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# REPORT #1

# STUDIES TO CONTROL ENDEMIC TYPHOID FEVER IN CHILE

## ANNUAL REPORT

Contract Period 7/15/81-12/31/82


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A multi-faceted program of applied research was undertaken to control endemic typhoid fever in Santiago, Chile. These studies included: 1) A Case/Control study to identify risk factors and incriminate specific vehicles involved in transmission of S. typhi. 2) Prevalence of chronic S. typhi carriers in persons with cholecystitis. 3) Simple serologic and other screening tests to identify chronic S. typhi carriers. 4) A simple, practical, non-surgical treatment for chronic S. typhi carriers. 5) An evaluation of the sensitivity and specificity of diagnosing acute typhoid fever by identifying Vi antigen in urine. 6) A field Trial of Ty21a attenuated S. typhi vaccine.

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SUMMARY

A multi-faceted program of applied research was undertaken intended to control endemic typhoid fever in Santiago, Chile. Information gained from these studies will be directly applicable to control of typhoid fever in U.S. military personnel deployed to endemic areas. Results of these studies are summarized below.

1) Case/Control Study to Identify Risk Factors and Incriminate Specific Vehicles Involved in Transmission of S. typhi.

Eighty-one culture-proven cases of typhoid fever in children 3-14 years of age in Area Oriente of Santiago and 81 age-matched neighborhood healthy control children were systematically questioned. The questionaire included inquires about eating habits, socioeconomic factors, history of contact with cases of typhoid fever, etc. Significant risk factors and vehicles among cases included:

i) Lack of a functioning refrigerator in the home.

ii) Habit of eating ice from street vendors.

iii) Eating lunch at school versus at home.

iv) Ingestion of plums.

Significant protective factors identified included:

i) Travel out of Santiago.

ii) Swimming in the ocean (implies a vacation out of Santiago).

iii) Swimming in a lake (implies a vacation out of Santiago).

iv) Swimming in a pool.

2) Prevalence of Chronic S. typhi Carriers in Persons with Cholecystitis.

Under the direction of Dr. Conrado Ristori, 1000 persons undergoing cholecystectomy in seven hospitals in Santiago had their bile and gall bladder tissue cultured. Among this high-risk group 38 were found to be infected with S. typhi, 5 with S. paratyphi A and 30 with S. paratyphi B. Using data on the prevalence of cholelithiasis in Chileans (derived from autopsy studies of

unexpected deaths), the age-specific populations of Santiago, and the prevalence of 3.8% for chronic S. typhi infection among persons with gall bladder disease, we estimate that there exist 22,121 female and 5,627 male chronic S. typhi carriers in Santiago.

3) Simple Serologic and Other Screening Tests to Identify Chronic S. typhi Carriers.

We are evaluating the passive hemagglutination test for Vi antibody as a screening test to detect chronic S. typhi carriers and gelatin string capsule devices (Enterotest$^{R}$) as a practical method to obtain bile-stained duodenal fluid for culture to confirm carriers.

All chronic carriers tested so far have manifested reciprocal titers $\geq$160 (geometric mean titer, GMT, = 523). Among cases of acute typhoid fever 38% had titers $\geq$160 (GMT = 53). Only one of 38 (3%) persons tested 12-24 months after acute typhoid fever (and known not to be carriers) had titers $\geq$160 (GMT = 19). In these preliminary studies of standarization, the Vi antibody assay appears to be highly promising.

Women age 25 years and older who were hospitalized at least 12 months earlier with acute typhoid fever are being contacted to arrange screening to compare the gelatin capsule string device, multiple stool culture and Vi serology in detecting chronic carriers. Among 39 women screened so far, two carriers have been identified (5%).

4) A Simple, Practical, Non-Surgical Treatment for Chronic S. typhi Carriers.

As we identify chronic carriers in the course of the above-mentioned studies we are offering them enrollment in a treatment study which involves ingestion of amoxicillin (2.0 gm thrice daily) and probenecid (0.5 gm thrice daily) for 28 days. Three chronic carriers have completed therapy so far and all three have had negative cultures since cessation of medication. One individual has remained culture-negative for 4 months so far. While the numbers are small and this study

is just in its very early stages, the preliminary results are highly encouraging.

5) Evaluation of the Sensitivity and Specificity of Diagnosing Acute Typhoid Fever by Identifying Vi Antigen in Urine.

We are collaborating with a team from the Centers for Disease Control who are evaluating the sensitivity and specificity of identifying Vi antigen in the urine as a method of diagnosing acute typhoid fever. Three methods of detecting Vi antigen are being compared including enzyme-linked immunosorbent assay (ELISA), counter-current immunoelectrophoresis, and Staphylococcus aureus co-agglutination. These studies were begun in January, 1982. At the time of preparation of this report approximately 50 patients have been evaluated.

6) Field-Trial of Ty21a Attenuated S. typhi Oral Vaccine.

Coded capsules of enteric-coated vaccine and placebo were shipped to Santiago and are in storage at 4°C. Preparations are underway to carry out the immunization of 80,000 to 120,000 children in Area Norte, Santiago between May 3 and June 18, 1982.

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## I. INTRODUCTION

Typhoid fever, the acute, often debilitating, febrile illness caused by Salmonella typhi is endemic in many less-developed areas of the world. Man is the sole reservoir and host of this infection, in contrast with the epidemiologic patterns of other salmonella serotypes which are typically zoonoses infecting domestic and herd animals. A few percent of acute infections result in chronic gall bladder infection; such chronic S. typhi carriers help to maintain the endemicity of the disease by contamination of food and water vehicles.

Typhoid fever is highly endemic in Santiago, Chile. This is a perplexing, as well as challenging, observation since in other ways Chile is a technologically-advanced, highly literate country whose demographic and health statistics would lead one to expect little typhoid fever or other enteric infections. The literacy rate in Chile exceeds 96%, the infant mortality rate is 30 per 1000 live births, measles and pertussis have been greatly reduced by immunization, and poliomyelitis has been eradicated. Yet the incidence rates of typhoid fever in Chile in recent years have ranged from 50 to >100 per $10^5$. The complexity of the epidemiologic situation leading to maintenance of urban endemic typhoid fever in Santiago is further highlighted by the occurrence of much disease in populations of the highest socioeconomic level and in a striking seasonal pattern.

In contrast, because of the relatively advanced technology and educational development of Chile and its population, there exist human resources of a level of sophistication and professional competence rarely found in other areas where typhoid fever is endemic. Thus, into this challenging and fascinating area we proceeded with our project "Studies to Control Endemic Typhoid Fever in Chile". The project comprises several distinct, yet mutually-related, components including:

1) A case/control study to identify risk factors and to incriminate vehicles of transmission of S. typhi.

2) A study to quantify the prevalence of chronic S. typhi carriers in adults with cholecystitis.

3) Development of serologic and other simple screening tests to identify chronic S. typhi carriers.

4) Evaluation of a simple, practical, non-surgical treatment for chronic S. typhi carriers.

5) Evaluation of the sensitivity and specificity of the diagnosis of acute typhoid fever by detection of Vi antigen in urine.

6) A large-scale field trial of the efficacy of Ty21a attenuated S. typhi oral vaccine formulated in enteric-coated capsules.

The progress of each of these components of the project will be summarized in the ensuing pages.

## II. A CASE/CONTROL STUDY TO IDENTIFY RISK FACTORS AND VEHICLES OF TRANSMISSION

### 1) Introduction

Typhoid fever is an endemic public health problem in Chile[1], presenting some interesting and, mostly unexplained, epidemiological features. It presents a marked seasonality with peak incidence during the summer months; it is remarkably infrequent below three years of age and seems to have a high incidence in both poor and wealthy children, even among those who apparently live in homes with excellent sanitary conditions.

So far, there is little known about the vehicles of transmission of typhoid fever in Chile. The two leading hypothesis suggest that spread is by means of food contaminated by foodhandlers who may be asymptomatic carriers of Salmonella typhi; or by food, mostly fruits and vegetables, contaminated by irrigation with sewage-containing water.

Regarding the first hypothesis, it is known that Chile has one of the highest rates of cholelithiasis in the world; it is also known that typhoid fever is endemic and those two factors combined suggest that the rate of biliary carriage might be high. This was confirmed in a recent study, in which 4.8% of the bile cultures of patients undergoing cholecystectomy for any reason were positive for S. typhi.[2]

The second hypothesis indicates that irrigation with water containing sewage might be important in the contamination of fruits and vegetables; most importantly, those that are eaten raw or improperly cooked. Sewage in Santiago is evacuated untreated into the Mapocho River and the presence of high coliform counts and multiple Salmonella strains has been demonstrated in that river.[3] Mostly for economic reasons, those waters are used to irrigate crops such as lettuce and strawberries.

The aim of the present study was to identify risk factors and vehicles of transmission of typhoid fever in the Area Oriente of Santiago, a predominantly middle- and upper class-subdivision which also has some slum areas.

