REVIEW OF LITERATURE

1.1 Historical overview of typhoid

Typhoid fever was first described as long back as 1659 by Thomas Willis. However, it was only in 1829 that Dr. P. Ch. A. Louis gave a clear definition of the disease, separating it from other fevers and associated the clinical expression of the infection with the essential pathologic lesions in the intestines, mesentric lymph nodes and spleen. He also described the rose spots, intestinal perforation and hemorrhage. William Budd in 1873 provided evidence that bowel discharges were the main source of infection; that the disease was water borne; that milk, food, contaminated linen and other fomites were sources of dissemination and insisted without direct proof that a specific germ caused typhoid. It was in 1884 that Gaffkey, a German, first cultivated and isolated Salmonella typhi as a pure culture from the spleens of infected patients. This was followed shortly by the cultivation of the organism from stool, urine, rose spots and gall bladder of the patients by other German investigators. Pfeiffer and Kolle in 1896 made the first vaccine for human use against typhoid with heat-killed organisms and demonstrated the development of antibodies that passively protected guinea pigs against experimental
infection. It was in that year that Gruber, Durham and Widal each independently reported that convalescent phase serum when mixed with *S. typhi* caused agglutination of the organisms. Thus came in existence the classic serologic test for infection by *S. typhi*, the Widal’s agglutination test. In 1903 Robert Koch outlined three logical methods for typhoid control: disinfect the excreta at its source, improve sewage handling and isolate convalescent patients until they become bacillus free. However, case fatality continued to range upto 30% until Theodore Woodward and his colleagues reported in 1948 that chloromycetin could sterilize blood cultures from typhoid patients. There started the era of antibiotics for treatment of typhoid fever.

1.2 Epidemiology

The first and the only attempt to quantitate the extent of typhoid fever throughout the contemporary world was reported in 1984 by Dr. Dhimon Barua in an international workshop on typhoid fever held at the Pan American Health Organisation in Washington, D.C. Dr. Barua gave an annual estimate of approximately 12.5 million cases in the world (excluding China). This estimate included 6.98 million cases per year in South and East Asia (population 1369 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 South Pacific Islands (population 369 million) and 23,000 in the developed world (population 1131 million).

The countries that report increased typhoid severity share several characteristics: 1) rapidly increasing population, 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 supply and 6) overburdened health care delivery systems (Gangarosa, 1982). All these factors together have led to increased numbers of people coming into contact with larger inocula of S. typhi. This has probably led to an absolute increase in number of cases of typhoid fever with an increase in the incidence and an increase in the prevalence and percentage of severe typhoid cases. The situation has been further complicated by the emergence of strains of S. typhi resistant to commonly used and inexpensive antibiotics (Taylor et al., 1983). In India, S. typhi is the major cause of enteric fevers. Of the 373,522 cases reported in 1979 by W.H.O., there were no fewer than 266,204 from India (Huckstep, 1985).

1.3 Bacteriology

Salmonella typhi is a gram negative, non-sporing
bacterium, about 2-4 \( \mu \) by 0.5 \( \mu \), actively motile with numerous long peritrichous flagella. Almost all the clinical isolates are encapsulated on primary isolation. It is an aerobe and facultative anaerobe and grows well on ordinary culture media. MacConkey's agar and Deoxycholate citrate agar (DCA) are the selective media.

1.4 Pathogenecity

*S. typhi* is primarily an intestinal parasite. It exhibits strong host specificity for man. A disease similar to typhoid fever in man can be produced in Chimpanzees if massive doses of living bacilli are administered orally. However, the incubation is shorter and ulceration of lymphoid tissue of the small intestine does not occur (Metchnikoff & Bersedka, 1911; Edsall et al., 1960; Gaines et al., 1968b). Oral administration of typhoid bacilli to small laboratory animals (rabbit, guinea pig, rat or mouse) does not give rise to any infection of this type or usually to any harmful effect. However, massive doses given intraperitoneally or intravenously would produce fatal infection.

1.5 Antigenic structure

The most extensively studied antigens of *S. typhi* which have been suggested to play a role in the pathogenesis or in the development of immune response are 1) the
somatic or the O-antigen 2) the flagellar or the H-antigen and 3) the capsular or the Vi antigen.

