells does occur. Thus, in AIDS the reversal of the T4:T8 ratio reflects a somewhat different absolute representation of these T cell subpopulations than is observed in other viral infections, although the effect on the relative proportion of T4* and T8* cells may be similar.

In contrast to the findings in patients with AIDS and acute viral infections, Kornfeld et al. [3] showed that healthy, promiscuous homosexual men had increased numbers of T8* cells and normal numbers of T4* cells. A similar observation was made by Lederman et al. [4] in hemophiliacs who had received lyophilized preparations of antihemophilic factor. In patients with the AIDS-related complex (ARC) of symptoms and physical findings, both an expansion of the T8* population and a reduction in the T4* lymphocyte subset have been observed [3]. Because patients with ARC have a greater risk of developing AIDS, it is crucial to determine the temporal relationship of these lymphocytic alterations and their relevance to the eventual progression to AIDS.

We therefore examined patients with AIDS and ARC to determine whether the profound immunosuppression seen in these patients may be associated with more specific alterations in suppressor T lymphocyte subpopulations. We found that AIDS patients and ARC patients differed significantly from normal subjects and from individuals suffering acute viral infections. These differences included an increase in subpopulations of T8* cells bearing an additional cell surface determinant, Leu7, and an increase in numbers of Leu1* cells. This finding was in marked contrast to the relative infrequency of T8*Leu7* cells in normal subjects [6], in which these cells constitute a minor subpopulation of T8* cells. Furthermore, the data suggest that evolution of the immunodeficiency state may include an expansion of the T8*Leu7* subpopulation in those patients with ARC who progress to the development of AIDS. When lymphopenia develops in AIDS patients, all subpopulations of lymphocytes are depleted and eventually only T8*Leu7* cells remain. These alterations in lymphocyte subpopulations may provide new clues to understanding the pathogenesis of AIDS.

Subjects and Methods

Subjects: Patients with AIDS, ARC, and viral infections were referred for cell surface phenotyping to the Howard Hughes Medical Institute flow cytometry facility. All patients with AIDS were diagnosed according to
MOLECULAR TECHNIQUES IN THE STUDY OF SALMONELLA TYPHI IN EPIDEMIOLOGIC STUDIES IN ENDEMIC AREAS: COMPARISON WITH VI PHAGE TYPING

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Abstract. We examined 141 Salmonella typhi strains of known phage type isolated during ongoing epidemiologic studies in Santiago, Chile, and Lima, Peru. Plasmids were present in 12 (17%) of 70 S. typhi isolates from Santiago and 5 (7%) of 71 isolates from Lima; these plasmids were not associated with antimicrobial resistance. Identical 21 kilobase (kb) plasmids (as defined by restriction endonuclease digest pattern) were present in 13 of the 17 plasmid-containing isolates. Virtually identical digest patterns were identified when chromosomal DNA of selected strains from Santiago, Lima, and the United States was extracted and then digested with restriction endonucleases. The similarities among plasmids and chromosomal digest patterns emphasize the homogeneity and possible clonal origin of S. typhi isolates; these data also suggest that there is only a limited role for plasmid and chromosomal analysis as a substitute for phage typing in epidemiologic studies.

Typhoid fever is a major urban health problem along the western coast of South America, with reported incidence rates of 150 and 212 cases/100,000 in Santiago and Lima, respectively. Studies of the epidemiology of typhoid fever in these areas are notoriously difficult because of the multiplicity of vehicles and risk factors present. The ability to differentiate strains based on specific epidemiologic markers is critical in such studies; unfortunately, few markers for Salmonella typhi have been described, with Vi phage typing currently providing the only useful means of distinguishing one S. typhi strain from another. Molecular genetic techniques, including plasmid analysis and examination of restriction endonuclease digest patterns of chromosomal DNA, have been found to be valuable tools in epidemiologic studies of certain other bacterial pathogens. We studied selected isolates from Chile and Peru to determine if these molecular techniques were useful in differentiating S. typhi strains beyond what could be accomplished with Vi phage typing alone.

Materials and Methods

S. typhi strains from Chile were randomly selected from strains isolated from pediatric patients seen at the Roberto Del Rio Hospital in Area Norte, Santiago, between January and June 1983. The identification of the isolates was confirmed by the Institute of Public Health, Santiago, and isolates were phage typed by the Vi phage typing scheme of Anderson and Williams at the Institute of Public Health, Santiago, and the Division of Enteric Pathogens, Central Public Health Laboratory, Colindale, England. Strains from Lima were isolated from pediatric and adult patients between February and December 1984 at the Universidad Peruana Cayetano Heredia, Lima, isolates were phage typed at the Central Public Health Laboratories, Colindale. American S. typhi strains were isolated from adult patients in Maryland and Texas. Plasmids were extracted from isolates using an alkaline extraction procedure. All strains containing plasmids were tested by disc diffusion for susceptibility to ampicillin, chloramphenicol, gentamicin, and trimethoprim/sulfamethoxazole. Chromosomal DNA was extracted from...
isolates using a phenol/chloroform extraction procedure. DNA was digested with restriction enzymes (EcoRI, HindIII, BamHI, or PvuII; Bethesda Research Laboratories, Inc.), and visualized under ultraviolet light after electrophoresis in 0.7% agarose gels and staining with ethidium bromide.

RESULTS

Eleven phage types were represented among 70 S. typhi strains isolated from patients in Santiago. Vi phage type E1 accounted for 43% and Vi phage type 46, 26% of isolates (Table 1). Plasmids were present in 12 (17%) of the 70 strains. Two distinct plasmid profiles were identified: 10 isolates had a single 21 kilobase (kb) plasmid and 2, a 57 kb plasmid. Isolates with the 21 kb plasmid were significantly more likely to be of Vi phage type 51, with four of six isolates of this phage type carrying the plasmid (P < 0.01. Fisher's exact test, two-tail). All plasmid-carrying strains were susceptible to the four antimicrobial agents tested. No correlation could be shown between specific plasmid profiles and age or sex of the patient from whom the strain was isolated.

Nine phage types were represented among the 71 S. typhi strains isolated from patients in Lima. Vi phage type M1 accounted for 30% and Vi phage type 46 18% of typable isolates (Table 1). Plasmids were present in 55 (71%) of the 77 strains. Three plasmid profiles were identified among the Peru isolates: 3 isolates had a single 21 kb plasmid, 1 a 57 kb plasmid, and 1 a 29 kb plasmid. Presence of the 21 kb plasmid was significantly associated with Vi phage type 35, with 3 of 7 isolates of this phage type carrying the plasmid (P < 0.01; no isolates of Vi phage type 51 were identified among the Peru isolates. All strains carrying plasmids were susceptible to antimicrobial agents tested. When cut with each of three

---

Table 1

<table>
<thead>
<tr>
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<th>21 kb</th>
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</tr>
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<td>51</td>
<td>4</td>
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<td>10 (100%)</td>
</tr>
<tr>
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<td>4</td>
<td>-</td>
<td>1</td>
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<td>-</td>
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<tr>
<td>Total</td>
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restriction endonucleases (HindIII, EcoRI, or BamHI) 21 kb plasmids identified in both Lima and Santiago had identical restriction fragments. Restriction fragments of the 57 kb plasmid identified in Santiago differed from those of the Lima 57 kb plasmid.

Chromosomal DNA was extracted from 32 S. typhi strains from Chile, 28 strains from Peru, and 3 strains from the United States. Restriction endonuclease digest profiles were identical for all strains after digestion with HindIII, EcoRI, or BamHI. DNA from 17 of these strains (9 from Chile, 7 from Peru, and 1 from the United States) was also cut with PvuII. After PvuII digestion it was possible to identify 2 slightly different restriction profiles (Fig. 1). Both profiles were pres-
ent among strains from Chile and Peru, with no apparent correlation between either of the profiles and phage type or source of the isolate; both profiles were present among isolates having the same phage type.

**DISCUSSION**

We found that less than 20% of antibiotic-sensitive *S. typhi* strains in Santiago and Lima carried plasmids, in keeping with previous studies of *S. typhi* from these and other geographic areas. The similarities in plasmid sizes among Santiago and Lima isolates prompted us to further characterize the plasmids based on restriction endonuclease digest pattern. While there were differences between the 57 kb plasmids present in the two cities, the 21 kb plasmids found in 13 of the 17 plasmid-containing strains appear to have been identical. These data emphasize the similarities among *S. typhi* strains in the two areas, and the apparent lack of diversity among plasmids not encoding resistance to antimicrobial agents. Similar observations have been made with *S. typhi* antimicrobial resistance plasmids of incompatibility group H1, with one study demonstrating that 8 resistant isolates from 4 different geographic areas either had identical plasmids, or had plasmids that were very similar based on sequence homology. Limited in vitro studies suggest that this lack of diversity, at least among resistance plasmids, is the result of plasmid instability in *S. typhi*, rather than an inherent barrier to the entry or establishment of foreign plasmid DNA.

In this study for the first time chromosomal restriction endonuclease digests of *S. typhi* strains were systematically examined. In contrast to observations made with other species, the chromosomal patterns of our isolates were almost identical. We were able to demonstrate differences between strains with only 1 of the 4 restriction enzymes used; differences that were observed were minor, with only 2 different patterns noted among the isolates studied. In contrast, each of 4 *S. paratyphi* A strains from Lima studied at the same time had a distinct digest pattern (K. O’D. Maher, personal communication). Previous investigators have noted the striking biochemical and serological similarities among *S. typhi* strains isolated in different geographic areas, and proposed that *S. typhi* strains represent a "clone" that has retained a remarkable degree of homogeneity, despite worldwide distribution of the disease; our observations support this concept.

While plasmid profiles may be of use in outbreak situations or in following transmission of a specific strain in a community (provided the strain carries a plasmid), our data make it clear that plasmid analysis cannot be a substitute for a general typing scheme such as phage typing. The association between plasmids and specific phage types is a further disadvantage from an epidemiologic viewpoint, with plasmid profiles providing little help in subdividing the major Vi phage groups such as E1. Similarly, chromosomal restriction endonuclease digests do not appear to be a useful epidemiologic tool for investigation of *S. typhi* outbreaks. However, further molecular studies, including studies of isolates from other geographic areas, may provide some insight into the observed lack of diversity among plasmids in antibiotic-sensitive *S. typhi* strains, and into the phylogeny and possible clonal origin of the organism.

