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Cholera—Cause and Remedy

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Vibrio cholerae and Infection

Historically, the original concept that the clinical cholera is the result of intoxication could be credited to Robert Koch's efforts beginning from 1884. In order to prove the validity of this discovery, scientists had to wait till suitable techniques, sophisticated equipments and chemicals were available.

When *Vibrio cholerae* enters the small intestine, it has to do so through the gateway of the stomach. The first enemy that it encounters is the acid, i.e., gastric juice, to which it readily succumbs and because of the acidity most of the people virtually protect themselves. The organism may have an easy foothold in people suffering from achlorhydria.

Normally, in the stomach the vibrios do not have the chance to secrete diarrhoeal toxin in appreciable quantity. If it does, the toxin is not effected by the pepsin—which is one of the important constituents of the juice (Dutta & Oza 1963) but the high acidity may affect its potency. The toxin is absorbed in the stomach though bulk of it is through the small intestine (Oza & Dutta 1963).

Vibrio in the Intestine

After this perilous journey, the bacterium could enter the small bowel where the alkaline medium helps their multiplication, provided they could overcome the immunity barrier, either acquired naturally or induced artificially.

Stools of normal individuals or those suffering from cholera, on many occasions contain microorganisms like *Salmonella*, *V. parahaemolyticus*, *Shigella*, *E. coli*, NAG (non-agglutinable vibrios), Streptococci, or fungi. Besides, there could be a variety of parasites. How far these organisms invade the small intestine is undeterminable at present. Do the flora generate mutual potentiation or inhibition of growth of the target organism? Are the effects of the flora to be classified as synergist, indifferent or detrimental? Comprehensive answers to these questions are not yet available.

The vibrios may thrive in the intestine but it does not necessarily mean that it can produce enough toxin to elicit the characteristic reaction in the host. Toxin-producing capacity varies from organism to organism and much depends on the

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favourable conditions, *V. cholerae* Inaba 569 B is the best known toxigenic strain of today (Oza & Dutta 1963).

It has been seen that even a poorly *in vitro* toxin produced may cause typical clinical cholera (Hernick et al. 1971).

In the intestine, the toxin comes in contact with the intestinal secretion containing pancreatin and phosphorylase. These enzymes may detoxicate the choloragenic toxin. Clinical symptoms appear when sufficient quantity of toxin is absorbed (Dutta & Oza 1963).

Animal Models

Most of our knowledge on the pathogenesis of cholera has been derived from animal models. Infant rabbits of 10 days or less, develop a state of diarrhoea with dehydration indistinguishable from clinical cholera, following instillation of viable vibrios (Dutta & Habbu 1935) or administration of enterotoxin, directly, into the small intestine or orally in the host, whose stomach has been lavaged to make empty (Oza & Dutta 1963).

After 6–8 hr of inoculation of isolated segments of the adult rabbit intestine with viable vibrio or cholera enterotoxin, a fluid was produced within the lumen of the bowel (De & Chatterjee 1953). Within 24 hr the fluid production may be so great that secondary changes in the bowel may occur due to the intra-lumen pressure of the fluid.

Sack et al. (1966) observed that the adult dog responded to the intestinal instillation of vibrios or enterotoxin similar to that of human cholera (Sack & Carpenter 1969).

During the last 80 years several other models have been employed for experimental studies of the changes following manifestation of the cholera disease and its avoidance but these findings are not conclusive enough since none of these animals exactly mimics the human disease.

Choloragenic Toxin

The secretion of a toxin (now commonly known as enterotoxin) by *V. cholerae* which is responsible for diarrhoeal symptoms in clinical cases, was first demonstrated with the help of the infant rabbit model (Oza & Dutta 1963). Later on, it was confirmed in human volunteers (Benyajati 1966). The proteinaceous toxin is thermolabile and acid-sensitive and its choloragenic property could be degraded by pronase. It is unstable at *p*H 6.5 or below and in an alkaline medium of *p*H 9.0. The purified toxin has a molecular weight of about 60,000. The potency of purified toxin is short-lived even when kept at low temperatures.

Besides classical and *el tor* vibrios there are other vibrios which can cause clinical cholera. In recent years, sporadic cases of cholera have been known to occur due to NAG in India (Dutta et al. 1963) and other places like Sudan and Czechoslovakia (Shakuntala & Dutta 1971).

