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# ORAL ENTERIC BACTERIAL VACCINES

Report of a WHO Scientific Group

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## WHO SCIENTIFIC GROUP ON ORAL ENTERIC BACTERIAL VACCINES

Geneva, 2-8 November 1971

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# ORAL ENTERIC BACTERIAL VACCINES

## Report of a WHO Scientific Group

A WHO Scientific Group on Oral Enteric Bacterial Vaccines met at the Institute of Hygiene, University of Geneva, from 2 to 8 November 1971. Dr M. Takabe, Director of the Division of Communicable Diseases, opening the meeting on behalf of the Director-General, briefly outlined the past activities of WHO in the field of enteric bacterial vaccines.

### 1. INTRODUCTION

The concept of oral vaccination against microbial disease is by no means new. This method of immunizing human beings was first contemplated over 70 years ago and has been used, somewhat fitfully, ever since. For reasons that have emerged only in the past few years, the original oral vaccines were not generally accepted because they failed to induce detectable levels of serum antibody. They were presumed, therefore, not to be capable of conferring immunity. It is realized now that this may be the worst criterion by which to judge the potential efficacy of an immunizing agent that is delivered *via* a mucosal surface.

The success of the live poliomyelitis vaccine has undoubtedly served as an important stimulus to the re-examination of oral vaccination against the important bacterial diseases of the human intestinal tract: enteric fever, shigellosis, cholera, and *Escherichia coli* enteritis. This report reviews what is currently known on the subject, correlates this knowledge with more fundamental information on the mechanisms of immunity operating in the intestinal tract, and finally suggests approaches that could lead to the development of effective oral prophylactic techniques.

There are cogent reasons for considering this approach to immunization. At the moment, none of the parenteral vaccines against enteric bacterial infections is wholly satisfactory: these vaccines are lacking in efficacy; the duration of the immunity that they confer is inadequate; they tend to cause side reactions; and the cost of using them to immunize the population most at risk is excessive. Oral immunizing procedures are potentially far less costly and make far less demands on public health facilities; it now remains to be determined whether they could be at least as effective as the vaccines now used, besides conferring more lasting protection and being safer to administer. At the moment these questions cannot be answered but the Group's recommendations for further research

on both the fundamental and the practical issues of oral immunization may well indicate whether these techniques are likely to be of universal interest and applicability.

## 2. PATHOGENESIS OF INTESTINAL INFECTIONS

The basic requirement for a rational approach to the development and use of immunizing procedures against microbial infections is a clear understanding of the way in which a pathogenic organism produces infection and, consequently, pathological changes in the tissues of its natural host. In tetanus, for example, the observation that the production of exotoxin is directly related to its physiopathological effects on the tissues of the central nervous system led to the development of one of the most effective immunizing agents currently available. In the case of the bacterial diseases of the intestinal tract, a whole spectrum of bacterial pathogenetic mechanisms, ranging from toxin production to invasion, may be involved. Furthermore, only in rare instances have the causative agents been found to naturally infect animals that can be used conveniently in laboratory studies. As a result, information on the pathogenesis of these diseases has been severely restricted by the inability to study in depth the dynamic features of microbial adsorption, invasion, and multiplication together with the inflammatory and other pathological changes that might occur in human patients as the infection runs its natural course. It is only recently that studies in volunteers have been made in an attempt to answer these fundamental questions.

As will be seen below, the alternative is to use experimental animal models. Whereas these may yield useful information in some instances, considerable caution must be exercised in extrapolating direct from them to the diseases occurring in man.

### 2.1 Enteric fevers

The Group restricted its discussions entirely to typhoid fever. The causative agent, *Salmonella typhi*, is naturally pathogenic only for man, although it will cause an enteric illness in subhuman primates when administered by the oral route. Whereas the organism is lethal to rodents when given intraperitoneally, death results solely from septicaemia and not from the disease itself.

Animals are naturally susceptible to many serotypes of *Salmonella* and in many instances may develop enteric-type illnesses bearing a resemblance to typhoid fever. For convenience, *S. typhimurium* and *S. enteritidis* infections of mice and rats have been used extensively for many years as models of typhoid fever and have yielded information which, as far as can be ascertained, correlates well with the pathogenesis of the human infection

(Orskov et al., 1928; Blanden et al., 1966). Irrespective of whether the infection is given intraperitoneally, intravenously, orally, or by other routes involving the intestinal tissues, it appears that the prerequisites for infection are bacteraemia, hepatosplenic multiplication, and secondary bacteraemia, the latter leading to multiplication in a variety of other tissues, particularly those of the intestinal tract. An essential characteristic of these infections is localization and multiplication of the organism within macrophage cells (Blanden et al., 1966).

Histological studies have shown gross depletion of lymphocytes in the reticuloendothelial and lymphoid tissues of animals dying from *Salmonella* infection (Stuart & Collee, 1967); it is perhaps noteworthy that in animals infected sublethally an initial acute inflammatory response occurs in the lymphoid tissues and is followed by lymphoid cell depletion. A marked histiocytic-mononuclear cell response develops within a matter of 3–5 days of the infection (G. N. Cooper — unpublished observations). Whether this represents the onset of an immune response has not been determined, yet it is interesting in view of the tissue response observed early in the development of typhoid fever (see below). Except in special instances, most infections in rodents terminate in death; this is related to the development of “lethal numbers” in the liver and spleen (Blanden et al., 1966). The exact cause of death of the animals has not been determined.

Studies in volunteers to whom *S. typhi* has been given orally have helped to clarify the gross features of the microbiology and pathology of enteric fever. Provided that the organisms evade the acidic barrier of the stomach, they initiate an inflammatory reaction in the small bowel. Midway through the incubation period—i.e., after 3–4 days—this reaction is characteristically of the mononuclear cell type. There is some evidence that strains containing Vi antigen may possess greater virulence for man than those that do not, as demonstrated by the incidence of clinical disease and by the excretion of the organisms in the faeces of volunteers who ingested standard doses of the organisms (Hornick et al., 1970). It is believed that the virulent strains penetrate the mucosa in the distal ileum and large bowel, but more detailed evidence of this is required. As in the experimental models, hepatic (and possibly splenic) localization and multiplication result, followed by bacteraemia and dissemination, especially to the intestinal tissues, where reinvasion, multiplication, and tissue necrosis (haemorrhage and/or perforation) may occur. Mononuclear granulomata have been observed in liver biopsies, as pathological studies of the intestines have shown.

Although certain features of typhoid pathology may be related to the activity of endotoxin (e.g., pyrexia, leucopenia, rose spots), it is still entirely uncertain to what extent this material contributes to the other characteristics of the disease (mononuclear cell infiltrations, intravascular coagulation, perforations, and haemorrhage). Despite the substantial evidence

on what might be regarded as the gross features of pathogenesis, there is much to be learned about the invasiveness of the bacteria, together with the physiological, biochemical, and immunological factors concerned in the inflammatory processes that are characteristic of the disease.

## 2.2 Shigellosis

Bacillary dysentery, which is caused by bacteria of the *Shigella* genus, is characterized by the production of copious quantities of blood and mucus in the stools, which leads to prostration. It is not uncommon to observe all grades of the infection ranging from asymptomatic carriage through mild diarrhoeic symptoms to the dysenteric form. Studies in volunteers have indicated that in shigellosis—in contrast to other enteric infections—the infectious dose is very small ( $10^1$ – $10^2$  organisms compared with  $10^5$ – $10^{10}$ ).