2) Materials and Methods

The study had a case-control design and was conducted from December 1980 through June 1981. Cases of typhoid fever where children of either sex from age three through 14 years who lived in the Area Oriente of Santiago, and who were diagnosed as having typhoid fever confirmed by blood and/or bone marrow cultures positive for S. typhi. These children were diagnosed and treated at the Calvo McKenna Hospital (serving a low socio-economic group) or at the private offices of approximately 20 pediatricians who also worked at the hospital but care for a more middle and upper-socio-economic class in their private practices.

Controls were children of the same sex and age (± one year of age) who lived in the same neighborhood as the case and were identified following a preselected route which started at the home of the case. Children who had a febrile illness suggestive of typhoid fever during the four weeks prior to their participation were excluded and a new control was selected.

Two public health nurses administered a questionnaire to cases and their matched controls, and the answers given by the children were verified with their mothers. The questionnnaire explored the following areas:

a) Demographic characteristics.

b) Socio-economic level.

c) Sanitary conditions at home.

d) Food and drink consumption, both at home and outside.

e) Existence of cooks and/or maids at home and their role in food preparation.

f) History of gall bladder disease among the foodhandler (s) at home.

g) Contact with known cases of typhoid fever.

h) Summer activities.

Two stool cultures were obtained from the primary foodhandler in the household of both cases and controls. The initial culture was obtained with a rectal swab following the interview. The rectal swabs were placed in Cary-Blair transport media and cultured the same day. The second sample was a passed stool obtained the next working day, preferentially during the morning of the day of collection, which was kept refrigerated and was cultured within six hours. Cultures of blood, bone marrow aspirates, and stools, as well as identification of S. typhi, were done using routine bacteriology techniques.

Statistical analysis was done using the McNemar's test for matched pairs.[4]

3) Results

A total of 81 children who met the criteria for inclusion in the study were identified. The sex distribution was even. The largest number of cases was among six year olds, with relatively few cases observed below this age and fairly uniform frequencies above it (Table 1). The monthly distribution of cases during the summer months is shown in Table 2.

i) Blood Cultures

Of 181 blood cultures obtained from private laboratories that participated in the study, 24 were positive for S. typhi; from those 10 were excluded due to their age or because of living outside Area Oriente. Thirty blood cultures were positive for other bacteria, most frequently S. paratyphi. From the Calvo McKenna Hospital 99 blood cultures grew S. typhi and from those 67 met the criteria for inclusion in the study.

ii) Stool Cultures

Rectal swabs were obtained in 78 of the 81 domestic foodhandlers and stool samples in 77 of them. Cultures of both were positive in three persons, including the mother of one case and the cook (female) of another. The third person with positive cultures was the mother of a control child whose own children were stool culture negative but whose occupation involved preparing ice cubes which she sold to neighborhood children, including the case matched with her child. The ages of those foodhandlers were 34, 37 and 55; none of them had a history of typhoid fever, two had a history of biliary colic and one of them was known to have gallstones. Eight additional foodhandlers had stool cultures positive for other enteropathogens (Table 3).

iii) Socio-Economic Status

It is fair to assume that the 14 cases diagnosed in private offices belonged to the middle or upper class, while most of the cases treated at the Calvo McKenna Hospital were of lower socio-economic status. It was thought to be inadvisable to include direct questions regarding income, as this

is not culturally acceptable. The results of several variables that might be associated with socio-economic status are shown in Table 4.

The source of water supply appeared to be significant when analyzed by McNemar's test in a three by three distribution. However, when this was collapsed into two times two tables the results were not significant, suggesting that the initial results were artifactual due to small numbers of non-concordant pairs. Lack of refrigerator remained significantly associated with a high risk of developing typhoid fever even after re-analysis.

iv) Food and Drink Consumption

Consumption patterns of 42 products (drinks, fruits, vegetables and other foods) were investigated. The consumption of ice cubes bought outside the home and eating plums were associated with a higher risk of developing typhoid fever (both $p<0.05$). When analyzed as two times two distributions, ice cubes seemed to be a risk factor when consumed frequently (three or more times per week); however, plum consumption lost statistical significance as a risk factor. None of the other frequently incriminated food items such as strawberries and lettuce showed a significant association with the development of typhoid fever.

It is interesting that when asked how they thought they had acquired the disease, the largest numbers answered "by eating ice cubes" (eight cases) or "by eating unripe plums" (six cases).

Two food items were associated with a lower risk of developing typhoid fever: the consumption of "mote con huesillos" (a local drink made from corn and apricots) ($p<0.01$) and cabbage ($p<0.05$). Consumption of "mote con huesillos" was still significant when re-analyzed as a two times two table comparing "never" versus "ever" comsumed and using two degrees of freedom, but consumption of cabbage was not.

v) Exposures

Cases and controls were questioned about 11 different exposures (Table 5). Eating lunch at the school cafeteria as well as sharing food with a classmate were associated with a higher risk of developing typhoid fever. When the analyses was limited to cases that occurred while school was in session, the trend persisted but this did not reach statistical significance, perhaps due to the relatively small number of case-control pairs. Cases had also a significantly higher frequency of relatives ill with typhoid fever during the two preceding months; this included the mother of one case, siblings in six cases, cousins in seven cases and an aunt in one case. It is not known whether "cousins" and "aunt" indicated members of the case's household.

vi) Travel

Travel outside Santiago as well as traveling to a lake, the ocean or going to a pool were significantly more frequent among the controls. Cases and controls showed no difference regarding swimming in a river, irrigation channel or "somewhere else" (Table 6).

4. Discussion

The epidemiology of endemic diseases is frequently complex and more than one vehicle of infection can be involved. This might well be the situation regarding typhoid fever in Santiago, Chile. The present study identified some factors associated with a nigh and a low risk of developing typhoid fever, which deserve to be analyzed in more detail.

Since more than 70 variables were investigated, it can be expected that by chance alone two or three of those variables may show significance at a p level of less than 0.05. In some instances, however, the statistical significance of the association was much greater. Furthermore, in the increased risk associated with ice cubes and decreased risk associated with "mote con huesillos", after the initial analysis on a three times three distribution,

the data was collapsed into two times two tables and the chi square was calculated using two degrees of freedom, a criterion more strict than usual.[4] Even then the significant associations held.

If ice cubes prove to be a vehicle of transmission of typhoid fever among children in Santiago, it will have to be established how the contamination of the ice cubes occurrs. One possibility is that the water is contaminated (our results weakly suggested consumption of non-piped water as a risk factor, but the number of case/control pairs was too small to fully evaluate this factor). Another hypothesis is that S. typhi carriers contaminate the containers, the water or the cubes while preparing them for sale in their homes. In this regard, it is pertinent that we identified a S. typhi carrier who had prepared and sold ice cubes to a child who subsequently developed typhoid fever.

We did not detect any clustering of cases in any given school; however, eating lunch at a school cafeteria and sharing food with classmates were both associated with a higher risk of typhoid fever. Since this increased risk appeared to be present even for cases who became ill during the summer school holiday, it is possible that these questions identified a tendency of certain children to eat food prepared outside of their own home. This would potentially expose these children to the food prepared by a wide variety of persons, including their classmates' mothers, who could be chronic carriers of S. typhi.

The higher risk of typhoid fever associated with the lack of a refrigerator in the household has several possible explanations. It could merely reflect socio-economic differences between cases and matched controls; however, this seems unlikely since they lived in the same neighborhood and did not differ in a number of other economic indicators. It is more likely that the lack of refrigeration per se is an important factor in the transmission of S. typhi.

Perhaps the increased risk is due to the inability to keep foods cold, thereby allowing multiplication of S. typhi in them. Furthermore, without a refrigerator some items for consumption, such as ice cubes, must be obtained from neighbors or small producers thereby exposing the family to potentially contaminated products.

The "protective effect" of cabbage had a borderline statistical significance and may represent an artifact. The significantly higher frequency of consumption of "mote con huesillos" among controls is intriguing, since popular belief assoicates it with the development of enteric infections due to the lack of hygiene with which it is often prepared and the fact that it is left at room temperature for several hours. Since it is not expensive, probably there is no association with socio-economic level. However, we can hypothesize that it prevents infection by colonizing the small bowel with a saprophytic bacteria that interferes with growth of Salmonella typhi.

The reduced risk of typhoid fever among children who went on vacation out of Santiago, either to the lakes or to the beach, may be a marker of socio-economic status, but also could indicate that they were removed from the source of infection, being safer away from Santiago. We did not ask for how long the children were out of town but further statistical analysis may help to differentiate among those two possibilities. The reduced risk associated with swimming in a pool may represent a certain economic level, because even when there are several public pools in the area, the admission ticket was relatively expensive.

