REVIEW OF LITERATURE

Typhoid Fever

Typhoid fever is caused by a gram-negative, motile, facultative rod named <u>Salmonella</u> <u>typhi</u> which is prevalent in developing countries with lack of sanitation and hygiene. The attack rate of <u>S.typhi</u> is equal among males and females, but is slightly greater in children than adults (3). The carrier state which can occur after infection with <u>S.typhi</u> is the most important factor in maintaining the disease.

Pathogenesis and Clinical Menifestations

When ingested, Salmonella organisms tend to multiply in the gastrointestinal tract. Stomach secretions probably provide some protection, because infections are more common after gastrectomy (5). While in the gut, the organisms cause inflammation of the mucosa, with cramping diarrhea, distention, nausea, and vomiting (5). <u>S.typhi</u> is a highly invasive organisms, so that it rapidly and effeciently penetrates the intestinal mucosa such as Peyer's patches. Having crossed the intestinal mucosa the organisms make their way throughout the reticuloendothelial system, where they multiply in their harbouring phagocytic cells and on

release produce a bacteremia, which is typhoid fever.

Several factors play an important role in the development of the disease following ingestion of salmonella organisms such as, size of inoculum, serotype, environment and the status of host (3). Hornick reported that following administration of S.typhi in volunteers, a dose of 10^5 organisms caused disease in about 35 % of subjects with an average incubation period of about 13.2 days. When the dose of organisms was increased to 10^9 , almost all volunteers become ill with an incubation period of 6 days. Doses of below 10^5 organisms did not cause disease. At times the incubation period can extend to 50 days (6).

On reaching the small intestine, S.typhi rapidly penetrate the mucosal epithelium to arrive in the lamina propria. In non-immune hosts the organisms elicit an influx of macrophages which ingest but are generally unable to kill them. Some S.typhi apparently remain in macrophages in the lymphoid tissue of the mucosal of small intestine, whereas other are drained to mesenteric lymphnodes, where further multiplication and ingestion by macrophages take place. Shortly after invasion of the intestinal mucosal, a primary bacteremia is belived to occur in which S.typhi are filtered from the circulation by phagocytic cells of the reticuloendothelial system. In the mucosa , however , transient bacteremia occurs within 20 hours of ingestion and bacteria reach the liver and spleen shortly thereafter

(7).It is believed that the main route by which S.typhi reach the blood stream in this early stage is by lymph drainage from mesenteric nodes entering the thoracic duct and then the general circulation (8).

The incubation period of typhoid fever is generally one to two weeks but it varies inversely with the size of inoculum and may be as short as three days or as long as two months. Non-specific symptoms, including anorexia, malaise, lethargy, nausea, vomiting and continuous headache, accompany the fever. Typhoid patients have been noted to have constipation rather than diarrhea.

Although some patients experience spontaneous remission of illness in one week or less, high sustained fevers characteristically occur during the second and third weeks. Typhoid fever has been associated traditionally with two late complications : significant intestinal hemorrage and frank perforation. These problems occur secondarily to bacterial invasion of the Peyer's patches, with in turn may lead to necrosis, ulceration and erosion of blood vessels. Perforation and massive hemorrhage are major causes of the reported mortality rate of about 10 % in untreated infections (9).

Relapse, which is a common complication usually occurs one to three weeks after therapy is discontinued. The signs and symptoms are similar to the first episode, but

relapse is usually a milder illness that responds promptly to antimicrobial drugs.

Immune Response in Typhoid Fever

The mechanism of acquired resistance against enteric fever in man is not at present well understood. In the animal model of typhoid which includes extensive studies in mice and rats, the existence of both type of immune response, cellular and humoral, are well established (10). The former seems to play a major role in recovery from primary infection, as well as in resistance to reinfection (11). Recent studies in man also indicated a possible protective role for the cell-mediated immune response (CMIR) in typhoid fever (12,13).

Antibody to O,H and Vi antigens are not relevant for protection (5) and reinfection or relapse are known to occur in spite of high O and H antibody levels (14).

1. Cell-mediated Immune Response (CMIR)

The causative organisms of typhoid fever is a facultative intracellular parasite, thus a cell-mediated immune response is essential for resistance to this organism.

It has been found from the studies in typhoid patients that CMIR appears after first week of illness and persist at least for 3 weeks, as estimated by lymphocyte migration inhibition (LMI). It was often found to be negative in complicated cases of typhoid fever and positive in uncomplicated cases (12,13) so that LMI positivity indicates a good prognosis (13).

2. Humoral Immune Response (HIR)

The humoral immune response in typhoid patients have been mainly determined by standard Widal agglutination. The levels of antibody to O and H antigens are related to the duration of illness. Although humoral antibodies show a lack of protection in general, it has been shown in the experimental mouse model that HIR limits the multiplication of the bacteria early in the course of infection (5).

Serum antibodies have been found to be three major classes of immunoglobulin IgA, IgM and IgG by radioimmunoassay (15) and by ELISA (16). Tsang reported that the determination of antibody to lipopolysaccharide (LPS) or protein antigen of S.typhi is more specific and sensitive than the Widal test for the diagnosis of typhoid fever (15).

Subsequently, Hohmann reported that intestinal IgA was produced most favourably following oral immunization with salmonella in a mouse model (17). Recently it has been

demonstrated that intestinal antibody specific to S.typhi antigens are developed in typhoid patients and at least, there are two classes of intestinal antibodies, IgM and IgA (18,19). Although the specific activity of these intestinal antibodies has not been firmly established, the possibility exsists that they might be the first line of defense mechanism following S.typhi infection.

Defense Mechanisms of the Intestinal Tract

The local immune system within the intestine provides a number of mechanisms where by pathogenic microorganisms are expelled from the gut, but in addition the intestinal tract possesses features of inate resistance which, since they act in synergy with those of immune system, must be considered in any discussion of immune mediated protection in the gut.