1.5.1 **Somatic antigen:** The somatic or the O-antigen forms the side chain component of the lipopolysaccharide present in bacterial cell envelope. It is composed of a polymer of oligosaccharide molecules in repeating units. Each serogroup within a genus of gram negative bacteria is characterized by a unique O specific chain structure. This is exploited in the Kauffman-White scheme for serogrouping of Salmonellae in which specific antisera are used to classify different Salmonella species (Kauffman, 1975). According to this scheme, S.typhi falls in the serogroup D and contains O antigens 9 and 12. The structure of O-antigenic polysaccharide complex of S.typhi LPS is shown in Figure 1. O9 consists of Tyvelose (dideoxyhexose) coupled to D mannose and O12 is constituted by a sequence in the chain 2 D-mannose $1\alpha \rightarrow 4$ L-rhamnose $1\alpha \rightarrow 3$ D-galactose. The O-antigen is linked through 2-keto-3-deoxy-D-manno-octonate (KDO) of the core oligosaccharide to lipid A as shown diagramatically in Fig 2.

1.5.2 **Flagellar antigen:** The flagellar antigen consists of a single protein of around 60,000 daltons molecular weight. It has both common antigenic determinants shared by different Salmonella species and serotype specific
Fig 1: Structure of O antigenic polysaccharide complex of S. typhi

(2 D-Mannose $\alpha\rightarrow$ 4 L-Rhamnose $\alpha\rightarrow$ 3 D-Galactose) $n=5-30$

Fig 2: Geometrical model of a Salmonella LPS molecule. The dimensions of the individual regions were derived from X-ray diffraction data (from Labischinsiki et al., 1984). HR = hydrophilic region (phosphorylated glucosamine disaccharide); LR = lipophilic region (acyl group) of lipid A; PS = polysaccharide portion of LPS.
determinants; the latter form the basis of Kauffman-White scheme for serotyping. In most Salmonellae, the flagellar antigens exist in two alternate phases, in phase 1 there are one or more antigens and in phase 2 two or more. The phase 1 antigen of \textit{S.typhi} is designated as d; it has no phase 2 antigens.

1.5.3 \textbf{Capsular antigen} : The Vi capsular polysaccharide of \textit{S.typhi} is a linear homopolymer of $1^\alpha \rightarrow 4$, 2 deoxy 2N-acetylgalacturonic acid, with variable O-acetylation to about 90% at the C3 position. It has a molecular weight of more than $5 \times 10^6$ daltons but less than $20 \times 10^6$ daltons (Robbins & Robbins, unpublished data). It has been shown to have two antigenic determinants, one constituted by the O-acetyl galacturonic acid moiety and the other by N-acetyl and carboxyl groups together (Szewczyk & Taylor, 1980).

1.6 \textbf{Pathogenesis}

\textit{S.typhi} enters the body by the oral route. The bacilli after passing through the stomach penetrate the intestinal mucosa to be carried to the blood stream. Primary colonization of the bacteria in the small intestine, as was thought earlier, has been ruled out after it was demonstrated that multiplication of bacteria in the blood stream precedes their
multiplication in the intestine (Christie, 1980). The bacilli have been isolated from the feces during the incubation period only on very rare occasions (Wilson, 1881; Ledingham & Arkwright, 1912). Their presence in large numbers in feces during the 2nd and 3rd weeks of an attack of typhoid fever is due in large part to bacteria re-entering the lumen from the biliary tract or from the intestinal lesions that develop later in the disease. The bacteria find their way into the bloodstream after entering the intestinal lymphatics perhaps via Peyer's patches. A transitory bacteraemia which apparently takes place within 24 to 72 hours after ingestion of bacteria is rapidly brought to an end by the removal of bacilli by macrophages and monocytes of the reticuloendothelial system. Nevertheless, viable bacilli are disseminated throughout the body and apparently persist within the reticuloendothelial cells. The patients are usually asymptomatic during this period. After this, a phase of secondary bacteraemia starts which is associated with the dissemination of the organisms throughout the tissues and a secondary invasion of the intestine. The organisms re-enter the bloodstream producing continuous bacteraemia for days or weeks and the symptomatic phase of infection starts. There is an initial hyperplasia of the endothelial cells which leads to lesions in Peyer's patches followed by
necrosis and sloughing. The gall bladder is one of the most frequent sites of infection and bacilli may remain latent for long periods of time. The organisms multiply in the bile to a high titre, usually without manifestations of cholecystitis and are excreted with bile into the intestinal tract. Stool cultures, which are usually negative for *S. typhi* during the incubation period and early phase of the disease, become positive in a large proportion of cases during the third or fourth week of illness.