Like classical or *el tor* strains, NAG vibrios belonging to the O Group I, have been known to produce enterotoxins and clinical symptoms of cholera. The enterotoxins of cholera and of NAG vibrios are immunologically closely-related.

Permeability Factor (PF)

Once it was thought that cholera enterotoxin alters the capillary permeability of the endothelium of the bowel. The idea originated from the fact that the injection of relatively crude enterotoxin (Panse & Dutta 1963) or sterile filtrate of cholera stools into the rabbit skin or other mammalian model caused delayed and prolonged change in the vascular perme-

ability (Basu Malik & Ganguli 1964). What takes place in the rabbit skin would happen in the small intestine too is difficult to predict but morphological picture of the capillaries in choleraic-patient does not indicate any abnormal feature. The question of the role of the PF factor in the production of diarrhoeal symptoms is still an open one.

There are indications that the permeability factor could be separated from the diarrhoeal constituent of the cholera toxin. Experimentally, differentiation has been observed when crude enterotoxins obtained from different strains of *V. cholerae* were administered to baby rabbits. These enterotoxins were tested for their permeability reactions and for diarrhoeal actions. Several preparations had only diarrhoeal effect while others caused permeability (PF) reaction and still others had both (Bhatia et al. 1969). Further support came from studies which provided some evidence that enterotoxic factor and permeability factors are identities (Lewis & Freeman 1969).

Following this lead, the scientists at the Haffkine Institute could separate the skin permeability factor and enterotoxic factor by fractionating the culture filtrate of Inaba 569 B using DEAE cellulose chromatography (Chatterjee et al. 1972). They could also demonstrate that all the three Sudanese NAG strains, which produced clinical cholera in individuals did not elaborate enterotoxins but all the three of them contained skin permeability factor. These strains produced fatal diarrhoea in infant rabbit too.

The immunological studies (Chatterjee et al. 1973) indicate that PF isolated from the three potentially diarrhoea-producing NAG strains showed a common antigen between them and also shared a common antigen with *V. cholerae*. Permeability factor produced antibodies against itself but no cross reaction with enterotoxin factor was observed.

Absorption of Toxins

In the intestine, billions of vibrios release significant quantity of enterotoxin, which when absorbed is responsible for the diarrhoea. The toxin has been localised in the stools of cholera patients. When sterile filtrates of such stools are instilled in the small intestine of infant rabbits, the animals suffer from choleraic diarrhoea (Panse & Dutta 1963). Crude enterotoxin given intra-intestinally to a human volunteer through a cathetor and Crossby capsule led to the development of severe form of cholera (Benyajati 1966).

Histological and EM studies indicate that the vibrios remain close to the surface of microvilli in an extracellular space (LaBree et al. 1965). The toxin secreted by the vibrios is rapidly absorbed through both the villi and the crypts of the epithelial cells. It first gets into the apex of the cell in the microvilli (Peterson et al. 1972).

After absorption, the enterotoxin acts locally to produce over secretion of the intestinal fluid. It is also absorbed into the vascular system and exerts its action from a remote place.

The 'syncase choleragan' (crude choloragenic toxin) has no effect on the baby rabbits when administered paraneterally or intra-peretoneally or when given by the intra-cardiac route in doses greater than those required to produce fatal diarrhoea when given orally (Finkelstein 1965).

Several studies demonstrate that toxin could not act from a remote place after being absorbed from the intestine. A small segment of the jejunum of the infant rabbit was separated off, with one

end blind and the other opening to the exterior as a fistula (Thiry 'loop) and the remainder of the gastrointestinal tract was restored to its continuity by anastomosis. When *V. cholerae* or enterotoxin was introduced into the separated segment with intact blood supply, the animal suffered from typical diarrhoeal symptom, thereby implying that enterotoxin had travelled by only the blood circulation. Further (Vanghan Williams et al. 1969) support came from another experiment where two baby rabbits were cross-circulated with one of them infected with *V. cholerae*. After some hours, the uninfected perfused acceptor had diarrhoea and fluid accumulation in the gut. Similar result was obtained when cholera enterotoxin was used instead of live vibrios (Vanghan Williams et al. 1969). Thus, the possibility of the local as well as systemic effects operating, simultaneously may not be over-emphasised, though the local action of the enterotoxin may be a predominant feature.