1. Non-specific Defense Mechanisms
(inate resistance)

1.1 **Gastric Acidity** Gastic juice, with its low pH, acts as an efficient bactericide limiting the number of viable bacteria and viruses which can enter the intestine. The bactericidal effects of gastric pH can also reduce the antigenicity of protein (20) and this is further affected by proteolytic enzymes within the small intestine (21).

1.2   **Mucus**   Mucus lubricates the epithelial surface, protects it from the low pH of the stomach and acts as a medium in which digestion and absorption can occur. It has direct antimicrobial effects by cleansing the epithelial surface. It can entrap bacteria and nematodes (22,23) and so facilitate their removal.

1.3   **Peristalsis**   Peristalsis is probably the major factor reducing bacterial numbers within the small intestine. Organisms introduced into the small intestine are rapidly cleared by peristalsis (24). So effective is peristalsis that it is proably essential for microorganisms that colonise the small intestine to adhere to the epithelial surface via specific receptor mechanisms (25,26) and inhibition of this adherence is a powerful immune strategem.

1.4   **Normal Flora**   The normal microflora of the small intestine represents a stable ecosystem which appears to be able to resist colonisation by pathogenic microorganisms. The normal bacteria do this either by successfully competing for essential nutrients or they may render the environment toxic to the newcomers by altering the pH or producing toxic agents such as colicins or volatile fatty acids.

In addition to these direct effects the intense competition that the indigeneous microflora provides will

amplify any small antibacterial effect so lead to the rapid displacement of a bacterial species which is selectively disadvantaged, even through the degree to which it is discriminated against may be quite small (27).

1.5   Lactoferrin, Lysozyme and   Interferon Lactoferrin is found in intestinal secretions. It has a bacteriostatic effect on E.coli by successfully competing for iron (28,29). Lysozyme with its powerful antibacterial effects is produced within small intestine (30) and may exert its effect in the mucus layer , which has been shown to possess phagocytic activity. Interferon has recently been demonstrated in the intestine of pigs following infection with transmissible gastroenteritis (TGE) virus and may play an important part in antiviral defense.

1.6 Epithelial Turnover The epithelium of the intestine is continually being renewed. The epithelial cells are produced in the depths of the intestinal crypts, migrate upwards through the length of the crypt and are shed from the villus not only from its tip but over its entire area. The time taken for this transit is dependent on many factors e.g. different stimuli, including hormones, intestinal secretion feeding, feed back signals from mature villous cells, aging and immune response but in man it is about 3 days in the ileum and 5-6 days in the duodenum.

2.   Specific Defense Mechanisms(immune resistance)

2.1   Humoral Immune Response (HIR)   Antibodies
that   protect the mucosa of the bowel   against   non-invasive
organisms   or   their   products   can   be   derived   from   two
sources,   namely   serum and plasma cells in   the   intestinal
lamina   propria. The mechanisms for deriving   antibody   from
serum   appears   inefficient because   protective   amounts   of
antibody are present only when serum antibody titer are high
and these can rarely be sustained. Serum antibody that makes
its   way   into   the lumen of the gut   is   predominanty   IgG.
Antibodies   produced locally by plasma cells in   the   lamina
propria,   however,   are usually IgA,   they   are   selectively
secreted   on   the mucosal surface by the   crypt   epithelium.
Since   secretory   IgA   resists   proteolysis   by   intestinal
enzymes,   it appears better designed for protection   of   the
mucosal surface than IgG (31).

2.2   IgA   and   secretory   IgA   Although   IgA
is   relatively   abundant   in serum   it   is   the   predominant
immunoglobulin   in   mucus secretion e.g.   oral,   urogenital,
nasal,   bronchial,   intestinal secretion, tears, and milk(32).
Congenital   deficiency   of   IgA   is one of the   most   common
genetic disorders in humans and almost certainly involves   a
defect in regulation of immunoglobulin production (33).

2.2.1   Structure and Chemistry of IgA   In
man,   the   molarity   of IgA is the monomer,   a   Y-shaped   7S

protein with a molecular weight of approximately 165,000. Between 5-15 % of the circulation IgA is dimeric, with two IgA monomer linked via the J chain. The biological significance of the J chain and the dimeric or higher polymeric configuration is that the polymers have the ability to combine with secretory component producing IgA (34,35). IgA has a half-life of about 5-6 days and is synthesized at a rate of about 22 mg/kg body weight per day and is equivalent to 225-255 mg/dl of serum (36).

In secretions, the predominant form is the dimer. The two proteins associated with the latter, are termed secretory compenent and J chain (Figure 2). Therefore the human secretory IgA is a much large molecule than serum IgA, has twice the serum IgA's molecular weight plus J chain and secretory component. Its M.W. is about 385,000.