*Sh. dysenteriae* type 1 is known to produce a highly potent neurotoxin and an identifiable enterotoxin. No other bacterium of the genus has been shown to possess either of these properties. Animal models of *Shigella* infections have been described, but in many instances probably bear little relation to the disease in man. Intraperitoneal infections produce fatal septicaemia; the organisms have been inoculated into the ligated ileal loops of rabbits, and administered orally to guinea-pigs or mice pre-treated with antibiotics and agents to decrease the motility of the small bowel. However, it is difficult to extrapolate information from these models direct to the natural infection (Formal et al., 1958; Cooper, 1959). The same may be said for corneal inoculation into guinea-pigs, though this technique may be used to indicate the potential virulence of the organisms (Sereney, 1957).

Studies in subhuman primates have yielded what is probably the most valuable information yet obtained on the pathogenesis of the disease. It seems that the organism may first proliferate in the upper regions of the small bowel, then penetrate the epithelial cells of the large intestine, multiplying subsequently within the mucosa and, perhaps, the lamina propria. This leads to inflammatory responses and the rapid destruction of areas of the intestinal epithelium. These factors are clearly responsible for the bloody diarrhoea observed in persons with dysentery (Formal et al., 1966). The organism rarely penetrates beyond these local intestinal sites. The mechanism involved in this destructive process is unknown. It is unlikely to be caused solely by endotoxin, since organisms such as the *Salmonellae* may also proliferate in similar sites without giving rise to the typical pathology of dysentery. Although few studies have been performed in human beings, it is believed that the infection in subhuman primates presents similar, if not identical, features.

### 2.3 Cholera

Natural infections due to *Vibrio cholerae* are limited to man; in infected areas, the organism is harboured in the bowel and gallbladder of asymptomatic carriers. During epidemics, a number of grades of the infection are likely to be seen, ranging from asymptomatic carriage, through mild-to-moderate diarrhoea, to the clinically recognizable form in which large quantities of fluid stools are passed, accompanied by vomiting and acute dehydration and shock. A broad spectrum of infections has been observed in studies in volunteers (Hornick et al., 1971).

Despite the absence of a natural animal host, experimental models of the disease have yielded much useful—indeed definitive—information on the pathogenetic mechanisms of the organism. The most successful models have been the ligated ileal loops of rabbits (De & Chatterje, 1953), newborn rabbits (Dutta & Habbu, 1955), mice (Ujiye et al., 1968), and adult dogs (Sack & Carpenter, 1969). It is now clear that, in each of these models, the evocation of choleraic symptoms depended on the production of an enterotoxin that had a direct effect on the actively proliferating epithelial cells at the base of the villus crypts. Typical choleraic symptoms may be induced by the intraluminal injection of cholera enterotoxin, thus providing a convenient method for assaying this substance and antitoxic antibodies. The chemical nature and pharmacological properties of cholera enterotoxin have recently been reviewed and need not be restated here (Pierce et al., 1971).

If cholera is to develop, either by way of natural infection or in animal models, the organism must pass through the gastric acid barrier and multiply rapidly within the intestinal lumen and, most probably, in the immediate vicinity of the mucosal surface. It is a curious but well established fact that many strains of cholera isolated from patients, and therefore presumed to be virulent, do not produce demonstrable cholera enterotoxin *in vitro*. Indeed, only a few strains—e.g., Inaba 569B—produce adequate quantities of this substance under laboratory conditions, and it is noteworthy that, in studies carried out in volunteers, this strain appears less virulent than Ogawa strain 365, which *in vitro* is an extremely poor enterotoxin producer (Hornick et al., 1970). The “micro-environment” in the immediate vicinity of the intestinal epithelium is probably important in determining whether toxin will be produced *in vivo*; as yet there is no information on the great variety of physiochemical factors that might influence toxin production. Evidence obtained from experiments with the ligated ileal loops of rabbits and from neonatal mouse models suggests that a percentage of the infecting organisms may be bound to the epithelial surfaces and, presumably, may multiply there. Though there is no direct evidence, it is possible that these organisms are responsible for the choleraic symptoms since they are conveniently situated to deliver

enterotoxin directly to the susceptible cells. The mechanisms by which *V. cholerae* organisms adhere to the intestinal epithelial surfaces are still unknown.

#### 2.4 *Escherichia coli* enteritis

*E. coli* infection of the intestinal tract is a major cause of infant mortality. Though these organisms may cause mild diarrhoeic symptoms in older children and adults, the neonate produces profuse watery diarrhoea and, because of its extreme susceptibility to fluid imbalance, rapidly suffers dehydration and shock. A number of specific *E. coli* serotypes have been recognized as enteropathogenic strains. However, some of these do not appear to possess the factors responsible for the disease process. The isolation of large numbers of a particular *E. coli* serotype in the stools of patients may be taken only as circumstantial evidence of its infectious nature; a more positive demonstration would be the detection of large numbers in the upper regions of the small intestine.

Human enteropathogenic *E. coli* strains may produce clinical disease in suckling mice. However, this disease does not resemble that seen in babies (Mushin et al., 1970), the murine infection being primarily a bacteraemia consisting in invasion and extraintestinal multiplication. In the natural disease, on the other hand, the organism is confined to the intestinal lumen. *E. coli* infections of the intestine occur naturally in some species of domestic animal, especially newborn pigs, calves, and lambs. These infections resemble the human disease except that the serotypes involved are distinct from human strains (Sojka, 1965, 1970). Nonetheless, it is now agreed that piglets infected with their natural *E. coli* enteropathogens constitute the most suitable experimental model for defining the pathogenetic mechanisms of *E. coli* diarrhoea. Under natural conditions or when infected experimentally by the oral route, piglets suffer a severe cholera-like disease with intense dehydration, which may lead to death in one or two days; curiously enough, the pig is also susceptible to *E. coli* infection in the immediate post-weaning period.

The advantages of the pig experimental model are considerable, especially for studying the dynamic aspects of the disease process; isolated ligated loops may be prepared and used to study certain aspects of the infectious process. The most characteristic feature of the infection is the rapid multiplication of enteropathogenic strains in the anterior small intestine. Although, *in extremis* or *post mortem*, organisms may be found in other organs, this fact has no significance in the pathogenesis of infection (Smith & Jones, 1963).

As is the case with cholera, the disease is dependent on two processes—i.e., (a) microbial multiplication and (b) production of an enterotoxin that possesses many characteristics in common with that of *V. cholerae*.

Genetic studies have shown that enterotoxin production by porcine strains is controlled by a transmissible plasmid (Ent) (Smith & Linggood, 1971), and recent evidence suggests that human enteropathogenic strains may possess the same genetic control of enterotoxin production (S. B. Formal—unpublished observations). A second plasmid identified in porcine strains controls the production of a capsular protein antigen (K88) that seems to be important in the adsorption of organisms to the intestinal epithelium, thus encouraging multiplication in the upper regions of the tract. No similar "antigen-controlling" plasmid has yet been identified in human enteropathogenic strains. Although enteropathogenic strains all liberate endotoxin material, there is no evidence that this plays a role in the pathogenesis of the disease.

Few studies of human *E. coli* infection have been carried out. However, as far as can be determined from studies in adult volunteers and in young babies, in which intubation was performed and the numbers of viable organisms in various regions of the small bowel were measured, the infection depends on the multiplication of the organisms and, most probably, the release of enterotoxin in the jejunum (Gorbach et al., 1971; Sack et al., 1971). No information is available on the doses that cause infection in young babies, who are normally susceptible to the disease.