The number of organisms ingested seems to be playing an important role in determining the chances of contracting the infection, with no significant effect on the clinical course of the disease (Hornick et al., 1970). Studies in volunteers have shown that with the Quailes strain (Vi +), the dose necessary to cause typhoid fever in 50 percent of the subjects was $10^7$ organisms, though a few volunteers remained well after ingesting 100 times this dose. Vi negative strains, though less infective, caused clinically typical disease in some of the volunteers and symptomless infection in others. Thus it is evident that different strains of typhoid bacilli vary considerably in their capacity to produce disease in humans.

An important protective mechanism against invasion by *S. typhi* resides in the upper intestinal tract. Studies
in volunteers have demonstrated that antimicrobial therapy a day or so before oral challenge with *S. typhi* markedly decreases the number of bacilli required to produce disease. It is possible that certain factors known to be associated with typhoid outbreaks, such as malnutrition, enhance susceptibility to typhoid infection by alterations in the intestinal flora or other host defenses.

The factors responsible for fever, leukopenia and other manifestations of typhoid have been inadequately defined. Biologically active lipopolysaccharides or endotoxins present in typhoid bacilli have been shown to produce fever, leukopenia, thrombocytopenia and hyperplasia of reticuloendothelial cells when injected into animals or humans (Morgan, 1943; Hornick et al., 1970; Greisman et al., 1963; Woodward, 1963; Morgan, 1941; Favorite & Morgan 1942). However, the evidence regarding the role of endotoxin in causing the manifestations of typhoid is inconclusive. The development of tolerance to pyrogenic effects of endotoxins during convalescence phase of illness in patients and volunteers infected with *S. typhi* suggested the release of endotoxins during infection (Greisman et al., 1963; Woodward, 1963; Neva, 1950). However, studies aimed at detection of circulating endotoxin in patients with typhoid fever have yielded conflicting
results (Butler et al., 1978; Maglilulo et al., 1976). The endotoxin tolerance was proposed as an important mechanism in recovery from typhoid fever (Greisman et al., 1963,64). But in subsequent studies it was shown that typhoid fever follows a normal course in volunteers rendered tolerant to endotoxins prior to challenge (Hornick et al., 1970; Greisman et al., 1964; Greisman et al., 1969; Woodward, 1963). Thus it has been suggested that endogenous pyrogens released by local inflammatory effects of *S. typhi* may sustain pyrexia in typhoid fever. The role of flagella in pathogenesis has been demonstrated in murine typhoid (Carsiotis et al., 1984; Weinstein et al., 1984). The studies suggested that flagella either protected *S. typhimurium* from intracellular killing mechanism of murine macrophages or that flagella enhanced the ability of *S. typhimurium* to multiply within the macrophages.

1.7 Pathology

The most prominent microscopic lesion in typhoid fever is proliferation of large mononuclear cells in many different tissues. Mononuclear hyperplasia leads to lymphadenopathy, splenomegaly and enlargement of lymphoid tissues in the intestines, especially in Peyer's patches. Proliferation of mononuclear cells may also be observed in bone marrow, liver and lung.
Necrosis in Peyer's patches may be associated with erosion of blood vessels in the lesions in the intestinal tract which leads to oozing of blood or massive hemorrhage. Lesions may extend deep into the intestinal wall and cause perforation, usually in the distal ileum. This takes place most often in the third febrile week.

The gall bladder and bile ducts are routinely infected during the disease. The biliary infection is mostly asymptomatic although acute cholecystitis may occur occasionally. In addition to intestinal perforation and hemorrhage other complications which could arise due to localized infection include meningitis, chondritis, periostitis, osteomyelitis, arthritis and pyelonephritis. However, these complications are very rare. Jaundice secondary to extensive infiltration of mononuclear cells in the liver and hepatic cell necrosis is again a rare complication. Acute renal failure leading to so called typhoid nephritis is also observed rarely.