Many reasons have been attributed for cessation or diminution of urinary output in cholera cases and the most common is the circulatory failure leading to ischaemic damage of the renal cortex. Whole cell toxin (containing both enterotoxin and endotoxin) when injected intra-peritoneally to normal hydrated-white rats resulted in anuria (rats do not suffer from diarrhoea due to the administration of toxin). Dialysis of the whole cell toxin had yielded an antidiuretic moiety which was protein-like and non-dialysable but toxic to mice. If one presume that the anuria could be attributed to this protein-like-principle then the effect of anuria could only be explained on the basis of the absorption of the toxin through the vascular system.

Secretion of Fluid

When the enterotoxin is absorbed in the epithelial cells, the process of excretion of the fluid begins. Does the enterotoxin alter the permeability of the endothelial cells of capillaries to give passage to the fluid? Or does the secretion of fluid is mediated through the vascular permeability factor (PF) referred to before. Or one has to look forward to the more drastic action of the toxins on the mucosal wall, that is, to rely on the earliest hypothesis that the outpouring of fluid occurs through damaged epithelium.

The vascular permeability factor (Shakuntala Kumar & Dutta 1971, Basu Malik & Ganguli 1964, Graig 1965) is said to act on the capillary endothelium, so that permeability to macromolecules such as albumin or fibrinogen is changed. In support of this, it has been shown that vascular permeability for dyes occur at the site of injection when cholera toxin or sterile filtrate of cholera stools are given intradermally to the rabbit. Increased histamine is also found in choleraic small intestine (Panse & Dutta 1963). The presence of meagre amount of protein in the rice-water stool and the little histological changes that may occur in the epithelial cells do not justify such an assumption.

Proponents to the theory of production of diarrhoeal fluid due to the injury to the capillary endothelium provided evidence of biochemical change or damage to the brush borders of the epithelial cell which has been observed in clinical cases (Chen et al. 1971). Chemical changes in the form of decrease in sialic acid, phospholipid, cholesterol, alkaline phosphatase and 5'-nucleotide in the epithelial cells have been offered as sign of damage to the cell though not visible microscopically. The sections of the intestine

in clinical cases displayed shortened villi, some of these thickened and even fused. A chronic mononuclear inflammatory reaction has often been seen in the *lamina propria*. One could not loose sight of the fact that bulk of the hospitalised patients came from the lower socio-economic strata of the society and whose level of nutrition and freedom from intestinal infections are questionable. It is not unusual to find, in many of them the chronic enteritis (inflammatory, degenerative or reparative) over which choleraic picture is superimposed.

Till a decade back, scientists by tradition were inclined to think that the action of toxins are associated with pathologic lesions only. Such thinking is not totally unjustified when one finds on the post-mortem table, intestines of numerous patients with eroded mucous surfaces. This line of thinking took a different turn when it was demonstrated that vibrios cause profuse loss of fluid from the intestine through automatically intact intestinal membrane (Gamgarosa et al. 1960).

There is little doubt that minimal morphologic changes occur in the small bowel, both in experimental or clinical cholera. There is increased discharge of mucus from the goblet cells of the epithelium and hyperaemia of the sub-epithelial capillaries. What have been said above is not enough to explain that the cellular alterations lead to the excretion of the fluid.

The Diarrhoeal Action

In cholera the fluid is withdrawn, as if, by a powerful force from the blood circulating in the capillaries of the intestinal wall and hurled into the bowel lumen. The vibrios are found all over in the intestinal canal, either freely moving in the fluid or clinging to the mucous membrane. The fluid drains out only from the small-intestine and the stomach and the colen does not participate in this process (Greenough 1965, Banwell et al. 1970). The small bowel secretes this fluid, at a rate which exceeds the absorptive capacity of the colon, so that the effluent is excreted out as rice-water stool. Curiously enough in such a situation the small-intestine retains the selective power of glucose absorption. This is the key factor in the oral-fluid therapy in cholera.

