Summary of an International Workshop on Typhoid Fever

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On November 29 and 30, 1984, a workshop on typhoid fever was held at the Pan American Health Organization in Washington, D.C. The workshop was sponsored by the Microbiology and Infectious Diseases Program of the National Institute of Allergy and Infectious Diseases (NIAID) and the Fogarty International Center of the National Institutes of Health (NIH), Bethesda, Maryland, and the Pan American Health Organization (PAHO). Thirty-one investigators from 13 countries discussed the epidemiology and clinical presentation, diagnosis and bacteriology, pathogenesis, therapy, immunology, and immunoprophylaxis of typhoid fever.

An extended international workshop on typhoid fever had never before been sponsored by the NIH or PAHO or, insofar as we are aware, by any other agency within recent memory. Because typhoid fever continues to be a serious public health problem in developing countries, the time was considered propitious to reexamine old “truths” and to discuss and critique the sizable amount of new information about typhoid fever. The workshop was designed to focus in particular on potential typhoid vaccines and to identify other promising areas for research.

Historical Overview of Typhoid Fever

The workshop was opened by Dr. Robert Edelman, NIAID Enteric Diseases Program Officer and Workshop Chairman, and by Dr. Ronald St. John, Coordinator, Epidemiology Unit, PAHO. Dr. Edelman provided a brief historical overview of typhoid fever.

In 1829, Dr. P. Ch. A. Louis in Paris described typhoid, clearly separating it from other fevers, and associated the clinical expression of the infection with the essential pathologic lesions in the intestines, mesenteric lymph nodes, and spleen. Dr. Louis discussed rose spots, intestinal perforation, and hemorrhage [1]. It can be argued that since 1829 no important clinical or gross pathologic facts have been added. Also, about this time, Drs. M. Bretonneau in France and N. Smith in the United States recognized the spread of the disease by contagion and insisted that immunity was conferred by an attack of the illness. William Budd, an Englishman, wrote his epidemiologic masterpiece in 1873, providing evidence that bowel discharges were the main source of infection; that the disease was waterborne; that milk, food, contaminated linen, and other fomites were sources of dissemination; and insisted without direct proof that a specific germ caused typhoid and deduced its capacity for multiplication [2]. In 1884, Gaffkey, a German, first cultivated and isolated Salmonella typhi in pure culture from the spleens of infected patients. There shortly followed in close order the cultivation of the organism from stool, urine, rose spots, and gall bladder by other German investigators.

Pfeiffer and Kalle in 1896 made the first vaccine for human use against typhoid with heat-killed organisms and demonstrated the development of pro-
tective antibodies that passively protected guinea pigs against experimental infection. In that year Gruber, Durham, and Widal each independently reported that convalescent-phase serum mixed with S. typhi caused the organisms to "stick together in large balls and lose their motility." Thus was born the term agglutinins and the classic serologic test for infection by S. typhi. In 1903 Robert Koch outlined three logical methods of typhoid control: disinfect the excreta at its source, improve sewage handling, and isolate convalescent patients until they become bacillus-free. In 1909 an American, Warren Coleman, established by accurate scientific methods the usefulness of high caloric, high protein diets to shorten the hitherto prolonged period of convalescence and to minimize the debilitation in typhoid. However, case fatality continued to range up to 30% until Theodore Woodward and his colleagues announced in 1948 that chloromycetin sterilized the blood cultures of 10 Malaysian typhoid patients [3], thus ushering in the modern era of antibiotics for treatment of typhoid fever.

**Epidemiology and Clinical Presentation**

Dr. Dhiman Barua (World Health Organization [WHO], Geneva) reminded us that typhoid fever continues to be a global health problem. Although one-quarter of the world’s population live in developed areas where the likelihood of acquiring the infection is extremely low, imported cases from the endemic regions continue to cause problems in non-endemic regions. Reliable systems of reporting that exist in most of the developed world indicate that in western Europe, Japan, and the United States, the annual incidence of typhoid fever has decreased to about 0.24-3.7 cases per 100,000 population and to about 4.3-14.5 cases per 100,000 in southern Europe.

No such data are available from the developing countries. In order to make an estimate of the typhoid problem in those countries, Dr. Barua used data on the incidence of typhoid fever in the control cohorts of recent typhoid vaccine trials in India, Egypt, and Chile. He also used data collected in Indonesia by active surveillance of bacteriologically confirmed cases, and for other countries, he employed a factor proportionally derived from surveys of diarrhea morbidity and mortality by the WHO Diarrhoeal Diseases Control Programme.

Using these estimates and 1980 census data, Dr. Barua estimated that 6.98 million cases occur per year in south and east Asia (population, 1.369 million), 749 thousand in west Asia (population, 98 million), 4.36 million in Africa (population, 427 million), 15 thousand in Egypt (population, 43 million), 406 thousand in Latin America and the South Pacific islands (population, 369 million), and 23,000 in the developed world (population, 1,131 million). By adding up these figures from different regions, he arrived at an annual estimate of approximately 12.5 million cases in the world (excluding China), an incidence of 365 cases per 100,000 of the total population (excluding China) and 540 cases per 100,000 (0.5%) of the population of the developing world. Although crude and liable to different interpretation, these figures represent the first and only attempt to quantitate the extent of typhoid fever throughout the contemporary world.

Dr. Paul A. Blake (Centers for Disease Control, Atlanta) reviewed typhoid fever outbreaks, importations, and laboratory-derived cases in the United States. Information about typhoid fever in the United States is available from three major sources. One source is the number of cases of typhoid reported by the states to the Centers for Disease Control. These data show that the incidence of typhoid fever in the United States decreased from about one case per 100,000 population in 1955 to about 0.2 cases per 100,000 in 1966 and has remained fairly stable at that level since then. The second source of information is the typhoid case-report form, which has been filled out by state and local governments since 1975. These forms have been completed for about half of the reported cases since 1975 and are the source of almost all of the detailed data presented below. The third source of data is a retrospective study by Rice et al. [4] of the cases of typhoid fever in the United States between 1967 and 1972.

Mortality from typhoid fever in the United States has been fairly low, with 1.3% of cases ending in death. The case-fatality rate has been <0.5% until age 40 years, increasing to 29% in persons ≥80 years of age. Antimicrobial resistance has been a minor problem, with only about 3% of strains resistant to chloramphenicol and 2.3% resistant to ampicillin. Relatively few strains were tested for resistance to trimethoprim-sulfamethoxazole, but of these about 7% were resistant.

Large outbreaks of typhoid fever are very unusual in the United States; only four outbreaks have involved more than 15 persons since 1973. None of these outbreaks occurred in populations that were clearly at high risk, and thus none were candidates
for typhoid vaccine. Laboratory workers are theoretically at relatively high risk for typhoid. Surveillance in the United States detected only 20 cases of typhoid fever acquired by laboratory workers in the United States during 1975–1984, but this clearly represents incomplete ascertainment. Blaser et al. conducted an intensive search for laboratory-associated cases occurring during a 33-month period beginning January 1977 and found 24 such cases [5]. Of interest, five of the 24 had current S. typhi immunization, and the severity of illness and the median incubation period (14 days) was about the same for vaccinated and unvaccinated patients. Only three of the 24 infections were caused by clinical isolates; the remainder resulted from proficiency tests (11 cases) and stock strains (10 cases). A variety of exposures were involved, but at least five of the 24 patients had not worked directly with the organisms; they were merely present in a laboratory while others were studying S. typhi. The risk of laboratory-acquired typhoid fever is hard to measure because the number of persons at risk is unknown; in three national proficiency programs, there was one case of typhoid fever for every 653 laboratories receiving S. typhi.

During 1967–1972, 33% of the cases of typhoid fever in the United States were imported. Review of the case-report forms for 2,468 cases of acute typhoid fever that occurred in the United States between 1975 and September 1984 showed that 58% of these were imported. The proportion of cases that are imported has varied from year to year, but the general trend has been upwards, with 68% and 74% of cases in 1983 and 1984, respectively, being imported.

During the decade 1975–1984, about 43% of the travel-associated cases occurred in persons who were not United States citizens. Forty-five percent of the imported cases in the United States came from Mexico, and 15%, from India. No other country was a prominent source. However, these data do not give us the risk of typhoid fever associated with travel to particular areas because data on numbers of travelers going to and from these areas are very difficult to obtain. Taylor et al. [6] were able to make some rough estimates of risk, although their data were imperfect because the denominators included only air travelers who were also United States residents. These data showed that the risks were highest for travelers to India and Pakistan, with >400 cases per one million travelers. Mexico, which accounted for almost half of the imported cases in the United States, presented a much smaller risk for the individual traveler, with 34 cases per one million travelers. The lowest rate was for northern Europe, with 0.6 cases per one million travelers.

In conclusion, in the United States a new typhoid vaccine is most likely to be useful in overseas travelers, especially those going to developing countries in Asia. It would be useful to have better denominator data on travelers so that better estimates of the risks associated with each country could be calculated. Among persons who do not travel, the vaccine would be most useful for workers in microbiology laboratories who work with S. typhi. When the efficacy and cost of the new vaccine are known, cost-benefit studies could be done to help develop recommendations.

Dr. Myron M. Levine (University of Maryland, Baltimore) described epidemiologic patterns of endemic typhoid fever and the usefulness of seroepidemiology. A seroepidemiologic tool is necessary to determine the prevalence of S. typhi infection in geographic areas where notification data for typhoid fever are not based on bacteriologic confirmation and where other infections exist (e.g., typhus, malaria) that may clinically resemble typhoid. Following clinical and subclinical infection with S. typhi, most persons develop significant rises in antibody titer to the flagellar H antigen (d). This antibody resides in the IgG class and is long lived. While approximately 70 other Salmonella bioserotypes in addition to S. typhi possess the d flagellar H antigen, these others are rarely associated with human infection. Since vaccination with parenteral killed whole cell typhoid vaccine also stimulates the appearance of flagellar antibodies, this serology is not useful in areas where parenteral vaccine is commonly used.