1.8 Clinical manifestations

The clinical manifestations show marked variation from one patient to another. The incubation period averages about two weeks but may go up to eight weeks depending upon the infecting dose. Mild form of the disease,
characterised primarily by fever, may last only a week. The onset of the disease is insidious with headache, malaise, anorexia and fever. The fever is remittant, frequently increasing in a step ladder like manner from day to day as the disease progresses. A relative bradycardia occurs in 30 to 40 percent of the patients. Mental dullness and delirium are associated with prolonged persistent fever. Abdominal pain and marked distention are usual. Constipation during the early phase of illness may give rise to diarrhea later in the course of the disease. The characteristic rash (rose spots) are often observed during the second week of the disease. The lesions are small, 2 to 4mm, erythromatous macules which occur in small numbers on the upper abdomen and anterior thorax. The lesions blanch on pressure and last only 2 to 3 days. The spleen and liver are frequently enlarged and palpable after the first week of illness. The spleen is palpable in about 75% of the patients. The liver may be tender and occasionally a friction rubber is audible over the spleen. The symptoms slowly abate after the third week and temperature returns to normal over a period of days. The incidence of relapse is 5 to 10 percent. It is usually milder and of shorter duration than the original illness.
1.9 Chronic carriers

About 3% of typhoid patients become chronic carriers and continue to excrete organisms in feces for years. The chronic carrier state is rare in children and occurs more commonly in women than men. The chronic biliary carrier is usually asymptomatic. Despite millions of organisms entering the intestine in each milliliter of bile, patients show no systemic manifestations.

1.10 Immune response during Typhoid fever

The mechanism of host defense in typhoid fever is not very well understood. Both humoral and cell mediated immune responses develop during the disease. A majority, if not all, of typhoid patients develop antibodies to somatic(O), flagellar(H) and capsular(Vi) antigens of S.typhi (Dham & Thompson, 1982; Olitzki, 1982). The antibodies start appearing about one week after the illness and gradually increase during the following days. Anti-0 antibodies appear earlier than anti-H antibodies and fall more quickly. Tsang et al., (1981) reported anti-LPS antibodies of all three classes (IgM,IgG,IgA) in patients suffering from typhoid fever. Antibodies to the flagellar antigen are predominantly of IgG type and persist for a long time even after the disease is over. These may also be stimulated by
subsequent non-specific febrile illness and are, therefore, of less diagnostic value than anti-0 antibodies. Antibodies to Vi capsular polysaccharide do not appear with any regularity during typhoid fever. However, these have been found to be very useful in detecting the carrier state (Lanata et al., 1983; Losonsky et al., 1987; Lin et al., 1988). In addition antibodies of IgM, IgG and IgA classes against protein antigens have also been reported but the nature of these antigens has not been defined (Tsang et al., 1981). Chau et al. (1984) have reported antibodies to non-O, non-H and non-Vi antigen in the sera of typhoid patients and carriers. Calderon et al. (1986) reported high antibody levels against outer membrane protein antigens (OMP) of S. typhi in sera from typhoid patients in acute phase and convalescent phase.

The antibodies to S. typhi do not seem to afford any protection in patients with naturally acquired typhoid infection (Dham & Thompson, 1982; Sarma et al., 1977). The levels of antibodies do not have any relationship with the severity of illness or relapses. However, it has been reported that patients with low antibody titres seem to suffer from intestinal perforation more often (McKendrick, 1978). This could be due to diminished local immune response (IgA synthesis) in these patients. High levels of IgA antibodies against LPS and protein
antigens of *S. typhi* have been reported in typhoid carriers (Chau et al., 1981).
The development of cell mediated immunity (CMI) and its possible role in protection in typhoid fever has been demonstrated by many workers. Dham & Thompson (1982) studied CMI and antibody response in typhoid patients and TAB (vaccine comprising of *S. typhi, S. paratyphi A* and *S. paratyphi B*) vaccinated subjects. CMI as assessed by lymphocyte migration inhibition (LMI) developed in all cases of typhoid fever in the first week of illness and was maintained during the progression of the disease. In some patients LMI could be seen even after a year. Five out of nine TAB vaccinated subjects also developed this response at the end of three weeks. Similar studies have been carried out by Kumar et al. (1974). Rajagopalan et al. (1982) studied LMI, blast transformation and E-rosetting in patients suffering from typhoid fever. Specific lymphocyte sensitivity could be demonstrated in the uncomplicated cases of typhoid fever during second week of illness. In complicated cases these tests were usually negative. However, the antibody levels were comparable in the two categories of patients. A relationship between complications and lack of cell mediated immune response was thus a reasonable assumption. The presence of high levels of circulating immune complexes (CICs) and high
levels of Fcγ bearing T cells has been suggested to be responsible for suppressed CMI in these patients (CICs are known to stimulate suppressor T cells and other suppressor factors - Perry et al., 1978).