It is now fairly established that the loss of fluid and electrolytes from the cells takes place through the mediation of cyclic-Adenosine Monophosphate(cAMP). Using the preparation of animal or human isolated pieces of small-intestines, suspended in using chamber and bathed in isotonic fluid, which could neutralise any physical, chemical or electrical gradient acting across the tissue, it could be demonstrated that normally, the sodium ion of salt move through the mucous membrane towards the blood side, corresponding to the absorption of salt and water by blood from the intestine. The addition of enterotoxin to the mucosal side reverses this process, that is, the chloride ion move towards the intestinal lumen side and sodium is no longer absorbed. Along with the sodium, the water moves out with the result that active secretion of sodium and decreased absorption of it take place (Field 1971, Field et al. 1972, Al-Awqati et al. 1970). Earlier, it had been shown that when theophylline, a drug known to stimulate accumulation of c-AMP in the cell is added to the luminal side of the gut, the results are identical to that of the diarrhoeal toxin. When c-AMP itself is added to the blood side of the normal intestinal segment then the active secretion of chloride and decreased absorption

of sodium from the lumen take place. When the intestinal tissue is exposed to the action of enterotoxin, there is an increase in the intracellular level of the c-AMP in the epithelial cells (Schafer et al. 1970). This elevated level is the result of the increased activity of the enzyme adenyl cyclase (Chen et al. 1972) which converts Adenosine Triphosphate (ATP) into c-AMP.

Hormones are known to stimulate the accumulation of c-AMP in the cell. One of the hormones, tested for this purpose, is prostaglandin. When this hormone is infused through the superior mesentric arteries in "Thiry Vella" small bowel loops in dogs, secretion of isotonic fluid with the electrolyte pattern similar to produced by the cholera enterotoxin takes place (Pierce et al. 1971). The response to the throphylline is similar to that of enterotoxin or prostaglandin in this type of dog model. It is conceivable that c-AMP is directly involved in the mediation of the secretion of the gut fluid due to the enterotoxin.

Further support to such an assumption comes from the fact that enterotoxin mimics the actions of c-AMP on other tissues. The enterotoxin increases lipolysis in rat edididymal lypocytes, stimulates glycogenolysis in the human platelets and in the rat liver cells and produces prolonged hyperglycaemia in dogs (Pierce et al. 1972).

Clinical Aspects of Cholera

The enterotoxin acts on the small bowel and the secretion is triggered by the interaction between the epithelial cells and the former. The small bowel secretes effluent which is an isotonic solution containing sodium, potassium bicarbonate and chloride, the proportion varies in children and in adults. The small bowel secretes at the rate which exceeds the absorption capacity of the colon, resulting in the watery stool. The clinical sign and the biochemical abnormalities could mainly be explained on the loss of isotonic fluid with low protein content (less than 200 mg / 100 ml). The rapid depletion of fluid results in hypovolaemic-shock, increased concentration of plasma protein, non-protein nitrogen and urea in the blood. The total body potassium-depletion occurs, and severe metabolic acidosis ensue. The death occurs, if these are not corrected, due to hypovolaemic shock, renal failure and uncompensated metabolic acidosis.

Antibacterial Immunity

Against the remarkable achievements in the field of pathogenesis and treatment of cholera, the progress made in the protection of an individual or the community against the disease by way of using immunising antigens has not been very impressive. But, during the last decade or so it has been possible to understand, at least, the status of cholera vaccines which are being currently used from a series of field trials which alone could give satisfactory answer against our knowledge derived from unsuitable techniques adopted in earlier years to evaluate vaccines. We are no longer under the false hope that all conventional cholera vaccines can give protection for any length of time to an adequate degree.

The discovery of enterotoxin (diarrhoeal toxin) stimulated our rethinking on antitoxic immunity as an additional support in the prevention of the disease.

There are many bacterial diseases that make one immune from the second attack. But there is meagre evidence to suggest that one attack of cholera will protect the individual against the second.

On the contrary, there are reports of the individual having more than one attacks. This being the trend one would realise to get an effective immunising agent that would protect, would by no means an easy task. So far it has proved to be so.

Epidemiological studies have shown that residents in the highly endemic cholera area acquire a fairly high degree of natural immunity with the advancing age. The problem of protecting children as well as those living in an non-endemic area are considered to be the main issue in the development of an effective vaccine. When the epidemic occurs in an non-endemic area the adult people are affected more than the children. The process is reversed in the endemic areas because the case index is higher in the children.