The serologic assay employs whole killed Salmonella virgini a as a source of flagellar antigen (this strain shares no O antigens with S. typhi) and agglutinations are carried out in tubes. An H antibody titer >1:40 was found in 38 (90%) of 42 Mexican patients <19 years of age with bacteriologically confirmed acute typhoid fever. In contrast, only one of 124 (0.8%) healthy residents of Baltimore <19 years of age was seropositive with titers >1:40. However, in areas of Peru where notifications revealed a high incidence of typhoid fever, H antibody titers >1:40 were common in healthy adolescents; 32% of 209 10 to 14 year olds and 80% of 102 healthy 15 to 19 year olds had H titers >1:40. H titers of 1:160 to 1:1,280 were not uncommon.
In Chile notification data in the 1970s suggested that typhoid fever was endemic in all socioeconomic segments of the population in Santiago, where seroprevalence data were high, whereas typhoid fever was rarely seen in the cool, wet, southern lakes region of the country, where the seroprevalence was low. Thus, measurement of *S. typhi* H antibody by the tube dilution method in populations where parenteral vaccine is not commonly used represents a rapid, simple, and practical tool for seroepidemiology.

Dr. Narain H. Punjabi (U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia) was asked if the clinical severity of typhoid fever was increasing in the developing world. In his thoughtful paper he replied that the question is unanswerable. The reported case-fatality ratios, ranging from 10% to 37% in the preantibiotic era [7], appeared to decrease to 1%–12% in the years after introduction of chloramphenicol [8]. However, in areas of some countries (Indonesia, India, Nigeria) case-fatality ratios of 9%–32% have been reported in the past 10–15 years [9]. Unfortunately, available clinical data are non-comparable over time and from place to place because of inconsistencies in case definitions and differences in methods of case counting, laboratory expertise, populations studied, and definitions of complications such as intestinal perforation, myocarditis, pneumonia, and central nervous system manifestations. The countries that report increased typhoid severity share several characteristics: (1) rapidly increasing populations, (2) rapidly increasing urbanization, (3) inadequate facilities for processing human wastes, (4) decreasing water supply per capita, (5) intimate contact between humans, food, and heavily contaminated water supplies, and (6) overburdened health care delivery systems [10]. It is likely that these factors have led to increased numbers of people coming into contact with larger inocula of *S. typhi*; this in turn has probably led to an absolute increase in number of cases of typhoid fever, with perhaps an increase in the incidence and an increase in the prevalence and percentage of severe typhoid cases. Incomplete antimicrobial therapy for typhoid has probably aggravated the situation.

Recent studies from Indonesia indicate that in some areas of the country, the incidence of typhoid fever is 10 cases per 1,000 population per year and that typhoid fever is among the five major causes of death. Typhoid kills young adults at the beginning of their productive years; these people frequently have several young children, so here the socioeconomic impact of the disease is magnified. A reliable estimate of the typhoid mortality rate is not yet available.

According to Dr. Punjabi the question that must be answered is not whether the clinical severity is increasing, but, rather, how much severe disease exists and whether there are differences in the severity of the disease in different parts of the world. These questions can only be answered by carefully designed, population-based prospective studies that place great emphasis on case definition, methods of case counting, and recognition of severe and fatal cases. Only when this information is available will we be able to assess the true impact of typhoid fever on the health of communities throughout the world and make rational decisions as to what resources must be allocated for the control of the disease.

Dr. Catterine Ferreccio (Ministry of Health, Santiago, Chile) reported on her studies of typhoid fever in children younger than two years of age in Chile. Because few cases of typhoid fever are reported in children under two years of age, Dr. Ferreccio thought it necessary to determine whether young children do not consume the vehicles that transmit *S. typhi* to older children or whether infection occurs but the infant host manifests an atypical illness not recognized clinically as typhoid. To assist in resolving this question, Dr. Ferreccio and colleagues systematically performed blood cultures for children under two years of age with fever who were seen at health centers in Santiago during the three peak months of the typhoid fever season in 1983 and 1984. By the end of March 1984, blood from 648 infants younger than 24 months old had been cultured.

Acute respiratory infection in 43%, diarrhea in 18%, and viral syndrome in 15% were the commonest 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 performed for any infant.

*S. typhi* was isolated from seven children (1.1%), *Salmonella paratyphi* B, from five (0.8%), and *S. paratyphi* A, from one infant (0.1%). No other blood cultures contained pathogenic bacteria. The clinical syndrome in these 13 infants was mild, consisting of one to five days of fever of <38.8°C and the presence in some patients of cough, pneumonitis, diarrhea, or vomiting. No infants had a rash or splenomegaly. Upon follow-up it was found that the
mothers had spontaneously discontinued the medication after one or two days because the infants appeared well. In each instance the infection resolved without complications. The Widal test was performed on convalescent-phase sera from nine of these young children with benign S. typhi and Salmonella paratyphi bacteremia; all but one serologic test were negative for antibodies against S. typhi and S. paratyphi A and B. Thus, it seems that infants in a hyperendemic area of Chile are infected more commonly than is appreciated and manifest a mild, bacteremic illness not recognized as enteric fever.

Dr. Claudio F. Lanata (Instituto de Investigación Nutricional, Lima, Peru) spoke on the role of chronic typhoid carriers in Santiago, Chile [11], and the diagnostic methods he and his colleagues used to identify such carriers. Typhoid fever is highly endemic in Santiago. Chile also has one of the highest prevalences of cholelithiasis in the world. The combination of these two factors has led Dr. Lanata and colleagues to assess the prevalence of chronic S. typhi carriers in Santiago. They used a reliable census, the data available from a large necropsy survey done in about 2,000 persons—most of whom died from accidental trauma—that showed the prevalence of cholelithiasis in Santiago and a recent report of the prevalence of S. typhi in bile from 1,000 persons undergoing cholecystectomy in seven major Santiago hospitals. Calculations reveal that in 1980 there were approximately 25,019 female chronic carriers and 4,575 male chronic carriers, a total of 29,594 chronic S. typhi carriers in Santiago, yielding a crude rate of 694 carriers per 100,000 population. The way in which these large numbers of chronic carriers transmit S. typhi to a susceptible population was the focus of Dr. Sears’ presentation, summarized elsewhere in this report.

The detection of chronic carriers of S. typhi is not easy. The traditional method, which has been the collection of a series of daily stool cultures, is expensive and only intermittently positive because S. typhi is inhibited by the normal colonic flora. One bile sample obtained by duodenal string with pH >6 and stained yellow was as sensitive in recovering S. typhi as three stool cultures.

To determine whether Vi serology might be useful to detect carriers, Dr. Lanata and colleagues compared stool and bile culture results with Vi antibody titers in the same Santiago residents. Dr. Lanata measured Vi antibody by a passive hemagglutination assay [12], utilizing a highly purified Vi antigen obtained from Citrobacter freundii provided by Dr. J. B. Robbins.

From 569 women who participated in the screening, 36 (6.3%) were chronic S. typhi carriers detected by culture. The geometric mean Vi antibody titer among the 36 carriers was 1:296, compared with 1:53 in 29 patients with documented acute typhoid fever, 1:21 in 388 women with past history of typhoid fever who were not carriers, and 1:16 among 59 healthy adult Chileans selected by a random sample. These differences were highly significant statistically. As expected, the sensitivity curve decreased with an increment of the screening Vi antibody titer, whereas the specificity increased [12]. Dr. Lanata concluded that the passive hemagglutination assay used to measure the Vi antibody level is very sensitive and specific for the screening of chronic carriers. This screening assay is practical and technologically appropriate for populations where typhoid fever is endemic.

Dr. J. Glenn Morris, Jr. (University of Maryland, Baltimore) and associates conducted family studies in Chile in an attempt to better understand typhoid transmission patterns [13]. They were interested in determining whether chronic carriers were present in the households where there were children with typhoid, how frequently concurrent and secondary cases occurred within households, and whether risk factors could be identified for persons with typhoid as compared with uninfected household members.

They collected data from 24 families having a family member younger than 16 years with culture-confirmed typhoid fever; complete clinical, laboratory, and epidemiologic data were available for 155 of the 161 family members in these 24 families. A chronic carrier was identified in only one household; two households had concurrent or secondary cases. Index cases were significantly more likely to eat outside of the household at least once per week than were other age-matched household members. Unexpectedly, they found that 17 of the 155 household members, from 13 different households, were culture-positive for nontyphoidal Salmonella or Shigella. These cases were distributed throughout all age groups, with no geographic clustering of cases and no household having more than one household member with the same species or serotype of Salmonella or Shigella.

These data suggest that chronic carriers within the household do not play an important role in transmission of typhoid in Santiago. Also, the inability to identify additional household cases suggests that
transmission within households, including acquisition of \textit{S. typhi} by consumption of a common vehicle, is not a frequent occurrence. The high isolation rate for nontyphoidal \textit{Salmonella} and \textit{Shigella} indicates that persons in the study population have a high general level of exposure to enteric pathogens; the failure to identify more than one person with the same species or serotype of either organism in any of the households again suggests that infections are acquired away from the home. Taken together, these data emphasize the importance of events occurring outside of the household in the epidemiology of typhoid (and other enteric pathogens) in Santiago.

Dr. Stephen D. Sears (University of Maryland, Baltimore) reported on his environmental bacteriology studies in Chile and their relevance for epidemiologic control of typhoid fever \cite{14}. Because Dr. Morris showed that most cases of typhoid fever were not associated with a chronic carrier in the home, Dr. Sears and his colleagues decided to look more closely at the water and sewerage system in Santiago. They learned that although three-fourths of households are 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 Zanjon 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). As the Zanjon and the Mapocho River reach the western most 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 that 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 epidemiologic observations: first, the striking seasonality of typhoid (irrigation is used in the summer when there are no rains); second, the low reported incidence in young children (raw vegetables are not an important food item for infants and toddlers); third, the high incidence of typhoid fever in high socioeconomic neighborhoods of Santiago (where salads are eaten in restaurants and at home); and fourth, the low incidence of typhoid fever in the lakes region of Chile (because of year round rains in this region, irrigation is not used).