**ACKNOWLEDGMENTS**

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**REFERENCES**

MOLECULAR CHARACTERIZATION OF TYPHUS

PROGRESS IN VACCINES AGAINST TYPHOID FEVER

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Running Head: New Typhoid Vaccines

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The widely available heat-phenol-inactivated whole cell typhoid vaccine, which provides approximately 65% protection, has limited usefulness because of the adverse reactions it evokes. In contrast, several new typhoid vaccines promise protection without reactogenicity. Attenuated oral vaccine Ty2la has been evaluated in three field trials of efficacy in Santiago, Chile, involving 450,000 schoolchildren. Three doses of Ty2la in an enteric-coated formulation given within one week provided 67% efficacy for at least three years. Less protection followed administration of fewer doses, while adding a fourth dose significantly enhanced protection; increasing the interval between doses did not improve protection. Large-scale vaccination with Ty2la appeared to cause a herd immunity effect. Ty2la has reached the stage of being a practical public health tool. Regarding other vaccines, the safety and immunogenicity of an auxotrophic (Aro-, Pur-) S. typhi mutant (strain 541Ty) has recently been demonstrated. Lastly, parenteral purified Vi polysaccharide of S. typhi, shown to be safe and immunogenic in young adults, is being evaluated for efficacy in controlled field trials.
INTRODUCTION

Typhoid fever remains an important public health problem in many less-developed regions of the world and poses a risk for travelers from industrialized countries who visit such endemic regions (1-4). In virtually all endemic areas the incidence rates for typhoid fever are highest in children 5-19 years of age, i.e. schoolchildren (5-9). This is of potential relevance in the control of typhoid, since schoolchildren represent a "captive" population amenable to school-based immunization programs.

Field Trials with Parenteral Killed Whole Cell Typhoid Vaccines

Parenteral killed whole cell typhoid vaccines, available since 1896 (10-12), have been used throughout this century. In the 1950s and 1960s the World Health Organization sponsored a series of large-scale field trials in several countries to assess the efficacy of various types of parenteral killed whole cell vaccines. In the first of these trials, in Yugoslavia, a fluid heat-inactivated, phenol-preserved parenteral vaccine was found to be superior in protective efficacy in comparison with an alcohol-inactivated and preserved vaccine (13-14).

Shortly after results of the above field trials in Yugoslavia became available, the Walter Reed Army Institute of Research in Washington, D.C. prepared for the World Health Organization two lyophilized reference vaccines for use in several additional field trials (15). These included a heat-phenol-inactivated and an acetone-inactivated vaccine, referred to respectively as L and K vaccines. The reference L and K vaccines were
evaluated together in randomized, controlled, double-blind trials in Yugoslavia and Guyana (16,17); in addition, the K vaccine was tested for efficacy in controlled trials in Poland and the L vaccine in the U.S.S.R. (18,19). Results of these trials are summarized in Table 1. While both reference vaccines conferred significant protection in the field trials in Yugoslavia and Guyana, the K vaccine was found to provide significantly superior protection than the L vaccine. In three separate trials, L vaccine conferred 51% (Yugoslavia), 66% (U.S.S.R.), and 67% (Guyana) protection (Table 1).

Although somewhat more efficacious, the acetone-inactivated vaccine is largely unavailable. Of the manufacturers of parenteral killed whole cell typhoid vaccines listed in the WHO's International List of Availability of Vaccines and Sera (21), 40 make the heat-phenol-inactivated variety while only three manufacture the lyophilized acetone-inactivated vaccine. Moreover, because of the high rates of adverse reaction that they elicit, parenteral killed whole cell typhoid vaccines are rarely used by any country in systematic typhoid fever control programs (with the possible exception of Thailand). A summary of the adverse reaction rates encountered in the WHO-sponsored field trials of K and L vaccines in Yugoslavia (16), Guyana (21), and the U.S.S.R. (19) are shown in Table 2.

**Oral Killed Whole Cell Vaccines**

It has been known for many decades that killed whole S. typhi can be safely given by the oral route without eliciting adverse reactions, in contrast with their reactogenicity when administered parenterally. However, in both experimental challenge studies in volunteers and in controlled field trials in endemic areas, killed whole cell vaccines given orally have provided little if any protective efficacy (22-26).
New Typhoid Vaccine Candidates

Several new candidate typhoid vaccines have emerged that offer the promise of significant protection without causing notable adverse reactions. These include two attenuated *S. typhi* strains used as live oral vaccines (strains Ty2la and 541Ty) and a purified subunit parenteral vaccine consisting of the Vi polysaccharide of *S. typhi*. The state of development of these vaccines is reviewed below.

DEVELOPMENT OF TY2LA LIVE ATTENUATED ORAL VACCINE

Volunteer Studies with Ty2la

An important advance for the potential control of typhoid fever was the development by Germanier and Furer (27) of an attenuated strain of *S. typhi*, Ty2la, that can be utilized as a live oral vaccine. In preliminary studies in adult volunteers in North America, Ty2la was found to cause no adverse reactions, to be genetically stable, and to significantly protect against experimental infection with an inoculum of pathogenic *S. typhi* that caused typhoid fever in 53% of control volunteers (28).

Egyptian Field Trial of Ty2la

Based on these highly encouraging observations in adult volunteers, Ty2la vaccine was evaluated for efficacy by Wahdan et al (29,30) in a placebo-controlled, randomized, double-blind trial in Alexandria, Egypt. In this trial, three doses of Ty2la vaccine (1-3 x 10⁶ viable vaccine organisms per dose) or placebo were given to schoolchildren on Monday, Wednesday, and Friday of one week. Prior to ingestion of vaccine or placebo, children chewed a tablet containing 1.0 gm of NaHCO₃ (to neutralize gastric acid). Each dose of lyophilized vaccine or placebo was contained within glass vials in vacuo. The vials were opened, the lyophilate reconstituted in the field with diluent, and the liquid vaccine
(or placebo) suspension given to the child a few minutes after the child ingested the NaHCO₃ tablet. Passive surveillance failed to identify notable adverse reactions in the Egyptian schoolchildren, corroborating the safety of the live oral vaccine.

During the 36 month period of surveillance in Alexandria, the vaccine efficacy was 96% (Table 3) (29).

**Field Trials of Ty2la in Santiago, Chile**

**Rationale**

Shortly after the Egyptian field trial established the biological safety and efficacy of Ty2la in schoolage children in an endemic area, the Swiss Serum and Vaccine Institute made a formulation of vaccine commercially available which consisted of two gelatin capsules each containing 0.4 gm of NaHCO₃ and a third gelatin capsule containing lyophilized vaccine. Although this formulation resembled that used in Alexandria, Egypt, it was clearly not identical. Despite the highly encouraging results in the first field trial in Egypt, it was obvious that additional information had to be obtained before the Ty2la live oral vaccine could be employed as a practical public health tool. Some of the critical questions yet to be answered included:

1) What was the efficacy of Ty2la when administered in a formulation such as enteric-coated capsules that does not require pretreatment with NaHCO₃?

2) Could fewer doses (one or two) than used in Alexandria provide a satisfactory level of protection?

3) What level of protection would Ty2la provide in areas with incidence rates of typhoid fever much higher than the 44-50 cases/10⁵/year that prevailed during the trial in Alexandria?
4) What was the efficacy of the commercial formulation consisting of gelatin capsules containing NaHCO₃ and lyophilized vaccine that was marketed after the Egyptian field trial?

5) Could prolongation of the interval between the doses enhance the immunogenicity of the vaccine?

6) Could an immunologic assay be identified that would correlate with levels of vaccine efficacy in field trials and could therefore be used to predict the effect of changes in formulation and immunization schedules?

In order to answer these questions, four separate field trials of efficacy were carried out in Santiago, Chile. These trials represent a collaborative effort involving the Ministry of Health, Santiago, Chile, the Center for Vaccine Development of the University of Maryland School of Medicine, the Pan American Health Organization, the World Health Organization, the Swiss Serum and Vaccine Institute, and the Walter Reed Army Institute of Research.

Field Trial Designs

The first two field trials were placebo-controlled and were initiated in the Northern (Area Norte) and Western (Area Occidente) administrative areas of Santiago in 1982 and 1983, respectively. The third field trial was begun in the Southern (Area Sur) and Central (Area Central) administrative areas of Santiago in 1984. Santiago, Chile was selected as the site for these field trials because of the combination of high endemicity of typhoid fever (the annual incidence rate from 1977 to 1981 exceeded 150 cases per 10⁵ population) (31), the presence of a renowned health care infrastructure (the National Health Service), a strong commitment on the part of the Ministry of Health towards innovative methods to control typhoid fever, and a long history of school-based
vaccination programs.

Only children of consenting parents entered the studies and were randomized to the various cells of the trials. Remaining children of non-consenting parents were also kept under surveillance and served as unvaccinated controls.

Since typhoid fever exhibits a marked seasonality (November to April) in conjunction with summer in Santiago (31), the vaccinations were limited to the cool months of the year (May to October). Computerized data files were generated from the completed class lists.

Only bacteriologically-confirmed cases (i.e. those from whom S. typhi was isolated from blood, bone marrow, or bile-stained duodenal fluid) were utilized in computations of vaccine efficacy. Therefore considerable resources were directed toward bacteriologic confirmation of suspect cases. Children admitted to hospital with a clinical suspicion of typhoid fever had three 4 ml blood cultures and one bone marrow culture obtained (32), while those presenting to the consultorios (health centers) as outpatients with suspect typhoid fever had two 6 ml blood cultures drawn 30 minutes apart.

Chronologically, the Area Norte field trial preceded the Area Occidente field trial. However, for purposes of clarity of presentation, the sequence of presentation of data will be Area Occidente, followed by Area Norte, and finally Area Sur and Central.

**Area Occidente Field Trial**

Parents of 96% of the 141,127 children in Area Occidente consented for their children to participate. These were thereupon randomized to one of five groups to receive:
Group 1 - Three doses of vaccine in enteric-coated capsules given with an interval of two days between the doses.

Group 2 - Three doses of vaccine with NaHCO₃ given with an interval of two days between the doses. The commercial gelatin capsule formulation was used which consisted of two gelatin capsules each containing 0.5 gm of NaHCO₃ and a third gelatin capsule containing lyophilized vaccine.

Group 3 - Three doses of vaccine in enteric-coated capsules with an interval of 21 days between the doses.

Group 4 - Three doses of the commercial gelatin capsule formulation with an interval of 21 days between the doses.

Group 5 - Three doses of placebo given with an interval of two days between the doses.