Other serotypes of *E. coli* may produce dysentery-like infections in all groups (DuPont et al., 1971a). The strains responsible for this disease seem to be capable of penetrating the intestinal submucosa in exactly the same fashion as virulent *Shigellae*, and they presumably proliferate in the same sites. No information is available on the mechanisms responsible for the damage to the intestinal epithelial surfaces that occurs during infections by these organisms.

### 3. GENERAL IMMUNE MECHANISMS OF THE INTESTINAL TRACT

A characteristic of all the diseases considered here is that infection takes place *via* the alimentary canal. Though typhoid fever is essentially a systemic infection, its portal of entry is almost certainly the small bowel; therefore it is likely to be susceptible to immune mechanisms that operate in the intestinal lumen or in the tissues in its immediate vicinity. The same immune mechanisms would be expected to operate in the diseases that are restricted to the intestine. Before considering the immune mechanisms operating in each individual disease (see section 4), it may be useful to discuss briefly the general aspects of immunity relating specifically to the intestinal tract.

### 3.1 Non-specific resistance mechanisms

For many years, non-specific resistance to infection has been the subject of extensive speculation and has been reviewed in the major texts on immunity (Wilson & Miles, 1964). However, the investigations carried out in this field have been of only limited value. Non-specific resistance may be simply, though not necessarily completely, regarded as the expression of a wide range of genetically endowed, relatively immutable host factors (many of them ill-defined) that may prevent invasion by, and/or multiplication of, pathogenic micro-organisms. In a few instances, identifiable genetic factors have been shown to correlate with differences in the susceptibility of various strains and species to infectious agents. However, the reasons for these differences remain largely unexplained. That they do exist is illustrated by the difficulty of establishing infection models of intestinal diseases in laboratory animals (see section 2).

Factors contributing to the degree of resistance in susceptible species include age, nutrition, and physiological state. The subject is far too complex to allow of generalizations and, as far as intestinal infections are concerned, has been investigated only fitfully and superficially.

Nevertheless, certain identifiable factors that may operate in the alimentary canal—the most obvious of which is gastric acidity—have been described. Clearly, if pathogenic organisms are to multiply in the lumen or invade the tissues of the small bowel, they must be able to survive gastric acidity. Evidence from studies in volunteers suggests that the infectious doses of *S. typhi* and *V. cholerae* may vary from  $10^6$  to  $10^{11}$  depending on whether these organisms can be protected against the highly acidic environment of the stomach. *S. typhi* is highly susceptible to pH values of 3.0 or below, whereas *V. cholerae* is adversely affected by a pH below 5.0. In contrast, *Shigella* species seem to be highly resistant to the acid conditions of the human stomach (R. B. Hornick—unpublished observations).

In these studies it was evident that the organisms (or, for that matter, living vaccines) can be readily protected by administering sodium bicarbonate immediately before their ingestion. Whether this agent protects by neutralizing the acid or by preventing closure of the pylorus is unknown. Probably, in natural conditions, the survival of infecting organisms depends heavily on their being protected by food or their rapid passage through the stomach and pyloric opening. The latter can only occur when the inoculum is ingested in fluids that are non-irritant or rapidly become isotonic.

Once the organisms have passed into the small bowel they are exposed to conditions suitable for multiplication. Here a variety of non-specific antibacterial mechanisms or substances are known to exist, though their protective role in intestinal infections is by no means established. They include the physical barrier of the mucus immediately above the intestinal

epithelium, enzymic materials such as lysozyme, and other antibacterial substances. Gastric and intestinal juices have been found to contain a protein known as lactoferrin, which has an extreme affinity for iron; it exerts a bacteriostatic effect by depriving the environment of ionized iron, and under experimental conditions has been found to be effective in restricting the growth of some bacteria (Masson et al., 1969). Its effect against intestinal pathogens is completely unknown. Another substance found in the stomach is  $\beta_2$ -glycoprotein (Faulk et al., 1971). It is also of some interest as it may possibly act as the proactivator in the  $C'_3$  by-pass system (Götze & Müller-Eberhard, 1971); its role in opsonization of organisms in the intestines is of special interest but, again, unknown. C-reactive protein, which may be bactericidal for some Gram-positive species, is released into the intestinal juices during inflammatory conditions; although this may have opsonizing properties for Gram-negative organisms, whether it plays a role as a defence mechanism is undetermined.

It is known that, during certain disease processes involving the intestinal tract, phagocytic cells may be detected in the stools. Again, it is not known whether these cells migrate to the intestinal lumen and play a defensive role in normal individuals, but this is likely to be the case.

In many animal infection models there is clear-cut evidence that susceptibility is increased markedly if some of the normal (autochthonous) flora of the intestinal tract can be eliminated or reduced (Bohnhoff et al., 1964). Recent studies have indicated that some of the autochthonous flora may produce volatile fatty acids that control or prevent the growth of Gram-negative bacteria (Lee & Gemmell, 1972). At the moment very little is known of the autochthonous flora in man; in particular, it is not known whether these bacteria may similarly, or by a variety of other physicochemical means, affect the rate of multiplication of intestinal pathogens.

### 3.2 Antibody-mediated mechanisms

In terms of lymphoid cell numbers, the intestinal tissues of human beings and animals could be considered as the most important immunological organ of the body. It may be conveniently visualized as a compendium of different categories of lymphoid tissue—e.g., the mesenteric lymph node system, the Peyer's patches and follicular accumulations of the submucosae, and the extensive, diffuse population of plasma cells and lymphocytes of the lamina propria. The first two systems have been shown to be important in localizing or restricting the spread of *Salmonellae* in experimental models (Collins, 1970; Cooper & Fahey, 1970); they also contribute to the pool of serum immunoglobulins (primarily IgM and IgG). In contrast, it is now evident that the plasma cells of the lamina propria are especially involved in the production of the immunoglobulins that are found in the

intestinal secretions (Heremans & Bazin, 1971). For this reason, their role in providing a specific barrier to infection in the intestinal lumen or tissues must be of the utmost importance.

The intestinal secretions of man and of animals are known to contain the immunoglobulin classes IgM, IgG, IgA, and possibly, in man, IgD and IgE. As both IgM and IgG immunoglobulins are susceptible to proteolytic enzymes in the intestinal juices, due care must be taken, when analysing intestinal secretions for immunoglobulins, to prepare the samples of these secretions so as to prevent such degradation. In contrast to its concentration in serum, IgA is the largest component of the intestinal secretions; in man, it may be present in concentrations of up to 30 mg per 100 ml of fluid. The IgG and IgM concentrations are somewhat lower (Girard & de Kalbermatten, 1970). The structure of the "secretory IgA" is now well documented: it is a dimer of 7S IgA to which a glycoprotein (the secretory component or SC piece) and a special polypeptide chain (the "J" chain) have been added (Heremans & Bazin, 1971; Halpern & Koshland, 1970).

In human beings, and probably in most animals, secretory IgA is produced locally and, in the case of the intestinal secretions, is synthesized by the plasma cells that underly the epithelial cells. It has been calculated that in the human duodenum at least  $4 \times 10^5$  IgA-producing cells are contained in 1 mm<sup>3</sup> of connective tissue. The arguments for the local production of secretory IgA are convincing and have recently been reviewed (Heremans & Bazin, 1971). It is known, however, that some excretion of plasma IgA may occur through epithelial barriers. It is less certain how IgM and IgG originate in the intestinal secretions, but this may be both from the intestinal tissues and by way of leakage from the plasma existing in the interstitial fluids at the apices of the villi. Certainly, this is likely to occur more readily in pathological conditions that damage the epithelial cells.