Prior to 1983, Chilean microbiologists carried out, unsuccessfully, a series of environmental microbiology studies designed to isolate \textit{S. typhi} from water of the Zanjon and the Mapocho River and from vegetables irrigated with untreated waste water. Dr. Sears initiated new environmental bacteriologic studies using Moore swabs to collect and concentrate samples. Moore swabs are thick wads of cotton gauze left as filters in the flowing waste water for two to three days. Although none of the 17 swabs placed in industrial areas yielded \textit{S. typhi}, eight of 76 swabs placed in agricultural areas contained \textit{S. typhi} (four of the 31 swabs from the Zanjon de la Aguada and four of 45 from the Mapocho River). Five of the eight isolates were phage types E1 and 46, the two commonest disease-causing types in Chile, one strain was untypable, and the other two were N and M1.

Because the sensitivity of the Moore swab is inversely related to the size of the waterway sampled, the isolation rate of 11\% from these large waterways is probably an underestimate. \textit{S. typhi} is fastidious, easily inhibited by coliforms, and usually present in relatively small numbers in environmental samples. The Moore swab, by acting as a filter, improves the chance of isolating rare \textit{S. typhi} among millions of coliforms, and Dr. Sears has shown it to be a practical, reliable tool for isolating \textit{S. typhi} from irrigation water in endemic areas. Finding \textit{S. typhi} of the same phage types in irrigation water as those causing disease supports the hypothesis that contaminated vegetables in Santiago serve as important vehicles of transmission.

Dr. Robert E. Black (University of Maryland, Baltimore) conducted a matched-pair case-control study of typhoid fever in Chile from December 1980 through June 1981. His aim was to identify risk factors and vehicles of typhoid fever in the eastern area of Santiago, an area that includes families of all economic strata but consists largely of middle- and high-income persons living in modern housing. Typhoid fever in Chile has a marked seasonality, with a peak in the summer months, and the highest incidence occurs in children between the ages of eight and 13 years. Furthermore, typhoid fever seems to have a high incidence among both poor and wealthy children, even those who apparently live in nearly optimal sanitary conditions.

Cases were defined as occurring among children of either sex from three through 14 years of age living in the eastern area of Santiago who were diag-
nosed as having typhoid fever confirmed by blood and/or bone marrow cultures positive for *S. typhi*. Controls were children of the same sex and age, plus or minus one year, who lived in the same neighborhood as a child with a case and who were identified by following a preselected route that started at the home of the case.

Dr. Black's study questionnaire covered the following topics: socioeconomic level (including house construction and ownership; number of rooms, beds, and persons in the house; presence of electricity or refrigerator in the house; and ownership of a car); sanitary conditions at home (including water source and bathroom facilities and food and drink consumption at home and outside of the home for the two weeks prior to the onset of illness in cases and for the same period for the matched control); existence of cooks and/or maids at home and their role in food preparation; frequency of eating food from street vendors, restaurants, or schools; history of gallbladder disease among the food handlers at home; contact with known cases of typhoid fever in the preceding two months; and travel and swimming activities in the month prior to onset of illness. Stool cultures were performed for the primary food handlers in the households of both cases and controls.

This study revealed that food handlers preparing food solely for their families were not the main source of *S. typhi* infection. Two stool cultures should identify most asymptomatic carriers, and by use of this technique only two carriers were found in the homes of 78 cases. Although chronic carriers are undoubtedly important in the transmission of *S. typhi*, these studies indicate that chronic carriers within the household could account for only a small fraction of typhoid fever cases. The only significant risk factor in cases compared with controls was the consumption of flavored ices sold by street vendors. The risk factors identified in this study are consistent with the hypothesis, also proposed by Dr. Morris, that most *S. typhi* contamination originates outside of the home. From these data it cannot be determined if this contamination results from preparation of food items by chronic *S. typhi* carriers outside of the home or from the use of raw foods contaminated with *S. typhi* from Santiago sewage.

Dr. Eduardo Gotuzzo (Universidad Peruana Cayetano Heredia, Lima, Peru) performed a case-control study of typhoid fever in Lima, incorporating an experimental design similar to that used by Dr. Black in Chile. In summary he found that eating out was a risk factor for children and adults, especially for those who ate from street vendors. He emphasized the difficulties in using the case-control epidemiologic design in endemic areas where multiple modes of *S. typhi* transmission undoubtedly exist. Furthermore, he found that the incubation period of typhoid fever is too long for persons to remember reliably what they may have eaten two weeks before.

**Diagnosis and Bacteriology**

Speakers from six countries reviewed and compared bacteriologic methods for the diagnosis of acute typhoid fever. From 1980 to 1984, Dr. Stephen L. Hoffman and associates (U.S. Naval Medical Research Unit No. 2, Jakarta, Indonesia) evaluated a number of bacterial isolation techniques used in more than 500 patients with culture-positive typhoid fever [15]. In Jakarta the bone marrow aspirate culture (BMAC) was the best method for isolating *Salmonella* from patients with typhoid and paratyphoid fever; it was positive in approximately 90% of patients and was at least 20% more sensitive than all other isolation methods tested. The BMAC specimen was easy to acquire from the iliac crest of children or the posterior superior iliac spine of adults; the procedure was well tolerated by patients, without significant adverse effects (two minor wound infections among more than 600 patients), and the aspirate was positive in culture more rapidly than were specimens from other sites. Chloramphenicol therapy did not interfere with recovery of *S. typhi* from bone marrow. The streptokinase clot culture was, if anything, inferior to the blood culture; the duodenal string bile culture was probably no better than a blood culture and certainly no better than the combination of rectal swab and blood culture. When a bone marrow sample cannot be acquired, increasing the amount of blood drawn, increasing the dilution of blood in broth, and combining the results of blood, bile, and rectal swab cultures will significantly improve isolation rates. In terms of culture media, there was no advantage in using trypticase soy broth with sodium polyanethol sulfonate as compared with use of 10% oxgall. Dr. Hoffman concluded that an improved method requiring less blood and broth is needed to improve *S. typhi* isolation rates from blood.

Dr. Eduardo Gotuzzo presented the joint diagnostic experience of several Peruvian investigators. In 616 Peruvian adults hospitalized with typhoid fever
in Lima from 1976 to 1983, the isolation rate varied according to the body site cultured; the bone marrow was positive in 87% of patients, bile in 72%, blood in 45%, and stool in 35% of patients [16, 17]. Culture of bone marrow in trypticase soy broth, Ruiz-Castaneda, and oxgall media showed no differences in isolation rates (90%–97% positive), but oxgall (62% positive) was superior to the other two media (40% and 47% positive) for the culture of blood. In oxgall medium, 77% of blood cultures were positive if no antibiotics were given before culture, but only 50% of blood cultures were positive if antibiotics had been used. By contrast, bone marrow cultures were not affected by prior antibiotic use. Blood cultures and blood clot cultures treated with streptokinase were equally sensitive. The experience of the operator seemed important in obtaining positive bone marrow cultures because the hematologist and chief resident were significantly more successful than the medical intern in culturing S. typhi (88% vs. 62% positive). In a separate study comparing stool cultures, 43 children were positive significantly more often than 48 adults (65% vs. 27%, respectively). A series of 91 pregnant women with typhoid had bacterial isolation rates similar to those for the adult population.

Dr. Joel Escamilla and Philippine colleagues (U.S. Naval Research Unit No. 2 and San Lazaro Hospital, Manila, the Philippines) evaluated duodenal string capsule (DSC) cultures for the diagnosis of enteric fever in Manila. Parallel cultures of DSC, blood, rectal swab, and urine were performed on a group of persons thought to have enteric fever to determine the sensitivity of each test under three situations: before in-hospital administration of antimicrobial agents, after three days of in-hospital antimicrobial treatment, and two days after the last dose of a 14-day antimicrobial treatment regimen.

Enteric fever was bacteriologically confirmed in 30 of 72 patients entered in the study; 18 patients presented with S. typhi and 12 had S. paratyphi A in at least one of the four pretreatment cultures. DSC and blood cultures were equally sensitive but were significantly more sensitive than rectal swabs and urine cultures. Twenty-eight patients of those having previously confirmed cases were restested during the course of antibiotic treatment. Sixteen (57%) were still culture-positive at this time, and three of 27 were still positive after treatment had been completed. Again, cultures of blood and DSC were the most useful. All S. typhi and S. paratyphi A strains were susceptible to chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole, whether they were isolated before, during, or after antibiotic therapy. Cultures of blood and DSC together confirmed 29 (96.7%) of 30 cases tested before treatment, and the combination confirmed all 19 cases that were positive during or after treatment. The excellent performance of blood cultures may have been due to the use of 10 ml of blood rather than a lesser volume, use of two types of culture media, the favorable dilution (1:11) of blood to broth, and incubation of cultures for 21 days before regarding them negative.

Although there was excellent agreement between results of the cultures and final diagnoses of patients, bone marrow cultures were not performed and such cultures are generally regarded as the most sensitive test for confirming enteric fever. Thus, the possibility exists that the overall culture-positive rates reported by Dr. Escamilla may have been low. Nonetheless, the results indicate that, regardless of status of antimicrobial treatment, there is no significant difference between the results of blood and DSC cultures in their patients and that the two types of cultures comprise a good test combination for the diagnosis of enteric fever.

Dr. Robert Gilman (Johns Hopkins University, Baltimore) cultured blood, urine, stool, bone marrow, and rose spots from 62 Mexican patients thought to have typhoid fever. Bone marrow culture was the single most effective method of isolation, considerably more so than blood culture. Culture of a rose spot was also a useful procedure, and in two patients (3%) it was the only culture positive for S. typhi. In Maryland volunteers with typhoid fever tested by Dr. Gilman, the string test was positive for S. typhi in four of seven patients. Also, in two of seven volunteers who became ill after challenge with S. typhi, the string test was positive before stool or blood cultures, suggesting a high rate of biliary infection early in the course of typhoid fever. In chronic Bangladeshi typhoid carriers, the string test was sampled after it had remained in patients overnight, and, if bile stained on retrieval, it was significantly more effective for isolation of S. typhi than was the stool culture.

Dr. Isidoro Horwitz and colleagues (Faculty of Medicine, University of Chile, Santiago) studied 80 children between four and 14 years old with bacteriologically confirmed enteric fevers. The combined sensitivity of two blood cultures was 71%, the bile culture obtained by string test was positive in 69%,
and the bone marrow culture was positive in 79% of the patients; these differences were not significant. In eight patients (10%), the bile culture was the only positive specimen. The combination of two blood cultures plus one bile culture proved to be as sensitive (92%) as that of two blood cultures plus one bone marrow culture (86%), and the procedure is less invasive. Dr. Horwitz recommends the combination of two blood cultures plus one bile culture as the most practical and efficient assay for the bacteriologic diagnosis of enteric fevers in children.