The J (for joining) chain has a molecular weight of 15,000 dal (37) and appears to be necessary for the proper assembly of dimeric IgA. J chain consists of 129 amino acid residues, this confers a high net negative electrical charge on the molecule. The amino acid composition of J-chains of human, rabbit, pig and dog origin is very similar and all have about 7.5 % carbohydrate. J chain is attached to the α - chain of secretory IgA by virture of disulfide bonding to the H chain. There is one J chain per two IgA units. Plasma cells that synthesize L chains also synthesize J chain, yet free J chain is scarce in these cell and is
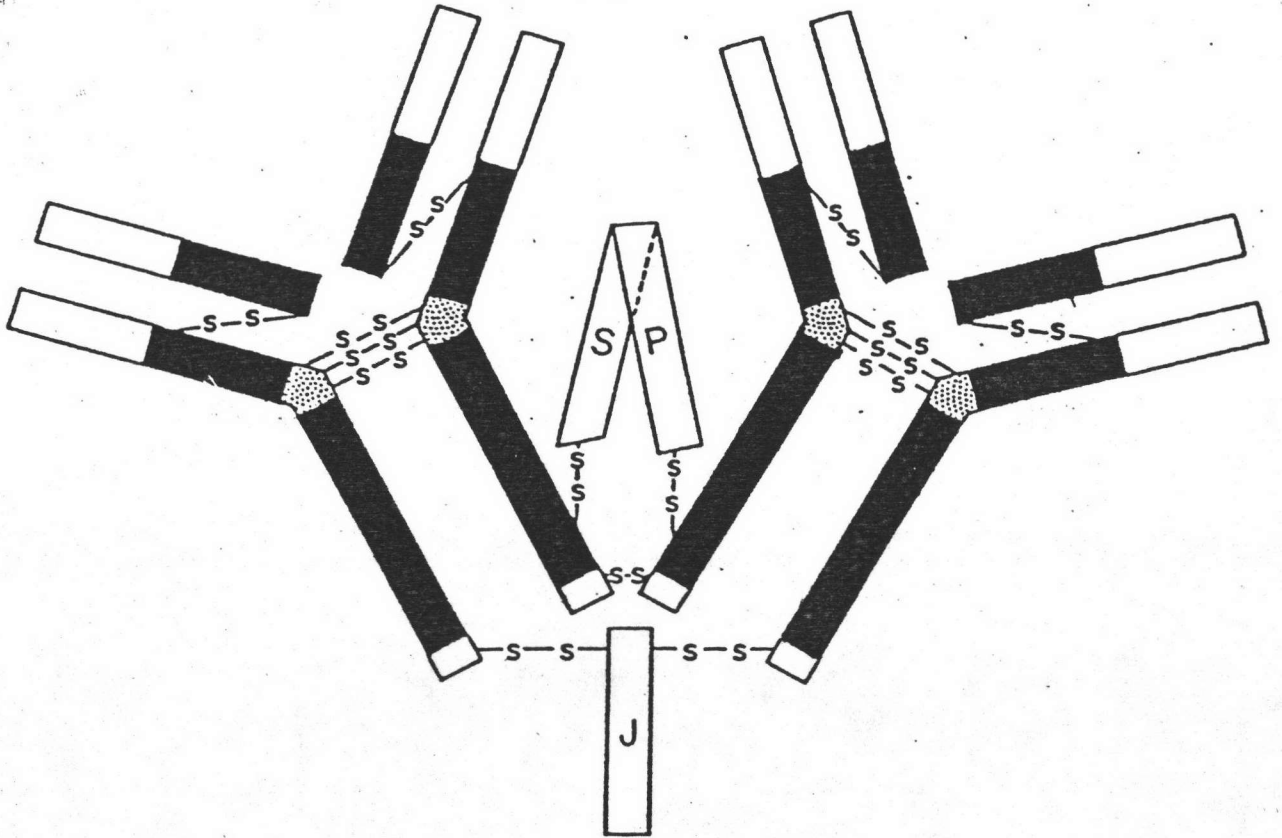
Figure 2   Schematic representation   of human IgA. Four
unique polypeptide chains are indicated ; heavy
and light immunoglobulin chain, J-chain and
secretory component.

(From Parker W.E. Structure and Function of Immunoglobulins.,
Clin. Immunol., 1, 8-9, 1980)

almost entirely bound into the immunoglobulin polymer. Synthesis of J chain is apparently the rate-limiting step in the synthesis of polymeric IgA and IgM and this is the reason it is rarely found as free protein.

Secretory component (SC) is an epithelial glycoprotein of about 83,000 daltons which contains 18-19 % carbohydrate by weight. SC found in secretions by Tomasi (38) as a part of the secretory IgA in all species examined, including dog, cow, sheep, goat, rabbit, mouse and chicken as well as the humans. It binds to a heavy chain through disulfide bonds and non-covalent bonds (39). Within the sIgA molecule, SC affords protection against attack by proteolytic enzymes (20) and contributes to the stabilization of the quarternary form of sIgA (40). SC appears to be synthesised in the glandular epithelial cells and it is probably added to the IgA dimer after crossees to the mucosal basement membrane (41) and it has been demonstrated that the binding of polymeric IgA to the surface or cytoplasm of epithelial cells requires the presence of SC and is inhibited by anti-SC (42).

2.2.2 <u>Function of IgA and sIgA</u>   sIgA has now been shown to have a variety of antibody activities directed towards bacteria, viruses, autoantigens, toxins and many protein and other antigens by neutralization, agglutination, coating infectious particles or preventing their adhesion to mucosal surface (43). Since the sIgA

molecule has four combining sites, it is more efficient in agglutination than IgG or 7S IgA (44). Native sIgA does not activate complement via either the classical or alternative pathway, although in aggregated form, it can activate the latter (45).

The sIgA antibody class is capable of blocking bacterial adherence to a variety of mucosal surfaces and thereby is able to prevent colonization (46). It has been amply demonstrated that, a mother's milk is endowed with IgA antibodies to microorganisms residing in the intestine and can thereby reduce the incidence of infantile diarrhoea (47).

For the various effector mechanisms to work efficiently, the presence of functional IgA antibody is continually required in secretions. Due to the presence of proteolytic enzymes in secretions, antibodies belonging to other immunoglobulin class would be largely destroyed. But sIgA, on the other hand would has a much better chance to survive because it is known to be considerably more resistant to proteolytic attack (48). The SC in sIgA is belived to be the component that confers resistance to such proteolytic attack.

2.3 <u>Cell-Mediated Immune Response</u> It should be emphasized that, secretory antibody is only one component of a complex enteric defense system. Although cell-mediated immunological defense mechanisms are poorly understood,

they clearly play a role of delayed-type hypersensitivity (DTH) responses has been observed in the intestine and evidence exists that lymphokine activated macrophage is contribute to local resistance to salmonellosis and listeriosis (49). Cytotoxic responses may also be of importance in the intestine, cytotoxic T-lymphocytes, natural killer (NK) and killer (K) cells have all been indentified among the intraepithelial lymphocytes population and lymphocytes from lamina propria (50). The preliminary evidence suggests that, cytotoxic reactions may be implicated in defense against several pathogens, such as Salmonella typhimurium in mice (51). While its too early to assess the significance of these results it is probable that CMIR responses contribute significantly to mucosal protection in a number of diseases.