Recent studies in experimental animals that had received antigen by the oral route have indicated that immune responses in the lamina propria exhibit sequential changes in the class of immunoglobulin produced, in the same manner as the systemic response to antigen infection. In the latter instance, IgM antibody is produced first and later there is a shift to IgG production (IgA possibly being a minor component); in the mucosa of the intestine, the early IgM response is followed by a response that is largely of the IgA type (Heremans & Bazin, 1971; Henry et al., 1970). It should be noted that, in some animal species, specific IgG production may also occur, although only as a minor component of the response. It is also noteworthy that approximately 1 in 4000 human beings may be constitutionally deficient in IgA. In such persons, the mucosal plasma cells seem to produce immunoglobulins that are primarily of the IgM type; curiously enough, these people seem to be no more susceptible to intestinal infections than normal individuals are (Heremans & Crabbé, 1967). At the moment it is not known whether repeated antigenic stimuli induce characteristic

secondary-type immune responses in the mucosal lymphoid system of the intestine.

It is certain that, in some animal species, the intestinal mucosal lymphoid cells are the major source of serum IgA. However, this may not be the case in man, and other tissues, e.g., mesenteric lymph nodes and spleen, may be the most important source. In man, in contrast to most other animal species, the IgA of plasma is unique in that it exists as a 7S monomer.

When antigens are administered by the oral route or delivered into the intestinal lumen by direct inoculation, the immune response is not confined to the local site. Specific serum antibodies may be identified by a variety of serological techniques. It is obvious that, depending on a number of factors (most of which are unidentifiable), the serum response may be of two forms. The first is one in which IgM and IgG predominate. In this case, antigen from the intestinal lumen has probably found its way to the mesenteric lymph nodes and the blood, possibly by the mechanism of persorption (Volkheimer & Schulz, 1968). In the second type of response, IgA antibodies are predominant. It is likely that in these cases the antibodies are derived both from the local intestinal tissue responses and from cells that have been stimulated at that site and have later migrated to the peripheral lymphoid tissues (Nash et al., 1969).

As immunoglobulins are present in the secretions, it may well be that, if they are specific for one or another of the antigens of intestinal pathogens, they may exert a protective effect. Much interest has centred on the biological functions of IgA antibody, which occurs in the highest concentration. There is, indeed, some evidence to suggest that resistance to viral infections at the mucosal surfaces after natural infections or immunization is causally related to the secretion of antibodies of this class.

In the case of the organisms responsible for the infections discussed here, only two mechanisms by which antibody may exert a protective (killing) effect have been clearly established so far.

### 3.2.1 *Complement-mediated bactericidal mechanisms*

There is no evidence to suggest that IgA antibody, in the presence of complement, is able to kill Gram-negative bacteria. Though it is now apparent that IgA antibody-antigen complexes may activate the C<sub>3</sub> by-pass system (Götze, O., Spiegelberg, H. & Müller-Eberhard, H. J. — to be published) it appears that the reaction is not completed. As a result, lytic or other major effects on the bacterial cell membranes are not likely to occur. The potentiating effect of lysozyme in the bactericidal effect of IgA and complement (Adinolfi et al., 1966) has not been established conclusively and could indeed be explained in other ways. At the moment it appears unlikely that IgA exerts its protective effects by a bactericidal mechanism.

### 3.2.2 Complement-dependent phagocytosis

Recent experiments have confirmed that IgA, in the presence of complement (possibly by activating by the C<sub>3</sub> by-pass system), may serve as an effective opsonin for Gram-negative micro-organisms (Knop et al., 1971; Wernet et al., 1971). The protective effects of specific IgA in the interstitial fluids of the intestinal lamina propria may well be explained by this mechanism. This immunoglobulin might readily opsonize invading organisms, which could then be ingested by cells of the reticuloendothelial system existing in the submucosa and other more organized lymphoid tissues of the intestinal system. At the moment, it is impossible to determine whether it acts in a similar manner in the intestinal lumen or at the epithelial surfaces, for there is no concrete evidence that phagocytic cells exist or are active in these sites. Until this can be established, it can be regarded as no more than a strong possibility that the protective role of IgA in the intestinal lumen is due to opsonic activity.

Although the above-mentioned mechanisms are the only known means by which antibody might destroy micro-organisms, the possibility that IgA protects by an entirely different mechanism cannot be excluded yet. If intestinal bacterial infection depends on multiplication of the organism in close apposition to the epithelial surface, or invasion through it, it is questionable whether the IgA antibody serves merely to prevent the organisms from being adsorbed to this surface (Wernet et al., 1971).

By the same token, the possibility cannot yet be excluded that IgM and even IgG antibody may exert a protective function in intestinal disease. To state that these immunoglobulins will be inactive because of their susceptibility to proteolytic enzymes in the intestinal tract seems to be begging the question. It could be argued that, if the protective effects are mediated at the very surface of the epithelium, the dynamic state of transfer of immunoglobulins to this site might ensure that sufficient amounts of them are available to exert direct bactericidal effects *via* the complement system. Until more is known of the pathogenetic mechanisms and the environment in the immediate vicinity of the intestinal epithelial surface, these questions cannot be answered.

In the case of the diseases that depend on enterotoxin production, the role of IgA antitoxic antibody as a protective mechanism is more readily understandable; however, curiously enough, it has not yet been established.

### 3.3 Cell-mediated immunity

In the present context, this form of immunity has been considered as that which is mediated by the specific activity of thymus-derived lymphocytes and their effects on macrophage cells. These cells may demonstrate non-specific hyperactivity after stimulation by "macrophage activation

factor" or other active factors, released as a result of reaction between the "sensitized" lymphocyte and its specific antigen (Lawrence & Landy, 1969). In animal models of *Salmonella* infection, the role of the thymus-dependent lymphocyte in cell-mediated immunity is now well established (Mackaness, 1971) and, in some, can be demonstrated to be the dominant immune mechanism. In this case, the immunity is generalized and operates as well in the spleen as in the intestinal tissues. The fact that, in the intestinal tissues, the preponderance of lymphocytes is thymus-derived (for the species in which such studies are possible) and that there is an extensive macrophage system associated with them might suggest that all the elements for cell-mediated immune reactions are present, particularly in respect of organisms that must invade the tissues in order to produce disease.

Unfortunately, the Group was unaware of any experiments indicating whether cell-mediated immunity plays a role in resistance to intestinal infection. It is noteworthy that a local cellular immunity, independent of the systemic cellular immune system, has been observed in the respiratory tract (Waldman & Henney, 1971), and similar studies of the gastrointestinal tract are imperative. Moreover, it is logical that markers of cell-mediated immunity (e.g., lymphocyte transformation, macrophage migration inhibition, and macrophage activation) should be sought in patients and volunteers suffering from intestinal infections or immunized by various means. Thus it may be doubted whether these studies should be related solely to the lipopolysaccharide antigens of the organisms: the possibility that protein antigens may be involved in these reactions must also be considered.