Dr. John Sippel and Egyptian associates (Naval Medical Research Unit No. 3, Cairo, and University of Alexandria, Alexandria, Egypt) estimated the extent to which _S. typhi_ lipopolysaccharide (LPS) antigens are involved in the immune response in pediatric typhoid fever by Western immunoblot analysis. Virtually no bands were observed when control sera were tested by immunoblot against LPS, whereas heavy, broad bands were produced with serum from typhoid patients. These bands were not present when the patients’ sera were absorbed with LPS. When whole cell _S. typhi_ antigens were immunoblotted, a few narrow bands were seen with the control sera, but both narrow and broad bands were produced with the patients’ sera. Again, the broad bands were eliminated when the sera were treated with LPS.

An ELISA that measured IgM and IgG antibodies against _S. typhi_ LPS antigen was compared with Widal agglutination for the diagnosis of typhoid fever in these pediatric patients (<12 years of age). Dr. Sippel compared 38 control children having Widal titers <1:80 with four patient groups: 46 culture-negative children with Widal titers <1:80 (group 1); 22 culture-negative children with Widal titers ≥1:160 (group 2); 28 _S. typhi_–positive children (group 3); and 12 _Salmonella_–positive, _S. typhi_–negative children (group 4). The percentage of patients in groups 1, 2, 3, and 4 who had IgG ELISA values higher than any of the control sera were 44%, 82%, 93%, and 92%, respectively. The corresponding IgM ELISA values for the four groups were 35%, 68%, 82%, and 92%, respectively. Dr. Sippel suggested that his anti-LPS ELISA assay may be useful for the serodiagnosis of typhoid fever in children in endemic areas.

Dr. T. Jacob John (Christian Medical College and Hospital, Vellore, India) critically reviewed the world literature on the rapid diagnosis of typhoid fever. Possible approaches to the rapid diagnosis of typhoid fever in which results are ready within a few hours include the rapid identification of cultured organisms, diagnostic titers of _S. typhi_ antibody, and detection of _S. typhi_ antigens in body fluids.

**Rapid identification of cultured organisms.** _S. typhi_ in blood culture may be rapidly identified by coagglutination (COAG) with a suspension of protein-A-bearing _Staphylococcus aureus_ sensitized with O and Vi antibodies [18]. _S. typhi_ antigen(s) in the culture broth could be detected by immunoprecipitation in counterimmunoelectrophoresis (CIE) [19]. In coprocultures the incorporation of _S. typhi_ antibodies in semisolid media will result in a halo of immunoprecipitate around _S. typhi_ colonies [20]. Alternatively, COAG may be applied directly on colonies on culture plates [21]. Because the latter two methods are only applicable to fecal culture, they are of limited value in rapid diagnosis.

**Rapid detection of antibody.** High titers of agglutinating antibody measured by the single-serum Widal test suggest recent infection with _S. typhi_, but this test usually requires overnight incubation. O and H antibody titration may be done more rapidly if samples are incubated at 52°C. Agglutinating antibodies may be titrated rapidly in slide tests [22]. Recently, a slide Widal test has been claimed to be highly specific and moderately sensitive [23, 24]. Precipitating antibody may be rapidly detected in CIE, but it is a qualitative test, highly specific but of variable sensitivity [25, 26]. RIA has been described to determine antibodies to LPS and protein, but it is not suitable for rapid diagnosis. ELISA has also been described by Dr. Sippel in this workshop and by others [27] for class-specific antibodies to LPS antigens. CIE and ELISA methods deserve further evaluation. IHA has also been used to quantitate O, H, and Vi antibodies. An indirect immunofluorescence (IF) method for quantitating Vi antibody has recently been described; a titer of ≥1:64 was found to be highly sensitive and specific [28].

**Detection of antigen in body fluids.** Urine and sera of typhoid fever patients and control patients have been investigated for the presence of bacterial antigens. Passive staphylococcal coagglutination has been used to detect O, H, and Vi antigens in urine; the results reported by Mr. Lesmana in this workshop were highly sensitive but not very specific [29]. An ELISA for Vi antigen in urine also lacked satisfactory specificity [30, 31]. CIE, used to detect antigen in serum [25], appears to be highly specific, but its sensitivity ranges from 36% to 92% in different studies [19, 25] (T. J. John, unpublished observations). Passive staphylococcal coagglutination also
has been described for the detection of O antigen in serum [32]. Initial results show high sensitivity and specificity, and further evaluation is warranted [19].

In conclusion, Dr. John believes that no single test can be recommended at the present time for rapid diagnosis of typhoid fever. Antibody detection by slide-Widal, CIE, ELISA, and IF deserves to be evaluated more extensively. Vi antibody measured by IF seems to be circulating even during the first week. Antibody measurement by slide-Widal and CIE are suitable during or after the second week of illness. Antigen detection in the serum by CIE and COAG appears to be a promising development, but antigenemia seems to be short-lived and the methods require further evaluation.

Mr. Murad Lesmana and colleagues (U.S. Naval Medical Research Unit No. 2 Detachment, Jakarta) summarized the current status of coagglutination tests for the diagnosis of typhoid fever. The COAG test employs the protein A-containing Cowan I strain of S. aureus sensitized with absorbed rabbit antisera prepared against S. typhi group D somatic antigen or Vi capsular polysaccharide antigen. The various culture materials or patient specimens usually need some treatment prior to COAG testing, such as heat treatment or dilution. When COAG tests are used to supplement current standard culture and enrichment techniques, they provide 94%–100% sensitivity and 83%–100% specificity; the presumptive results can be available as early as one day after receipt of the sample and can eliminate several days of processing time. However, Mr. Lesmana emphasized that the COAG test as a culture supplement cannot substantially improve the sensitivity or specificity of any given culture system; it only provides results more rapidly and at less cost in materials and technician time. Unfortunately, the detection of S. typhi by a direct COAG test of a body fluid still lacks the necessary sensitivity and specificity required of a truly useful rapid diagnostic test.

Dr. P. Y. Chau (Department of Microbiology, University of Hong Kong) spoke on his studies of the role of protein antigens from S. typhi in serology and immunity [33]. He analyzed antibodies to S. typhi in sera obtained from typhoid patients and from carriers by crossed immunoelectrophoresis (XIE). He used a veronal buffer extract of S. typhi as the reference antigen and its corresponding rabbit antiserum as the reference antibody with sera from typhoid patients and carriers in the intermediate gel. Three precipitating antibodies to the protein antigens of S. typhi were regularly detected in sera from typhoid patients and carriers. The first antibody was directed against an antigen (antigen 19) common to most gram-negative bacteria, including Pseudomonas; the second antibody reacted with an antigen (antigen 14) common to most members of the Enterobacteriaceae, and the third antibody was directed against an antigen (antigen 28, or the Tp antigen) that appeared to be specific to S. typhi.

Dr. Chau determined by XIE the prevalence of antibodies to these three protein antigens of S. typhi in sera obtained from various patients. He found antibodies to antigen 19 in almost all sera from patients recovering from systemic infections caused by gram-negative bacteria. Antibody to antigen 14 was found in most sera from patients with systemic infections due to Enterobacteriaceae (excluding Pseudomonas) and, occasionally, in sera from normal individuals. Antibodies to the Tp antigen were detected in most sera from typhoid patients but not in sera from patients with other gram-negative bacterial infections, with paratyphoid fever, or in sera obtained from volunteers immunized with an oral, live typhoid vaccine strain Ty 21a. Some vaccinees, however, did develop antibodies to antigen 19, antigen 14, or both antigens after oral immunization with vaccine Ty 21a.

Dr. Chau studied the distribution of Tp antigen in multiple strains of S. typhi. Tp antigen was present in the reference strain Ty 2 and in all of the tested S. typhi strains, either freshly isolated from typhoid patients or stored in the laboratory for some time. S. typhi Ty 2 is the parent strain of the two candidate strains for the live, oral typhoid vaccines: Ty 21a and 620 Ty, an aromatic amino acid–histidine–, and purine-dependent auxotrophic mutant. Ty 21a is Vi-negative, and 620 Ty is Vi-positive. Tp antigen is consistently present in Ty 21a cultured in the presence of galactose but is less regularly present in Ty 21a when cultured in galactose-free media. By contrast, Tp antigen is regularly present in strain 620 Ty cultured in all test media. Thus, Dr. Chau suggests that 620 Ty might be a useful alternative live vaccine to Ty 21a because the Vi and the Tp antigens are better preserved in strain 620 Ty.

Dr. Barbara E. Murray (University of Texas, Houston) summarized studies of antibiotic resistance plasmids in S. typhi. In general S. typhi seems to have remained remarkably susceptible to antimicrobial agents, particularly when one compares their resistance to that of other enteric pathogens, such as Shigella species and nontyphoidal Salmonella. Some
exceptions to this experience, of course, are the multiresistant epidemic *S. typhi* strains isolated in Mexico and Vietnam a decade ago; the sporadic reports of strains resistant to chloramphenicol, trimethoprim-sulfamethoxazole, and other antimicrobial agents reported over the past five years; and the current outbreak of multiresistant strains in Peru.

Dr. Murray examined 100 Chilean strains of *S. typhi* within two to 52 weeks of isolation in order to minimize spontaneous plasmid loss. The strains belonged to 10 different phage types. Not one of the 100 strains was resistant to any of the seven antibiotics tested. Moreover, in studies by others, 99.5% of 4,000 strains examined in Chile over the past five years were susceptible to all antimicrobial agents tested, despite the fact that several of the drugs had been used for more than 30 years. Ninety-two of the strains had no plasmids identified by one of three different methods of lysis. Dr. Murray also screened 50 strains of *S. typhi* from Thailand, and 47 of these lacked plasmids.