Indeed macrophages from mice susceptible to S.typhimurium that have been activated by suitable oral feeding, as shown by a marked increase in esterase activity or by enhanced phagocytosis, are able to kill S.typhimurium to the same extent as macrophages obtained from mouse strains immune to S.typhimurium infection (52). Since the infection cycle of S.typhi and S.typhimurium each require a period of proliferation in the phagocytic cells their activation to " killer " cells status may well tip the balance.

Reports on cell-mediated immunity (CMI) in humans

typhoid are scarce. Kumar et al (12), Nyerges (53,54) and Balakrishna Sarma (13) showed that a CMIR as measured by the leukocyte migration inhibition test (LMI) develops during typhoid fever. Balakrishna Sarma et al (13) suggested that the development of a CMIR correlated with recovery and the absence of complication.

The Mucosal Immunity

The mucosal surface is in constant contact with a myriad of substances such as infectious agents, toxins, enzymes, microorganisms, macromolecules, intestinal break down products' etc. In order to combat antigens which cross the mucosal barriers, the animal host has created an elaborate system of defense mechanisms on the luminal surface. Such systems comprise specifically-immune as well as a number of important non-immune systems conferring an innate resistance.

As a result, responses to potentially pathogenic stimuli could conceivably be swamped by response to an over-whelming array of inconsequential material. For this reason it is not suprising to find that the mucosal immune system has powerful regulatory mechanisms that allows it to react selectively to many or most substances found in the mucosal environment.

We will now emphasize the immunologic defense of the

local immunologic system and outline the structure and function of gastrointestinal immune system (which is the one of mucosal surface) and discuss the nature and function of mucosal antibodies.

1. Mucosa-Associated Lymphoid Tissue (MALT)

Lymphoid tissue in mucosae occurs as loosely constituted isolated lymphocytes of both T and B varieties, scattered throughout the interstitial spaces but particularly in the lamina propria of the intestinal and respiratory tract. In the former they are known as gut-associated lymphoid tissue (GALT) and in the latter as bronchus-associated lymphoid tissue (BALT) (55). In both sites their morphologic, macroscopic, microscopic appearence and function are very similar (56). A similar generalization holds true for the mucosal surface of the nose, the salivary, lachrymal and mammary glands.

The similarity of these features has been commented on before and provides a basis for our initial speculation that these form a commom mucosal immune system. GALT and BALT are also contained in follicles which occur as solitary lymphoid nodules (SLN) throughout the length of the intestine and in much the upper and lower respiratory tract (57). They contain predominantly B cells in which the major immunoglobulin classes synthesized are IgM and IgA. The majority of these B cell have IgA surface-immunoglobulin

having binding sites for the peanut lectin, characteristic of germinal centers and early thymocytes (58).

2. Gut-Associated Lymphoid Tissue (GALT)

Gut-associated lymphoid tissue (GALT) is the one type of mucosa-associated lymphoid tissue (MALT) which is located in the gastrointestinal tract. The term gut-associated lymphoid tissue (GALT) refers to such structures as the appendix (man, rabbit),Peyer's patches (man, rabbit, rat, mice, calf), tonsils (man) and sacculus rotundus (rabbit) (59).

3. Peyer's Patches

These structures were first described by Peyer in 1667. They are covered by specialized epithelial cells which have no microvilli, but whose surface seems wrinkled or folded under the scanning electron - microscope (60). Peyer's patches extend through the lamina propria and submucosa of the small intestine, and crypts and villi are sparse in the overlying epithelium. That part of the epithelium which is immediately above the nodules in the Peyer's patches and heavily infiltrated with lymphocytes is know as the dome area (Figure 3). The subepithelial zone contains many macrophages and plasmas cells. The epithelium over the dome area differs from that eleswhere, in being composed of cuboidal rather than columnar cells and
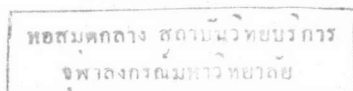
containing few of globlet cells but a large number of intraepithelial lymphocytes (IEL) (61).

The microfold area contains vesicles that actively transport particulate antigens from the lumen into dome area, where T and B cell mix freely with the microfold, which is called " M cell " (Figure 4) (62). Macromolecules are transported by M cells from the lumen into the space between cells surrounding the migrating lymphocytes (63).

During foetal development, gut-associated lymphoid tissue (GALT) is organized into follicles made up of macrophages and lymphocytes. At birth, when these follicles are exposed to antigen, germinal centers develop in which the replicating lymphocytes replenish migrating cells which pass via the lymphatic vessels to the circulation.