Even though our numbers are relatively small, we are under the impression that the foodhandlers preparing food solely for their family were not the main source of S. typhi infection. Two stool cultures should identify a fair proportion of asymptomatic carriers, and only two carriers were found in the homes of the 81 cases. The risk factors identified in this study are consistent with the hypothesis that endemic typhoid fever in Santiago is

largely spread by exposure to items for consumption that are prepared in private homes and are sold to or shared with children during the summer months. From this study, it cannot be determined if S. typhi contamination of these items is a result of their preparation by chronic S. typhi carriers or because the raw foods (and perhaps water) contain S. typhi from pollution by Santiago sewage. Further epidemiologic and bacteriologic studies are planned to provide definitive answers to these questions on transmission of endemic typhoid fever.

## III. THE PREVALENCE OF CHRONIC S. TYPHI CARRIERS IN ADULTS WITH CHOLECYSTITIS

### 1) Introduction

Chronic gall bladder infection with S. typhi, resulting in an asymptomatic persistent carrier state, is a consequence of acute S. typhi infection in a few percent of cases. The propensity to become a chronic carrier following acute S. typhi infection augments with increasing age at the time of infection and is greater in females than males.[5,6] In these features chronic S. typhi infection resembles the prevalence of cholecystitis which is greater in females and also increases with age.[7-9] It appears that acute infection of the gall bladder probably occurs in all cases of acute disease. Those individuals who have pre-existent gall bladder disease are at particular risk of the S. typhi infection becoming chronic.

Since Chile manifests an extraordinarily high prevalence of cholecystitis and cholelithiasis[7-9] (indeed one of the highest in the world!), and the incidence of typhoid fever is also high, one would expect to find a high prevalence of chronic S. typhi carriers in Chile. One would also expect such carriers to play an important role as a reservoir in maintaining the endemicity of the disease.

Since persons with cholecystitis should be considered a high risk group with respect to having a high prevalence of chronic S. typhi carriers,

studies were carried in this population in an attempt to quantitate the prevalence of chronic S. typhi carriers in Chile.

2) Methods

Dr. Conrado Ristori of the Chilean Ministry of Health and Dr. Luis Cisneros of the Center for Vaccine Development (CVD) made arrangements with surgeons at the seven major hospitals in Santiago for cultures of bile and gall bladder tissue to be collected at the time of cholecystectomy. Cultures were sent to the Institute of Public Health where they were plated both directly onto selective media and after incubation for 18 hours in Selenite F broth. Salmonella typhi were identified by biochemical and serologic confirmation of suspicious colonies.

3) Results

One thousand consecutive unselected patients undergoing cholecystectomy at these seven hospitals were cultured. Four-fifths (79.6%) of the patients were females (Table 7), of whom 58.7% were over 35 years of age; 75% of the males were more than 35 years old.

As shown in Table 7, S. typhi were recovered from 38 individuals, S. paratyphi A from five and S. paratyphi B from 30. In total, pathogens responsible for enteric fever (typhoid or paratyphoid fever) were recovered from 73 persons with chronic gall bladder disease.

IV. DEVELOPMENT OF SEROLOGIC AND OTHER SIMPLE SCREENING TESTS TO IDENTIFY CHRONIC S. TYPHI CARRIERS

1) Introduction

The role that chronic carriers play in providing a reservoir of S. typhi and in serving to contaminate food and water vehicles has been discussed. Simple yet reliable screening tests are required to allow public health authorities to rapidly identify carriers and thence offer them treatment or ensure that they do not work as foodhandlers.

Heretofore two methods were generally available to screen for carriers and another two methods were ordinarily used for confirmation. The screening tests included:

i) Culture of at least three consecutive stools passed.

ii) Tests for Vi antibody using partially purified Vi antigen or whole Vi-containing bacteria.[10-17]

Each of these screening tests had significant drawbacks. For example, known carriers commonly have negative stool cultures if the stool specimens are fully formed.[18-21] This is because the short chain fatty acids produced as end products of metabolism by normal anaerobic and aerobic colonic flora are highly inhibitory to salmonella; so a delayed intestinal transit time can result in suppression of the organism. In the past, the practice used to overcome this was to administer oral purgatives such as $MgSO_4$ to induce watery stools accompanied by a rapid intestinal transit.[18,21] In this way bile and upper intestinal fluid rapidly reached the colon and were excreted. Induction of bouts of diarrhea on one or more occasions was not well-appreciated by the suspect carrier.

The other method of confirming the existence of chronic gall bladder carriage was to have individuals ingest intestinal tubes leading to intubation of the duodenum or jejunum wherein bile-stained material could be collected for culture.[18,19] Prior to development of the Enterotest$^R$ gelatin string capsule device, intestinal intubation was a procedure that was uncomfortable, time-consuming and, depending on the tube and method, required considerable clinical supervision.

Serologic screening tests for carriers based on measurement of Vi antibody were until recently highly variable and the subject of much acrimonious disagreement.[10-17] This was so because highly purified preparations of Vi antigen[22] were not yet available as a reagent.

Two significant advances have recently been described that offer

great promise as screening and confirmatory tests for detection of S. typhi carriers:

i) Chau and Chan[23], and Nolan et al.[24,25] have developed a passive hemagglutination assay (PHA) for detection of Vi serum antibodies that utilizes highly purified Vi antigen.[22] This test has been shown to be quite sensitive and specific in preliminary reports involving small numbers of individuals tested.[23-25] This PHA Vi antibody test offers great potential as a simple screening test to detect asymptomatic chronic carriers when large numbers of individuals must be screened.

ii) Gilman et al.[26] have described use of the gelatin string capsule device as a simple, practical, and non-discomforting way to obtain bile-stained duodenal fluid for culture. In this way suspect chronic carriers can be confirmed by direct culture of S. typhi from proximal small intestinal fluid.

2) Detection of Carriers

We have undertaken studies to evaluate each of these two tests. In seeking to detect chronic S. typhi carriers, one of our project public health nurses has reviewed the records of admissions to the Hospital Enfermedades Infecciosas (the Infectious Disease Hospital) of Santiago. She has been collecting the names and addresses of women admitted with typhoid fever at least 12 months earlier who were 25 years of age or older. Letters are being sent to these women requesting that they visit our nurse at the hospital for collection of specimens. Each woman is being asked to swallow a gelatin string capsule device to collect duodenal fluid, to provide three stool specimens for culture and to allow a blood sample to be obtained for Vi antibody tests.

So far the nurse has reviewed 1208 case records to detect women age 25 years or older who were admitted 12-24 months earlier with a diagnosis of typhoid fever. Among these 1208 cases, 102 (8.4%) fell into the proper

category. Letters on Ministry of Health stationary were sent to all 102 women; 39 of these 102 (38%) came for screening, among whom two chronic carriers were detected. These two carriers had very high Vi antibody titers, and positive bile and stool cultures.

3) Vi Serology

Using highly purified Vi antigen kindly supplied by Dr. John Robbins of the Bureau of Biologics, Bethesda, Md., we adopted the PHA assay for Vi antibody as described by Nolan et al.[24,25]

Studies were begun to assess the sensitivity, specificity and repeatability of the assay with respect to adapting it for use in Chile.

Preliminary studies were begun with sera from the following populations:

i) Six known, culture-proven, chronic S. typhi carriers (four Chilean two U.S.).

ii) Twenty-nine Chileans with acute typhoid fever (sera drawn four to 10 days following onset).

iii) Thirty-eight Chileans who had acute typhoid fever 12-24 months earlier and were shown not to be chronic carriers.

iv) Twelve U.S. medical students who 21-28 days earlier had ingested one to three doses of Ty21a attenuated S. typhi vaccine (this strain lacks Vi antigen).

v) Fifty-nine healthy Chilean adults and children living in areas of Santiago where typhoid fever is highly endemic.

Results of these data are summarized in Table 8. All six chronic carriers had reciprocal titers $\geq$ 160 with geometric mean titer (GMT) of 523. The Chilean patients with acute typhoid fever also typically had elevated titers. Indeed 21 of 29 had some measureable antibody (titer $\geq$ 20) and 15 of 29 (52%) had titers $\geq$ 80; only 11 (38%) had titers $\geq$ 160. It is of interest to note that within 12-24 months after typhoid fever, the GMT drops and now

only three of 38 (8%) had reciprocal titers $\geq$ 80; furthermore, only one (3%) had a titer $\geq$ 160. Lastly, reciprocal titers $\geq$ 80 were rare among Ty21a vaccinees and healthy Chileans living in Santiago. These data suggest that if someone has a Vi antobody titer of $\geq$ 160 measured by PHA he/she is either a chronic carrier or has had acute typhoid fever in the very recent past.