After systematic phage typing, analyses of antimicrobial resistance and plasmids, plasmid transfer experiments between *Escherichia coli* and *S. typhi*, and in vitro growth experiments, Dr. Murray reached the following conclusions: (1) Most strains of *S. typhi* do not have plasmids, and thus the possible existence of a virulence plasmid in this species seems unlikely. (2) If a plasmid is present, even though cryptic, it provides a useful marker for epidemiologic purposes. (3) *S. typhi* in Chile and Thailand remain susceptible to antimicrobial agents despite marked resistance in other Enterobacteriaceae. (4) Studies examining possible reasons for the lack of resistance in *S. typhi* reveal that there does not appear to be any exclusion by *S. typhi* of incoming R factor DNA; that growth rates of *S. typhi*, already slow in comparison with those of *E. coli*, are further slowed by the presence of at least some R factors; and, finally, that the stability of four of five R factors was markedly less in *S. typhi* than in the *E. coli* host strains (all clinical isolates) used as donors and the *E. coli* recipient strain J53. The lack of stability of R factors in *S. typhi* and, possibly, the diminished growth rate of these strains may explain why *S. typhi* have in general remained more susceptible to antimicrobial agents than other Enterobacteriaceae.

In the final paper in the workshop session on diagnosis and bacteriology, Dr. Bernard Rowe (Central Public Health Laboratory, London) provided an update on the uses of phage typing in the control of typhoid fever. Phage typing of *S. typhi* provides a highly discriminatory system of strain identification. When carried out in experienced reference laboratories, its reliability is unquestioned and has been proved by numerous epidemiologic studies throughout the world. The results from the system are readily reproducible, and the standardization of reagents and methodology is ensured because the WHO Collaborating Centre at the Division of Enteric Pathogens, Central Public Health Laboratory (Colindale, U.K.) is responsible for training, distributing reagents, and validating all new phage types.

The phage-typing scheme depends on the Vi antigen of *S. typhi* and makes use of the remarkable ability of Vi phage II to adapt to new host strains. In 1938 workers recognized 18 Vi phage types. Since that date successive additions have brought the total to 106. Reagents for the last 10 new Vi phage types are currently being issued by Dr. Rowe. Phage types are designated A to T (with numerous subtypes, e.g., D11, L1) and 25 to 61; some designations have been deleted for technical reasons. Untypable strains possessing the Vi antigen should be forwarded to the WHO Centre, where they are designated UVS (untypable Vi strain) and given a code number. If they recur with meaningful frequency, they may be given a definitive designation and incorporated in the scheme.

Because of the network of national phage-typing laboratories, it has been possible to obtain a global appraisal of the distribution of phage types. Some are cosmopolitan, e.g., A1 and E1, whereas others have a more limited geographic distribution. A notable example was phage type 34 in the Aberdeen typhoid outbreak in 1964; it was known that this phage type was not indigenous to the British Isles but had been found in South America. This fact helped to identify corned beef imported from Argentina as the vehicle of infection. Dr. Rowe concluded that Vi phage typing of *S. typhi* approaches the ideal characteristics of an epidemiologically orientated scheme. The network of national centers needs to be expanded in regions of high typhoid endemicity.

**Pathogenesis**

The workshop session on pathogenesis opened with a paper by Dr. Stephen L. Hoffman (U.S. Naval Medical Research Unit No. 2, Jakarta) on endo-
toxin, soluble mediators, and possible immunosuppression in the pathogenesis of typhoid fever. Dr. Hoffman pointed out that the pathogenesis of typhoid fever is poorly understood. Until the early 1970s, most manifestations of the disease were attributed to the effects of circulating endotoxin. However, studies in volunteers by Hornick, Greisman, and co-workers in the United States suggested that the manifestations of typhoid were not caused by circulating endotoxin, and studies in patients in Vietnam showed that typhoid patients had no detectable endotoxin in their plasma. It was then hypothesized that endotoxin contributed to the pathogenesis of typhoid fever by enhancing local inflammatory responses at the tissue sites of S. typhi multiplication.

In 1980 Dr. Hoffman and colleagues noted that in Jakarta typhoid patients with diminished level of consciousness, delirium, or other abnormal mental status had an extremely poor prognosis. Since then these investigators have been working to elucidate the pathogenesis of delirium and other systemic manifestations of typhoid and to reduce mortality in severe typhoid fever. Among 392 patients with typhoid fever who had enhanced bone marrow aspirate cultures, 90% of the cultures were positive in patients who died (STD, n = 29), 75% were positive in severe typhoid patients who lived (STL, n = 90), and 56% were positive in patients with non-severe typhoid (NST, n = 273). There were no differences in phage types between STD, STL, and NST. The routine CSF examination was normal in >70% of patients with severe typhoid. The limulus assay for endotoxin activity in the CSF was positive in 88% of patients with STD and in 54% of patients with STL (P < .05). Patients with STD had much lower levels of Vi antibody and circulating immune complexes (CIq binding) than did STL and NST patients, and there were no apparent differences among subgroups in regard to plasma levels or macrophage production of thromboxane B2.

On the basis of these preliminary findings, Dr. Hoffman formulated the following hypothesis. Most manifestations of typhoid fever are caused by arachidonic acid metabolites, free oxygen radicals, and other mediators released from mononuclear cells, primarily macrophages. Patients who develop severe disease ingest higher numbers of organisms, which after localization in macrophages, lead to production of a variety of mediators and a suppressor factor. The mediators cause the manifestations of the disease, and the suppressor factor leads to immunosuppression. This immunosuppression allows for relatively unbridled growth of the organisms and release of endotoxin intra- and extracellularly, which leads to some immune response and increased production of mediators and suppressor factors and, eventually, death. Dexamethasone therapy dramatically reduces mortality in severe typhoid fever [34] and may do so by reducing the production, release, or end organ effects of mononuclear cell-derived mediators and suppressor factor.

Dr. Alison D. O'Brien (Uniformed Services University of the Health Sciences, Bethesda, Maryland) reported studies of the flagella of S. typhimurium, which represent a virulence factor in the murine typhoid model [35, 36]. She and her associates determined whether flagella, chemotaxis, and motility of S. typhimurium are virulence factors in infected C57BL/6J mice. She did this by constructing three different pairs of S. typhimurium, each pair differing by the presence or absence of flagella, the presence or absence of functional flagella, or the presence or absence of flagella that responded to a chemotactic stimulus. No additional differences between members of a pair were evident. The virulence of each pair of bacteria was then assessed in mice challenged orally, intraperitoneally, or intravenously. The results established that flagella, whether functional or nonfunctional as organelles of motility, were Salmonella virulence factors and that neither chemotaxis nor motility were required for virulence.

Dr. O'Brien next evaluated how flagella enhance the pathogenicity of S. typhimurium in C57BL/6J mice. When animals were infected orally with flagellated or nonflagellated Salmonella, equivalent numbers of bacteria colonized the animals' gastrointestinal tracts, but the number of flagellated organisms increased faster once in the spleens and livers. To evaluate this differential rate of Salmonella growth, she compared the rate of blood clearance and the kinetics of net multiplication of salmonellae in splenic tissue of mice after intravenous challenge. She found that clearance of bacteria from the blood was the same for flagellated or nonflagellated strains. However, the number of flagellated bacteria in the spleen increased logarithmically until death of the animals, whereas the numbers of nonflagellated salmonellae increased only slightly. In contrast, both flagellated and nonflagellated strains grew exponentially in the spleens of mice pretreated with silica,
an agent toxic to macrophages. In an in vitro macrophage assay, flagellated salmonellae survived longer than did nonflagellated organisms. These results indicate that flagella either protect *S. typhimurium* from the intracellular killing mechanisms of murine macrophages or that flagella enhance the ability of *S. typhimurium* to multiply within murine macrophages.

Dr. Toby K. Eisenstein (Temple University School of Medicine, Philadelphia) summarized her studies of typhoid in mice and the relevance of such studies to human typhoid vaccine strategies [37–39]. In order to probe the mechanisms of immunity to typhoid fever, she has employed murine infection with *S. typhimurium* as a model system and has tested a panel of nonviable *Salmonella* preparations, as well as a live, avirulent organism, to assess vaccine efficacy. Killed vaccines included phenol-water–purified LPS, trichloroacetic acid–extracted LPS, endotoxin protein, a subcellular, ribosome-rich extract, and acetone-killed and dried cells of a virulent strain. A living attenuated strain, designated SL3235, was an auxotrophic *S. typhimurium* derivative blocked at the *aro A* gene. (Dr. Bruce Stocker speaks more about auxotrophic *Salmonella* vaccines below.) The vaccines were tested in strains of mice with widely differing susceptibilities to *Salmonella* infection, ranging from a theoretical LD<sub>50</sub> of one cell to an LD<sub>50</sub> of 10<sup>7</sup> cells. Hypersusceptible mice included the LPS-hyporesponsive C3H/HeJ strain and the closely related LPS-sensitive strain, C3HeB/FeJ. Inherently resistant strains of mice were the C3H/HeNcr1BR and the outbred Swiss CD-1.

Dr. Eisenstein concluded from 15 years of detailed mouse typhoid studies that antibody is sufficient to protect inherently resistant mice against *Salmonella* infection, but antibody is ineffective or poorly effective in protecting inherently hypersusceptible mice. Nonviable vaccines are apparently effective in resistant mice and of marginal efficacy in susceptible mice because their mode of action is by induction of antibody. In contrast, a live, avirulent strain is effective in both resistant and susceptible mouse strains, apparently because it induces cellular immunity. Dr. Eisenstein emphasized that in the evaluation of *Salmonella* vaccines in mice, there is not one mouse model but a spectrum of models, depending on the innate resistance or susceptibility of the strains of mice used. Which mouse model most closely simulates human immunity to typhoid fever is not known with certainty. However, because humans are protected by acetone-killed and dried *S. typhi*, humans seem more similar to the inherently resistant mice than to the hypersusceptible mice.

**Therapy**

The workshop session on therapy for typhoid fever was opened by Dr. Stephen L. Hoffman (U.S. Naval Medical Research Unit No. 2, Jakarta), who detailed the studies of his collaborative group of Indonesian and American investigators on the use of high-dose dexamethasone in the management of severe typhoid fever [34]. Ninety-seven Indonesian patients who were culture-positive for *S. typhi* and who were delirious, obtunded, stuporous, comatose, or in shock were considered to have severe typhoid fever. The case-fatality ratio in such patients treated with standard antimicrobial therapy but with no dexamethasone was 51.4%. By contrast, the case-fatality ratio was only 16.7% in patients with severe typhoid fever treated with standard antimicrobial therapy and high-dose dexamethasone (initial dose, 3 μg/kg over 30 min, followed by eight doses of 1 μg/kg given at 6-hr intervals for a total of 48 hr). There was no significant difference in medical or surgical complications among survivors in both treatment groups. Of the severely ill typhoid patients, 88% had been ill more than four days, 72% were older than four years of age, 93% had <10,000 leukocytes/μl blood, and 30% had an abnormal CSF.