Peyer's patches are found in all mammals, the number varies considerably according to species from around 3 to 9 follicles throughout the small intestine in rats or mice (64) but in humans, there may be 200 in the terminal ileum (5). These structures have been most carefully studied in the Peyer's patches in mice, rat, rabbits and humans ; it is assumed that comparable structures would be found in the tonsils and sacculus rotundus.
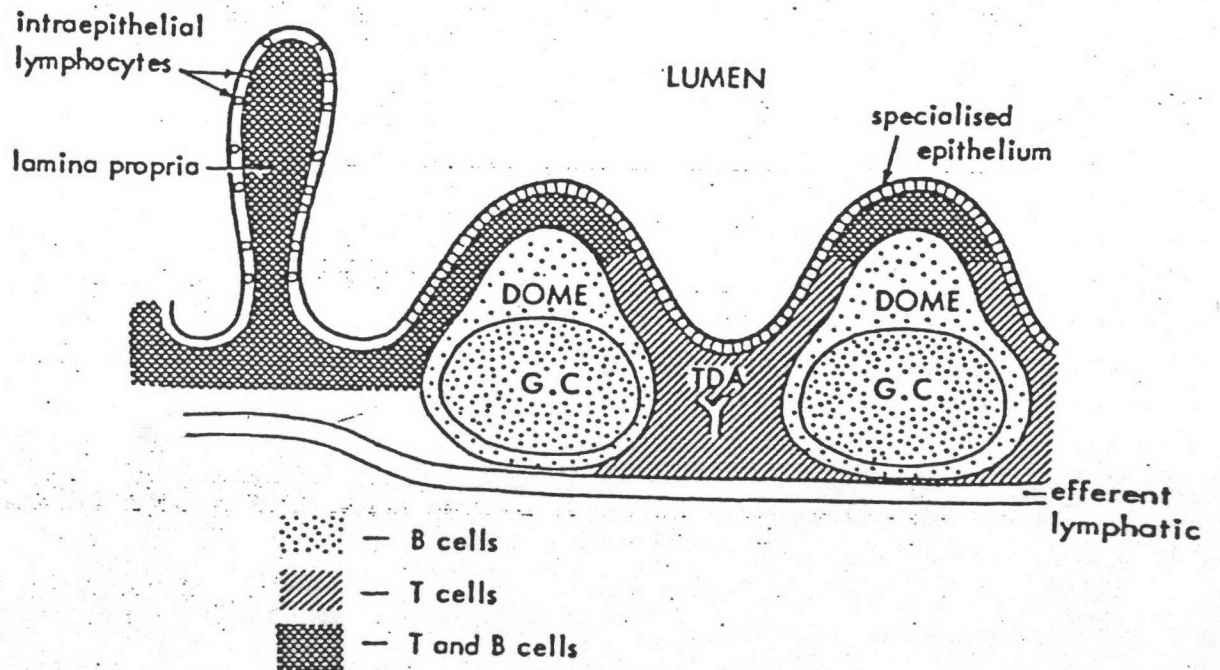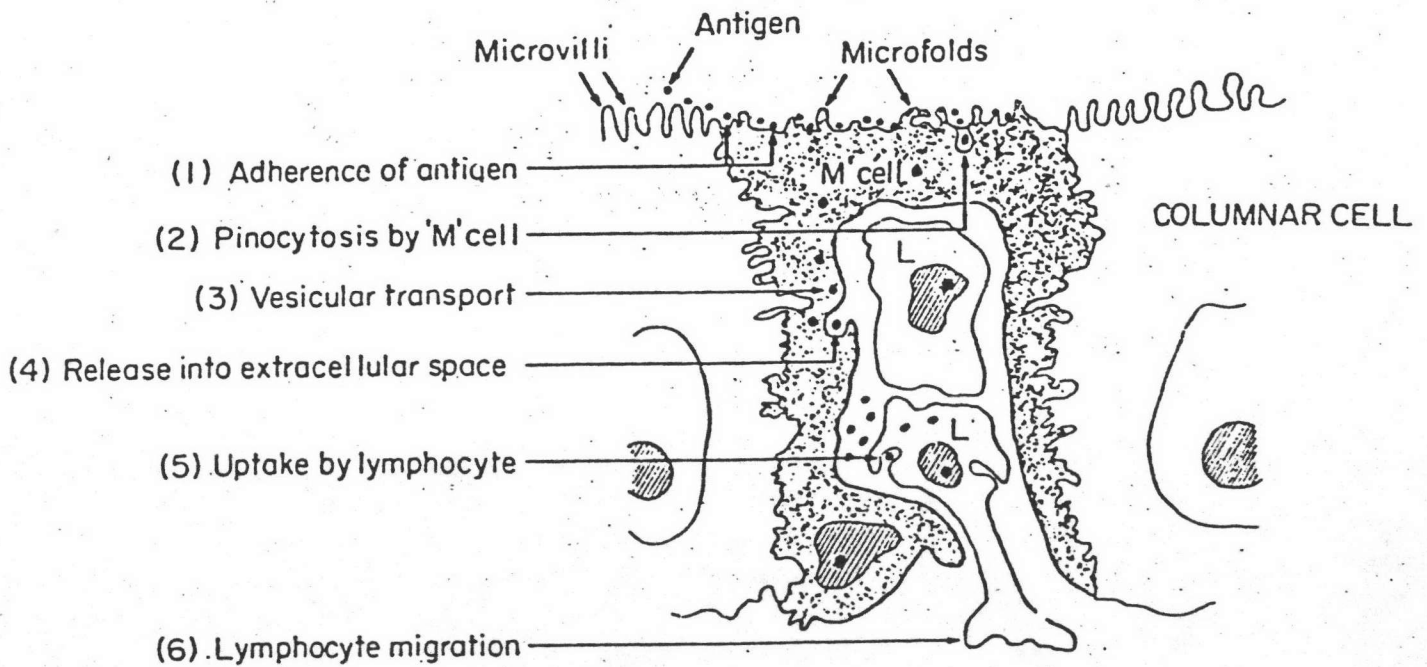
Figure 3   Diagram of Peyer's patches structure in the

            mouse.

            G.C.   =   Germinal center

            T.D.A. =   Thymus-dependent area

(From Parrot D.M.V. The Gut as a lymphoid organ., Clin.

Gastroenterology., 5(2), 211, 1976)

Figure 4   A summary of the stages involved in the transport of antigen by the " M cell " from the intestinal lumen into the extracellular space where it is take up by T lymphocytes (L) for subsequen presentation to B cell.

(From Chapel H.and H.Hneney (1984). Gastrointestinal and Liver Disease, Essentials of Clinical Immunology Blackwell Scientific Publication)

4. Antigen Uptake by GALT

It has been an axiom of local immunity that responses are best stimulated by the local application of antigen and clearly an understanding of route by which orally administered material is taken up and processed by the intestinal immune system is essential to an appreciation of the process of oral immunization.

Macromolecular and even particulate uptake across the intestine into the circulation is now well established. The lymphoepithelium, overlaping MALT, which contains the specialized " M cell " , selectively samples the environment and passes potentially antigenic material to lymphocytes below the epithelium (63). M-cells have a rudimentary brush border and comprise approximately 15 % of the total cell population of the higher regions of the dome, where they are closely associated with lymphoid cells (65). After particulate antigens are passed to the Peyer's patches, they are degraded by macrophages and then pass to the mesenteric lymph node via lymphatic vessels or to the circulation via the portal vein. Passage through the specialized epithelium of lymphoid follicles, rather than leakage into intracellular spaces, may determine whether a secretory immunoglobulin A response will result rather than an immunoglobulin G response. Although mucosal IgA response occurs when antigen is presented locally to GALT (or BALT), parenterally administered antigen may also prime or boost

such a response (66). For parenteral priming to be effective, the antigen must be given via the intraperitoneal route for GALT or intranasal route for BALT and often must be accompanied by an appropiate adjuvant (67).