Mass administration of vaccine (containing 1-3 x 10⁹ viable vaccine organisms per dose) or placebo was carried out between mid July and mid September, 1983 and surveillance began on September 21, 1983. In total, 109,594 children received all three scheduled doses of vaccine or placebo.

Results of three years of surveillance in the Area Occidente field trial are shown in Tables 4 and 5. The main points are:

1) The enteric-coated formulation was very significantly superior to the gelatin capsule/NaHCO₃ formulation (Table 4).

2) Increasing the interval between doses to 21 days offered no advantage to administering all three doses within one week (Table 4).

3) The level of protection (67% vaccine efficacy) conferred by the best regimen in the Occidente field trial (three doses of enteric-coated capsules given within one week) persisted for at least three years of surveillance (Table 5).

Surveillance is being maintained in Area Occidente to determine if the
efficacy of Ty2la can endure for more than three years. This information is critical for public health authorities to design typhoid control programs based on the systematic use of Ty2la.

**Area Norte Field Trial**

Parents of 92,356 of the 137,697 schoolchildren in Area Norte consented for their children to participate and they were randomized to one of three groups to receive:

1) Two doses of Ty2la vaccine in enteric-coated capsules (1-3 x 10\(^9\) organisms per dose).
2) One dose of vaccine and one dose of identical appearing placebo.
3) Two doses of placebo.

The two doses of vaccine or placebo were given to the children one week apart in May and June, 1982 and surveillance began on July 1, 1982.

Results of the Area Norte field trial are shown in Table 6. The main points include:

1) Two doses of enteric-coated vaccine provided moderate (48-72%) protection for a period of two years. However, the efficacy then dropped to 21% in the third season and was non-existent by the fourth season of surveillance.

2) A single dose of vaccine in enteric-coated capsules provided low levels of protection (16-39%) for two years but by the third year of surveillance no further efficacy was demonstrable.

These data demonstrate that, when administered in enteric-coated capsules, Ty2la provides insufficient levels of protection when given as only one or two doses.

**Area Sur and Area Central Field Trials**

A third field trial was undertaken in 1984 in Areas Sur and Central
where 247,561 children were randomized to receive either two, three or four doses of Ty21a vaccine (1-3 x 10^9 viable vaccine organisms per dose) in enteric-coated capsules with all doses of vaccine being administered within a period of eight days in September and October, 1984. No placebo control group was included in this trial in which surveillance began on November 1, 1984.

Results of surveillance of typhoid fever through two seasons are shown in Table 7. In this trial the incidence of typhoid fever in recipients of three doses of Ty21a in enteric-coated capsules was only slightly lower than the incidence in children who received two doses of vaccine. In contrast, the incidence of typhoid fever in recipients of four doses of vaccine was very significantly lower than the rates in children who received two or three doses.

**Area Sur Oriente Trial**

In October, 1986, a fourth field trial was initiated in the Area Sur Oriente and Area Norte administrative areas where children received within one week three doses of Ty21a or placebo in either enteric-coated capsules or in a liquid formulation. Results of this trial (available in 1988) should answer the question of whether a liquid formulation of Ty21a, similar to what was used in Egypt, is inherently superior to enteric-coated capsules. This trial will also provide information on the absolute efficacy conferred by each formulation of vaccine.

A field trial similar in design to the above, using the identical liquid and enteric-coated capsule formulations of Ty21a, is concomitantly being carried out in Plaju, Indonesia, under the auspices of the Indonesian National Institute of Health and Ministry of Health with collaboration of the U.S. Naval Medical Research Unit, Djakarta, the World
Health Organization, and the Swiss Serum and Vaccine Institute.

Epidemiologic Evidence for a "Herd Immunity" Effect Consequent to the Broad Application of Ty2la Vaccine

Analysis of the incidence rate of typhoid fever in the placebo control group in the first field trial of Ty2la in Area Norte, Santiago provides some fascinating insights on what might be expected from the systematic wide-scale application of Ty2la live oral vaccine in typhoid fever control programs. As seen in Table 6, the incidence rate in the randomized control group in the first year of surveillance was 210 cases/10^5 schoolchildren. This rate of culture-confirmed cases is similar to the reported rate for schoolchildren in Area Norte in the period 1977-1981, prior to the field trial; however, at that time cases were not bacteriologically confirmed.

Surveillance of the second typhoid season in Area Norte took place after most of the children in adjacent Area Occidente had been given vaccine as part of the second field trial of Ty2la. The incidence rate in the placebo control group in Area Norte in this second year of surveillance fell to 141 cases/10^5 (Table 6).

Shortly before the third typhoid season of surveillance began in Area Norte, more than 247,000 children in Areas Sur and Central were given two, three or four doses of vaccine. In this third year of surveillance the incidence in the placebo group in Area Norte fell even further to 69 cases/10^5 (Table 6). A rate this low had not been encountered in Area Norte for decades.

The fourth year of surveillance in the Area Norte field area occurred during a year when no further trials were carried out in Santiago. Notably, in that fourth year the incidence of typhoid fever in the placebo
control group did not fall further. Rather, the incidence, 78 cases/10^5, closely resembled that of the previous year (Table 6).

In the course of the first three field trials in Santiago, approximately 65% of the schoolchildren in the city have participated, many having received an efficacious formulation and number of doses of vaccine. Thus, an interpretation of the sharp decrease in incidence rates in the placebo control group in the Area Norte trial is that this is the consequence of the mass application of Ty2la vaccine in schoolchildren.

Correlation of IgG ELISA S. typhi 0 Antibody with Efficacy in Field Trials

Serologic studies have been carried out in healthy Chileans, age 17-21 years, who received Ty2la in one of two formulations and in various immunization schedules. Serum IgG and IgA antibodies to S. typhi 0 antigen have been measured before and after vaccination by an ELISA that has been described in detail (32). Now that results of the field trials are available, it has become possible to relate seroconversion rates to vaccine efficacy; these comparisons are summarized in Table 8. It is obvious that there exists a positive correlation between the seroconversion rate of IgG S. typhi 0 antibody and vaccine efficacy in the field.

Ty2la Vaccine in Perspective

The great advantage of Ty2la live oral typhoid vaccine, in comparison with parenteral killed whole cell vaccines, is that it provides significant protection without causing adverse reactions (34). A wealth of evidence from volunteer studies (28) and from some of the largest vaccine field trials ever carried out attest to the biological activity of
this attenuated strain in providing protection against typhoid fever. Considerable resources have been expended in attempts to identify an effective and practical formulation and dosage schedule for Ty21a. After a series of field trials in Egypt and Chile, information has now been accrued demonstrating both the advantages as well as the limitations of Ty21a.

Field trials in Chile have shown that Ty21a in enteric-coated capsules is significantly more protective than vaccine administered in the gelatin capsule/NaHCO₃ formulation. These results corroborate a retrospective study reporting poor efficacy for the gelatin capsule/NaHCO₃ formulation (35) which prior to the Chilean trial had not been field tested. Following results of the Chilean field trials, production of the gelatin capsule/NaHCO₃ formulation was discontinued and replaced commercially by the enteric-coated capsule formulation.

In the Chilean trials, three doses of an enteric-coated formulation of Ty21a given within one week provided 67% protection for at least three years (Table 5). This level of vaccine efficacy is equal to the protection conferred by the highly reactogenic liquid heat-phenol-inactivated parenteral vaccine, the only other widely available effective vaccine (16,17,19, Table 1). The phenol-inactivated vaccine, which causes notable adverse reactions in approximately 25% of recipients, must be administered by needle and syringe or jet gun. Thus, Ty21a is distinctly more advantageous because it causes no discernible adverse reactions and is easy to administer to schoolchildren in mass vaccinations (34). In our estimation, this clearly makes Ty21a at present the vaccine of choice for any country intending to embark on a systematic typhoid fever control program.
The 67% protection conferred for at least three years by three doses of enteric-coated capsules given within one week in Area Occidente in Santiago, Chile is less than the impressive 96% efficacy over three years provided by a liquid formulation in Alexandria, Egypt. Besides the obvious differences in vaccine formulation and genetic constitution of the populations, other factors may have contributed to the difference in results. For example, the mean annual incidence rate in the placebo control group in the Occidente trial (103/10^5/year) was twice as high as the mean annual incidence rate in the placebo group in the Alexandria trial (46/10^5), suggesting that force of infection and modes of transmission may differ between the two sites. A fourth field trial currently underway in Chile, and a trial of similar design in Indonesia, will directly answer the question of the relative efficacy of enteric coated capsules versus a liquid formulation.

Widespread vaccination with Ty21a apparently created a "herd immunity" effect in which the incidence increasingly dropped in the control group in the first field trial area as children in other areas of the city were vaccinated. These observations support the contention that Ty21a live oral vaccine, while not the ideal anti-typhoid vaccine, is nevertheless a credible weapon to be employed in systematic typhoid fever control programs. Since man is the only reservoir, as well as the only natural host, of this infection, this approach is epidemiologically rational.

The multiple field trials of efficacy of Ty21a that have been required so far to generate the information necessary to determine how to use this vaccine as a public health tool are reminiscent of the series of field trials undertaken by WHO over a period of more than 15 years to accrue similar information for the parenteral killed whole cell vaccines. Until
a superior formulation of Ty2la is identified, or Ty2la is surpassed by
another typhoid vaccine, the information now available should allow public
health authorities to utilize Ty2la in enteric-coated capsule formulation
as a tool in national typhoid fever control programs.

DEVELOPMENT OF ADNUTROPHIC ARO-, FUR- MUTANTS OF S. TYPHI AS LIVE ORAL
VACCINES

Vaccine strain 541Ty was derived by Stocker and coworkers (36) from a
wild strain of S. typhi by transducing deletions in two separate genes,
each previously characterized in S. typhimurium and affecting a different
pathway such that the mutations cause requirements for metabolites that
are unavailable in mammalian tissues and intercellular fluid. The
deletion mutation of gene aroA creates a requirement for several aromatic
compounds, including p-aminobenzoic acid and 2,4-dihydroxybenzoic acid,
which are not mammalian metabolites. The second deletion mutation, at
gene purA, causes a specific requirement for adenine (or an assimilable
compound such as adenosine) (37). These nutritional requirements render
strain 541Ty unable to sustain growth in mammalian tissues. Strain 543Ty
is a derivative of 541Ty that lacks the Vi antigen.