On the basis of an experience of >500 typhoid patients, Dr. Hoffman and his colleagues have developed the following recommendations for the optimal use of high-dose dexamethasone in Indonesian patients: (1) Dexamethasone is only to be used in severe typhoid fever; more than 80% of typhoid patients in Jakarta do not require it. (2) All patients in Jakarta with fever and shock or abnormal mental status must be suspected of having typhoid fever. (3) All such patients should have a lumbar puncture (if there are >35 polymorphonuclear or mononuclear cells/μl of CSF it is highly unlikely that patients have typhoid). (4) All patients with suspected severe typhoid should receive chloramphenicol and high-dose dexamethasone as soon as possible after admission. (5) If patients deteriorate clinically after initiation of therapy or are suspected of having intestinal perforation, an antimicrobial agent effective against gram-negative organisms resistant to chloramphen-
icolon should be added to the chloramphenicol and dexamethasone regimen. (6) Careful attention should be paid to nursing care, fluid and electrolyte balance, and signs of gastrointestinal bleeding, intestinal perforation, anemia, and shock. Dr. Hoffman concluded that if high-dose dexamethasone is used appropriately and promptly, deaths from severe typhoid fever can be reduced markedly.

Dr. Claudio F. Lanata described his recent attempt to treat chronic S. typhi carriers with antibiotics. In the past, cholecystectomy alone provided a cure rate of 40%–70%, and in one small study in Italy, ampicillin given intravenously for two weeks cured 100% of carriers. In general the combination of surgery and intravenous ampicillin is very successful but is invasive, costly, and unpopular among patients and is not a practical public health regimen in endemic countries. In order to confirm that 6 g of amoxicillin plus 1.5 g of probenecid ingested daily for 28 days was an effective and practical oral regimen for the nonsurgical treatment of S. typhi carriers, regardless of the presence of gallstones, Dr. Lanata conducted a clinical trial in Santiago, an area hyperendemic for typhoid fever and cholelithiasis.

Using this regimen Dr. Lanata was able to cure only 58% of 26 chronic S. typhi carriers. All failures occurred within the first four months of follow-up. Poor compliance and amoxicillin-resistant S. typhi were not causes of treatment failure. A more likely cause was gall bladder disease, present in all 12 of the carriers who had oral cholecystograms. The serum amoxicillin level was compared in each of 12 carriers who were cured with that in eight carriers who were not cured. Because the mean amoxicillin level measured at 4 hr after dosing was significantly lower in the failed group, Dr. Lanata speculates that carriers could have failed because they metabolized amoxicillin faster, with subsequent lower serum and bile levels. Dr. Lanata urged that the search for an effective and practical ambulatory treatment of chronic S. typhi carriers should continue, especially now that specific and practical screening techniques for such carriers exist.

Immunology and Prevention

Dr. Stefan B. Svenson (National Bacteriological Laboratory, Stockholm) reviewed the purification and characterization of O-antigen polysaccharides and synthesis of O-antigen-specific glycoconjugates of Salmonella. He prepared a series of saccharides representative of the O-antigenic polysaccharide side chains of different Salmonella serotypes and covalently linked them to different carrier proteins as haptenic groups. Rabbits immunized with the saccharide-protein conjugates readily responded with antisaccharide hapten antibody titers nearly as high as those elicited by injection of whole, heat-killed cell vaccines. Antibodies elicited in rabbits against the saccharide-protein conjugates, when passively transferred to mice, protected them against experimentally induced mouse typhoid. The protection was shown to be serotype specific. In another set of experiments, monoclonal antibodies with specificity for the O antigen serotype–specific determinant O4, but not the shared antigen determinant O12, were highly protective in the same experimental mouse typhoid system. These findings emphasize the importance of structural and conformational studies of bacterial polysaccharide antigens in efforts to find the specific epitopes important for protective immunity.

Dr. Svenson also covalently linked the O antigen–specific oligosaccharides to straight carbon aliphats to yield artificial glycolipids. Such glycolipids, injected intradermally, were highly efficient in eliciting O antigen–specific delayed-type hypersensitivity skin reactions in calves experimentally infected previously with the Salmonella serotype bearing the specific oligosaccharide epitope. Uninfected control calves showed no delayed skin reactions to the test antigens as measured by gross skin swelling or by histologic examination of skin biopsies. Intradermal injection of the O antigenically homologous poly- or oligosaccharides in the absence of lipid carrier gave no skin reactions. Dr. Svenson concluded that his artificial, Salmonella O antigen glycoproteins represent promising synthetic, nontoxic vaccines, and his synthetic glycolipids provide useful diagnostic skin test antigens.

Dr. James R. Murphy (University of Maryland School of Medicine, Baltimore) summarized his studies of cell-mediated immunity in human Salmonella infections. The literature on human cellular immune responses to S. typhi infection or vaccination contains conflicting results. Studies from Denmark showed moderate levels of enhanced cellular responsiveness from soon after infection through almost 40 years, whereas studies in India have documented markedly decreased immunoresponsiveness.

Dr. Murphy used O antigen polysaccharides (O PS) from S. typhi, Salmonella enteritidis, and Salmonella thompson, prepared by Lindberg and col-
leagues in Sweden, in a five-day blast transformation assay to test for sensitized lymphocytes in residents of the United States who were nonimmune, immunized with the live attenuated Ty 21a vaccine, or recovered from S. typhi infection. He also tested residents of Santiago, who had recovered from S. typhi infection or claimed not to have had typhoid fever or received vaccination against typhoid. With his assay Dr. Murphy did not detect immunocomversion of United States residents following vaccination or infection with S. typhi. Residents of Santiago showed a uniformly higher level of lymphocyte responsiveness to O PS when compared with United States residents; however, the test could not discriminate between putatively nonimmune Chileans and Chileans recovered from known infection.

In other investigations of cell-mediated immunity, Dr. Eisenstein measured delayed-type hypersensitivity (DTH) in experimental mouse typhoid [38, 40]. She immunized four different strains of mice with a live, avirulent strain of S. typhimurium prepared by Dr. Stocker, who blocked aromatic synthesis, thus rendering the strain purine dependent. In a series of elegant experiments [37, 38], Dr. Eisenstein showed that (1) there are marked differences among strains of mice in their ability to display a DTH response in their foot-pads to a Salmonella elicin; (2) ability to mount a DTH response is related to the innate susceptibility or resistance of the mice to virulent Salmonella infection; (3) the anergic characteristic is not related to the H-2 histocompatibility loci; (4) the anergy is not related to the presence or absence of LPS reactivity in strains of mice; (5) anergy is not caused by antigen overload in the nonreactive strains; (6) foot-pad anergy can occur concomitant with protective immunity, even in responsive mouse strains, if the immunizing dose of the vaccine is low. As high levels of protection can occur without a positive DTH reaction as measured by foot-pad swelling, Dr. Eisenstein concluded that this test of DTH, or DTH itself, may be a relatively insensitive measure of protective immunity.

Dr. Suttipant Sarasombath (Siriraj Hospital, Mahidol University, Bangkok, Thailand) measured specific secretory IgA (SIgA) and secretory IgM (SIgM) responses in the intestinal lavage fluid of 25 typhoid vaccinees and six unvaccinated, culture-positive typhoid patients. The vaccinees and the patients were men, aged 19–26 years. The vaccinees had no previous history of typhoid or paratyphoid fever, no typhoid or paratyphoid vaccination for at least seven years prior to this study, and negative results for cell-mediated immunity and circulatory antibodies to S. typhi before vaccination. The vaccinees were divided randomly into three groups according to the type of vaccine administered, namely, the oral, attenuated S. typhi Ty 21a vaccine, a parenteral acetone-inactivated S. typhi Ty 2 vaccine, and a parenteral heat-inactivated, phenol-preserved S. typhi Ty 2 vaccine. The oral attenuated typhoid vaccine was given in single capsule with sodium bicarbonate on days 1, 3, and 5; the acetone- and heat-inactivated vaccines were given subcutaneously only once. Dr. Suttipant obtained intestinal secretions repeatedly for 48 weeks after vaccination or after the onset of fever in the typhoid patients. In order to assay intestinal lavage fluids, she employed an ELISA, using a protein extract of S. typhi as the specific antigen.

Specific SIgA was detected in each vaccinee before vaccination, an observation suggesting that these Thai vaccinees were naturally primed to S. typhi. The oral attenuated vaccine boosted S. typhi SIgA better than did the parenteral vaccines, as measured by the magnitude of the secretory antibody response and its duration in intestinal wash fluids. The peak SIgA response in vaccinees was seen at about the first week, and antibodies persisted for at least 48 weeks after vaccination. Specific IgM antibody was not measured in the intestinal fluid of vaccinees. Four of the six typhoid patients mounted a detectable intestinal SIgA and IgM response that peaked by four weeks after the onset of fever. The IgM declined more rapidly and was barely detectable by eight to 20 weeks, whereas the SIgA persisted for at least 48 weeks. Dr. Suttipant believes that both SIgA and IgM contribute to protection. She quantitated S. typhi SIgA intestinal antibody and found it to be higher after natural infection than after vaccination. Serum immunoglobulin class-specific anti-S. typhi levels were not reported.

Vaccines

The last session of the workshop focused on a review of the status of three experimental S. typhi vaccine formulations, the attenuated gal E mutant strain Ty 21a, the purified capsular polysaccharide Vi antigen, and auxotrophic mutants of Vi-positive and Vi-negative S. typhi.