Macrophages are found both in organized mucosal lymphoid tissue such as MALT and also in the lamina propria (68). It is only very recently that the role of the mucosal macrophage has begun to receive increasing attention (69). The concentration in these cells of noxious enviromental agents is obviously of great biological importance. Carragenan in the diet can cause mucosal ulceration and is found in mucosal macrophages (70). The possibility that mucosal macrophages have a regulated migration pattern and themselves traffic between mucosal tissues would require careful examination and only then will it become clear how we balance our contact with our enviroment; such as the balance between antigen access to the circulation and the local immune response which can provide antibodies capable of blocking such antigen uptake by the gut (71). Brandtzaeg and co-workers have shown that whereas antibody within the mucosa can depress the uptake of intact homologous antigen, immune reactions within the mucosa may enhance the penetration of unrelated macromolecules (72). Another factor which may be important in the subsequent immune reactivity of the host, is the nature of the antigen and the site of its processing. For example, Hunter showed that Salmonella flagellin appeared on intravenous administration

to selectivity localized in bronchial and intestinal lamina propria (73). It may well be that binding of antigen to the mucosal epithelium is a crucial first step in promoting a predominantly secretory immune response.

5. Differentiation and Homing of Mucosal Lymphocytes

Although it has been recognised for a long time that most secretory or glandular tissue are populated predominantly by IgA-producing plasma cells, questions as to the origin of these cells and the mechanisms responsible for their selective homing to these mucosal sites have not been answered until very recently (74). The studies designed to answer these questions must also consider the fact that some secretions such as colostrum and milk, have antibodies against antigens present at remote mucosal sites even though these antibodies cannot be readily detected in the serum. Different lines of evidence currently available indicate that, following appropriate antigenic stimulation both B and T cells, say, in Peyer's patches migrate from the original site for further differentiation and maturation (74,75). They pass to the blood circulation via thoracic duct after which they "home" not only to their original mucosal site but to a less extent also to more remote mucosal sites (Figure 5).

The development of the secretory immune system is thymus dependent. Therefore, because of the peculiar
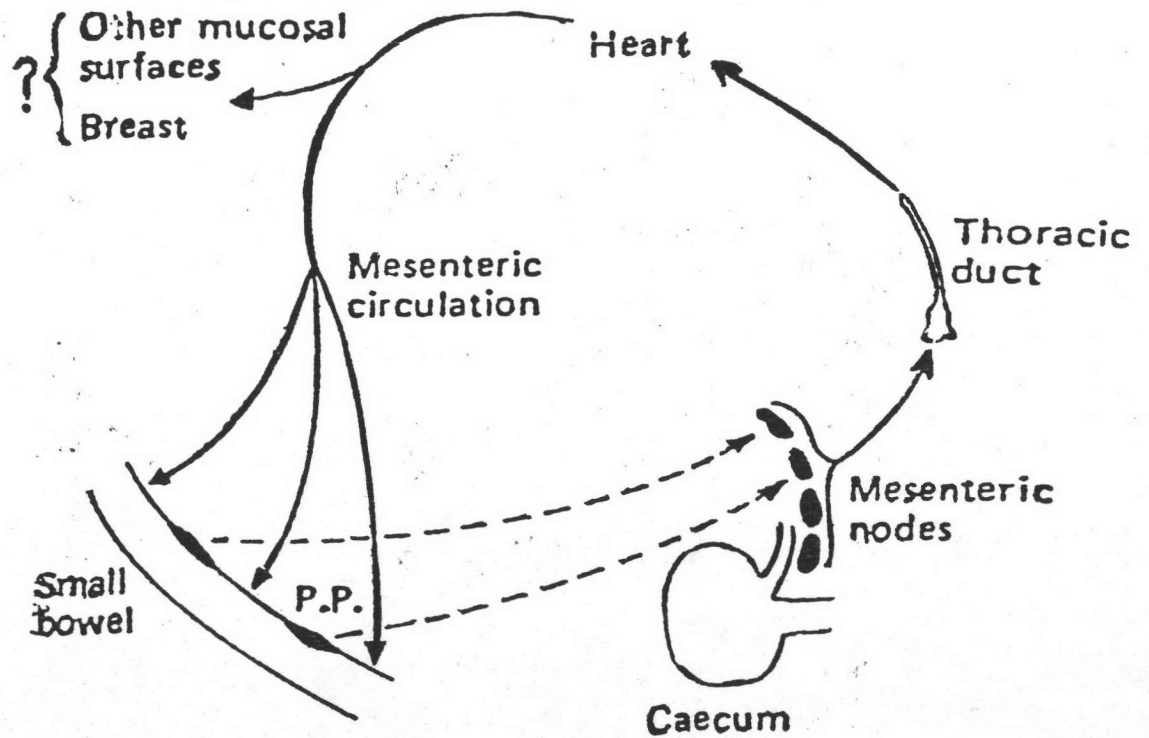
Figure 5   Circulation of enteric immunocytes. Immunoblasts
            originate in Peyer's patches (PP) or mesenteric
            nodes, migrate through the thoracic duct, and
            enter the systemic circulation. Homing is
            predominantly to the intestine but possibly also
            to other mucosal surface or to the breast. In
            the lamina propria immunoblasts appear as
            plasma cells.