Strain 541Ty or 543Ty were administered orally with NaHCO₃ to 33
healthy young adult volunteers in single doses of 10⁸, 10⁹, or 10¹⁰
organisms, while four additional vaccinees ingested two 2 x 10⁹ organism
doses four days apart, in preliminary evaluations of the safety and
immunogenicity of the vaccine strains (33). No notable adverse reactions
such as fever, diarrhea, vomiting, or abdominal discomfort were observed
during 15 days of surveillance in a Research Ward or for two weeks
thereafter. Vaccine organisms were recovered from coprocultures of 29 of
37 vaccinees (78%) and from duodenal cultures of two individuals; in
contrast, repeated blood cultures were negative. The humoral antibody
response to S. typhi O and H antigens in serum and intestinal fluid was
meager; no vaccinees had rises in serum antibody to S. typhi Vi or lysate
antigen. However, all vaccinees manifested cell-mediated immune
responses. After vaccination, 72% of recipients of doses of $>10^9$
vaccine organisms responded to S. typhi particulate or purified O
polysaccharide antigens in lymphocyte replication studies but not to
antigens of other Salmonella or Escherichia coli. All individuals, after
vaccination, demonstrated a significant plasma-dependent mononuclear cell
inhibition of wild S. typhi. These preliminary results suggest that Aro-
auxotrophic mutants of S. typhi are safe and immunogenic oral vaccines
in man and are worthy of expanded clinical trials. The possible advantage of
strain 541Ty, should it prove to be protective, is that its method of
preparation involves the creation of precise deletion mutations in
specific genes that do not otherwise affect the antigenic make-up of the
S. typhi.

ATTENUATED S. TYPHI VACCINE STRAINS EXPRESSING GENES OF OTHER ORGANISMS

Because they are so well-tolerated and stimulate both humoral and
cell-mediated immune responses (33, 38-41), attenuated S. typhi oral
vaccines are attractive as carriers of critical genes of other organisms.
The expression of foreign genes in strains such as Ty2la results in
bivalent vaccines. Ty2la, for example, has been modified to express
Shigella sonnei O antigen (42), the B subunit of E. coli heat-labile
enterotoxin (43), colonization factor antigen I (44), and Vibrio cholerae
antigens (45). In each of these instances Ty2la contains a plasmid
encoding the relevant antigen of another enteropathogen. The bivalent
typhi/S. sonnei vaccine has undergone extensive clinical testing in humans
and is safe, immunogenic and protective (although lot-to-lot variation has been described) (46,47). Ty2la expressing \textit{V. cholerae} Inaba O antigen has been found to be well-tolerated and to stimulate circulating and local intestinal antibodies to both typhi and \textit{V. cholerae} O antigens (48).

**DEVELOPMENT OF HIGHLY PURIFIED VI POLYSACCHARIDE AS A PARENTERAL TYPHOID VACCINE**

The Vi polysaccharide of \textit{S. typhi} is a homopolymer of alpha-1,4 2-deoxy-2-N-acetyl galacturonic acid that covers the bacteria as a capsular antigen and is a known virulence property. The Vi antibody response following acute illness is usually modest, detectable in only a minority of patients, and short-lived, except in chronic biliary carriers of \textit{S. typhi} who maintain very elevated levels of Vi antibody (49,50). Historically, several investigators have proposed that protection against typhoid fever may be feasible if high titers of Vi antibody can be elicited. It is known, however, that highly significant protection against \textit{S. typhi} can be exhibited in the absence of Vi antibody, since Ty2la lacks Vi antigen and does not stimulate Vi antibody.

In the early 1950s, Landy (51) prepared purified Vi polysaccharide from acetone-inactivated bacteria by multiple extractions with saline, ethanol, and acetic acid. This early method may have partially denatured the antigen, resulting in a loss of O-acetyl and N-acetyl moieties (52,53). Landy's Vi preparation was immunogenic (51,54) but did not provide significant protection to volunteers in a small experimental challenge study carried out by Hornick et al (55) in the 1960s.

In attempts to purify Vi under non-denaturing conditions, Wong et al (56) and Robbins and Robbins (53), treated \textit{S. typhi} with hexadecyltrimethylammonium bromide, a detergent that was previously
instrumental in the preparation of purified meningococcal polysaccharide vaccines (57). Two separate lots of Vi vaccine prepared by this procedure, one made at the National Institutes of Health in the U.S.A. (Lot 53226) and the other made at the Merieux Institute, Lyon, France (Lot IMS 1569) were evaluated for safety and immunogenicity (58). The NIH vaccine contained approximately 5% residual lipopolysaccharide (LPS), while the French vaccine had only 0.2% LPS. Both vaccines elicited significant rises in Vi antibody in circa 90% of recipients but the NIH preparation caused some systemic and local adverse reactions. The occurrence of significant rises in O antibody in 83% of recipients of the NIH vaccine, suggest that residual LPS was responsible for the untoward reactions.

Vi vaccine prepared by the Merieux Institute is currently being evaluated in controlled field trials of efficacy in Nepal and South Africa. Preliminary results from these trials should be available in 1987 (personal communications, J.B. Robbins and H. Koornhoff).
REFERENCES


Table 1. Results of controlled field trials of lyophilized acetone-inactivated and heat phenol-inactivated reference vaccines

<table>
<thead>
<tr>
<th>Field</th>
<th>Age</th>
<th>Vaccine</th>
<th>No. of Vaccinated</th>
<th>Duration of Surveillance</th>
<th>Incidence of typhoid per 105 Vaccine Efficiency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yugoslavia, 1960-63</td>
<td>2-50 yrs.</td>
<td>K (2)</td>
<td>5028</td>
<td>2 1/2 yrs.</td>
<td>318&lt;sup&gt;b&lt;/sup&gt;</td>
<td>79%</td>
</tr>
<tr>
<td></td>
<td>Mostly</td>
<td>L (2)</td>
<td>5068</td>
<td>2 1/2 yrs.</td>
<td>72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>51%</td>
</tr>
<tr>
<td></td>
<td>Schoolchildren</td>
<td>Control (2)</td>
<td>5039</td>
<td>2 1/2 yrs.</td>
<td>1480&lt;sup&gt;d&lt;/sup&gt;</td>
<td>---</td>
</tr>
<tr>
<td>Guyana, 1960-67</td>
<td>5-15 yrs.</td>
<td>K (2)</td>
<td>24,046</td>
<td>7 yrs.</td>
<td>71&lt;sup&gt;d&lt;/sup&gt;</td>
<td>88%</td>
</tr>
<tr>
<td>(Schoolchildren)</td>
<td>L (2)</td>
<td></td>
<td>24,241</td>
<td>7 yrs.</td>
<td>159&lt;sup&gt;e&lt;/sup&gt;</td>
<td>87%</td>
</tr>
<tr>
<td></td>
<td>Control (2)</td>
<td></td>
<td>27,756</td>
<td>7 yrs.</td>
<td>605&lt;sup&gt;f&lt;/sup&gt;</td>
<td>---</td>
</tr>
<tr>
<td>Poland, 1961-64</td>
<td>5-14 yrs.</td>
<td>K (2)</td>
<td>81,534</td>
<td>3 yrs.</td>
<td>78&lt;sup&gt;g&lt;/sup&gt;</td>
<td>88%</td>
</tr>
<tr>
<td>(Schoolchildren)</td>
<td>Control (2)</td>
<td></td>
<td>83,734</td>
<td>3 yrs.</td>
<td>47&lt;sup&gt;h&lt;/sup&gt;</td>
<td>---</td>
</tr>
<tr>
<td>USSR, 1962-65</td>
<td>Schoolchildren</td>
<td>L (2)</td>
<td>36,112</td>
<td>2 1/2 yrs.</td>
<td>55&lt;sup&gt;i&lt;/sup&gt;</td>
<td>66%</td>
</tr>
<tr>
<td>and young adults</td>
<td>Control (2)</td>
<td></td>
<td>36,999</td>
<td>2 1/2 yrs.</td>
<td>62&lt;sup&gt;j&lt;/sup&gt;</td>
<td>---</td>
</tr>
<tr>
<td>(92% age 7-15 yrs.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> vs <sup>c</sup>, p = 0.00001  
<sup>b</sup> vs <sup>a</sup>, p = 0.0064  
<sup>c</sup> vs <sup>f</sup>, p < 0.000005  
<sup>d</sup> vs <sup>f</sup>, p < 0.000001  
<sup>e</sup> vs <sup>e</sup>, p = 0.000046  
<sup>g</sup> vs <sup>h</sup>, p = 0.0000025  
<sup>b</sup> vs <sup>c</sup>, p < 0.00004  
<sup>d</sup> vs <sup>f</sup>, p < 0.0000001  
<sup>f</sup> vs <sup>e</sup>, p = 0.0000046  
<sup>i</sup> vs <sup>j</sup>, p = 0.000021, all comparisons by Chi square.
Table 2. The frequency of fever, malaise and pain at the injection site approximately 24 hours following subcutaneous inoculation with heat-phenol-inactivated (vaccine L) or acetone-inactivated (vaccine K) whole cell typhoid vaccines or tetanus toxoid

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>No. of Vaccinees</th>
<th>Fever after Vaccination (%)</th>
<th>Inability to Work (%)</th>
<th>Local Pain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yugoslavia</td>
<td>Guyana</td>
<td>USSR</td>
<td>Yugoslavia</td>
</tr>
<tr>
<td>Heat-phenol-inactivated</td>
<td>345</td>
<td>86</td>
<td>1656</td>
<td>24</td>
</tr>
<tr>
<td>Acetone-inactivated</td>
<td>326</td>
<td>80</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>Tetanus toxoid</td>
<td>328</td>
<td>86</td>
<td>1757</td>
<td>3</td>
</tr>
</tbody>
</table>

* > 37°C
+ > 37.3°C
++ > 37.5°C

Data summarized from references 16, 19 and 21
Table 3. Field trial of efficacy of three doses of a liquid formulation of Ty21a vaccine given with NaHCO₃ to six and seven year old schoolchildren in Alexandria, Egypt. Results of three years of surveillance.