Dr. Rene Germanier (Swiss Serum and Vaccine Institute, Berne, Switzerland) summarized the laboratory development and results of volunteer trials of
the oral, attenuated Ty 21a strain vaccine [41-43]. The vaccine is safe and protects adult volunteers challenged with S. typhi, Egyptian schoolchildren, and in current field trials, Chilean schoolchildren (discussed by Drs. Black and Ferreccio below). Dr. Germanier emphasized that the vaccine must be given in enteric-coated capsules because the strain is killed rapidly by acidic stomach fluids. Also, the shelf life of the vaccine is reduced by residual moisture. He ascribed vaccine failures in Swiss tourists to a suboptimal formulation and improper storage and dosages of the vaccine (called Vivotif) licensed for use in Europe. Attempts are underway now to determine an optimal formulation and immunization schedule for the Ty 21a vaccine.

Dr. Robert E. Black described the 1982–1983 Ty 21a vaccine field trial in Chile. The incidence of typhoid is five to 10 times higher in Santiago than it is in Alexandria, the site of the first field trial, where the annual incidence was only 44 per 100,000 schoolchildren. Furthermore, the formulation used in Egypt (three doses over one week of 10^9 organisms resuspended in buffer just before administration together with 1-g chewable bicarbonate tablets) is impractical for mass immunization. It was important to learn whether a simpler formulation and fewer than three doses of vaccine could provide protection in an area hyperendemic for typhoid fever.

Thus, Dr. Black and his colleagues initiated a randomized double-blind field trial of vaccine efficacy. Because the highest rates of typhoid fever are in school-aged children in Santiago, they conducted the study in this age group. Children were randomized into three groups—one group to receive two doses of an enteric-coated vaccine one week apart, one group to receive one dose of vaccine and one dose of an identical placebo, and one group to receive two doses of placebo. The enteric-coated formulation was found to be very practical for mass vaccination; 92,000 participating schoolchildren received vaccine or placebo within one week without notable adverse reactions. Following vaccination, bacteriologically confirmed cases of typhoid fever were identified in schoolchildren. In the first year of surveillance after vaccination, 67 cases of typhoid fever were identified in children who received placebo, an incidence of 211 cases per 100,000 children. In contrast, 58 cases were identified in children who had received one dose of vaccine and only 29 cases in children who had received two doses of vaccine. Thus, one dose resulted in 16% vaccine efficacy and two doses in 50% efficacy for the first year. In the second year after vaccination, there were 45 cases in the placebo group. The vaccine efficacy was 42% with one dose and 72% with two doses. When one examines the efficacy of two doses of vaccine for three-month periods, it is apparent that there was 60%–90% protection for each quarterly period except for April to June of the first year, when the protective efficacy nearly disappeared for unknown reasons.

Salmonella paratyphi B causes approximately 10% of the enteric fever illnesses in Santiago. Since this organism shares O antigen 12 with S. typhi, Dr. Black was interested to determine if Ty 21a provided some protection against S. paratyphi B illness. In the two years after vaccination, 56 cases of enteric fever with blood or bone marrow cultures positive for S. paratyphi B were identified. One dose of Ty 21a vaccine resulted in 22% efficacy against S. paratyphi B, and two doses resulted in 54% efficacy.

The level of protection against typhoid fever found in the first Santiago field trial was substantially lower than the efficacy of 95% observed in the Egyptian field trial of Ty 21a. Several reasons for the reduced efficacy were considered. First, the vaccine dose schedule was changed from three doses within one week to two doses one week apart or one dose only. Second, the vaccine organisms were administered in lyophilized form contained within enteric-coated capsules, instead of being ingested as a liquid, after neutralization of gastric acid with sodium bicarbonate. Third, the incidence of bacteriologically confirmed typhoid fever in Santiago schoolchildren was five times that of Egyptian children, raising the question of whether protective efficacy could be less in heavily endemic areas where children may ingest large doses of S. typhi. In addition to these considerations, it was important to determine if protection could be enhanced by increased spacing between vaccine doses, possibly resulting in a boosting of immunity. Thus, a second field trial was conducted in 1983–1984 in Santiago, and the results were reported by Dr. Ferreccio.

The 1983–1984 field trial involved five groups of children vaccinated as follows: (1) three doses of enteric-coated vaccine given every other day; (2) three doses of vaccine in gelatin capsules plus 1 g of bicarbonate given together every other day; (3) three doses of enteric-coated vaccine given every 21 days; (4) three doses of vaccine in gelatin capsules plus 1 g of bicarbonate given together every 21 days; (5) three doses of placebo given every other day. Ap-
proximately 30,000 children were randomized into each of the five groups. Sixth and seventh groups of about 5,000 children each received only two doses of enteric-coated vaccine or vaccine with bicarbonate because of school absenteeism. Dr. Ferreccio vaccinated her cohort between July and September 1983 and initiated surveillance for typhoid fever in September 1983. In April 1984 she broke the code for the field trial and prepared a preliminary analysis of the data, which led to the following conclusions. The enteric-coated formulation had a vaccine efficacy of 75% with the short immunization schedule and 71% with the long-interval schedule. The vaccine in gelatin capsules plus bicarbonate provided only 11% efficacy given over seven days and 30% efficacy given over 42 days. The efficacy of the enteric-coated vaccine was similar in children given three or two doses, although the latter group was not randomly assigned and was too small to permit statistical significance. Children who were >15 years of age were better protected than were children five to nine years old. A third large-scale field trial is underway to compare the efficacy of two, three, or four doses of enteric-coated vaccine given within one week.

Dr. Bruce A. D. Stocker (Stanford University School of Medicine, Stanford, California) described the development in his laboratory of strains of *Salmonella* made nonvirulent by a nutritional requirement; such strains, since they are of wild-type antigenic character, should be effective live vaccines. Dr. Eisenstein had given a single intraperitoneal dose of one of these aromatic-dependent strains (*S. typhimurium* SL3235) to hypersusceptible mice, and it proved safe and protected well against later challenge with a virulent *S. typhimurium* strain. Bacteria such as *E. coli* and *S. typhimurium*, unlike mammals, can make all essential aromatic metabolites via chorismic acid, the product of the aromatic biosynthesis (aro) pathway. A complete block in this pathway makes a bacterial strain dependent on external supplies of both the three aromatic amino acids and of several minor aromatic compounds. Two of the latter, *p*-aminobenzoate (for making folate) and 2, 3-dihydroxybenzoate (for making the iron-capturing compound enterochelin), are not vertebrate metabolites and are not expected to be available in host tissues. Dr. Stocker made mouse-virulent *S. typhimurium* strains aromatic dependent by selection of *aroA*::Tn10 transductants, i.e., by replacement of gene *aroA* by a copy of the gene inactivated by insertion of transposon Tn10 (which confers resistance to tetracycline). Such *aroA*::Tn10 transductants (grown in broth with tetracycline to keep up selection for the transposon) were nonvirulent for mice, compared with the highly virulent parent strain.

Strains with a gene inactivated by insertion of a transposon can at low frequency (~10⁻⁶/bacterium/generation) recover the lost gene function by "clean excision" of the transposon. To eliminate the risk of such reversion to *aro*⁺ (and so to virulence), tetracycline-sensitive mutants of the *aroA*::Tn10 strains were selected and tested for ability to produce colonies on medium with growth-limiting tetracycline content. Mutants giving no colonies in tests able to detect reversion at 10⁻¹¹/bacterium/generation were inferred to have transposon-generated deletion or inversion mutations at *aroA* and so to be totally unable to revert.

Such stable *aroA* derivatives of mouse-virulent *S. typhimurium* strains proved effective as live vaccines in mice. Thus BALB/c mice given a single intraperitoneal injection or one feeding of 10⁶ cfu of live *aro* bacteria survived challenge 30 days later with 25,000 LD₅₀ of a virulent *S. typhimurium* strain given intraperitoneally or with 100 LD₅₀ given orally. Stable *aro* strains of *S. typhimurium* were tested for ability to protect calves against a severe oral challenge with the same species. The *aro*⁻ strain SL1479, given in two doses either orally or intramuscularly, gave substantial protection; two other strains, derived from different wild-type parents, were much less effective for unknown reasons [44, 45]. In a trial in Sweden [46], strain SL1479, in three oral doses, protected calves against challenge with oral *S. typhimurium* much more effectively than did three subcutaneous doses of a killed vaccine. An *aro*⁻ strain of *S. dublin* given intramuscularly in two doses was likewise effective.

Stable *aro*⁻ derivatives of Vi-positive *S. typhi* have now been made as candidate oral vaccines. *S. typhi* strains were first given a requirement for serine, pyridoxin, and aromatic metabolites (serC⁺ *aro*⁻ phenotype) by transducing in a Tn10 insertion in the proximal part of the serC-*aroA* operon. A genetically analyzed *aroA* deletion mutation in *S. typhimurium* was next transferred to the serC⁺ *aro*⁻ *S. typhi* recipients by cotransduction with serC⁺. The resulting stable *aro*⁻ strains were given a his (histidine requirement) gene by two steps of transduction, to further distinguish them from all "wild" strains. As an additional guarantee of safety, a proven deletion in
a pur (purine biosynthesis) gene was next transferred, by cotransduction with an adjacent silent Tn10 insertion, from an S. typhimurium donor into an S. typhi strain made aro'(stable)his. The tetracycline resistance caused by the silent Tn10 insertion was eliminated and a Vi-negative mutant was selected. In mouse tests either the aro mutation alone or the pur mutation alone sufficed to cause nonvirulence. Dr. Stocker now proposes the aro his pur strain, in Vi-positive and Vi-negative form, for testing in volunteers as an oral, live S. typhi vaccine.

Dr. John B. Robbins (National Institute of Child Health and Human Development, NIH, Bethesda, Maryland) reevaluated the immunopathogenic role of the capsular polysaccharide of S. typhi (Vi antigen) in typhoid fever [47]. This reevaluation, summarized below, has also prompted interest in testing the Vi antigen as a candidate as reported by Dr. Carol O. Tacket.

The Vi antigen is a polysaccharide capsular surface antigen composed of a linear homopolymer with a repeating unit of α-1→4,2-deoxy-2-N-acetyl galacturonic acid. Virtually all S. typhi isolated from the blood of patients with typhoid fever are encapsulated with the Vi antigen. Occasional S. typhi from the stool, especially from asymptomatic carriers, may be unencapsulated.