#### 4. IMMUNE MECHANISMS OF THE INTESTINAL TRACT IN SPECIFIC DISEASES

##### 4.1 Enteric fevers

Over the past half-century, numerous studies of *Salmonella* infections have been carried out, using animal models. In many of these studies, e.g., of various forms of parenteral inoculation of *Salmonella* species in mice, the infection bore little resemblance to enteric fever in man and little light was thrown on the immune mechanisms. On the other hand, oral infection with natural rodent pathogens may be more informative. A generation ago, Topley's notable studies of natural infection and reinfection in mice gave little hope for the development of vaccines against *Salmonella* infections, and the high incidence of second infections of typhoid fever in British troops during the 1940s was equally discouraging (Marmion et al., 1953). Recent studies in which volunteers were rechallenged with *S. typhi* gave somewhat similar results. The attack rate for the infection

was 25% of the individuals exposed and when these 25% were rechallenged the attack rate was again 25%. However, this result can be interpreted in two ways: either there was no protection in this group and the expected attack rate occurred again; or else, on rechallenge, 75% were protected and only 25% became ill (Hornick et al., 1970).

Recent studies in animal models suggest that resistance to reinfection with this group of systemic intracellular infections is better achieved with living than with killed vaccines. Killed vaccines will protect against death from enteric fevers and seem to do this by delaying the establishment of the organisms in the target organs (liver and spleen) rather than by preventing their multiplication. Living vaccines have the effects of restricting the spread of virulent organisms from the intestinal tissues and arresting their growth in these sites (Collins, 1970; Cooper & Fahey, 1970). It is likely that similar methods will yield the most significant results in studies relating to the basic mechanisms of action of oral immunizing agents or in the more pragmatic assessment of their usefulness as prophylactic agents.

Despite these and numerous other studies in experimental models, little has actually been learned about the mechanisms of immunity in typhoid fever; even less is known about the role, if any, of local intestinal immunity in this disease. The biology of the typhoid carrier suggests that some form of local gut immunity may well exist, but specific data are lacking. Numerous extensive field trials (Cvjetanović & Uemura, 1965; Hejfec et al., 1966) have shown that most killed whole typhoid vaccines administered parenterally are effective in reducing the incidence of this disease in endemic areas. However, efforts to determine the immune mechanism responsible, either by serological studies (Benenson, 1964) or by immunological characterization of the vaccines used (Spaun & Uemura, 1964), have yielded virtually no useful information. Although there are slight correlations between serological titres in man (especially "H" antibodies) and the protective effect of vaccines, the occurrence of relapses in subjects with high serum antibody titres casts doubt on the importance of serum-borne antibodies in this disease (Watson, 1957). Moreover, studies in volunteers have shown that a person without demonstrable antibodies is just as likely not to develop disease as one with high titres of serum bactericidal activity; moreover, serum antibody titres did not correlate with the prevention of relapses (Hornick et al., 1970).

On the other hand, though it has often been suggested that immunity in typhoid fever is cell-mediated, there is little evidence available to support this view. Although macrophages are rarely seen in sections of normal enteric tissue outside the organized lymphoid tissues, it has been clearly shown that in early typhoid infection these cells are concentrated in the subepithelial lymphoid tissues of the small intestine. However, it is not clear whether this reflects the intracellular invasion process, a protective immune process, or both. Thus the information on immune mechanisms

in this infection remains virtually limited to (a) extensive but to a large extent uninterpretable serological data ; (b) evidence that most recovered patients and vaccinees are resistant to reinfection ; and (c) the inadequately investigated phenomenon of the immune carrier.

#### 4.2 Shigellosis

Equally little is known about immune mechanisms in shigellosis. Although this syndrome is generally characterized by bacterial penetration of the intestinal wall, numerous attempts over many years to immunize with killed vaccines given parenterally have failed to produce demonstrable protection against *Shigella* infection. On the other hand, a variety of studies in both animals and man (see section 5) have shown the development of type-specific resistance following the oral administration of live attenuated *Shigella* organisms. In addition, the epidemiology of shigellosis (together with a few controlled observations) indicates that type-specific resistance to reinfection may often be a normal sequel of an initial infection. Therefore, it seems that type-specific immunity to shigellosis can be induced either naturally or artificially *via* the enteric route, although there is virtually no information regarding the mechanism by which this immunity is conferred.

#### 4.3 Cholera

In contrast to shigellosis, there is a plethora of information concerning immune mechanisms in cholera. Epidemiologically, a fair correlation between the prevalence and/or titres of serum vibriocidal antibodies on the one hand and resistance to cholera on the other has been demonstrated in endemic areas. To a limited extent, the development of intestinal antibacterial antibodies to *V. cholerae* has been shown to follow cholera infection (Waldman et al., 1971). Current data thus support the hypothesis that immunity to cholera may result from either antibacterial or antitoxic antibody action. It has been shown repeatedly that certain cholera vaccines, containing virtually no toxin or "toxoid" and inducing no antitoxic antibodies, will induce a significant degree of resistance to cholera, lasting at least several months. This resistance is largely (though not entirely) type-specific, suggesting that the cell-wall antigens characteristic of the two major serotypes—Inaba and Ogawa—play a major role in the production of antimicrobial immunity. However, a protein antigen found in both major serotypes is immunogenic and may conceivably play a role in cholera immunity since, on the basis of weight, its antibodies are at least as protective as those directed against cell-wall lipopolysaccharides in the infant mouse model (Neoh & Rowley, 1971).

The data supporting the role of antitoxic immunity in cholera are less complete. Toxoids prepared from concentrated and relatively highly purified cholera enterotoxin are protective in various experimental animal models of infection, whereas actively (Finkelstein & Hollingsworth, 1970) or passively induced antitoxic immunity is also effective against toxin challenge. Although antitoxic immunity does not regularly prevent the intrainestinal multiplication of vibrios in animal models (in contrast to antibacterial antibodies), it is noteworthy that a reasonably well purified cholera toxoid, administered to volunteers, gave fairly good protection. However, the sera of these individuals contained vibriocidal, agglutinating, and toxin-neutralizing antibodies in significant titres; there was no correlation between the severity of the disease and prechallenge antitoxin levels.

A recent finding by Hornick and his associates, which is of potential importance as far as live vaccines are concerned, is that a significant degree of resistance was observed in volunteers who had experienced a choleraic infection some 12 months previously. This is the first specifically documented demonstration of infection-immunity in this disease; how it is mediated is at present unknown.

In summary, some evidence points to the importance of *vibriocidal* antibodies in resistance to cholera while the limited experimental data (as well as the apparent pathogenesis of the disease) suggest that this immunity is most probably mediated directly by mucosal antibodies in the intestinal lumen. On the other hand, *antitoxic* immunity has been clearly demonstrated experimentally. This appears to operate *via* the humoral route and it is possible (though by no means proved) that it could be employed to provide resistance to clinical cholera (Finkelstein & Hollingsworth, 1970).

#### 4.4 *E. Coli* enteritis

Immune mechanisms in *E. coli* infection of the intestinal tract have only recently begun to be classified, largely as a result of the new understanding of their pathogenesis described in section 2.

Weanling piglets have been given antiserum parenterally or orally and then infected orally. A definite protective effect against diarrhoea was obtained provided that the antiserum used had been prepared against the infecting strains—i.e., it was of a specific character. Protection was best when the serum was given orally. All the evidence indicates that the protection was antibacterial rather than antienterotoxic (H. W. Smith—to be published). Some evidence suggests that antibodies directed against the enterotoxin of *E. coli* may also be protective against the porcine disease (Kohler & Cross, 1971; Miniats et al., 1970).