<table>
<thead>
<tr>
<th>Year of observation</th>
<th>Confirmed cases of typhoid fever</th>
<th>Annual incidence per 10⁷</th>
<th>Vaccine efficacy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1978-1979</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vaccinees*</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>placebo</td>
<td>7</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>1979-1980</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vaccinees</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>placebo</td>
<td>8</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>1980-1981</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vaccinees</td>
<td>1</td>
<td>6</td>
<td>86</td>
</tr>
<tr>
<td>placebo</td>
<td>7</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Total 1978-1981</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vaccinees</td>
<td>1</td>
<td>-</td>
<td>96</td>
</tr>
<tr>
<td>placebo</td>
<td>22</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Data from reference 29
* n = 16, 486
+ n = 15, 502
Table 4. Comparison of the efficacy of two different formulations of Ty21a vaccine administered in two different immunization schedules in Area Occidente, Santiago, Chile. Results of 36 months of surveillance, 9/83 - 8/86

<table>
<thead>
<tr>
<th></th>
<th>Enteric-Coated Capsules</th>
<th>Gelatin Capsules with NaHCO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Long</td>
<td>Short</td>
</tr>
<tr>
<td>Interval</td>
<td>Interval*</td>
<td></td>
</tr>
<tr>
<td>(22,170)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>34</td>
<td>23</td>
</tr>
<tr>
<td>Incidence/10⁵</td>
<td>157.4ᵃ</td>
<td>103.7ᵇ</td>
</tr>
<tr>
<td>Efficacy</td>
<td>49.3</td>
<td>66.6</td>
</tr>
</tbody>
</table>

* 3 doses, 21 days between doses
+ 3 doses, 1-2 days between doses

a vs e, p = 0.0006   a vs c, p = 0.23
b vs e, p < 0.00001  b vs d, p = 0.00052
c vs e, p = 0.023    a + b vs c + d, p = 0.001
d vs e, p = 0.21

All statistical comparisons by Chi square
Table 5. Duration of the efficacy conferred by three doses of the enteric-coated capsule formulation of Ty21a live oral vaccine given within one week in Area Occidente, Santiago, Chile

<table>
<thead>
<tr>
<th>Year 1 (9/83-8/84)</th>
<th>Vaccine* (22,170)</th>
<th>Placebo* (21,906)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>Incidence/10^5</td>
<td>31.6</td>
<td>109.6</td>
</tr>
<tr>
<td>Efficacy</td>
<td>71.2</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year 2 (9/84-8/85)</th>
<th>Vaccine* (22,170)</th>
<th>Placebo* (21,906)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>8</td>
<td>20</td>
</tr>
<tr>
<td>Incidence/10^5</td>
<td>36.1</td>
<td>91.3</td>
</tr>
<tr>
<td>Efficacy</td>
<td>60.5</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year 3 (9/85-8/86)</th>
<th>Vaccine* (22,170)</th>
<th>Placebo* (21,906)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>8</td>
<td>24</td>
</tr>
<tr>
<td>Incidence/10^5</td>
<td>36.1</td>
<td>109.6</td>
</tr>
<tr>
<td>Efficacy</td>
<td>67.1</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Years 1-3 (9/83-8/86)</th>
<th>Vaccine* (22,170)</th>
<th>Placebo* (21,906)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>23</td>
<td>68</td>
</tr>
<tr>
<td>Incidence</td>
<td>103.7^a</td>
<td>310.4^b</td>
</tr>
<tr>
<td>Efficacy</td>
<td>66.5</td>
<td>-</td>
</tr>
</tbody>
</table>

* 3 doses, 1-2 days between doses

a vs b, p < 0.00001, Chi square
Table 6. Comparison of the efficacy of one versus two doses of Ty2la live oral typhoid vaccine given in enteric-coated capsule formulation.

Randomized, controlled, double-blind trial in Area Norte, Santiago, Chile

<table>
<thead>
<tr>
<th>Year</th>
<th>One Dose (32,788)</th>
<th>Two Doses (27,620)</th>
<th>Placebo (31,948)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Year 1</strong> (7/82-6/83)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>58</td>
<td>30</td>
<td>67</td>
</tr>
<tr>
<td>Incidence/10^5</td>
<td>176.9^a</td>
<td>108.6^b</td>
<td>209.7^c</td>
</tr>
<tr>
<td>Efficacy</td>
<td>15.6%</td>
<td>48.2%</td>
<td>-</td>
</tr>
<tr>
<td><strong>Year 2</strong> (7/83-6/84)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>28</td>
<td>11</td>
<td>45</td>
</tr>
<tr>
<td>Incidence/10^5</td>
<td>85.4</td>
<td>39.8</td>
<td>140.8</td>
</tr>
<tr>
<td>Efficacy</td>
<td>39.3%</td>
<td>71.7%</td>
<td>-</td>
</tr>
<tr>
<td><strong>Year 3</strong> (7/84-6/85)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>23</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>Incidence/10^5</td>
<td>70.1</td>
<td>54.3</td>
<td>68.9</td>
</tr>
<tr>
<td>Efficacy</td>
<td>0%</td>
<td>21.2%</td>
<td>-</td>
</tr>
<tr>
<td><strong>Year 4</strong> (7/85-6/86)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>33</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>Incidence/10^5</td>
<td>100.6</td>
<td>79.6</td>
<td>78.3</td>
</tr>
<tr>
<td>Efficacy</td>
<td>0%</td>
<td>0%</td>
<td>-</td>
</tr>
</tbody>
</table>

a vs c, p = 0.42
a vs b, p = 0.037
b vs c, p = 0.0032
Comparisons by Chi square
Table 7. Comparison of the efficacy of two, three, and four doses of Ty2la vaccine in enteric-coated capsule formulation. Results of a randomized field trial in Area Sur and Area Central, Santiago, Chile.

<table>
<thead>
<tr>
<th>Surveillance from</th>
<th>Two Doses*</th>
<th>Three Doses*</th>
<th>Four Doses*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10/84 to 9/86</td>
<td>93,942</td>
<td>95,196</td>
<td>58,421</td>
</tr>
<tr>
<td>No. of Vaccinees</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases</td>
<td>126</td>
<td>117</td>
<td>34</td>
</tr>
<tr>
<td>Incidence/10^5</td>
<td>134.1^a</td>
<td>122.9^b</td>
<td>58.2^c</td>
</tr>
</tbody>
</table>

* Vaccine given within eight days with 1-2 days between doses

a vs c, p < 0.0001
b vs c, p < 0.0002
a vs b, p = 0.49
All comparisons by Chi square
Table 8. Rates of seroconversion of IgG-ELISA S. TYPH 0 antibody following one to three oral doses of Ty21a live oral typhoid vaccine given within one week. Comparison of two different formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>No. Doses</th>
<th>Seroconversion Rate (%)</th>
<th>Vaccine Efficacy in Controlled Field Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteric-coated capsules</td>
<td>3</td>
<td>61/96 (64)</td>
<td>67%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22/50 (44)</td>
<td>47%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>9/50 (18)</td>
<td>21%</td>
</tr>
<tr>
<td>Vaccine + NaHCO₃ in gelatin capsules</td>
<td>3</td>
<td>99/195 (50)</td>
<td>19%</td>
</tr>
</tbody>
</table>

*Data from first 36 months of surveillance in field trials in Area Norte and Area Occidente, Santiago, Chile.*
LARGE-SCALE FIELD TRIAL OF TY21A LIVE ORAL TYPHOID VACCINE IN ENTERIC-COATED CAPSULE FORMULATION

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ABSTRACT

Three doses, given within one week, of Ty2la attenuated Salmonella typhi oral vaccine in an enteric-coated capsule formulation provided 67% efficacy for at least three years in a randomized, placebo-controlled field trial involving 109,000 schoolchildren in Santiago, Chile. Increasing the interval between doses to 21 days did not enhance protection. Significantly less protection followed administration of vaccine in gelatin capsules with NaBD₄. Ty2la provides the same level of protection as the heat-phenol-inactivated whole cell parenteral vaccine but in contrast does not cause adverse reactions. Ty2la may now be considered a practical public health tool.
Typhoid fever remains an important public health problem in many less-developed regions of the world and poses a risk for travelers. In most endemic areas the incidence of typhoid fever is highest in children 5-19 years of age. This is of potential relevance in the control of typhoid, since schoolchildren represent a "captive" population amenable to school-based immunization programs.

Although heat-phenol-inactivated and acetone-inactivated parenteral killed whole cell typhoid vaccines confer significant protection, they are rarely used by any country in systematic typhoid fever control programs because of the high rates of adverse reaction that they elicit.

An important advance in immunization against typhoid fever was the development by Germanier and Purser of an attenuated strain of S. typhi, Ty21a, that can be utilized as a live oral vaccine. In preliminary studies in adult volunteers in North America, Ty21a caused no adverse reactions, was genetically stable, and significantly protected against experimental infection.

Wahdan et al carried out a placebo-controlled, randomized, double-blind trial of Ty21a in Alexandria, Egypt where three doses of vaccine (1-3 x 10^9 viable vaccine organisms per dose) or placebo were given to 32,000 schoolchildren on Monday, Wednesday, and Friday of one week. Prior to ingestion of liquid (reconstituted lyophilate) vaccine or placebo, each child chewed a 1.0 gm NaHCO₃ tablet to neutralize gastric acid. Notable adverse reactions were not detected, corroborating the safety of the live oral vaccine. During 36 months of surveillance the vaccine efficacy was 96%.18
Shortly after the Egyptian field trial, the Swiss Serum and Vaccine Institute made available commercially a formulation of vaccine consisting of two gelatin capsules each containing \( \text{NaHCO}_3 \) and a third gelatin capsule containing lyophilized vaccine. This first commercial formulation resembled, but was not identical to, that used in Alexandria.

Despite the highly encouraging results in the field trial in Egypt, additional information had to be obtained before Ty21a could be employed as a practical public health tool. Several critical questions had yet to be answered. What would be the efficacy of the commercial gelatin capsule/\( \text{NaHCO}_3 \) formulation of lyophilized vaccine that was marketed after the Egyptian field trial? What would be the efficacy of Ty21a in a formulation, such as enteric-coated capsules, that does not require \( \text{NaHCO}_3 \)? What level of protection would Ty21a provide in areas with incidence rates of typhoid fever much higher than the 44-50 cases/10^5/year that prevailed during the trial in Alexandria? Could prolongation of the interval between the doses enhance the immunogenicity of the vaccine?

To answer these questions, a randomized, placebo-controlled field trial of efficacy was carried out in Santiago, Chile. This trial represented a collaborative effort involving the Ministry of Health, Santiago, Chile, the Center for Vaccine Development of the University of Maryland School of Medicine, the Pan American Health Organization, the World Health Organization (WHO), the Walter Reed Army Institute of Research and the Swiss Serum and Vaccine Institute.