Studies on efficacy of typhoid vaccine by Nath et al. (1977) have shown that TAB vaccine could induce good anti-O and anti-H response three weeks after vaccination but no cell mediated immune response. Moreover, vaccination produced transient unresponsiveness in LMI positive subjects; the subjects became LMI negative and reverted back to normalcy within eight weeks. This would imply that typhoid vaccine should be avoided during epidemics.

In an experimental model of typhoid, mice depleted of T cells were able to survive the infection with Salmonella enteritidis but could not get rid of bacilli suggesting that the defect in CMI may have something to do with the carrier state, at least in mice. These mice were not protected with live Salmonella vaccine against subsequent challenge with virulent strains of Salmonellae (Davies & Kotlarski, 1976). Eisenstein et al. (1984) and Killar & Eisenstein (1984) have shown in mouse model of typhoid that antibody is sufficient to protect inherently resistant mice (C3H/HeN.Cr.1BR) against the infection but is ineffective or poorly effective in protecting inherently susceptible mice.
(C3H/HeJ). However, since mouse is not a natural host of *S. typhi*, it is difficult to argue about any correlation between these studies and typhoid fever in man. Udhayakumar and Muthukkaruppan (1987) have shown that outer membrane proteins of *S. typhimurium* could elicit a long lasting protective response to infective doses equivalent to 50 times the half-lethal dose. Recently Armando Isibase et al. (1988) have demonstrated that mice immunized with outer membrane proteins isolated from *S. typhi* 9,12 d Vi were protected against *Salmonella typhi* infection.

Natural antibacterial activity against *S. typhi* by human T4 lymphocytes armed with IgA antibodies has been demonstrated by Tagliabue et al. (1985). Antimicrobial treatment does not affect lymphocyte migration inhibition (Sarma et al., 1977). There are conflicting reports about the effect of chloramphenicol therapy on the development of anti-O and anti-H antibodies (Kumar et al., 1974; Gulati et al., 1968).

1.11 Diagnosis

1.11.1 **Bacteriological culture:** The most reliable way of establishing a definite diagnosis of typhoid fever is by blood culture. About 70-90 percent of the patients show positive blood culture in the first week of illness and about 30-40% during the third week (Guerrant, 1987).
Blood cultures are frequently positive during relapses. The number of organisms in the blood during typhoid fever is generally very small and may be as low as one to ten organisms per ml of blood. Therefore, sometimes the blood sample has to be incubated for a longer time.

Bone marrow culture has been shown to increase the sensitivity of diagnosis especially in cases where the patients are admitted while on antibiotic treatment (Sing et al., 1948). In a study carried out by Gilman et al. (1975), it was found that diagnosis would have been missed in 24 of 62 cases if cultures of bone marrow and of skin biopsies from rose spots had not been undertaken along with blood cultures.