Data on the immune mechanism or mechanisms in man are extremely limited. It has been clearly demonstrated that human enteritis may be induced by coliform organisms possessing either primarily invasive or

primarily enterotoxic properties, or possibly—as in the swine disease—both properties (Dupont et al., 1971a). Therefore it may be assumed that the dual mechanisms of bacterial proliferation and toxin production, so clearly defined and genetically accounted for in swine disease, will prove to have their parallel in human enterocolitis. However, it should be noted that human disease has been shown to be caused by a great variety of capsular (K) and somatic (O) serotypes, and immunity against these enteropathogenic organisms is almost certainly (as with the *Shigellae*) serotype-specific. Of interest in this connexion is the finding that *E. coli* enteritis in the newborn might, on occasion, be caused by serotypes not classed as enteropathogenic (Young et al., 1960). Thus, even though a “common antigen” has been described for the *E. coli* group and other *Enterobacteriaceae* (Neter et al., 1962), the serotypic complexity of the *E. coli* diarrhoeas may represent their most important characteristic as regards immunity and immunization.

## 5. ORAL PROPHYLACTIC AGENTS

### 5.1 Killed whole vaccines

Their simplicity of preparation and ease of administration, the relatively insignificant side reactions that they produce, and the absence of various hazards associated with their administration have given killed whole vaccines designed for oral administration a popularity that far outweighs the evidence for their efficacy. However, as noted in section 1, irrelevant laboratory criteria for assessing these vaccines may have been used in many early studies. Moreover, some of the early trials lacked adequate controls of safety: the first attempt to immunize American soldiers against typhoid fever with a “killed” oral vaccine induced more disease than it would ever have prevented (Tigertt, 1959). Although numerous comparisons between groups that received oral killed typhoid vaccines and other non-treated groups have been made, the apparently favourable findings (Raettig, 1962) from such uncontrolled observations cannot be accepted as conclusive. Whereas one field trial of a killed whole typhoid vaccine showed a marginal (25%) reduction in the disease among vaccinees (Chuttani et al., 1971), a similar preparation used in another trial gave no protection (C. S. Chuttani—unpublished observations). When such a preparation was given to volunteers, but in twice the dose recommended by the manufacturer, an encouraging reduction in the infection rate was observed; the recommended dose had no significant effect (Hornick et al., 1970; Dupont et al., 1971b).

Recently it has been reported that mice immunized with doses of  $10^9$  or  $10^{10}$  killed *S. typhimurium* administered from 1 to 10 times intragastrically with a blunt needle, or doses of  $10^9$  organisms given 3 times

subcutaneously or intraperitoneally, show some immunity to *S. typhimurium* infection. In all groups a marked reduction in the numbers of organisms in the spleen (compared with the placebo control group) and an apparent reduction in the intestinal *S. typhimurium* count were seen in the orally vaccinated groups (Waldman & Senterfitt, 1971). The ability of killed organisms to induce a systemic immune response transferable to the young in the infant mouse model of *V. cholerae* infection underlines the now extensive evidence that oral immunization can produce a systemic antibody response (Chaicumpa & Rowley, 1971).

These and other observations (limited as they are) suggest that this approach may be worth while and that, if enough organisms are given over a sufficient period of time, protection at the intestinal level may be achieved.

## 5.2 Live vaccines

Numerous approaches have been tried, both in experimental animals and in man, to the development of live oral vaccines against each of the four disease groups under consideration. Various techniques have been employed in order to derive live vaccine strains of acceptable safety that are nevertheless effective. Apart from the empirical approaches used since the time of Pasteur, the following list indicates an extensive, though not necessarily exhaustive, list of strains that have been studied and are considered as potentially useful live vaccines:

### 5.2.1 Strains that are avirulent because of poor growth in the host

(a) Biochemically deficient mutants—e.g., adenine-requiring mutants of *S. typhimurium* (Furness & Rowley, 1956); purine-requiring and amino-acid-requiring mutants of *S. typhi* (Bacon et al., 1951).

(b) Streptomycin-dependent mutants—e.g., of *V. cholerae* (Olitzki & Olitzki, 1955; Felsenfeld et al., 1970), *Shigella* spp. (Mel et al., 1965), *S. enteritidis* (Kishimoto, 1965), and *S. typhi* (Hornick et al., 1970; Reitman, 1967; Cvjetanović et al., 1970).

(c) Temperature-sensitive mutants—e.g., of *S. enteritidis* (Fahey & Cooper, 1970), *S. typhi*, *V. cholerae* (G. N. Cooper—unpublished observations).

(d) Dwarf colony mutants—e.g., of *V. cholerae* (Bhaskaran & Sinha, 1967).

(e) Interspecies hybrids—e.g., *E. coli* and *Shigella* hybrids (Formal et al., 1965).

### 5.2.2 *Avirulence due to loss of somatic antigens*

- (a) Smooth-to-rough mutations in *Salmonella*, *Shigella*, and *Vibrio*.
- (b) Quantitative diminution of somatic antigens—e.g., Strain M206 of *S. typhimurium* (Archer & Rowley, 1969).

### 5.2.3 *Avirulence because of impaired epithelial cell penetration*

E.g., colony variants of *Sh. flexneri* 2a (Schneider & Formal, 1963).

### 5.2.4 *Avirulence due to poor toxin production*

E.g., apathogenic strains of *V. cholerae*, biotype El Tor (Mukerjee, 1963; Blachman et al., 1970); *V. cholerae* mutants (Howard, 1971).

The most prolonged and extensive of such studies in man have been those of Mel and his associates, who have developed and used streptomycin-dependent strains of *Shigella flexneri* over a period of 8 years. Nearly 20 000 adults aged 21, and approximately 10 000 children aged 2 to 8 years, all living in areas known to be endemic or hyperendemic, were vaccinated. The strains used belonged to *Sh. flexneri* serotypes 1, 2a, 3, 4, and *Sh. sonnei*. The selection of the mutants, the safety tests carried out in adults and children, and the methods of preparation, standardization, and administration of the live vaccine tested for immunogenicity in man have been described in detail (Mel et al., 1965, 1968, 1971). The vaccine was given in 4 or 5 doses at intervals of 2–4 days; in each case it was essential to pretreat the vaccinees with sodium bicarbonate.

Although, *in vitro*, reversions have been observed in some of the mutants, no revertants were identified in 3 200 stool specimens from vaccinated adults and children. Of the 128 000 doses given so far to 30 000 subjects, none has produced any untoward effects that could be related to reversion *in vivo*.

Postvaccinal reactions, manifested by 1–4 soft or liquid stools per day, disappeared spontaneously within 24 hours and were observed in less than 1% of adults. Postvaccinal reactions in children varied in frequency from 2.6% at the age of 8 years to 20.8% at the age of 2 years. Oral booster doses given 1 year after the primary vaccination produced only mild postvaccinal reactions, with a frequency of about 0.6%. Most noteworthy of all is the protection, ranging between 85% and 100%, observed in a series of studies carried out over the past 8 years. The vaccine is now undergoing further trial for its acceptability and effectiveness.