4) Estimations of the Prevalence of Carriers in Santiago

We have two sets of data that allow us to make estimates of the number of chronic S. typhi carriers in Santiago. One estimate can be derived based on the prevalence of S. typhi carriers among persons with cholecystitis. The prevalence of cholelithiasis and cholecystitis in a cross-section of residents of Santiago of different ages is available from autopsy studies carried out by the Medico-Legal Institute of Santiago.[7-9] Autopsies carried out there consist mainly of persons involved in fatal accidents, trauma, etc. The population of Santiago by age groups is shown in Table 9. The percentage of individuals of each age group who have evidence of gall bladder disease (based on Medico-Legal Institute figures) is also listed, from which an estimate can be made of the total number of persons at any given age with gall bladder disease. The estimate of persons with gall bladder disease can be multiplied by 3.8% (based on the data of Ristori et al.[2] reported on p. 10-11) to give the number of S. typhi carriers. By this analysis we calculate that in the population 10 years of age or older, there exist approximately 22,121 female and 5627 male chronic S. typhi carriers in Santiago.

Another rough estimate of the number and extent of S. typhi carriers in Santiago can be made based on the incidence of acute S. typhi infection and the frequency with which acute infections result in a chronic gall bladder infection.

Approximately 4000 cases of typhoid fever are reported in Santiago annually. However, at least 10 to 15 sub-clinical cases must occur for each

clinical case, based on the comparison between notification data and the prevalence of antibody determined by seroepidemiologic surveys (see Figure on p. 72 of original research proposal). Thus, in fact, 40,000 to 60,000 infections including asymptomatic ones are occurring yearly. Of these, approximately one-third (13,200-19,000) occur in individuals over 19 years of age, the group in whom gall bladder disease is common. Our initial studies investigating the development of the chronic carrier state in women over 25 years of age who had typhoid fever showed that two of 39 women (5%) in this category became carriers following acute infection. Our preliminary data are very similar to the results of a study by Armijo et al.[6] who detected chronic carriers in 7% of 460 women over 15 years of age followed after discharge from Hospital Enfermedades Infecciosas following acute typhoid fever. Using the rate of 5%, one would expect approximately 660-990 new chronic carriers to enter the population yearly from the ranks of the 13,200-19,800 annual S. typhi infection cases in Santiago that occur in persons over 19 years of age.

## V. A SIMPLE, PRACTICAL, NON-SURGICAL TREATMENT FOR CHRONIC S. TYPHI CARRIERS

### 1) Introduction

Once chronic S. typhi carriers are identified, one would like to be able to successfully eradicate the carrier state, thereby assuring that the individual no longer poses a risk to others as well as releasing the person from various restrictions. Until recently, medical (i.e. antibiotic) therapy alone was largely ineffective in curing the chronic S. typhi infection, particularly if gallstones were present.[27-29] Particularly disappointing in efficacy were the antibiotics chloramphenicol and trimethoprim/sulfamethoxazole which are in contrast, so superior in treating acute infections. The combination of cholecystectomy plus several weeks of antibiotic therapy is highly successful in eradicating the chronic carrier state.[30] However, gall bladder surgery

is neither a practical nor inexpensive alternative and is often refused by carriers.

In 1972 Scioli et al.[31] reported successful treatment of 19 chronic S. typhi carriers by medical therapy alone by use of 15 consecutive days of intravenous ampicillin (1.0 gm every eight hours) in hospitalized patients. Although intravenous ampicillin is also not a practical form of therapy, this paper established that the carrier state could be eradicated without surgery if sufficiently high concentrations of an effective antibiotic could be achieved in the gall bladder. Ampicillin is concentrated in bile and is bactericidal in vitro against S. typhi.

Amoxicillin is an analogue of ampicillin that is superbly absorbed following oral administration (giving two to three times the serum levels of a comparable dose of oral ampicillin) and is concentrated in the bile. Amoxicillin is therefore attractive as a possible oral therapy for chronic S. typhi carriers. In one report[32], 15 carriers were treated with oral amoxicillin (2.0 gm three times daily) for 28 days. Nine of 10 patients who were able to complete therapy were cured. In five patients the dosage had to be cut in half; of these, only two were cured.

2) Methods and Results

We have undertaken to evaluate the efficacy of combined therapy with oral amoxicillin (2.0 gm thrice daily) and probenecid (0.5 gm thrice daily) in eradicating the chronic S. typhi carrier state. So far, three chronic gall bladder carriers have completed the course of treatment; the results are summarized in Table 10. Two carriers are from Chile and one is from Maryland. Two experienced mild diarrhea during the first seven to ten days of therapy but not of a severity requiring cessation or alteration of treatment. None complained of cramps, nausea or vomiting. All three have had negative bile and stool cultures for the period for observation

since cessation of therapy. These preliminary results are extremely encouraging.

Samples of serum and urine have been collected from the patients at various intervals during therapy to measure amoxicillin levels. These specimens will be tested shortly.

VI. DIAGNOSIS OF ACUTE TYPHOID FEVER BY DETECTION OF Vi ANTIGEN IN URINE

With few exceptions, S. typhi strains isolated from patients with acute typhoid fever produce polysaccharide Vi antigen. A report from Indonesia[33] demonstrated that patients with acute typhoid fever excrete Vi antigen in their urine which can be detected by the Staphylococcus aureus coagglutination test. Drs. D. Taylor and J. Harris of CDC are examining urine of suspect and culture-proven cases of typhoid fever for the presence of Vi antigen. They will compare the sensitivity and specificity of three assays for Vi antigen including S. aureus coagglutination, ELISA and counter-current immunoelectrophoresis. In early January 1981 Drs. M. Levine and R. Black went to Santiago, Chile with Dr. D. Taylor to initiate this component of the project. Arrangements were made to set up Dr. Taylor's laboratory in Hospital Roberto del Rio. This is the pediatric hospital located in Area Norte, the site selected for the vaccine field trial. Intensive bacteriologic surveillance has been initiated at this hospital which includes collection of multiple blood cultures and a bone marrow from every suspect case of typhoid fever. So one major source of specimens for the Vi antigenuria study will come from this population of pediatric cases. We also made arrangements for Dr. Taylor to acquire specimens from adolescent and young adult patients admitted to Hospital Enfermedades Infecciosas in Area Sur. Our clinical collaborators in that hospital admit 20-25 cases of acute typhoid fever daily during the peak summer season, so there is no shortage of clinical material.

## VII. LARGE-SCALE FIELD TRIAL OF Ty21a ATTENUATED S. TYPHI ORAL VACCINE

Ty21a, a stable attenuated mutant of S. typhi, is a safe, protective oral vaccine when three doses of $10^9$ cells in saline are taken after neutralization of gastric acidity by 1 g $NaHCO_3$.[34] To identify a more convenient method to administer vaccine and to determine the feasibility of immunizing with a single dose in a large-scale field trial, we studied the immune response of children and adults to different formulations of Ty21a with a newly developed, sensitive, enzyme-linked immunosorbent assay (ELISA). The wells of polystyrene microtitration plates were coated for one hour at 37°C with a 10 µg/ml concentration of Westphal lipopolysaccharide preparation from Salmonella typhi 0901 (lot control 678325, Difco Laboratories, Detroit, Michigan) in sodium carbonate coating buffer, pH 9.6. Test sera were diluted 1:100 in phosphate buffered saline with 0.05% Tween 20 (polyoxyethylene [20] sorbitan monolaurate; J.T. Baker Chemical Co., Phillipsburg, N.J. 08865) and 1% heat inactivated fetal calf serum and then were tested for class G or M specific immunoglobulin by an enzyme-linked immunosorbent assay as described by Young et al.[35] In this ELISA, rise in S. typhi O antibody was defined as a change in net optical density of $\geq$ 0.15 (more than three SD from the mean difference in optical density in a negative control population). Although the mechanism of immunity stimulated by strain Ty21a is believed to be cell-mediated, the humoral O antibody response has previously shown to be of value in comparing different formulations of this vaccine in populations.[36]

### 1) Pilot Study in Chilean Schoolchildren

In 1980 we carried out a study in Santiago involving 335 schoolchildren ages 6-9 years who received three doses (Monday-Thursday-Monday) of Ty21a S. typhi oral vaccine ($10^9$ vaccine organisms/dose). One hundred and sixty-eight ingested vaccine in enteric-coated capsules, while the remaining 167 drank vaccine that had been reconstituted in 150 ml of milk with

0.8 gm of sodium bicarbonate. Based on experiences with oral attenuated Shigella vaccines in children,[37] this milk/bicarbonate method should be regarded as a highly efficacious and practical method of successfully delivering attenuated bacterial vaccines to children. The enteric-coated vaccine had been stored for one year at 4°C prior to use.