In cholera, it is conceivable to have both the anti-bacterial and the anti-enterotoxic (anti-diarrhoeal) immunity. The evidence that vibriocidal antibody titre is directly related to immunity is shown through several sources. The close correlation of vibriocidal titres with protection from cholera suggests that the immunity that develops naturally in the endemic population as well as produced by the currently used whole-cell cholera vaccines is primarily anti-bacterial. This is what the vibrios have to encounter first in the hosts' intestinal tissues. The currently used vaccines are the expression of antibacterial antigens rather than antitoxic immunogens.

Antitoxic Immunity

What role the antitoxic immunity holds for future will only be known in the field trial of a suitable antigen. In infant rabbits, antitoxin (from adult rabbits) raised against enterotoxin when administered paraneterally, provides no protection against oral challenge of the enterotoxin but the antisera prepared out of live vibrios remarkably, prevents the disease (Panse et al. 1964). When there is an infection in a family, with increasing vibriocidal titres in family contacts, infection rate decreases (Mosely et al. 1968) but the chances of developing diarrhoeal symptoms are not decreased. It has also been observed from hospital records that prior vaccination does not significantly alter the clinical course of the disease (McCormack et al. 1969).

The interest in the antidiarrhoeal immunity is of recent origin as the cause of diarrhoeal symptom was not known before. Toxoid prepared from cholera toxin has been shown to produce high titres diarrhoeal factor, neutralising antibodies in adult rabbits or in dogs and to protect them from challenges of enterotoxin or viable vibrios (Finkelstein 1970, Pierce et al. 1972).

Using the experimental model of isolated rabbit gut it is possible to measure quantitatively, the degree of antitoxic immunity acquired naturally or induced artificially (Kasai & Burrows 1966). Further, using this technique one can measure the serum antibody level in the convalescent carriers as well as in the persons suffering from asymptomatic cholera which were fairly high. However, one would not know yet that the high degree of antitoxic antibody in the plasma in an asymptomatic subject would mean failure on the part of the vibrio to establish itself or neutralisation of the enterotoxin liberated by the vibrios.

The procedures employed presently, for the manufacture of conventional vaccines give no chance of forming antidiarrhoeal antigen, for the method of killing the vibrios and their preservation lead to the destruction of the whatever amount of enterotoxin might have formed.

In none of the field trials effort was made to measure the antitoxic immunity, probably, the method for quantitative assay did not exists in earlier years. Again, to gauge the antidiarrhoeal immunity status in a community it is necessary to undertake large-scale epidemiological studies. The complexicity of the problem should be realised.

Vaccines and Immunity

The reliability of vaccine should finally, come from the results of field trials. There are few vaccines in recent years which have undergone so much scrutiny in the field than cholera vaccine. Since 1963, nine field trials have been undertaken. Of these, two are of recent origin and their full results are yet to come.

The types of antigens tested in the field are (i) Agar-grown, phenol-inactivated and preserved biotype Inaba and Ogawa (classical), (ii) Purified LPS (lipopolysaccharide) antigen, *el tor* biotype Ogawa, (iii) Agar-grown, formalin-inactivated, freeze-dried, classical biotype Ogawa, (iv) Agar-grown, formalin-inactivated, freeze-dried, classical biotype Inaba, (v) Purified protein LPS antigen *el tor* biotype Inaba, (vi) Agar-grown, formalin-inactivated, freeze-dried, classical biotype Inaba and Ogawa, (vii) Fluid medium-grown, formalin-inactivated, phenyl-mercuric nitrate-preserved, classical biotype Inaba and Ogawa, (viii) Agar-grown, heat-inactivated, phenol-preserved, *el tor* biotype Inaba and Ogawa, (ix) Agar-grown, heat-inactivated phenol-preserved, classical biotype Inaba and Ogawa, and (x) Oil-adjuvant, classical biotype Inaba and Ogawa.

While evaluating the results of all these vaccines, consideration was given to the age factor of the subject, the nature of the antigen, the endemicity of the area, the number of doses administered, local and systemic reactions and population at risk.

On summing up the results of the field trials undertaken so far, it seems that the degree of protection which is expected of a conventional vaccine is about 50–60%. There are extreme cases wherein the vaccine gave no protection and on the other hand degree of protection reached a level of 90%. From the end of three months, the level of immunity steadily falls till to the end of six months, after which it ceases to be of any significant value.