Conflicting results have been obtained with a streptomycin-dependent strain of *S. typhi*. In one study in volunteers a marked reduction of infection was shown in vaccinated individuals, assessed both by the incidence of disease and by the number of positive stools (Hornick et al., 1970). A second trial, only recently completed and identical to the first except for minor

technical variations, showed no effect associated with the vaccines. In studies of vaccination against *E. coli* O<sub>111</sub>, a streptomycin-dependent strain has been tried by a group in Jena, using mice, rabbits, and a series of infants (Linde et al., 1970). Furthermore, in *patas* monkeys, a similar mixture of 3 strains gave good protection and was associated with an increase, in the faeces, of precipitable antibodies against the serotypes used.<sup>1</sup>

Interspecies hybrids of *Shigella* and *Escherichia* strains have been extensively studied also (Formal et al., 1965). In this procedure, selected segments of donor *E. coli* chromosome are incorporated into the recipient *Shigella* genome. The resulting hybrid strains are tested for their ability to evoke disease, in the hope of finding hybrids that have stably replaced one or more of their virulent genes with avirulent alleles of the *E. coli* donor. Hybrids of virulent *Sh. flexneri* strains, which incorporated the xylose-rhamnose region of *E. coli* chromosome into their genome after mating with *E. coli* donors, retained their capacity to penetrate the intestinal epithelium but failed to cause disease in laboratory models. Monkeys fed with  $1 \times 10^{11}$  of these organisms exhibited no disease or adverse reactions, whereas a single dose of  $5 \times 10^{10}$  cells rendered these animals resistant to experimental challenge.

Using sophisticated genetic techniques, Bhaskaran & Sinha (1971) have isolated hybrids of *V. cholerae* that provide a choice of toxigenic strains of varying antigenic composition. Such methods can facilitate the production of specifically desired immunizing components of cholera vaccines, the preparation of pure components for immunization, and the development of live attenuated strains.

Thus the variety of potential live attenuated vaccine strains for immunization against enteric infections, and the multitude of ways in which they can be derived, hold out encouraging prospects for further trials of their safety and efficacy in man.

### 5.3 Extracts and fractions of organisms

The somatic antigens of the *Enterobacteriaceae* have been characterized in many studies. This characterization applies to *Salmonella*, *Shigella*, and *Escherichia* vaccines (Lüderitz et al., 1966, 1968). Most interesting are the observations that antibodies to the "core" antigen common to this large group are active even against the highly virulent serotypes supposedly coated with virulent antigens that block access to the core antigen (Lüderitz & Westphal, 1966; Lüderitz, 1970). The "Lipid A" core of *S. minnesota* is immunogenic and can evoke, in mice and rabbits, opsonic antibodies against antigenically distinct "smooth" virulent organisms such

<sup>1</sup> Felsenfeld, O., Wolf, R. H., Greer, W. E., Parrott, M. W., Brannon, R. B. & Stegherr-Barrios, A. (1971). Unpublished document WHO/ENT/WP/71.4.

as *E. coli* O<sub>111</sub>:B4 (Galanos et al., 1971). These findings suggest that a broadly effective vaccine might be derived from a chemical fraction of the somatic antigen of any suitable strain from this group.

In studies directed towards human immunization, an *E. coli* antigen, extracted with desoxycholate, that produces group O antibodies in rabbits and rats has been extensively studied. After it had been demonstrated that oral immunization with this extract reduced the number of deaths in challenged mice (Ocklitz et al., 1967), a large-scale field trial was undertaken in infants, using a similar vaccine prepared from several of the most prevalent enteropathogenic types of *E. coli* (Ocklitz et al., 1970). The vaccine was well tolerated by newborn babies and gave 40% serological conversion; whereas over 11 000 infants have been immunized with this preparation, no epidemic that could be used to assess its effectiveness has yet occurred. More recently a similar vaccine extract has been tried in a small group of children, but without yielding conclusive results (Girard et al., 1971). Vaccines based on ribosomal extracts of *Salmonellae* have been shown to be highly effective in animal models (Venneman & Bigley, 1969), but have not so far been investigated in human beings.

#### 5.4 Criteria and methods of assessment

Clearly, the most critical problems are associated with live attenuated vaccines. In order to be considered for human use such preparations should meet the following requirements:

- (a) they should be incapable of causing disease yet must subsist long enough in the tissues to serve as a strong immunogenic stimulus;
- (b) ordinarily they should not be capable of spreading to non-immunized individuals by natural means;
- (c) they should be genetically stable, thus obviating the risks of reversion to a virulent form; and
- (d) they should possess characteristics that readily distinguish them from wild-type virulent strains existing in the natural state.

Operationally, any potentially suitable vaccine should induce some measurable response that can be used to evaluate its presumptive effect. Detailed criteria for the assessment of safety will have to be designed to fit the vaccine under study. Obviously—unless its efficacy is overwhelmingly superior—the vaccine should be at least as innocuous as any currently used vaccine that may exist. On the other hand, where the disease involved carries a major risk and cannot be adequately treated, the balance between innocuity, efficacy, and convenience of administration may be different.

Ultimately, the efficacy of a potential vaccine will have to be evaluated in controlled field trials. This may present special problems as regards the selection of trial areas and the development of techniques for identifying cases and, in the case of live vaccines, distinguishing vaccine strains from wild strains. The special problem of evaluating cases or outbreaks of disease considered as "vaccine-associated" must be considered. The extensive experience accumulated in previous field studies, notably those on the use of live attenuated polio vaccine, will be pertinent here.

Finally it must be emphasized that, although many laboratory tests that appear to provide a logical basis for evaluating the efficacy or safety of new vaccines may be developed, no such test can be regarded as suitable for use in the selection or rejection of batches of the vaccine in question until it has been clearly established that there is an acceptable co-ordination between the results of the laboratory test and the effect of the vaccine as seen in controlled field trials.

### 5.5 Oral passive immunization

Recent studies have indicated that purified bovine colostrum antibodies derived from immunized animals might have therapeutic effects in acute enterocolitis of infants. Such preparations consist largely of enzyme-resistant (IgG<sub>1</sub>) antibodies and may be stored for long periods. The potential usefulness of such material in the course of epidemics is obvious and gives rise to the possibility that they might also be used for short-term prophylaxis in high-risk situations, particularly those associated with *E. coli* and possibly other acute enteric infections.

## 6. CONCLUSIONS AND RECOMMENDATIONS FOR RESEARCH

As stated in section 1, oral immunization techniques have been contemplated and occasionally used over a period of many years. Yet the convening of this meeting has been one of the first serious attempts to bring together the body of experimental and clinical information currently available on the subject of oral prophylaxis in intestinal infections. With the rapidly accumulating basic information on the nature of immune responses at or in the proximity of mucosal surfaces, and the availability of many sophisticated techniques for studying the physicochemical and biological characteristics of antibodies and antigens, the time is now ripe for a complete and determined reappraisal of the potential value of oral prophylactics against these diseases, which, collectively, must be regarded as the most serious threat to the health of the majority of the world's population.

Whereas it must be conceded that the most effective and, in the long run, cheapest means of limiting the incidence of intestinal infections is through education and the implementation of simple measures of sanitation, as well as of public and personal hygiene, it is equally clear that the chances of achieving this within the next four or five decades are minimal. The only alternative is to raise the immune status of the populations at risk by widespread and effective immunization. From a mechanistic point of view, oral immunization methods offer many advantages over the conventional parenteral techniques that are currently available. The basic question is, of course, whether oral prophylaxis is likely to be effective in achieving the desired level of immunity.