Both formulations were well-tolerated by the Chilean children. Based on blood specimens collected prior to and 14 and 21 days after immunization, 5% of children in each group had seroconversions of S. typhi O antibody measured by Widal test. By ELISA, 41% of children in the enteric-coated capsule group and 42% who received vaccine in milk with bicarbonate had seroconversions of S. typhi O antibody documented by IgG ELISA (Table 11). Furthermore, the mean antibody levels were virtually identical (net O.D. of 0.37). In addition, 12% of children who received enteric-coated vaccine and 16% who received vaccine in milk had seroconversions by IgM ELISA (Table 12). From these data the following conclusions can be drawn:

i) The ELISA test for S. typhi O antibody is much more sensitive than Widal.

ii) There was no difference in frequency or magnitude of antibody response in groups of children immunized with enteric-coated capsules or with vaccine given in milk with bicarbonate.

iii) Properly prepared enteric-coated capsules kept at 4°C maintained the viability and immunogenicity of the Ty21a S. typhi for at least one year.

iv) Based on the frequent and vigorous IgG antibody response it appears that many Chilean schoolchildren of this age group are immunologically primed to respond to S. typhi O antigen, presumably by prior exposure to S. typhi, since parenteral typhoid fever vaccine is used rarely in Santiago.

2) Study in U.S. Volunteers of Three Doses of Ty21a in Two Different Formulations

Sixteen healthy U.S. adults ingested three doses of enteric-coated

vaccine, while 15 took vaccine in gelatin capsules (preceded by ingestion of two gelatin capsules each containing 0.4 gm $NaHCO_3$). As shown in Table 13, with IgG ELISA, the frequency of seroconversions (on day 28 after immunization) in each group (19 and 20%) were lower than in Chilean schoolchildren. On the assumption that this was due to lack of immunologic familiarity with this antigen, sera collected 10 days after immunization were examined by IgM ELISA. In this instance significant rises of O antibody were documented in 25% of those who received enteric-coated capsules and in 20% who got gelatin capsules (Table 14).

From these observations one can conclude:

i) Three doses of enteric-coated capsules appear as effective in delivering vaccine as three doses of gelatin capsules.

ii) U.S. adults do not have the same immunologic familiarity with S. typhi O antigen as Chilean children. U.S. adults respond to immunization as often with a primary (IgM) as with a secondary (IgG) antibody response.

3) Study in U.S. volunteers of One Dose of Ty21a in Two Different Formulations

Twenty-nine U.S. adults were given a single dose of Ty21a vaccine by the gelatin capsule formulation. IgM ELISA O antibody rises occurred in seven of 29 (24%) and the mean level of antibody was similar to that encountered in the group that ingested three doses of vaccine via gelatin capsule formulation. Twenty-one volunteers ingested a single dose of vaccine in enteric-coated capsules. IgM antibody rises occurred in only two of 21 (10%) but this was not significantly different from the rates of seroconversion in the other groups of volunteers receiving either one or three doses of vaccine. These data suggest the following conclusions:

i) One dose of vaccine given in a gelatin capsule with $NaHCO_3$ was as successful as three doses of vaccine in stimulating O antibody responses.

ii) One dose of Ty21a vaccine in enteric-coated capsules appeared somewhat less efficacious is stimulating O antibody responses. Although in the numbers of volunteers tested the difference was not significant.

iii) Further studies should be carried out in volunteers to increase the numbers who have received one dose of enteric-coated or gelatin capsule vaccine and to compare these with a group who have received two doses of enteric-coated vaccine.

The Ty21a vaccine as used in Egypt was highly impractical, although extraordinarily efficacious. In Egypt individual vials containing lyophilized vaccine (or placebo) were reconstituted and were given to children with 1.0 gm of $NaHCO_3$. Three doses were given to each child, one dose every other day.

The two formulations tested in volunteers both represent improvements over the method used in Egypt. The enteric-coated capsule, in particular, makes mass immunization of schoolchildren practical, particularly if one dose would suffice. Even if two doses are required, enteric-coated capsules represent an attractive formulation from the point of view of logistics and practicality.

4) Preparations for the Large-Scale Field Trial in Santiago, Chile

Upon notification that funds from the U.S. Army Medical R & D Command and from WHO would be forthcoming in July or August 1981 to support the large-scale field trial, the Swiss Serum and Vaccine Institute sent 96,000 doses of vaccine and 96,000 doses of placebo in coded capsules to Chile in early July 1981. Vaccine was put into storage at $4^{o}C$ in the central refrigerated storage facility of the Ministry of Health. Because of the late start, a race against time began in which two objectives had to be met:

i) The Institute of Public Health of Chile had to complete various protocol tests assuring the purity, safety, and potency of the field trial

lot of vaccine. This Institute in Chile has the same function in such matters as the U.S. Bureau of Biologics.

ii) Epidemiologic field work had to be completed which included obtaining informed consent from the parents of at least 80,000 of the 120,000 schoolchildren in Area Norte, arranging cooperation of the schools, and training and scheduling the health auxiliaries who were going to assist in the vaccination campaign.

From early July 1981, CVD staff worked closely with the Ministry of Health personnel to accomplish all the epidemiologic objectives. These objectives were successfully met: 1) Cooperation of the school teachers and school directors was obtained. 2) A census was made of the entire school child population. 3) Consent was solicited from parents of the 120,000 schoolchildren in Area Norte, of whom 80,000 gave informed consent. 4) The classes in each school were randomized to receive preparation A or B. 5) Capsules were apportioned according to class and inserted into bags which were then labelled and prepared for shipment to the schools. 6) Twenty health auxiliaries from the Area Norte health districts were assigned to assist full-time during the immunization campaign. These workers were intesively trained.

The epidemiologic field work and logistical preparations were completed in time for the immunization campaign to begin on August 17, 1981. Unfortunately, the various tests that the Institute of Public Health was carrying out were not completed in time to commence vaccination in mid-August. Indeed, the tests were not completed until late October, making it impossible to re-initiate and complete the vaccination campaign before onset of the Chilean school summer recess (late November or early December, depending on the particular school). Furthermore, immunizing in late November and early December would have meant immunizing well into the high season of typhoid fever. For these reasons, we elected to wait for the start of the new school

year (which begins on March 8, 1982) in order to carry out the immunization in the most organized and orderly fashion possible. Drs. M. Levine and R. Black visited Chile in January 1982 to re-affirm the commitment of the Ministry of Health to proceed with the vaccine field trial (Appendix A) and to prepare a strict timetable to be followed (Appendix B). The timetable, which was designed in conjunction with Ministry of Health collaborators (Appendix B), calls for preparations for the vaccination campaign to be carried out between late February and late April leading to the program of vaccination between May 3 and June 18, 1982.

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TABLE 1

AGE AND SEX DISTRIBUTION OF 81 PEDIATRIC CASES OF TYPHOID FEVER, AREA ORIENTE OF SANTIAGO, DEC. 80-JUNE 81

| AGE | MALE | FEMALE | TOTAL |
|---|---|---|---|
| 3 YRS. | - | 2 | 2 |
| 4 | 2 | 2 | 4 |
| 5 | 2 | 1 | 3 |
| 6 | 4 | 7 | 11 |
| 7 | 4 | 5 | 9 |
| 8 | 5 | 4 | 9 |
| 9 | 5 | 4 | 9 |
| 10 | 5 | 3 | 8 |
| 11 | 3 | 3 | 6 |
| 12 | 4 | 2 | 6 |
| 13 | 7 | 1 | 8 |
| 14 | 1 | 5 | 6 |
| TOTAL | 42 | 39 | 81 |

## TABLE 2

### MONTHLY DISTRIBUTION OF POSITIVE BLOOD CULTURES FOR *SALMONELLA TYPHI*

| MONTH | NO. OF CASES |
|---|---|
| NOVEMBER '80 | 8 |
| DECEMBER | 11 |
| JANUARY '81 | 17 |
| FEBRUARY | 11 |
| MARCH | 17 |
| APRIL | 11 |
| MAY | 6 |
| TOTAL | 81 |

TABLE 5

CHARACTERISTICS OF DOMESTIC FOODHANDLERS WHOSE STOOL CULTURES WERE POSITIVE FOR ENTERIC PATHOGENS

| Household | Relation | Age | Hx Typhoid | Hx Gallstones | Hx Biliary Colic | Isolate | Rectal Swab | Passed Stools |
|---|---|---|---|---|---|---|---|---|
| Control #4 | Mother | 34 | NO | YES | YES | *S. typhi* | + | + |
| Case #13 | Cook (female) | 55 | NO | NO | NO | *S. typhi* | + | + |
| Case #38 | Mother | 37 | NO | NO | YES | *S. typhi* | + | + |
| Case #61 | Mother | 27 | NO | NO | YES | *S. paratyphi* B | + | + |
| Case #62 | Mother | 33 | NO | NO | NO | *S. paratyphi* B | + | - |
| Control #62 | Mother | 38 | NO | NO | NO | *S. paratyphi* B | + | + |
| Case #17 | Maid | ? | NO | NO | NO | *S. typhimurium* | + | - |
| Case #11 | Grandmother | 60 | NO | YES | YES | *S. enteritidis* | + | - |
| Control #45 | Sister | 23 | NO | NO | NO | *S. enteritidis* | + | - |
| Control #39 | Mother | 36 | NO | NO | NO | Shigella | + | - |

TABLE 4

LIVING CONDITIONS OF *SALMONELLA TYPHI* CASES COMPARED WITH CONTROLS

| CHARACTERISTIC | CHI SQUARE* | P |
|---|---|---|
| WATER SUPPLY | 6.5000 | <0.05 |
| REFRIGERATOR | 4.6538* | <0.05 |
| SEWAGE | 3.8571 | >0.1 |
| AUTOMOBILE | 2.7692* | >0.1 |
| ELECTRICITY | 2.0000 | >0.25 |
| NO. PERSONS AT HOME | .9808 | >0.5 |
| NO. OF BATHROOMS | .6809 | >0.5 |
| SINK, KITCHEN | .3636* | >0.5 |
| SINK, BATHROOM | .0000* | - |

*ONE DEGREE OF FREEDOM; ALL OTHERS TWO DEGREES OF FREEDOM.