**MATERIALS AND METHODS**

Santiago was selected for the field trial because of the high endemicity of typhoid fever (the annual incidence rate from 1977 to 1981
exceeded 150 cases per $10^5$ population), the presence of an excellent health care infrastructure (the System of National Health Services), a strong commitment on the part of the Ministry of Health towards innovative methods to control typhoid fever, and a long history of school-based vaccination programs. The trial design and consent procedures were approved by ethical review committees of the University of Maryland and WHO. The field trial was initiated in Area Occidente of Santiago in 1983. The Ministries of Health and Education collaborated to ensure that, with cooperation of the teachers in all schools, parents were informed of the trial (by means of health education brochures) and permission to enroll their child was requested through consent forms and their response recorded.

Peak transmission of typhoid fever and the vast majority of cases occur during the summer (school holiday) season in Santiago (mid-December to mid-March) while schools are not in session. Therefore, randomization was carried out by classroom (i.e. all children in a class received the same vaccine regimen).

Only children of consenting parents were randomized to one of the five cells of the trial. Group 1 received three doses of vaccine with NaHCO$_3$ given with an interval of two days between the doses. The commercial gelatin capsule formulation was used which consisted of two gelatin capsules each containing 0.5 gm of NaHCO$_3$ and a third gelatin capsule containing lyophilized vaccine. Group 2 ingested three doses of lyophilized vaccine in enteric-coated capsules, with an interval of two days between the doses. Hydroxypropyl-methyl-cellulose-phthalate was the enteric-coating used to make the gelatin capsules acid-resistant. In vitro the capsules resisted gastric acid (pH 1.5) for at least two hours.
but dissolved within 10 minutes in artificial intestinal fluid of pH > 6.0.

Group 3 received three doses of vaccine in enteric-coated capsules with an interval of 21 days between the doses. Group 4 ingested three doses of the commercial gelatin capsule/NaHCO₃ formulation with an interval of 21 days between the doses, while Group 5 received three doses of placebo (in identical capsules as described above) given at an interval of two days between the doses. The identity of which coded preparation contained placebo was unknown to the vaccinators, the schoolchildren and the health care providers.

The administration of vaccine (containing 1-3 x 10⁹ viable vaccine organisms per dose) or placebo by trained health workers was carried out in the classrooms in the cool, non-typhoid season, mid-July to early September, 1983; surveillance began on September 21, 1983. Computerized data files were generated from the completed class lists.

Approximately 90% of health care visits in Area Occidente occur in facilities of the System of National Health Services where intensive surveillance could be maintained; the remaining visits involve private physicians. Physicians and nurses were kept aware of the importance of obtaining cultures from suspect cases of typhoid fever by means of letters, clinical conferences and weekly visits by surveillance nurses from the Ministry of Health. Only cases confirmed bacteriologically (i.e. those from whom S. typhi was isolated from blood, bone marrow, or bile-stained duodenal fluid) were utilized in computations of vaccine efficacy. Therefore, considerable resources were directed toward bacteriologic confirmation of suspect cases. Three 4 ml blood cultures and one bone marrow culture were obtained from children admitted to hospital with a clinical suspicion of typhoid fever. Two 6 ml blood
cultures, drawn 30 minutes apart, were collected from outpatients presenting to the consultorios (health centers) with suspected typhoid fever. Suspicious colonies were confirmed by standard biochemical and serological techniques.22

The code for this blinded study was kept in Berne and Geneva. After breaking the code, the results were analyzed by Chi square.

RESULTS

Parents of 96% of the 141,127 children in Area Occidente gave consent for their children's participation. In total, 109,594 schoolchildren 6-21 years of age (99% were 6-19 years old) received all three scheduled doses of vaccine or placebo. During the vaccination period in the schools there was no increased absenteeism or notable increase in febrile or intestinal illnesses and no cases of typhoid fever were recorded among the participating children.

Results of three years of surveillance in the Area Occidente field trial are summarized in Table 1 where incidence is presented both as cases per 10^5 schoolchildren as well as by classes with cases per 100 classes vaccinated (since randomization was done by class). The 227 confirmed cases of typhoid occurred in 221 separate classes. For those few classes with more than one case, they occurred in different years of surveillance; thus there were no clusters of cases. Vaccine efficacy was virtually identical whether calculated on the basis of incidence per 10^5 schoolchildren or classes with typhoid per 100 classes. The enteric-coated capsule formulation gave significantly better protection than the gelatin capsule/NaHCO₃ formulation. The best protection occurred in the group which received vaccine in enteric-coated capsules with all three doses given within one week (as in the Egyptian trial);
prolonging the interval between doses to 21 days did not enhance efficacy.

For the regimen (enteric-coated capsules, short interval) with the best protection (67% efficacy), the efficacy during each year of surveillance is presented in Table 2 and shows that the level of protection remained similar for all three years (61-71%). Surveillance is being maintained in Area Occidente to determine whether protection endures beyond three years. With this regimen fewer cases of enteric fever due to *S. paratyphi* B (10 cases, 45.1 cases/10^5) were observed than in the placebo group (17 cases, 77.6 cases/10^5; 45% vaccine efficacy) but the difference was not significant (p=0.24).

The relationship between age at vaccination and level of efficacy is shown in Table 3 for the group who received three doses of enteric-coated vaccine given within one week. While significant protection occurred in all age groups, there was a clear-cut trend suggesting that the level of protection increased with age at the time of vaccination; however, the differences in efficacy were not statistically significant.

**DISCUSSION**

The great advantage of *Ty2la* live oral typhoid vaccine, compared to parenteral killed whole cell vaccines, is that it provides significant protection without causing adverse reactions. A wealth of evidence from volunteer studies and from large vaccine field trials attests to the protective activity of this attenuated strain. The field trial from Chile, reported herein, evaluating different formulations and immunization schedules, provides practical information on both the advantages and the limitations of *Ty2la* as a possible control measure against typhoid fever in endemic areas.

The field trial in Area Occidente has shown that *Ty2la* in
enteric-coated capsules is significantly more protective than vaccine in the gelatin capsule/NaHCO₃ formulation (Table 1). These results corroborate a retrospective study reporting poor efficacy for the gelatin capsule/NaHCO₃ formulation, which prior to the Chilean trial had not previously been field tested. Based on the Area Occidente results, production and marketing of the gelatin capsule/NaHCO₃ formulation was discontinued and replaced by the enteric-coated capsule formulation.

In Area Occidente, three doses of enteric-coated Ty21a given within one week provided 67% protection for at least three years (Table 2). This level of vaccine efficacy is equal to the protection conferred by the heat–phenol-inactivated parenteral vaccine (Table 4), the only other widely available effective typhoid vaccine. The phenol-inactivated vaccine, however, causes notable adverse reactions in approximately 25% of recipients and must be administered by needle and syringe or jet gun. Thus Ty21a is distinctly more advantageous because it causes no discernible adverse reactions and is easy to administer to schoolchildren in mass oral vaccinations. In our estimation, this clearly makes Ty21a at present the vaccine of choice for any country intending to embark upon a systematic typhoid fever control program.

The 67% protection conferred for at least three years by three doses of Ty21a in enteric-coated capsules given within one week in Area Occidente is less than the impressive 96% efficacy over three years provided by a liquid formulation used in Egypt (Table 4). Besides the obvious differences in vaccine formulation and genetic constitution of the populations, other factors may have contributed to the difference in results. For example, the mean annual incidence in the placebo group in the Occidente trial (103/10⁵/year) was twice as high as the mean annual
incidence in the placebo group in the Alexandria trial (46/10^5), suggesting that force of infection and modes of transmission may differ between the two sites. Another WHO-sponsored field trial currently underway in Area Sur Oriente of Santiago and a similar trial in Indonesia, are directly comparing the relative efficacy of enteric coated capsules versus a liquid formulation.

Ty2la may also prove useful in the future in immunization against other infections. Because Ty2la stimulates cell-mediated as well as humoral immunity,\textsuperscript{24,25,28-30} it is attractive as a carrier of relevant genes from other organisms. Ty2la, for example, has been modified to express \textit{Shigella sonnei} O antigen,\textsuperscript{31,32} the B subunit of \textit{E. coli} heat-labile enterotoxin,\textsuperscript{33} colonization factor antigen I,\textsuperscript{34} and \textit{Vibrio cholerae} antigens.

The multiple field trials that have been required to generate the information on how to use Ty2la as a public health tool are reminiscent of the series of field trials undertaken by WHO over more than 15 years to accrue similar information for the parenteral killed whole cell vaccines.\textsuperscript{10-13} Until a superior formulation of Ty2la is identified, or it is surpassed by another typhoid vaccine, the information now available should allow public health authorities to utilize Ty2la in enteric-coated capsules in national typhoid fever control programs.
Acknowledgments:

The field trial was supported by grants from the World Health Organization and the Pan American Health Organization and by Research Contract DAMD 17-C-1115 from the U.S. Army Medical Research and Development Command. We are indebted to Viviana Sotomayor, Leonor Atroza, Gloria Berrios, Cecilia Rivera, Maria Rosa Aguirre, Conrado Ristori, Samuel B. Formal, Michael Merson, Nathaniel Pierce and Inma Canepa for their assistance. Dr. Rene Germanier, developer of Ty2la, died on December 25, 1986.
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    bivalent vaccine for typhoid fever and cholera- Escherichia

Table 1. Comparison of the efficacy of two different formulations of Ty21a vaccine administered in two different immunization schedules in Area Occidente, Santiago, Chile. Results of 36 months of surveillance, 9/83 - 8/86

<table>
<thead>
<tr>
<th></th>
<th>Enteric-Coated Capsules</th>
<th>Gelatin Capsules with NaHCO₃</th>
<th>Gelatin Capsules with NaHCO₃</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Long Interval</td>
<td>Long Interval</td>
<td>Short Interval</td>
<td>Short Interval</td>
</tr>
<tr>
<td>No. children</td>
<td>21,598</td>
<td>21,541</td>
<td>22,170</td>
<td>22,379</td>
</tr>
<tr>
<td>No. classes</td>
<td>861</td>
<td>864</td>
<td>863</td>
<td>862</td>
</tr>
<tr>
<td>Cases</td>
<td>34</td>
<td>46</td>
<td>56</td>
<td>68</td>
</tr>
<tr>
<td>Incidence/10⁵</td>
<td>57.4^a</td>
<td>213.5^c</td>
<td>250.3^d</td>
<td>310.4^e</td>
</tr>
<tr>
<td>Efficacy</td>
<td>49%</td>
<td>3%</td>
<td>(24-66%)</td>
<td>(0-52%)</td>
</tr>
<tr>
<td></td>
<td>(47-79%)</td>
<td>(0-43%)</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td>Classes with typhoid</td>
<td>34</td>
<td>46</td>
<td>54</td>
<td>64</td>
</tr>
<tr>
<td>Classes with typhoid/100 classes</td>
<td>3.95f</td>
<td>5.32^h</td>
<td>6.26^i</td>
<td>7.42^j</td>
</tr>
<tr>
<td>Efficacy</td>
<td>47%</td>
<td>28%</td>
<td>16%</td>
<td>---</td>
</tr>
</tbody>
</table>