The success of oral *Shigella* vaccines in Yugoslavia (Mel et al., 1968) over a period of almost 10 years must be regarded as a portent. If a successful vaccine can be developed on the basis of the limited knowledge of pathogenetic and immunological mechanisms operating in bacillary dysentery, might not much more success be achieved through the application of detailed fundamental knowledge on these and other factors relating to the behaviour of pathogenic organisms and their hosts *in all enteric bacterial diseases*? Oral prophylaxis is likely to have many advantages and could ultimately become the most desirable method of immunization. However, it is all too obvious that the information currently available is too fragmentary to allow such a prediction to be made with confidence and conviction.

Perhaps the most important single conclusion reached by the Group was that there is not enough well substantiated basic information on immunization *via* the intestinal mucosa. As a corollary, it must be concluded that, until this information is to hand, the fundamental questions regarding the use of oral vaccines in human populations cannot be answered specifically. In the hope that this report may serve as a stimulus in this field, a number of immediate research objectives and problems have been considered. Although the list is by no means exhaustive, the early solution of these problems would allow a valid assessment of oral immunizing procedures.

For convenience, the problems of a general nature and those of specific enteric diseases have been differentiated. Inevitably, there is overlapping and possibly some repetition in the following list. Furthermore, no attempt has been made to indicate priorities, for these may well be determined on a subjective rather than on an objective basis.

### 6.1 Immunity in the intestinal tract

(a) Physiological, histological, biochemical, and immunological studies on the inflammatory responses of intestinal tissues following microbial invasion. These should include studies on the classes and distribution of immunoglobulins at inflammatory sites, the nature of non-specific

antibacterial activities, and the role of bacterial products (exotoxins, endotoxins, and other antigens) in these processes.

(b) The nature of the autochthonous intestinal flora of man, its ecological interrelationships with host tissues and its potential role as a non-specific mediator of resistance to microbial infection.

(c) Enzyme and chemical inhibitors of microbial activity that operate at the intestinal surfaces or in the lumen.

(d) The role of phagocytic cells in the intestinal tissues and lumen.

(e) The mechanisms and efficiency of delivery of different immunoglobulin classes to the intestinal lumen.

(f) The responses of the intestinal mucosal lymphoid system to single and repeated antigenic stimuli; the duration of responses and the characteristics of secondary and anamnestic responses.

(g) The development of quantitative techniques for measuring specific immunoglobulins produced in the intestine; ideally, these techniques should be based on functional activities related directly to the organisms or antigens involved.

(h) Studies to determine whether segmental antigenic stimulation in the gut is likely to induce general responses (or priming) throughout the intestinal lymphoid system.

(i) The biological activity of specific IgA antibody against intestinal bacterial pathogens.

(j) Determination of the biologically most effective class of immunoglobulin capable of operating in the intestine, and methods for directing antibody synthesis to ensure that this class of immunoglobulin is delivered to the intestine.

(k) Detailed studies on the chemistry of microbial antigens and the methods by which these could be delivered to the intestinal tissues in an immunogenic form.

(l) The use of immunization techniques involving mucosal surfaces, and of substances to enhance immunity.

(m) Identification, quantification, and assessment of the role of cell-mediated immune mechanisms originating and/or operating within the intestinal tissues.

(n) Extension of well controlled studies in volunteers, designed to assess pathogenetic mechanisms and host-immune responses in man and to correlate them with those illustrated by animal infection-immunity models.

(o) Development of suitable methods for assessing safety, achieving biological standardization, and defining criteria for the immunogenicity and efficacy of oral immunizing agents.

(p) Studies on methods of deriving large concentrations of specific, proteolytic, enzyme-resistant antibodies that could be used for short-term prophylaxis or immunotherapy when given *via* the oral route.

(q) Detailed studies on the physiology and pharmacology of adsorption of immunogenic macromolecules in the digestive tract, and their fate.

## 6.2 Enteric fevers

(a) More complete characterization of the basic pathogenetic mechanisms of *S. typhi*.

(b) Detailed studies on the immunological status of *S. typhi* carriers and convalescent patient-volunteers. These studies should be related particularly to the functional activity of serum and intestinal antibodies, and to local (intestinal) and general cell-mediated immune mechanisms.

(c) From (a) and (b) identification of the most effective markers of the "immune state" and their relation to chemically defined antigens of the organism.

(d) Further genetic studies involving the derivation and appraisal of mutant strains of *S. typhi* that may be considered for possible use in studies of live vaccines.

(e) Further studies on the use of killed organisms, immunogenic extracts, and synthetic immunogens as oral immunizing agents.

(f) Evaluation, in studies in volunteers, of the safety, immunogenicity, and efficacy of potentially suitable live vaccines and other non-viable agents. It is implicit that this will demand consideration of the criteria that would be used as a basis for testing these agents in controlled field trials.

## 6.3 Shigellosis

(a) Clarification of the mechanisms by which *Shigella* organisms invade the intestinal epithelium and cause its destruction.

(b) The identification, by studies in human beings and in non-human primates, of the antibacterial or other immune mechanisms that are protective in the intestinal tract.

(c) Genetic studies relating to reversion of streptomycin-dependent mutant vaccines and the derivation of other, perhaps more stable, strains potentially suitable for the preparation of oral live vaccines; these would

include *E. coli-Shigella* hybrids, biochemically deficient mutants, and temperature-sensitive strains.

(d) Further field trials of currently available live vaccines in areas where dysenteric infections are highly endemic.

(e) Methods for the biological standardization of live *Shigella* vaccines.

#### 6.4 Cholera

Many of the recommendations of the WHO Scientific Group on Cholera Immunology (1969) are still relevant and need not be restated in detail. The Group considered that the most important questions to be investigated and tasks to be performed are :

(a) The relative effectiveness of antibacterial and antitoxic immunity, which might be ascertained by using a combination of sophisticated microbial genetic techniques, animal models of cholera infection, and studies in human volunteers.

(b) The derivation and characterization of mutant or hybrid strains of *V. cholerae* from which live oral vaccines might be prepared.

(c) The development of animal models for assessing the protective capacity of orally administered vaccines, followed by expanded studies on the efficacy of vaccines in volunteers.

(d) The more detailed chemical analysis of the determinants of the somatic antigen or antigens of *V. cholerae* and their pharmacological activities.

(e) An examination of methods for oral immunization with purified extracts (lipopolysaccharides, proteins, enterotoxins) of *V. cholerae*.

(f) Consideration of the use of oral passive immunization techniques for short-term protection during epidemics, using proteolytic enzyme-resistant antibodies.

#### 6.5 *E. coli* enteritis

(a) Detailed epidemiological, clinical, and bacteriological studies of *E. coli* infection in order to identify further enteropathogenic serotypes and to determine their incidence and significance in different age groups.

(b) Because neonates are particularly susceptible, it is essential to determine the age at which immunological responsiveness of the intestinal tissues to *E. coli* antigens develops.

(c) Once this age has been determined, the potential value of passive protection by orally administered antibodies must be assessed.

(d) Fundamental work on the pathogenesis and immunity of *E. coli* infection of the gastrointestinal tract must be continued in the piglet model. It is important to determine the relative importance of antitoxic and antibacterial immune mechanisms in this disease.

(e) Further genetic studies in *E. coli*, particularly in relation to the transmissible plasmids that control enterotoxin production and to the factors responsible for adsorption to epithelial cells in the small intestine. It is essential both to characterize the human enteropathogenic strains completely and to develop living mutant vaccines that might retain the most desirable immunogenic properties and yet remain non-infective for infants.

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