## TABLE 5

### EXPOSURES OF *SALMONELLA TYPHI* CASES COMPARED WITH CONTROLS

| EXPOSURE | CHI SQUARE | P VALUE |
|---|---|---|
| SHARING FOOD WITH CLASSMATES | 7.2789 | <0.05 |
| LUNCH AT SCHOOL CAFETERIA | 7.8387 | <0.025 |
| HISTORY OF TYPHOID, RELATIVE* | 4.7600** | <0.05 |
| EATING FROM STREET VENDOR | 5.5000 | >0.05 |
| HISTORY OF TYPHOID, DOMEST. FOODHANDLER | 2.8000 | >0.11 |
| MEETING WITH TYPHOID PATIENT | 2.7727 | >0.1 |
| RESTAURANT, 2 PRIOR WEEKS | 2.200 | >0.25 |
| EATING AT SCHOOL KIOSKS | 2.0243 | >0.25 |
| HISTORY GALLSTONES, DOMESTIC FOODHANDLER | 1.4848 | >0.25 |
| BREAKFAST AT SCHOOL CAFETERIA | 0.6950 | >0.5 |
| HISTORY TYPHOID, CLASSMATE | CNBC+ | |

*COUSINS: 7, SIBLINGS: 6, MOTHER: 1, AUNT: 1.

**ONE DEGREE OF FREEDOM, TWO DEGREES OF FREEDOM FOR ALL OTHERS.

+CAN NOT BE CALCULATED.

## TABLE 6

### TRAVEL AND WATER EXPOSURES OF *SALMONELLA TYPHI* CASES COMPARED WITH CONTROLS

| ITEM | CHI SQUARE* | P VALUE |
|---|---|---|
| TRAVEL OUTSIDE SANTIAGO | 5.3333 | <0.025 |
| GOING TO A LAKE | 10.250 | <0.005 |
| GOING TO A POOL | 5.7600 | <0.025 |
| GOING TO THE OCEAN | 4.2667 | <0.05 |
| GOING TO A RIVER | .9412 | >0.25 |
| GOING TO IRRIGATION CHANNEL | .5714 | >0.25 |
| SWIMMING ELSEWHERE | .1250 | >0.5 |

*ONE DEGREE OF FREEDOM.

TABLE 7

AGE AND SEX DISTRIBUTION AND FREQUENCY OF POSITIVE BILE CULTURES FOR SALMONELLA TYPHI AND PARATYPHI IN 1000 CONSECUTIVE PERSONS UNDERGOING CHOLECYSTECTOMY IN SANTIAGO, CHILE

| MALES | 25 | 25-34 | 35-44 | 45-55 | 55 | TOTAL |
|---|---|---|---|---|---|---|
| NO. OF CHOLECYSTECTOMIES (%) | 14 | 37 | 44 | 42 | 67 | 204 |
| NO. POSITIVE FOR: | | | | | | |
| S. TYPHI | 1 (7)* | 0 | 5 (11) | 0 | 0 | 6 (2.9) |
| S. PARATYPHI A | 1 | 1 | 1 | 2 | 1 | 5 |
| S. PARATYPHI B | 0 | 5 | 8 | 8 | 16 | 39 |
| FEMALES | | | | | | |
| NO. CHOLECYSTECTOMIES (%) | 144 | 185 | 195 | 116 | 156 | 796 |
| NO. POSITIVE FOR: | | | | | | |
| S. TYPHI | 8 (6)* | 8 (4) | 5 (3) | 5 (4) | 6 (3.8) | 32 (4.0) |
| S. PARATYPHI A | 0 | 2 | 0 | 0 | 1 | 3 |
| S. PARATYPHI B | 8 | 3 | 4 | 4 | 6 | 25 |

* % POS. OF NUMBER IN THAT AGE GROUP.

TABLE 8

OCCURRENCE OF Vi ANTIBODY* IN CHRONIC SALMONELLA TYPHI CARRIERS, ACUTE TYPHOID FEVER, AND HEALTHY POPULATIONS

| GROUP | N | GEOMETRIC MEAN TITER | PERCENT WITH RECIPROCAL TITER: <80 | ≥80 | ≥160 |
|---|---|---|---|---|---|
| CHRONIC S. TYPHI CARRIERS | 6 | 523 | 0 | 100 | 100 |
| | | p<0.01 | | | |
| CHILEAN ACUTE TYPHOID FEVER PATIENTS | 29 | 53 | 48 | 52 | 38 |
| | | p<0.01 | | | |
| CHILEANS WHO HAD ACUTE TYPHOID FEVER 12-14 MONTHS EARLIER (NON-CARRIERS) | 38 | 19 | 92 | 8 | 3 |
| HEALTHY CHILEAN ADULTS | 59 | 16 | 85 | 15 | 3 |
| HEALTHY U.S. MEDICAL STUDENTS | 12 | 12 | 73 | 7 | 7 |

*MEASURED BY PASSIVE HEMAGGLUTINATION USING HIGHLY PRUIFIED VI ANTIGEN.

TABLE 9

AN ESTIMATE OF THE NUMBER OF CHRONIC SALMONELLA TYPHI CARRIERS IN SANTIAGO, CHILE BASED ON THE PREVALENCE OF GALL BLADDER DISEASE IN THE POPULATION AND THE PREVALENCE OF CHRONIC S. TYPHI INFECTION IN PERSONS WITH CHOLECYSTITIS

| | Age Group (years) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 10-19 | 20-29 | 30-39 | 40-49 | 50-59 | 60-69 | 70-79 | ≥80 | Total |
| Estimated Female population in Santiago | 452,523 | 384,038 | 288,645 | 205,177 | 153,560 | 106,652 | 59,005 | 20,016 | 1,669,616 |
| % with Cholelithiasis based on Medical Legal Institute autopsy data | 9.7 | 23.4 | 43.1 | 51.7 | 60.0 | 69.2 | 69.2 | 55.5 | |
| No. with Cholelithiasis | 43,895 | 89,865 | 124,406 | 106,077 | 92,136 | 73,803 | 40,831 | 11,109 | 582,122 |
| Estimated S. typhi Carriers | 1,668 | 3,415 | 4,727 | 4,031 | 3,501 | 2,805 | 1,552 | 422 | 22,121 |
| Estimated Male Population in Santiago | 437,960 | 315,656 | 270,689 | 187,468 | 131,302 | 80,366 | 37,998 | 9,781 | 1,471,220 |
| % with Cholelithiasis | 0 | 4.5 | 13.4 | 16.7 | 19.8 | 24.7 | 43.5 | 40 | |
| No. with Cholelithiasis | 0 | 14,205 | 36,272 | 31,307 | 25,998 | 19,850 | 16,529 | 3,912 | 148,073 |
| No. estimated S. typhi Carriers | 0 | 540 | 1,378 | 1.190 | 988 | 754 | 628 | 149 | 5,627 |

TABLE 10

SUMMARY OF TREATMENT OF CHRONIC *SALMONELLA TYPHI* CARRIERS WITH 28 DAYS OF COMBINED ORAL AMOXICILLIN (2.0GM THREE TIMES DAILY) AND PROBENICID (0.5GM THREE TIMES DAILY).

| | | | | Cultures Pre-Therapy | | | Cultures Post-Therapy | | |
|---|---|---|---|---|---|---|---|---|---|
| Carrier | Age | Sex | Gallstones | Stool | Duodenal Fluid | Adverse Reactions During Therapy | Wks. Post-Rx | Stool | Duodenal Fluid |
| J.G. | 40 | M | - | + | + | Occasional mild diarrhea | 1 | - | - |
| | | | | | | | 5 | - | - |
| | | | | | | | 9 | - | - |
| | | | | | | | 14 | - | - |
| M.R. | 35 | F | Results pending | + | + | Occasional mild diarrhea | 1 | - | - |
| | | | | | | | 4 | - | - |
| | | | | | | | 12 | - | - |
| M.C. | 37 | F | Results pending | + | N.T.* | Rash on days 19-23 | 3 | - | - |

*NOT TESTED

NUMBER AND PERCENTAGE OF CHILEAN SCHOOL CHILDREN HAVING A SEROCONVERSION BY SALMONELLA TYPHI O ANTIBODY BY WIDAL AND BY IgG ELISA AND MEAN NET OPTICAL DENSITY WITH SEROCONVERSIONS, AFTER INGESTION OF THREE $10^9$ ORGANISM DOSES OF TY21A *S. TYPHI* VACCINE IN TWO DIFFERENT FORMULATIONS

| FORMULATION | NO. CHILDREN VACCINATED | NO. (%) SEROCONVERSIONS | | MEAN (SEM) NET OPTICAL DENSITY* WITH SEROCONVERSIONS |
|---|---|---|---|---|
| | | WIDAL | ELISA | |
| ENTERIC-COATED CAPSULES | 168 | 8 (5%) | 69 (41%) | 0.38 (0.02) |
| VACCINE RECONSTITUTED IN MILK WITH BICARBONATE | 167 | 8 (5%) | 70 (42%) | 0.37 (0.01) |

*NET OPTICAL DENSITY AT 400NM IS A DIRECT MEASURE OF IgG ELISA *S. TYPHI* O ANTIBODY.