(From Pierce N.F. Intestinal Antibodies., J.Infect.Dis.,137,
661-662, 1978).

compartmentalised phenomenon of IgA B cells as well as the differentiation and proliferation of these cells in secretory tissues and it is logical to expect that stimulation of the mucosal immune system must be well regulated, most likely by an array of immunoregulatory T cells. One of these regulatory T cells, a suppressor T cell, has been found in high proportion in the mucosae. Like other mucosal lymphocytes, these cells migrate out of the secretory site and suppress systemic immune responses. The second type of immunoregulatory T cell associated, particularly with the mucosal immune response, is a contrasuppressor inducer T cell which has the ability to regulate suppressor T cells activity (76). In addition, there is yet another regulatory T-cell present in the mucosae,a switch T cell, which cause surface IgA positive (sIgA) B-cells to differentiate preferentially to sIgA B cells. While these switch T cells switch sIgA cells directly to sIgA B cells, they do not per se, facilitate the terminal maturation of sIgA B cell into IgA secreting plasma cells (77).

The latter process requires an additional type of regulatory T cell, namely helper T cells which are present in many other lymphoid tissue outside Peyer's patches including the mesenteric lymph nodes and spleen. Thus, switch T cells formed from conventional helper T cells which bring about the maturation of B cells already committed to a given isotype. The presence of these switch T cells in large

numbers in Peyer's patches may explain why the latter are a major source of IgA precursor cells.

Typhoid Vaccines

Vaccination against typhoid fever has been practiced for well over fifty years (78). Although it has been used extensively, especially with armies, a satisfactory vaccine that provides a really adequate protection against infection has not been realized.

The results obtained by Wright and co-workers, stimulated many other scientists to find new vaccines for protection against typhoid fever. One can divide typhoid vaccines used so far into two groups as follows (79) :

I. **Parenteral-inactivated typhoid vaccines**
   1. Whole-cell vaccines
       1.1 Heat-phenol inactivated
       1.2 Acetone-inactivated
       1.3 Formalin-inactivated
       1.4 Alcohol-inactivated
   2. Cell-free vaccines such as endotoxin and Vi antigen

II. **Oral typhoid vaccines**
   1. Killed or inactivated vaccines
   2. Live or attenuated vaccines

2.1 Streptomycin-dependent

   S.typhi (strain 20 SD/27V/SmD)

2.2 UDP-galactose-4-epimerase deficient

   S.typhi (strain Ty 21a)

Parenteral-killed typhoid vaccine

The early trials of Wright and collaborators of typhoid vaccine gave results which were generally accepted as proof of the protective value of the heat-killed vaccine inspite of unfavourable criticism by some contemporary authors (79). In 1954-1960 a Yugoslav typhoid commission carried out in the first controlled field trial of typhoid vaccines , showed that heat-phenol inactivated typhoid vaccine gave a relatively high and long-lasting immunity, but this liquid vaccine preparation was unstable.

In the period of 1960-1964 , World Health Organization has sponsored and assisted several field trials of typhoid vaccine in several countries , namely Yugoslavia (78,80), Poland (81), USSR (82,83) and British Guiana (84). These field trials can be divided into two groups for the sake of convenience. Some trials are concerned with the study of the effectiveness of typhoid vaccines that has been established as international reference preparations, namely acetone-inactivated vaccine (K-type) and heat-phenol inactivated vaccine (L-type). Other trials deal with various other types of vaccine, combined typhoid-paratyphoid antigen

or with alcohol-inactivated and formalin-inactivated bacteria. It was found that, L-type vaccine was superior to the K-type vaccine (85).

Oral typhoid vaccine

Although the currently available parenteral vaccines are effective, they frequently cause adverse reactions such as fever, intense local inflammation, headache, malaise and swelling (86,87). So the experimental focus in volunteers has turned to the oral route of immunization in an attempt to stimulate resistance of the intestinal barrier such as coproantibody or cellular immune factors in the lamina propria (14). Hornick and co-worker used two types of oral vaccine containing killed typhoid bacilli in keratinized tablets, one was an Taboral[R],a monovalent vaccine containing S.typhi (strain Ty 2), the other one was Typhoral[R], containing heat and acetone-killed S.typhi and S.paratyphi A and B bacilli. After ingestion of the infectious dose, orally immunized volunteers showed slightly less resistance to infection than did those vaccinated by the parenteral route (5,88).

Reitman produced a streptomycin-dependent mutant strain of S.typhi, strain S27, as an live oral vaccine. It was shown to be efficacious in mice and rabbits. It had some protective effect when ingested orally in humans but it lost efficacy after lyophilization and also required the

addition of streptomycin (86,89).