* 3 doses, 21 days between doses
** 3 doses, 1-2 days between doses

(95% confidence intervals of vaccine efficacy)

a vs c, p = 0.0006   a vs o, p = 0.23
b vs c, p < 0.00001  b vs d, p = 0.00052
c vs e, p = 0.023   a + b vs c + d, p = 0.001
d vs e, p = 0.21

f vs j, p = 0.00135   g vs i, p = 0.00032
f vs g vs h+i, p = 0.00024

g vs j, p = 0.0000031
h vs j, p = 0.07
l vs j, p = 0.35

All statistical comparisons by Chi square
Table 2. Duration of the efficacy conferred by three doses of the enteric-coated capsule formulation of Ty21a live oral vaccine given within one week in Area Occidente, Santiago, Chile

<table>
<thead>
<tr>
<th>Year</th>
<th>Vaccine*</th>
<th>Placebo*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982-6/821</td>
<td>122,120l</td>
<td>121,400l</td>
</tr>
</tbody>
</table>

**Year 1**

<table>
<thead>
<tr>
<th>Cases</th>
<th>Incidence/10k</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>31.6</td>
<td>71%</td>
</tr>
</tbody>
</table>

(36-87%)**

**Year 2**

<table>
<thead>
<tr>
<th>Cases</th>
<th>Incidence/10k</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>38.1</td>
<td>61%</td>
</tr>
</tbody>
</table>

(12-82%)**

**Year 3**

<table>
<thead>
<tr>
<th>Cases</th>
<th>Incidence/10k</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>38.1</td>
<td>67%</td>
</tr>
</tbody>
</table>

(28-85%)**

**Total Years 1-3**

<table>
<thead>
<tr>
<th>Cases</th>
<th>Incidence</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>103.7</td>
<td>67%</td>
</tr>
</tbody>
</table>

(47-79%)**

* 3 doses, 1-2 days between doses

** 95% confidence intervals of vaccine efficacy

vs p, p < 0.00001, Chi square
### Table 1. Efficacy of three doses of Ty21a in enteric-coated capsules given within one week in relation to age of vaccinated children

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Placebo</th>
<th>Ty21a</th>
<th>E-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-9 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. children</td>
<td>7193</td>
<td>7034</td>
<td></td>
</tr>
<tr>
<td>No. cases</td>
<td>28</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Incidence/10</td>
<td>347.6</td>
<td>142.2</td>
<td>0.021</td>
</tr>
<tr>
<td>Efficacy</td>
<td>-</td>
<td>65%</td>
<td>(16-80%)</td>
</tr>
<tr>
<td>10-14 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. children</td>
<td>9710</td>
<td>9992</td>
<td></td>
</tr>
<tr>
<td>No. cases</td>
<td>32</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Incidence/10</td>
<td>329.8</td>
<td>110.1</td>
<td>0.0016</td>
</tr>
<tr>
<td>Efficacy</td>
<td>-</td>
<td>67%</td>
<td>(35-83%)</td>
</tr>
<tr>
<td>15 years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. children</td>
<td>5001</td>
<td>5142</td>
<td></td>
</tr>
<tr>
<td>No. cases</td>
<td>13</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Incidence/10</td>
<td>260.9</td>
<td>38.9</td>
<td>0.0082</td>
</tr>
<tr>
<td>Efficacy</td>
<td>-</td>
<td>85%</td>
<td>(42-98%)</td>
</tr>
</tbody>
</table>

* Results of 36 months of surveillance
** (95% confidence intervals of vaccine efficacy)
Table 4. Comparison of results of WHO-sponsored controlled field trials of lyophilized heat-phenol-inactivated parenteral killed whole cell typhoid vaccine (L) with results of field trials of attenuated *S. typhi* Ty21a live oral vaccine

<table>
<thead>
<tr>
<th>Field Site, Dates</th>
<th>Age</th>
<th>Vaccine</th>
<th>No. (No. Doses)</th>
<th>Vaccinated</th>
<th>Duration of Surveillance</th>
<th>Incidence of typhoid per 105</th>
<th>Vaccine Efficiency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yugoslavia, 1960-63</td>
<td>Mostly Schoolchildren 2-50 yrs.</td>
<td>L (2) 5028</td>
<td>2 1/2 yrs.</td>
<td>727b</td>
<td>5%</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guyana, 1960-67</td>
<td>5-15 yrs. (Schoolchildren)</td>
<td>L (2) 24,241</td>
<td>7 yrs.</td>
<td>198c</td>
<td>67%</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>USSR, 1962-65</td>
<td>Schoolchildren and young adults 92% age 7-15 yrs.</td>
<td>L (2) 36,112</td>
<td>2 1/2 yrs.</td>
<td>55e</td>
<td>66%</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egypt, 1978-81</td>
<td>Schoolchildren 6-7 yrs.</td>
<td>Ty21a (3)* 16,486</td>
<td>3 yrs.</td>
<td>6g</td>
<td>96%** (77-99%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chile, 1983-86</td>
<td>Schoolchildren 6-21 yrs.</td>
<td>Ty21a (3)* 22,170</td>
<td>3 yrs.</td>
<td>104i</td>
<td>67%** (47-79%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Liquid formulation, three doses given within one week

** Enteric-coated capsule formulation, three doses given within one week

(95% confidence interval of vaccine efficacy)

a vs b, p < 0.0004
c vs d, p < 0.000005
e vs f, p = 0.000021
b vs e, p < 0.000021,
i vs j, p < 0.00001, all comparisons by Chi square.
Safety and Immunogenicity of Two *Salmonella typhi* Vi Capsular Polysaccharide Vaccines

Typhoid fever remains a public health problem in many developing areas of the world. An easily administered, well-tolerated vaccine that produces a long duration of immunity after a single dose would be an important advance in controlling this disease. Inactivated whole-cell typhoid vaccines are effective but cause high rates of adverse reactions and require two injections for maximum protection in younger children [1]. Live, attenuated *Salmonella typhi* vaccine strain Ty21a is orally administered, well tolerated, and provides *70%* (Chile) to *95%* (Egypt) protection; however, this vaccine is limited in usefulness because it requires several doses to achieve this level of effectiveness [2].

The Vi capsular polysaccharide (CPS) is a linear homopolymer of 1-4-2-deoxy-2-N-acetyl galactosamine; it is variably O-acetylated at C3 [3]. Measuring serum antibodies to Vi provides a sensitive and specific screening test for identifying chronic, asymptomatic carriers of *S. typhi* [4]. Some evidence suggests that Vi may be a protective immunogen. In an experimental challenge, volunteers immunized with Vi CPS were not protected against challenge with up to *10^8* *S. typhi* organisms [5]. The Vi CPS used in this study, however, was prepared under conditions that altered its structure [6]. New purification techniques using detergents that do not alter the structure of CPS led to the development of meningococcal and pneumococcal CPS vaccines. Vi CPS purified by a similar non-denaturing technique was evaluated in a small number of volunteers; the vaccine elicited higher antibody responses to Vi and less-severe reactions than did the acetone-killed, whole-cell vaccine [7, 8]. In this report we describe further studies assessing the immunogenicity and side effects of two Vi preparations in volunteers from nonendemic areas (Maryland and France) and from a hyperendemic area (Chile).

### Subjects and Methods

**Preparation and characterization of Vi CPS.** *S. typhi* strain Ty2 was cultivated in modified Frantz medium until stationary growth was reached [9]. For lot 3326, the culture was heated to 60 °C for 1 hr, and *1%* hexadecyltrimethylammonium bromide (Cetavlon; Eastman Chemicals, Rochester, NY) was added. The suspension was centrifuged at 10,000 g for 20-30 min, and Vi was extracted from the pellet [9]. Lot IMS1569 was similarly prepared, except that *S. typhi* was replaced by centrifugation and Cetavlon added to the supernatant; the resultant suspension was collected by centrifugation [10]. Vi CPS for passive HA and RIA was prepared from *Citrobacter freundii* strain WR7011 (provided by Dr. Louis Baron, Walter Reed Army Institutes of Research, Washington, DC). The two lots of Vi polysaccharide differed only in lipopolysaccharide (LPS) content (% in lot: 3326 26.0% in lot IMS1569) and in the minimal dose that was pyrogenic in rabbits (0.05 μg for lot 3326 and 0.5 μg for lot IMS1569).

**Volunteers.** Healthy volunteers were recruited at the University of Maryland at Baltimore. Forty-eight students (21-32 years of age) received by random assignment either 50 μg of Vi lot 3326 or 50 μg of meningococcal polysaccharide groups A, C, Y, and W135 combined vaccine (Squibb Connaught, Princeton, NJ) by jet-injection. Volunteers were interviewed about symptoms (mild, moderate, or severe) and examined 24 and 48 hr after injection in a double-blind fashion. Temperatures were recorded for volunteers who complained of feverishness in the first 24 hr and for all volunteers 48 hr after vaccination.

In May, during the low incidence season for typhoid fever in Chile, 150 Air Force recruits (18-21 years of age) volunteered for our study. One hundred thirty-six were randomly assigned to receive 50 μg of Vi lot 3326 and 34 to receive 50 μg of the tetranivalent meningococcal polysaccharide vaccine by jet-injection. Temperatures were recorded every 12 hr, and volunteers were interviewed about symptoms and their severity; and were examined 24 and 48 hr after injection in a double-blind fashion.

In Tours, France, 19 healthy medical students (20-24 years of age) were injected by syringe with 50 μg of Vi lot IMS1569. Rectal temperatures were taken 6, 24, 48, and 72 hr later. Symptoms and local reactions were recorded by the volunteers for 72 hr after injection.

**Serology.** Blood specimens from the Maryland students and the Chilean recruits were obtained before and 28 days after vaccination; blood from French volunteers was obtained before and 25 days after vaccination.