TABLE 12

NUMBER AND PERCENTAGE OF CHILEAN SCHOOLCHILDREN HAVING A SEROCONVERSION BY SALMONELLA TYPHI O ANTIBODY BY IgG AND IgM ELISA AFTER INGESTION OF THREE $10^9$ ORGANISM DOSES OF Ty21a S. TYPHI VACCINE IN TWO DIFFERENT FORMULATIONS

| FORMULATION | NO. CHILDREN VACCINATED | NO. (%) SEROCONVERSIONS | | TOTAL NO. (%) SEROCONVERSIONS |
|---|---|---|---|---|
| | | IgG | IgM | |
| ENTERIC-COATED CAPSULES | 168 | 69 (41%) | 20 (12%) | 89 (53%) |
| VACCINE RECONSTITUTED IN MILK WITH BICARBONATE | 167 | 70 (42%) | 26 (16%) | 96 (57%) |

TABLE 13

NUMBER AND PERCENTAGE OF U.S. ADULTS HAVING A SEROCONVERSION IN IgG ELISA *SALMONELLA TYPHI* O ANTIBODY AFTER INGESTION OF THREE $10^9$ DOSES OF Ty21a *S. TYPHI* VACCINE IN TWO DIFFERENT FORMULATIONS

| FORMULATION | No. ADULTS VACCINATED | No. (%) SEROCONVERSIONS |
|---|---|---|
| THREE DOSE ENTERIC-COATED CAPSULES | 16 | 3 (19%) |
| THREE DOSE GELATIN/$NaHCO_3$ CAPSULES* | 15 | 3 (20%) |

*GELATIN CAPSULE CONTAINING VACCINE TAKEN AFTER 2 CAPSULES WITH 0.4 G $NaHCO_3$ EACH

TABLE 14

NUMBER AND PERCENTAGE OF U.S. ADULTS HAVING A SEROCONVERSION IN IgM ELISA *SALMONELLA TYPHI* O ANTIBODY AND MEAN NET OPTICAL DENSITY WITH SEROCONVERSIONS AFTER INGESTION OF ONE OR THREE $10^9$ ORGANISM DOSES OF Ty21a *S. TYPHI* VACCINE IN TWO DIFFERENT FORMULATIONS

| Dose and Formulation | No. Adults Vaccinated | No. (%) Seroconversions | Mean (SEM) Net Optical Density* With Seroconversions |
|---|---|---|---|
| Three dose enteric-coated capsules | 16 | 4 (25%) | 0.22 (0.03) |
| Three dose gelatin/$NaHCO_3$ capsules† | 15 | 3 (20%) | 0.41 (0.16) |
| One dose enteric-coated capsules | 21 | 2 (10%) | 0.26 (0.10) |
| One dose gelatin/$NaHCO_3$ capsules† | 29 | 7 (24%) | 0.34 (0.19) |

*Net optical density at 400nm is a direct measure of IgM ELISA *S. TYPHI* O antibody

†Gelatin capsule containing vaccine taken after 2 capsules with 0.4 g $NaHCO_3$ each

# APPENDIX A1

**REPUBLICA DE CHILE**
MINISTERIO DE SALUD

SANTIAGO, 6 de enero de 1982.

Dr.
Myron M. Levine
Director
Center for Vaccine Development
University of Maryland School
of Medicine
Presente.

Estimado Dr. Levine:

Este Ministerio tiene el agrado de comunicar a Ud. su decisión de efectuar la prueba de campo con la vacuna antitífica oral Cepa TY-21 A, durante 1982. De acuerdo con el cronograma establecido en la reunión de fecha 6 de enero de 1982 en Santiago con Usted y sus colaboradores, estas actividades comenzarán en febrero próximo, y culminarán en mayo de 1982 con la vacunación de a lo menos de 80.000 niños escolares en el área norte de Santiago.

Como es de su conocimiento esta experiencia debió ser postergada, porque este Ministerio estimó necesario efectuar previamente exámenes bactereológicos de inocuidad y potencia de la vacuna en el Instituto de Salud Pública de Chile, los que se terminaron en noviembre de 1981, cuando el año escolar había terminado, y que confirmaron la inacuidad y potencia de este producto - biológico.

Finalmente, quisieramos reiterar a Ud. el interés de este Ministerio, para que en forma mancomunada efectuemos con éxito esta importante experiencia que contribuirá al control - de la fiebre tifoídea en nuestro país y en el mundo entero.

Saluda atentamente a Ud.,

HERNAN BUCHI BUC
MINISTRO DE SALUD.
SUBROGANTE.

# APPENDIX A2

Santiago, January 6, 1982

Dear Dr. Levine:

This Ministry has the pleasure of informing you of its decision to carry out the field study of oral attenuated Ty21a vaccine in 1982. In accord with the timetable decided upon in the meeting held on January 6, 1982 in Santiago with you and your collaborators, these activities will begin in February and will culminate in May 1982 with the vaccination of at least 80,000 schoolchildren in the north section of Santiago.

As you know, this field trial was delayed because this Ministry believed it necessary to carry out beforehand bacteriological tests of the vaccine's safety and efficacy at the Institute of Public Health of Chile, and the tests were not completed until November 1981 when the school year had ended; the safety and efficacy of this biological product were confirmed.

Finally, we would like to reiterate the intention of this Ministry to collaborate in carrying out successfully this important field trial that will contribute to the control of typhoid fever in our country and in the entire world.

Sincerely,

Hernan Buchi Buc
Subsecretary of Health

# APPENDIX B1

## ENSAYO EN EL CAMPO DE LA VACUNA TIFICA; CEPA ATENUADA DE S. TYPHI USADA COMO VACUNA ORAL.

| Fecha | Tarea | Responsable |
|---|---|---|
| 1-5 febrero/82 | Comunicar a Seremi Reg. Metropolitana y Director SSM Norte la realización de la vacunación. | Subsecretario |
| 1-5- febr.82 | Reunión entre autoridades del Ministerio de Salud y Ministerio de Educación<br>a) Comunicar al M. de Educación la iniciación el Programa de Vacunación.<br>b) Solicitar cooperación.<br>c) Comunicar la necesidad de obtener la aprobación de los padres a través de una comunicación en la libreta del alumno.<br>d) Plantear la necesidad de realizar una reunión con los directores de la Escuela del Area Norte.<br>e) Pedir apoyo del M. de Educación para informar a los profesores de las Escuelas del Area Norte respecto a sus responsabilidades en este programa:<br>1. Asegurar que los escolares escriban en su libreta de comunicaciones el mensaje a los padres:<br>- Preparar la planilla de su curso, la cual deberá incluir los nombres de los alumnos y la aprobación de los padres.<br>- Colaborar en la administración de la vacuna oral.<br>f) Solicitar lista de las escuelas del Area Norte con su dirección y el nombre y número de teléfono de los directores de las escuelas. | Jefe Ofic.Planif. y Presupuesto. |
| 22-26 febr.82 | Reunión entre los directores de las Escuelas del Area Norte y responsables del M. de Salud.<br>En esta reunión:<br>- Se explicará la campaña y la responsabilidad de los profesores.<br>- Se distribuirá los mensajes para los padres y apoderados.<br>- Se distribuirá las planillas que deberán ser llenadas por los profesores. | Jefe Depto. Apoyo Programas, Dr. C. Ristori, Dra. P.Vicent, Dr. A.Morales y profesionales del SSM Norte. |