Salmonella infections of mice has been extensively used as a model for typhoid fever. Studies in such models have shown that live attenuated vaccines provide mice with better protection when the oral route of administration was used (90,91). However, not all attenuated strains of Salmonella are equally suitable for use as a live vaccine. Of the various avirulent mutants of S.typhimurium, examimed only gal E mutants afford mice protection comparable to that induced by a sublethal infection with a virulent, smooth strain (92). Gal E mutants are rough-type strains that are characterized by a block in the enzyme uridine diphosphate (UDP)-galactose-4-epimerase. They owe their outstanding protective capacity as a live vaccine to the fact that, when galactose is supplied exogenously (as occurs in vivo), for that time, cell wall lipopolysaccharides of the smooth type are synthesied (93). The avirulence of gal E mutants is ultimately due to the strong bacterial lysis that follows due to the accumulation of galactose 1-phosphate and UDP-galactose with the cell (94). Both the virulence and the protective capacity of gal E mutants thus depend on the activity of all of enzymes responsible for metabolism of galactose and its distribution with in the bacterial cell (93).
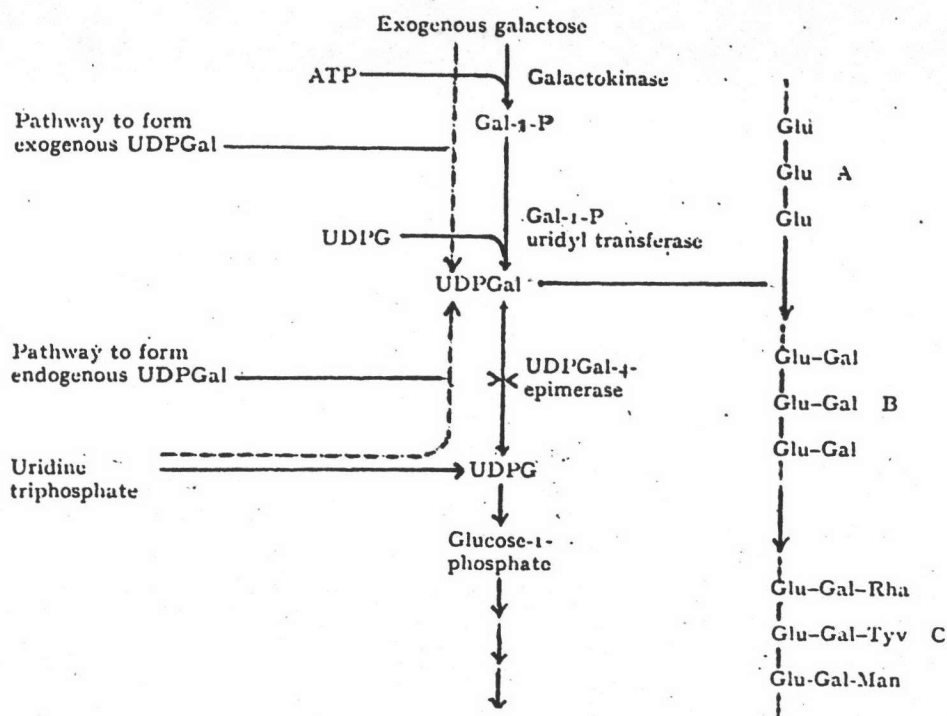
Because S.typhi is pathogenic only for man no true infection can be established with this species of Salmonella

in any laboratory animal, except for several primates.
Therefore, the selection of a live vaccine in further
studies was based on results obtained with mice (90).

Salmonella typhi strain Ty 21a (gal E mutant)

Salmonella typhi strain Ty2 (pathogenic strain) was
as the parent strain for the production of the gal-E mutant
by mutagenic treatment of Ty2 cell with N-methyl-N-
nitrosoguanidine (NG), JCR-170 or ultraviolet light (UV).
The resulting Ty 21a strain is lacking in the biosynthesis
of complete cell wall lipopolysaccharide, which is an
important factor in both the immunogenicity and virulence of
salmonella. The activity of the other enzymes involved in
galactose metabolism, galactose permease, galactokinase and
galactose-1-phosphate-uridyl transferase in strain Ty 21a
are also decreased (95).

When Ty21a was grown in the presence of galactose,
A Figure 6 represents the poly-glucose structure of Ty 21a
lipopolysaccharide ; Ty 21a cannot make endogenous
UDP-galactose owing to their defect in UDP-gal-4-epimerase.
In the wide type S.typhi able to make UDP-gal inserts gal-1-
phosphate in its polysaccharide , as in B Figure 6 (94).
Other sugars are then transfered finally resulting in the
formation of wild type polysaccharide, C Figure 6. However,
in the Ty 21a auxotroph the galactokinase increases the
internal level of galactose, gal-1-phosphate and UDP-gal

Exogenous galactose

ATP —— Galactokinase

Pathway to form
exogenous UDPGal ——

Gal-1-P

Glu
Glu A
Glu

UDPG

Gal-1-P
uridyl transferase

UDPGal

Pathway to form
endogenous UDPGal ——

UDPGal-4-
epimerase

Glu-Gal

Glu-Gal B

Glu-Gal

Uridine
triphosphate

UDPG

Glucose-1-
phosphate

Glu-Gal-Rha

Glu-Gal-Tyv C

Glu-Gal-Man

Figure 6   Schematic representation of the metabolic events
presumed to occur when Ty 21a was grown in the
presence of galactose . A represents the
poly-glucose structure of Ty 21a
lipopolysaccharide ; Ty 21a cannot make
endogenous UDP-Gal owing to their defect in
UDP-Gal-4-epimerase. But they transfer
accumulated UDP-Gal (originating from
exogenously fed galactose) to this
polysaccharide to make intermediate B.   Other
sugars are then transferred, finally resulting
in the formation of wild-type like
polysaccharide C compose of five sugars . The
formulae of this polysaccherides are
hypothetical, Glu, Gal, Man, Rha, and Tyv denote
glucose,galactose,manose,rhamnose and tyvelose,
respectively ; x = location of the enzymatic
block.

such that they eventually cause the bacteriolysis of the cell (90).

Controlled Field Trial of Oral Typhoid Vaccine

The well concieved and conducted field trial of live Ty 21a strain oral vaccine in Alexandria, Egypt by Wahdan and co-workers, showed striking protective efficacy. Three doses of vaccine or placebo were given to 32,388 children. The population was carefully monitored for three years each suspected case of typhoid was investigated bacteriologically and serologically. After three years, the incidence of typhoid fever was 4.9 cases per 10,000 children per year in the control group, and 0.2 cases per 10,000 children per year in the vaccinated group (96 % efficacy). The result from these studies by W.H.O. indicated that the Ty 21a mutant strain is stable and safe and is highly protective for a period of at least three years (88,96).

Recently a large field trial in Santiago and Chile, the oral vaccine Ty 21a has been given in an enteric coated capsule in one or two doses. Again, no-untoward side-effect had been noted. In volunteers studies the vaccine had an 87 % efficacy rate (97).

Reports on CMIR in humans after oral vaccination with live Ty 21a are scarce. Tagliabue (98) studied the cellular immunity against S.typhi after immunization with

live oral typhoid vaccine. They found that administration of live oral vaccine against S.typhi results in the induction of specific cellular immunity which is expressed at the peripheral level. Similarly Sarasombath et al (99), studied the CMIR after oral vaccination with different typhoid vaccines, the leukocyte migration inhibition test was used for the measurement of systemic CMIR. A significant differences in the LMI-index before and after vaccination was noted at 4 weeks, through 24 weeks of the study.