As far as it is known, S. typhi is pathogenic only for humans. This fact has limited research into the immunopathogenic mechanisms in typhoid fever. In animal models (including chimpanzees) and in humans experimentally challenged with live S. typhi, Vi-positive strains are more virulent than Vi-negative strains. It was proposed that the Vi antigen exerted a pathogenic role by shielding S. typhi from the actions of complement. This shielding may be illustrated by the observations that Vi-positive (encapsulated) S. typhi are not agglutinated by O antibodies. Vi antibodies have a higher specific complement-dependent bactericidal activity than do O antibodies. Immunization of mice with Vi-positive, but not Vi-negative, S. typhi or with C. freundii confers immunity against lethal challenge with S. typhi. The significance of this is not entirely clear, however, because mice are not natural hosts for S. typhi infection and do not develop a generalized infection resembling enteric fever. Passive immunization of mice with Vi antibodies of both animal and human origin confers protection against lethal challenge with S. typhi. Originally, Vi antibodies were measured in human sera by agglutination of bacterial cells. Dr. Robbins proposed that studies using this assay, which lacks specificity and sensitivity, led to conflicting results and conclusions regarding the presence of Vi antibodies in relation to typhoid fever or to asymptomatic carriage of S. typhi. Serologic assays using highly purified Vi polysaccharide as an antigen have yielded reliable results that clarify the situation. In fact, the measurement of Vi antibodies using purified antigen has proved to be a reliable index of chronic asymptomatic carriage of S. typhi (see Dr. Lanata's findings above).

Vaccines composed of S. typhi cells and standardized by the mucin-enhanced lethal intraperitoneal challenge assay have conferred immunity against typhoid fever. Vaccines prepared by methods that retained high levels of Vi antigen (acetone-inactivated and designated "K-type") were more protective than were heat-inactivated and phenol- or formalin-preserved vaccines ("L-type" or Vi-poor). The mouse protection test, as used in the United States, is indirectly an assay of the immunogenicity of the Vi polysaccharide in the typhoid vaccine under test. A Vi polysaccharide, extracted from C. freundii, was prepared by Landy and his collaborators in the 1950s. The Vi polysaccharide preparation was subjected to refluxing in 1.0 N acetic acid at 100°C for 24 hr to reduce the levels of endotoxin. This Vi preparation was active in the mouse protection test but failed to elicit Vi antibodies or to protect chimpanzees against bacteremic S. typhi infection. Volunteers immunized with this Vi polysaccharide preparation were similarly not protected against experimental typhoid fever. Dr. Robbins has shown that this reflux treatment depolymerizes the Vi polysaccharide and removes all of its O-acetyl and some of its N-acetyl moieties. Both of these treatments have been shown to reduce the immunogenicity and protective actions of other bacterial capsular polysaccharides as well as the Vi capsular polysaccharide in the mouse model. Vi capsular polysaccharides, prepared by nondenaturating methods, elicit higher levels of Vi antibodies and have a higher protective activity against lethal challenge with S. typhi in mice. The Vi polysaccharide elicited higher levels of Vi antibodies than did the U.S. Standard (K-type or Vi-rich) acetone-inactivated whole cell typhoid vaccine.

Dr. Robbins summarized by saying that the effectiveness of inactivated whole cell vaccines in preventing typhoid fever can be related to their content of immunogenic Vi antigen. The accurate and sensi-
tive measurement of Vi antibodies has provided a reliable method for detecting chronic infection with *S. typhi*. Methods for preparing Vi polysaccharide have improved so that higher molecular weights and lower LPS contents can be achieved. In Dr. Robbins’ opinion, there is sufficient evidence for both the pathogenic and immunologic roles of the Vi capsular polysaccharide of *S. typhi* to reevaluate its potential as a vaccine for the prevention of typhoid fever.

Dr. Robbins has prepared Vi polysaccharides from both *C. freundii* and *S. typhi* that have high molecular weight and low endotoxin (LPS) content. The molecular weights of these Vi preparations are among the highest of bacterial capsular polysaccharides used for vaccines. Initial studies of their safety and antigenicity in volunteers were reported by Dr. Carol O. Tacket (University of Maryland School of Medicine, Baltimore).

Dr. Tacket studied the effects of 50 µg of parenteral Vi antigen supplied by Dr. Robbins in 24 University of Maryland students and 136 Chilean Air Force recruits, comparing adverse effects with those of 24 students and 53 recruits who received 50 µg of meningococcal polysaccharide vaccine. In both groups, recipients of Vi polysaccharide had more local and systemic reactions than did recipients of meningococcal polysaccharide.

[Authors’ comment: It is also clear that effective protection against *S. typhi* can occur in the absence of Vi antibody because the protective Ty 21a oral vaccine lacks Vi antigen.]

Paired sera were examined for antibody to Vi and LPS O antigen. Fifty-eight percent of Maryland students and 69% of Chilean recruits had fourfold or greater rises in Vi hemagglutinating antibody. However, 83% in both groups also had rises in antibody to *S. typhi* LPS O antigen by ELISA. Tests confirmed that this antibody did not represent a response to possible Vi antigen contaminating the LPS antigen in the ELISA but rather a host response to residual LPS in the Vi vaccine. Parenteral Vi antigen produced fewer reactions than have been reported for whole cell vaccine but more reactions than for meningococcal vaccine or for oral Ty 21a vaccine. The residual LPS may have been responsible for the adverse local and systemic reactions. Further studies with a more highly purified Vi preparation are planned.

Summary and Conclusions

Dr. Myron M. Levine summarized the workshop. He noted that workshop papers on the epidemiology of typhoid fever stressed the difficulty in quantifying typhoid fever as a public health problem in many areas of the world because precise incidence and prevalence data were lacking. However, serologic tests for the prevalence of H antibodies to *S. typhi* provide a means of estimating the relative importance of typhoid fever in such countries. Populations cited as being at high risk for typhoid fever were school-aged children in developing countries, visitors from industrialized countries who travel to endemic areas, and technicians in clinical microbiology laboratories. These populations represent potential candidates for immunization with new, improved typhoid vaccines.

Attention was focused on the apparent worldwide differences in case-fatality rates and in clinical severity of typhoid fever in different geographic areas. It is not known if such differences are due to geographic variations in strains of *S. typhi*, promptness of therapy, or host factors.

Several speakers conducted case-control and family-based investigations in only partially successful attempts to unravel the complexities of transmission of *S. typhi* in endemic areas. The long incubation period of typhoid fever and the high infection-to-case ratio represent major impediments to such epidemiologic studies.

A systematic study from Santiago, involving blood cultures of several hundred infants with low-grade fever, demonstrated that *S. typhi* and *S. paratyphi* A and B can cause a mild, self-limited bacteremic illness. These data imply that in infants *S. typhi* infection is commoner than previously appreciated and, for reasons that are not entirely clear, may not present clinically as enteric fever. High-dose steroids have proved successful in the treatment of severe typhoid fever occurring in Indonesia.

Progress has been made in improving the simplicity and accuracy of identifying chronic *S. typhi* carriers. A simple passive hemagglutination test utilizing highly purified Vi antigen was 75% sensitive and at least 92% specific in identifying chronic biliary carriers of *S. typhi* in an endemic area (Santiago). A four-week course of oral amoxicillin and probenecid cured only 55% of chronic carriers with biliary tract disease. Thus, other antibiotic regimens must be sought that will achieve higher cure rates before such domiciliary therapy can be considered a practical public health tool.

Speakers from six countries, comparing bacteri-
ologic methods for the diagnosis of acute typhoid fever, agreed that bone marrow culture represents the single most sensitive procedure for the recovery of *S. typhi* and that clot culture offers no advantage over blood culture. Further investigations of the usefulness of duodenal string cultures are warranted, particularly in children. A review of the status of immunoassays for the rapid diagnosis of typhoid fever leads to the conclusion that a test does not yet exist that satisfactorily combines sensitivity, specificity, simplicity, rapidity, and economy for the rapid immunodiagnosis of typhoid fever.

An enigmatic feature of the epidemiology and microbiology of typhoid fever is the relatively low prevalence of antibiotic-resistant *S. typhi* strains in endemic areas, despite the widespread use of antibiotics and the high-grade antibiotic resistance of many other Enterobacteriaceae. Studies undertaken to investigate this phenomenon found that in vitro *S. typhi* readily accepts R factors bearing antibiotic resistance. However, the growth of these *S. typhi* strains is diminished, and their plasmids are somewhat less stable than they are in *E. coli*. These observations in part explain the low prevalence of antibiotic-resistant *S. typhi* strains.

Several papers on murine typhoid described results of experiments with *S. typhimurium* that may have relevance to *S. typhi* infections in humans. These include the demonstration of flagellae as a virulence factor and the use of mice with various genetic defects for testing the efficacy of live and killed *S. typhimurium* vaccines. In strains of mice that are inherently resistant to *Salmonella*, killed whole cell parenteral vaccines that induce antibody are highly efficacious. However, in hypersusceptible mice, only a live attenuated *S. typhimurium* strain used as an oral vaccine is able to confer protection, presumably because it induces cellular immunity.

Measurement of cell-mediated immunity using newly developed antigens, such as artificial glycolipids, represents one of the exciting areas of research in typhoid fever. Synthetic antigens of *Salmonella* may be particularly important in measuring human immune responses to live oral typhoid vaccines.

As for vaccines, the large-scale field trials in Santiago have shown moderate efficacy for two or three doses of live attenuated vaccine strain Ty 21a given in a highly practical enteric-coated formulation. Other live vaccine candidates, the *aro*-*pur* auxotrophic mutants, have reached the point where they are ready for trials in humans. A different approach to immunization against typhoid fever involves the use of purified Vi polysaccharide antigen as a parenteral vaccine.

The exciting new research presented in this multidisciplinary workshop engendered a sense of optimism among participants for improved, worldwide control of typhoid fever.

References

1. Louis PChA. Recherches anatomiques, pathologiques et thérapeutiques sur la maladie connue sous les noms de gastroentérite, fièvre putride, dynamiagnie, ataxique, typhoïde, etc., etc., comparée avec les maladies aigues les plus ordinaires. 2 vols. Paris: J-B Bailliere, 1829

2. Budd W. Typhoid fever: its nature, mode of spreading, and prevention. London: Longmans, 1873


15. Hoffman SL, Punjabi NH, Rockhill RC, Sutomo A, Rivai