Only about 10-15% of typhoid patients have positive stool cultures during the first week of illness. However, the frequency of positive stool culture increases as the disease progresses, with about 75% of the cases showing positive stool culture during third or fourth week of illness. After that it starts declining so that by eight week 10% of the patients are positive except for the chronic carriers who secrete the organisms in the feces even after one year. The incidence of positive urine cultures varies markedly during the course of typhoid fever and parallels the frequency of positive stool cultures.
1.11.2 **Serodiagnosis**: The most commonly used serological test for the diagnosis of typhoid fever is Widal's agglutination test (Widal, 1896). The test is based upon detection of serum antibodies against O and H antigens of *S. typhi*, capable of agglutinating fixed, killed bacteria. A fourfold increase in anti-O antibody titre is very much suggestive of *S. typhi* infection (% titre is regarded of little diagnostic value). Since a rise in titre must be demonstrated, at least two samples taken a week or more apart are required. The test becomes positive only a week after the onset of fever and lacks specificity. Due to high endemicity of the disease agglutinins are frequently found in normal and febrile non-typhoidal sera. The antibodies are also produced in response to vaccination. Hence the value of a single sample obtained from a patient in a typhoid endemic area or a putative typhoid patient who has been vaccinated is limited. The value of this test has been questioned time and again (Abraham et al., 1981; Levine et al., 1978) although it still remains the most widely used diagnostic test.

Many workers have reported more sensitive antibody-based tests for diagnosis of typhoid fever. Carlsson et al. (1975) used enzyme linked immunosorbent assay for the detection of anti-O antibodies. The assay although more sensitive and more reproducible did not offer any
advantage over Widal's agglutination test in terms of specificity. Antibodies bound to LPS from serogroup D as well as serogroup B Salmonellae. Several other workers have used enzyme immunoassays (Beasley et al., 1981; Nordiello et al., 1984) for serodiagnosis of enteric fevers. Tsang et al. (1981) demonstrated high levels of antibodies against lipopolysaccharide and protein antigens of *S. typhi* in the sera of typhoid patients, by radioimmunoassay. Chau et al. (1981) showed the presence of IgM and IgA class of antibodies in the intestinal lavage of typhoid patients and IgA type of antibodies in typhoid carriers.

Lim and Ho (1983) used a competitive enzyme immunoassay based upon anti-09 monoclonal antibody for the diagnosis of typhoid fever. However, even this assay could not differentiate patients, carriers and TAB vaccinated subjects nor could it discriminate typhoid fever from paratyphoid.

When combined with the determination of class of immunoglobulins, the antibody-based assay may provide a more sensitive means of diagnosing typhoid fever. However, since the antibodies appear 5 to 6 days after the onset of fever and are also produced in response to vaccination, antibody-based test may not be ideal for an early and definite diagnosis of the disease.

A number of studies have shown the presence of
circulating antigen in typhoid fever, using counterimmunoelectrophoresis (CIE) and coagglutination assays based upon polyclonal antibodies (Harish & Sambasiva Rao, 1983; John et al., 1984; Gupta & Rao, 1979). CIE, however, is less sensitive and cannot be performed routinely in primary health centres. Coagglutination test, though less time consuming needs prior absorption of the serum with Staphylococcus aureus. Rockhill et al. (1980) demonstrated, by slide agglutination, the presence of D, d and Vi antigens in urine from typhoid patients. Barrett et al. (1982) used ELISA for the detection of Vi in the urine. Taylor et al. (1983) compared these two assays for Vi antigen detection in urine samples obtained from patients with acute typhoid fever, paratyphoid fever, other febrile illnesses and afebrile control subjects. Both the tests were found to have low sensitivity and specificity and were, therefore, of little value for the diagnosis of typhoid fever. In both the assays false positivity with paratyphoid fevers and febrile non-typhoidal subjects was very high. The relative lack of specificity of the antibodies was suggested to be responsible for low sensitivity and specificity. In fact in all the polyclonal antibody-based assays for antigen detection it has to be made sure that the antiserum is extensively absorbed with related bacteria otherwise one would end
up with high false positivity owing to extensive cross-reactivity amongst the gram negative bacteria.

Recently attempts have been made to produce monoclonal antibodies against *S. typhi* and use them for diagnostic purposes. Lim and Fok (1987) used anti-09 monoclonal antibody for the detection of group D *Salmonella* in blood culture broths. Tsang and Chau (1987) raised monoclonal antibodies against Vi and propose to use them for diagnosis of typhoid fever. Monoclonal antibodies have also been generated against a 34Kd protein antigen of *S. typhi* and may find application in the serodiagnosis of typhoid (Sarasombath et al., 1988; Appasakij, 1987). An 8.6 kilobase DNA probe encoding a genetic locus vía B involved in the synthesis of Vi antigen has been developed by Frank Rubin (1985) and his colleagues. Initial studies with bacterial isolates from blood, bile, bone marrow and stool specimen from patients in Peru and Indonesia, using in situ hybridization, have shown the probe to be highly sensitive and specific. Attempts to detect *S. typhi* directly in specimens of blood and stool are in progress (Rubin et al., 1988).