**Passive HA.** Washed, glutaraldehyde-treated sheep erythrocytes were sensitized with 10 μg of purified Vi antigen from *C. freundii* strain WR7011 [4]. After adsorbing overnight with fresh sheep erythrocytes, sera were diluted from 1:20 to 1:2,560 in Fe-sensitized erythrocytes. As a control, sera were diluted in nonsensitized erythrocytes. Plates were incu-
HA. Appropriate positive and negative control sera were used.

R.A. Tyramine (30 mg/ml; Sigma, St. Louis) was added to 10 mg of Vi polysaccharide/ml, and the pH was adjusted to 4.9. The water-soluble carbodiimide EDAC (0.05 M; BioRad, Richmond, Calif.) was added, and the pH was maintained at 4.9-5.1 for 3 hr. The reaction mixture was dialyzed and passed through G-100 Sephadex® (Pharmacia, Piscataway, NJ) equilibrated in water; the void volume was freeze-dried. The final product contained 1.1% tyramine. A burro antiserum to Vi was used as a reference, and antibodies to Vi were determined by a modified Farr assay [9]. The sensitivity of the assay was 0.05 μg of antibody/ml, and the variability was <15%. Seroconversion was defined as an increase in specific antibody to Vi >0.15 μg/ml. (This increase is >3 SD from the mean difference of paired sera from controls. The control population consisted of the same 30 individuals described above.)

Sera were also assayed by ELISA for O-specific antibody using antigen prepared from S. enteridis bioserotype enteridis (Difco) to assure that antibodies to S. typhi LPS were O-specific and were not antibodies to Vi antigen that might have contaminated the LPS preparation. The LPS of S. typhi and S. enteridis bioserotype enteridis are serologically indistinguishable [12]; however, the latter organism lacks Vi antigen. Counterimmunoelectrophoresis using rabbit (Centers for Disease Control, Atlanta) and burro immunosurumne S. typhi antigen did not detect Vi antigen (sensitivity, 1.0 μg of Vi/ml) in either of these two LPS preparations at 1 mg/ml.

**Results**

**Clinical responses.** Table 1 shows the frequency of reactions in recipients of Vi lot 53226 and meningococcal vaccine. Vi preparation produced a higher incidence of local and systemic reactions (rated moderate to severe) than did meningococcal vaccine (P = .005 for local reactions; the difference was not significant for systemic reactions, Fisher's exact test). Two Maryland students who received Vi had temperatures >37.5°C during the 48-hr observation period; one of these had a temperature of 39.1°C with malaise and myalgia and required bed rest.

<table>
<thead>
<tr>
<th>Table 1. Reactions to two S. typhi Vi vaccine candidates.</th>
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<tbody>
<tr>
<td><strong>University of Maryland volunteers</strong></td>
</tr>
<tr>
<td>Vi lot</td>
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<tr>
<td>Systemic</td>
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<td>Malaise</td>
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<td>Fever*</td>
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<td>Went to bed</td>
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<td>Local</td>
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<td>Local pain</td>
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<tr>
<td>Tenderness</td>
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<tr>
<td>Erythema (&gt;2 cm)</td>
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<td>Induration (&gt;1 cm)</td>
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</table>

**NOTE:** Data are percentage of volunteers with moderate-to-severe reactions.

* For Maryland volunteers, the presence of fever indicated subjective fever or temperature >37.5°C (taken orally) 48 hr after vaccination; for Chilean volunteers, fever indicated subjective fever or temperature >37.5°C (taken orally) when measured every 12 hr for 48 hr after vaccination; for French volunteers, fever indicated temperature >37.5°C (taken rectally) when measured at 6, 24, 48, and 72 hr after vaccination.

1. P = .005 by Fisher's exact test comparing recipients of Vi with recipients of meningococcal vaccine.
2. P < .05 by Fisher's exact test comparing recipients of Vi with recipients of meningococcal vaccine.
3. P < .001 by χ² test comparing recipients of Vi with recipients of meningococcal vaccine.
Table 2. Immune response to two S. typhi Vi vaccine candidates.

<table>
<thead>
<tr>
<th>Lot</th>
<th>No. of volunteers</th>
<th>GNT GMT</th>
<th>HA Pre</th>
<th>Post</th>
<th>Pre Seroconversions (%)</th>
<th>Post Seroconversions (%)</th>
<th>GNT GMT</th>
<th>RIA Pre</th>
<th>Post</th>
<th>Pre Seroconversions (%)</th>
<th>Post Seroconversions (%)</th>
<th>GNT GMT</th>
<th>ELISA Pre</th>
<th>Post</th>
<th>Pre Seroconversions (%)</th>
<th>Post Seroconversions (%)</th>
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<tbody>
<tr>
<td>Lot 53226</td>
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<tr>
<td>Maryland students</td>
<td>24</td>
<td>14.14</td>
<td>109.28</td>
<td>85</td>
<td>2.5%</td>
<td>100</td>
<td>11.14</td>
<td>6.75</td>
<td>33</td>
<td>11.14</td>
<td>6.75</td>
<td>11.14</td>
<td>6.75</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chilean recruits</td>
<td>133</td>
<td>11.18</td>
<td>69.35</td>
<td>87</td>
<td></td>
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<td>Lot 1M51659</td>
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</tr>
<tr>
<td>French volunteers</td>
<td>19</td>
<td>12.45</td>
<td>82.97</td>
<td>89</td>
<td>2.7%</td>
<td>95</td>
<td>0.12</td>
<td>0.22</td>
<td>26</td>
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</table>

NOTE. GMT, geometric mean titer; Pre, before vaccination; Post, after vaccination (see Subjects and Methods for details.

* Fourfold or greater rise in titer of antibody
* Increase in titer of antibody \( \geq 10\) µg/ml.
* Increase in OD \( \geq 0.15\).

\( \chi^2 = 28.3, P < .0001 \), compared with recipients of lot 53226.

Eleven (8%) of 136 Chilean recruits who received Vi antigen had temperatures >37.5°C, one had a temperature of 39.6°C with malaise, myalgia, headache, chills, and dizziness. Four (3%) recruits required bed rest in the 48 hr after vaccination. Vi more commonly produced local erythematous reactions (P < .05 vs. meningococcal vaccine). Fisher's exact test, with a larger area of erythema. Table 1 also shows that no recipient of Vi lot 1M51659 had a temperature >37.6°C or any other systemic reaction.

Serological responses. Antibodies to Vi by passive HA. Paired serum specimens were available from 24 Maryland students, 133 Chilean recruits, and 19 French students. Table 2 shows the serological responses to the two Vi preparations. Eighty-five percent of the Maryland students and 87% of the Chilean recruits who received Vi lot 53226 had fourfold or greater rises in titer of antibody to Vi. A similar serological response was observed among recipients of Vi lot 1M51659.

Antibodies to Vi by RIA. Paired sera were available for RIA from 24 Maryland students and 19 French volunteers. The rate of seroconversions measured by RIA was higher (95%–100%) among volunteers who received either Vi preparation (table 2).

O-specific antibodies. Vi lot 53226 elicited seroconversion in 20 (83%) of 24 Maryland students and in 111 (83%) of 133 Chilean recruits (table 2). Identical responses were observed with S. enteritidis LPS (data not shown). Vi lot 1M51659 elicited both lower rates of seroconversion and lower titers of O-specific antibodies. Only five (26%) of 19 volunteers had significant rises in S. typhi O-specific antibodies (\( \chi^2 = 28.3, P < .0001 \), for recipients of lot 53226 vs. lot 1M51659).

Discussion

In these clinical trials, Vi CPS lot 53226 (administered by jet-gun injections) produced fewer reactions than were reported for parenteral inactivated, whole-cell vaccine but more reactions than were reported for parenteral meningococcal vaccine or for oral Ty21a typhoid vaccine [1, 2]. These reactions—fever, malaise, and myalgia—were typical of those seen after inactivated, whole-cell vaccination. The number and severity of reactions probably render this preparation unacceptable for further clinical trials. Because different observers in different countries evaluated the reactions of volunteers to Vi lot 1M51659 and because the preparation was administered by syringe and needle instead of jet gun, we cannot fairly compare reactions with those of volunteers who received Vi lot 53226. The jet gun may increase the rate and intensity of local and systemic reactions [31]. Nevertheless, the incidence of fever and other systemic reactions was less among recipients of Vi lot 1M51659.

Vi lot 53226 contained sufficient LPS (55%) to induce significant rises in O-specific antibodies in most of the volunteers. LPS is a pyrogen and elicits both local and systemic inflammatory reactions. The residual LPS was probably responsible for the fever, malaise, and local reactions. Vi lot 1M51659, however, had considerably lower levels of LPS (0.2 µg/mL), induced a lower seroconversion rate of O antibodies, and produced fewer adverse reactions.

Reduction of LPS content to 0.25% in Vi lot 1M51659 did not completely inhibit its immunogenicity. 26% of volunteers responded with increases in titters of O-specific antibodies. Accordingly, immunization with Vi polysaccharide vaccine may interfere with the use of O-specific antisera for clinical and epidemiological studies. The use of Vi eluted from C. freundii, with its serologically different LPS, may circumvent this problem.
dentia was equivalent to that in the immune population (Chileans). In addition, the antibody responses to Vi eluted by lot 33226 with 15% LPS and by lot IN1569 with 0.27% LPS were similar, which confirmed an earlier observation that LPS content of meningococcal polysaccharide vaccine did not affect the level of antibodies to CPS antigens [14].

The role of antibodies to Vi in providing immunity to typhoid fever in humans is unknown. In mice, immunization with Vi CPS confers a high degree of protection against challenge with S. typhi [15]. The significance of this is unclear, however, because mice are not natural hosts for S. typhi infections and do not develop a generalized infection resembling enteric fever. Protection against typhoid fever can be induced without antibodies to VI, as shown by the experience with Ty2la vaccine, which lacks VI antigen.

Thirty years ago, Hornick et al. carried out experimental challenge studies with S. typhi in volunteers previously immunized with a Vi vaccine prepared by Landy et al. [5]. This Vi was subjected to an acid treatment that removes all of the O-acetyl and part of the N-acetyl moieties and partially polymerizes the Vi polysaccharide [6]. The lack of these moieties may in part account for the poor protection provided by this preparation [6].

Vi in lots IN1569 induced high titers of antibodies to Vi in healthy volunteers and produced a diverse systemic reaction. The effects of age, nutrition, and chronic illnesses on production of antibodies to Vi after immunization are unknown. Further studies are needed to assess the role of serum antibodies to VI in protection against typhoid fever and to demonstrate the efficacy of a Vi vaccine.

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