| Fecha | Tarea | Responsable |
|---|---|---|
| 15-21 febr. 82 | Notificar al personal de los consultorios la realización de la campaña. | Profesionales del Depto. Apoyo Programas y Dr. A.Morales. |
| 1-5 marz. 82 | Reunión de cada director de escuela con sus profesores para explicar las responsabilidades y distribuir mensajes y planillas. | Directores de las escuelas. |
| 1-5 marz. 82 | Comunicación masiva a la prensa para informar sobre la campaña. | M. de Salud |
| 8 marzo 82 | 1°Envío de mensaje a los padres y apoderados. | Profesores |
| | 2°Preparar planilla e indicar si los padres aceptaron o no. | Profesores. |
| 15-26 marz.82 | Visita a las escuelas por las auxiliares con el fin de: | Aux. Area Norte |
| | • Revisar las planillas y modificar las incorrectas o incompletas. | |
| | •• Preparar un resumen de cada escuela indicando por curso el número total de niños que se vacunarán y los que rechazaron la vacuna. | |
| | ••• Entrega del resumen a la enfermera jefe del Consultorio respectivo. | |
| 22-26 marz.82 | Recolección de los resumenes de los consultorios para su revisión y análisis en el M. de Salud. | Sra. I. Cánepa y Sra. V. Sotomayor. |
| 29-Marz.-2-Abr. | - Cálculo del número de escolares participantes. | Depto. Apoyo Programas y P. A. Morales |
| | - Decisión del diseño de ensayo:<br>• Si 105 mil o más escolares participan se puede comparar la eficaciá de una dosis y 2 dosis con vacuna con placebo. | |
| | •• Si menos de 105 mil niños participan se puede comparar solamente 2 grupos. Uno Vacunado con una sola dosis de vacuna y otro inoculado con placebo. | |
| | - Preparación de listas y asignación de cada clase a un grupo en relación con el siguiente diseño de ensayo:<br>Si comparamos una dosis, dos dosis y placebo vamos a tener 3 grupos | |

| :ha | Tarea | Responsables. |
|---|---|---|

Vacunados dos dosis con formulación A ó B.

Dosis Una y Dosis Dos

| | | |
|---|---|---|
| A. | A. | Una formulación contiene va- |
| A. | B. | cuna y la otra placebo. |
| B. | B. | |

Sin embargo para asegurar que realizamos las cifras correctas de escolares en cada grupo, necesitamos administrar cápsulas de una manera tal que por cada cien cursos el 33% van a rec ibir AA., otro 33% BB y el otro 33% AB.

**Si se usa un diseño donde se compara una sola dosis con placebo se tendrá dos grupos de escolares que recibirán formulación A ó B en números más o menos iguales.

| | | |
|---|---|---|
| } abril 82 | Randomización de las clases de acuerdo con las guías mencionadas anteriormente. | Dr. R. Black. |
| -16 Abr.82 | 1) Preparar y marcar las bolsas con las cápsulas respectivas. (7 mil bolsas para los cursos y 440 para las ecuelas). | Dr. R. Black. Sra. I. Cánepa y Sra. V. Sotomayor. |
| | 2) Preparar de cronogramas específicos por consultorios, indicando fecha y horario de vacunación por cada escuela. | |
| -30 abr.82 | Entrega de las bolsas a los consultorios respectivos. | |
| mayo 82. a | Administración de las cápsulas a los niños. Las tareas serán las siguientes: | |
| -junio 82 | - Cada enfermera jefe entregará las bolsas a las auxiliares. | |
| | - Cada auxiliar irá a sus escuelas asignadas empleando transportes proporcionado por el Ministerio. (13 vebículos). | |
| | - Las auxiliares entregarán las bolsas a cada profesor y realizarán una demostración sobre el modo como cada escolar debe ingerir la cápsula. | |
| | - Cada profesor deberá administrar las cápsulas a sus alumnos y deberá registrarlo en la planilla. | |
| | - Las auxiliares revisarán las planillas de cada curso. | |

Una semana después se repetirá la administración de las cápsulas a los escolares (si se hace un estudio comparado, una dosis, dos dosis y placebo).

# APPENDIX B2

## TIMETABLE

## FIELD TRIAL OF TY21a STRAIN ATTENUATED

## S. TYPHI ORAL VACCINE

| te | Task | Responsible Ministry Personnel |
|---|---|---|
| ɔ. 1-5, 1982 | To communicate to the senior staff and Director of SSM Norte Santiago Metropolitan Health Region that the field trial will be carried out. | Subsecretary |
| b. 1-5 | Meeting between authorities of the Ministry of Health and the Ministry of Education.<br><br>a) To communicate to the Ministry of Education that the field trial will be proceeding.<br>b) To solicit the cooperation of the Ministry of Education.<br>c) To explain to the Ministry of Education the necessity to utilize the school-children's "libretas de comunicacion" (school notebooks) to send the message to parents to solicit informed consent.<br>d) To explain the necessity of arranging meetings with the directors of schools in Area Norte.<br>e) To request assistance of the Ministry of Education in informing the teachers of Area Norte with respect to their responsibilities in this program which include:<br>1) To assure that the message to the parents to solicit informed consent is put into the children's libretas.<br>2) To prepare a line list of each class which will include the names of the children and those whose parents gave consent.<br>3) Assistance in administering the oral vaccine.<br>f) To solicit an up to date list of all the schools in Area Norte, then addresses and the names of school directors. | Chief of the Office of Planning and Budget |
| ɔb. 22-26, )82 | Meeting between the directors of schools in Area Norte and Ministry of Health personnel.<br>In this meeting:<br>- The field trial will be described to the teachers and their responsibilities will be outlined.<br>- The messages and information for | Chief of the Dept. of Program Support (Epidemiology). Drs. C. Ristori, P. Vincent, A. Morales and Area Norte staff. |

| Date | Task | Responsible Ministry Personnel |
|---|---|---|
| | the parents will be distributed.<br>- The blank forms for the line lists to be prepared by the teachers will be distributed (one list per class). | |
| Feb. 15-21 | Notification of the personnel of the consultorios (health centers) of Area Norte that the field trial will be proceeding. | as above. |
| March 1-5 | Meetings between each school director and his teachers to explain the teachers responsibilities and to distribute to the teachers the messages for the parents and the blank line list forms. | School directors. |
| March 1-5 | Mass media and press publicity to explain the field trial in an effort to enhance the rate of informed consent. | Min. of Health |
| March 8 | 1) Sending of information and messages to the parents to solicit consent.<br>2) Preparation of line lists for each class indicating which children's parents have given consent and which have rejected participation. | Teachers<br>Teachers |
| March 15-26 | A visit to the schools by health auxiliaries with the objectives of:<br>- Reviewing the line lists to assure completeness and correctness.<br>- Preparing a summary for each school indicating for each class the total number of children who will be participating in the field trial.<br>- Delivery of the school summaries to the Head Nurse in the appropriate Consultorio. | Auxiliaries of Area Norte |
| March 22-26 | Collation, review and analysis of the summaries. | Ms. I. Canepa and V. Sotomayor |
| March 29 - April 2 | - Calculation of the precise number of participating schoolchildren.<br>- Decision regarding field trial design:<br>i) If 105,000 or more children participate we can compare the efficacy of one dose with two doses of vaccine versus placebo.<br>ii) If less than 105,000 children participate we can compare only | Dept. of Program Support.<br>Dr. A. Morales. |

| Date | Task | Responsible Ministry Personnel |
|---|---|---|
| | two groups (one given one dose of vaccine, the other placebo).<br>- Preparation of lists and assignment of each class to a group according to the following study designs:<br>i) If we compare one dose, two doses and placebo we will have a three cell trial.<br>Two coded formulations exist labelled A or B. One is vaccine, the other placebo and their identity is not known to us.<br><br>In a three cell trial, classes will receive:<br>First Dose — Second Dose<br>A — A<br>or<br>A — B<br>or<br>B — B<br>Randomization will proceed according to a formula such that approximately 33% of schoolchildren will receive one dose, 33% two doses and 33% placebo.<br>ii) If we use a two cell design where we compare one dose versus placebo, children will receive a single dose of A or B. | |
| April 5-9 | Randomization of the classes according to the above-mentioned guides. | Dr. R. Black |
| April 12-16 | 1) Careful preparation and labelling of bags containing capsules. (7000 bags for separate classes and 440 for schools if we have a three cell trial).<br>2) Preparation of specific timetables for each Consultorio indicating the date and time that each school in that Consultorio's district will be vaccinated. | Dr. R. Black, MS. I. Canepa, V. Sotomayor |
| April 15-30 | Delivery of the bags with the capsules to each consultorio. | |
| May 3 to June 18 | Administration of capsules to the children. Specific tasks will include:<br>i) Each Consultorio Head Nurse will deliver bags to the vaccination auxiliaries.<br>ii) Each auxiliary will go to the schools using vehicles assigned and provided by the Ministry of Health (13 vehicles).<br>iii) The auxiliaries will deliver the bags of capsules to each teacher and will | |

| Date | Task | Pesponsible Ministry Personnel |
|---|---|---|
| | demonstrate to the class how the child must swallow the capsule.<br>iv) Each teacher will supervise that the child takes the capsule and will make note of it in the line list.<br>v) The auxiliaries will review the line lists of each class.<br>One week after administration of the first dose, these procedures will be repeated to administer the second dose (if we carry out a threc cell trial). | |