1.12 Treatment

Chloramphenicol remains the drug of choice for the treatment of typhoid fever. It has been consistently shown to be more effective than other antimicrobial
agents in terminating the febrile toxic course of the disease in the greatest proportion of patients in the shortest period of time. This antibiotic is bacteriostatic. Bacteraemia usually clears within hours after therapy is instituted but occasionally organisms can be recovered from blood 24 to 48 hours after the treatment (Guerrant, 1987). Bone marrow damage and hemolytic crisis in persons with Glucose-6-phosphate dehydrogenase deficiency are the two major drawbacks of this drug (Brauer & Damashe, 1967; Wallerstein et al., 1969; Galpine, 1949). Chloramphenicol resistant strains have been reported since 1972 from many parts of the world especially Mexico, South-east Asia and India (Anderson, 1975; Panikar & Vimala, 1972; Sharma et al., 1979; Vongsthongsri & Tharavanij, 1980; Taylor, Pollard & Blake, 1983). Resistance is due to a transferable R factor which also codes for resistance to sulfonamides, tetracycline and streptomycin. Chloramphenicol does not work with typhoid carriers. Ampicillin, amoxicillin and trimethoprin-sulfamethoxazole are other useful drugs which have been found to be effective in the treatment of typhoid fever (Guerrant, 1987). Ampicillin is the drug of choice for the treatment of typhoid carriers (Christie, 1964; Scioli et al., 1972). In severe cases of typhoid fever administration of dexamethasone along with chloramphenicol has been found to reduce the
mortality (Hoffman et al., 1984).

1.13 Vaccines

The first vaccine for human use against typhoid was made by Pfeiffer and Kolle (1896), with heat killed organisms. However, it was not before 1960s that well controlled field trials were carried out to study the efficacy of the vaccines against typhoid fever. The first trial in Yugoslavia (W.H.O. report, 1957,62) showed that two doses, at three week interval, of heat killed phenolized vaccine were followed during the next year by a fall in the annual average attack rate per ten thousand from 19.2 to 6.5 but that two doses of alcoholized vaccine made from the same strain resulted in an attack rate of 14.1. The next trial in Guiana (W.H.O. report, 1964) compared a heat killed phenolized vaccine designated as L and acetone killed vaccine designated as K (acetone was used with the objective of preserving Vi antigen). Both the vaccines were found to be effective, with K vaccine slightly better. This was followed by efficacy studies in Yugoslavia, Guiana, Poland and U.S.S.R. (Cvjetanovic & Uemura, 1965). The efficacy of these vaccines was confirmed by the protection studies with typhoid bacilli. With an inoculum of $10^5$ organisms per os of a strain that gave 40 percent attack rate in unvaccinated subjects, the
percentage effectiveness of L vaccine was 67 percent and that of K vaccine 75 percent (Hornick & Woodward, 1967; Woodward, 1980). However, it was found that vaccination was not effective when the volunteers were infected with $10^7$ organisms. Furthermore these vaccines provoked unpleasant side reactions and there was no relationship between antibody levels against O,H and Vi antigens and resistance to the disease, relapses or reinfection. The vaccine had no effect on the severity and course of illness compared to the disease in the unvaccinated persons.

The killed oral vaccines have been found to confer only minimal protection against experimental infection in man (Woodward, 1980; Chutani et al., 1971). Infact, their effectiveness has been questioned even more than the killed parenteral vaccine.

The present human typhoid vaccine consisting of heat killed bacteria ($S. typhi$, $S. paratyphi$ A and $S. paratyphi$ B) confers only short term immunity and does not prevent relapses (Cvjetanovic, 1978). Little is known about the efficacy of its paratyphoid A component and since paratyphoid B is seldom of much importance in the geographical areas in which typhoid infection is prevalent (Santiago is one exception - Edelman & Levine, 1986), opinion now favours the use of a vaccine composed only of typhoid bacilli.
Work has been going on to study the efficacy of live oral vaccines for prophylaxis against typhoid fever for the last ten years or so. The results seem to be quite promising. The first to be studied was a streptomycin-dependent mutant of *S.typhi*. When freshly harvested and given orally it was effective in 66 to 78 percent of the subjects challenged with a dose of $10^5$ organisms (Levine et al., 1976). However, this vaccine lost its efficacy on freeze drying. In 1975 Germanier and Furer described a stable double mutant of the virulent but Vi negative strain Ty2 that lacks the enzyme UDP-galactose-4-epimerase. This galE mutant designated Ty2la, proved to be safe to administer and stable in human body and when given orally was 87 percent effective in preventing experimental typhoid fever in man (Gilman et al., 1977). A large scale field trial of the vaccine was conducted in school children in Egypt (Wahdan et al., 1980,82). Three doses of the vaccine (freeze dried material reconstituted in sucrose phosphate buffer) were given at two day intervals, 10 minutes after a tablet of sodium citrate had been chewed. The vaccine given to 19,000 children had 96 percent efficacy. Another large scale field trial was conducted in 1982-83 in school children in Santiago, Chile. The level of protection against typhoid fever was found to be substantially lower (60 to 90 percent) than the efficacy of 95 percent in Egyptian
field trial. Several reasons were considered for this reduced efficacy. 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 children was five times that of Egyptian children. Thus a second field trial was conducted in 1983-84 in Santiago with some modifications, in about 30,000 children. Three doses of enteric-coated vaccine given every other day had an efficacy of 75 percent while the one given every 21 days was 71 percent efficient. Children who were > 15 years of age were better protected than were children 5 to 9 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.

The vaccine *S. typhi* Ty2la described above was made by extensive non-specific mutagenesis which induced mutations other than gal E. These include i) a *via* mutation blocking Vi antigen synthesis (Germanier & Furer, 1983) ii) one or more mutations giving Ty2la a growth rate half that of its parent Ty2 (Germanier &
Furer, 1975) iii) a mutation(s) causing a requirement for valine and isoleucine (unpublished observation) and iv) an inability to produce H₂S (Germanier & Furer, 1983). Some of these mutations and perhaps others yet to be detected, may contribute to the attenuation of Ty2la. More recently one or more mutations of molecular character have been introduced to convert a virulent into a non-virulent strain. These strains are obtained by procuring non-reverting mutations causing requirement for certain metabolites. Two such strains S.typhi 541 Ty, strain bearing deletions in pur A and aro A and its Vi negative derivative S.typhi 543 Ty, have been tested for safety and immunogenecity in humans (Levine et al., 1987). The mutation in aro A made these strains dependent on external supply of essential aromatic metabolites and the one in pur A resulted in the loss of enzyme activity for three steps in purine biosynthesis pathway before inosine monophosphate thus causing a purine requirement. The two together led to a virtually complete loss of virulence. Both the S.typhi strains induced good CMI response but virtually no antibody response. Since the vaccinees were not challenged with virulent S.typhi, the protective efficacy of these vaccines remains unknown. Similar derivatives of S.typhimurium have been found to be efficient vaccines in mice and calves (Stocker et al., 1983; Smith et al.,
Work is in progress to construct better live attenuated oral vaccines against typhoid fever (Stocker, 1988).

Another candidate vaccine which has been shown to confer protection against typhoid fever is the Vi capsular polysaccharide (Hornick, 1985; Robbins & Robbins, 1984; Landy, 1954). The Vi vaccine elicited a four-fold or greater rise in serum antibodies in 75% of the children and adults in Nepal and in Eastern Transvaal, Republic of South Africa (Acharya et al., 1987; Klugman et al., 1987). The protective efficacy of the Vi antigen in these two trials was approximately 70%. In contrast, same Vi elicited >4 fold antibody rise in 97% of young adults in France and United States (Tacket et al., 1986). Studies have been undertaken to increase the immunogenicity of Vi by coupling it to a carrier protein like tetanus toxoid or cholera toxin (Szu et al., 1987) which might be more protective in high risk populations. It might be worth mentioning here that effective protection against S. typhi can occur in the absence of Vi antibody because the protective Ty21a oral vaccine lacks Vi antigen.