The vaccine, given in single doses in children is less effective than when given twice though, this is not the constant feature in all the trials. In adults, a single dose could generally, evoke the required degree of immunity.

Vaccine produced by classical *V. cholerae* strain protects against diseases caused by biotype *el tor*. Although, there is no evidence from the results of field trials that whole cell vaccine prepared from *el tor* could protect against classical biotype strains yet purified LPS Ogawa and protein LPS Inaba antigens prepared from *el tor* biotype strains confer protection against classical biotype Inaba and Ogawa strains.

Cross protection between Inaba and Ogawa gives varying results. In the second Bangladesh trial, the purified LPS Ogawa antigen protected adult well, but the children rather poorly against heterologous Inaba infection. In the fourth Bangladesh trial, the whole-cell Ogawa vaccine was ineffective against the Inaba infection.

The passive immunization test with infant rabbits demonstrated that monovalent cholera vaccines prepared from classical strains of *V. cholerae* or *el tor* vibrios of serotype Inaba and Ogawa are protective against challenge of hetero-

logous strains. But, vaccine made from *V. cholerae* serotype Inaba is effective in protecting against challenge of *V. cholerae* serotype Ogawa, *el tor* vibrios serotype Inaba as well as homologous challenge (Dutta et al. 1966).

Recent field trials of parenteral cholera vaccine have shown that prophylactic immunisation is feasible though, the degree of effectiveness may not be to the standard expected. Any conclusion other than this involves a fair amount of speculation. In one Dacca trial, significant protection for about 18 months was noted. The explanation offered is that the population at risk have had a continuous exposure to cholera vibrios (Benenson et al. 1968). This is supported by serological data which show that normal adult population in Dacca study area was relatively immune due to periodical exposure to the vibrios. The successful result is regarded as a booster immunisation superimposed on a significant degree of natural immunity.

The results of the field trials have demonstrated that the cholera vaccines are of some value in protecting individuals against the disease. How these immunological reactions are mediated? It has been observed that protection of vibrios is related to vibriocidal titres of the serum. One of the vaccines used in the field is shown to induce certain degree of antitoxic immunity in the rabbits (Kasai & Burrows 1966). But this had necessitated hyperimmunisation of the animals. In the human field trials, it is unlikely that a single dose of vaccine would confer a significant protection as it did in one of the trials on the basis of antitoxic immunity. It has been shown that passive immunisation (Panse et al. 1964) with antidiarrhoeal sera did not protect infant rabbits against challenge of live bacteria, while similar immunisation with antibacterial sera was effective (Freter & Gangarosa 1963). Moreover, close correlation of the vibriocidal titres with protection from cholera suggests that the immunity which develops naturally in endemic population as that produced by the cholera vaccine is primarily antibacterial rather than antidiarrhoeal.

Coproantibody

The field trials did not really give a chance to assess the nature of immune reactions involved in the protection. The evidence that immune response is associated with vibriocidal antibody levels but at the same time the protection afforded in Dacca trials did not necessarily imply that the vibriocidal serum *per se* has a protective effect. The protection could be provided by serum antibody or coproantibody or both. For the present, one has to take recourse to experimental findings if one has to assess these.

It has been demonstrated in human volunteers that parenteral vaccination induces both serum and coproantibodies and the latter could be maintained by periodic exposures to vibrios orally (Freter & Gangarosa 1963). It is suggested that the significant protection induced at the field trials at Dacca might have been "due to coproantibody response which was originally induced by the vaccine and was maintained, subsequently, by periodic oral revaccination of the population with vibrios from the environment".

Such an hypothesis is supported by studying a model of rabbit ileum, suspended in Krebs-Ringer solution which shows that clinging of vibrios to the mucosal surface reduce antisera; most of the vibrios are forced to swim within the intestinal canal and out they go along with the rice-water stools. It is

further shown that in unvaccinated population, 50% of the vibrios are attached to the mucous surface and the remaining ones are free within the bowel. The role of coproantibody needs further elucidation.

Live Parenteral Vaccine

As far back in 1885, live cholera vaccine was tried. Following this it was again "successfully" used in the field by Haffkine (1892). When the killed vaccine was developed, which was much safer to use, the live vaccine receded to the background. During the last decade the question of the use of live vaccine was revived in view of the impressive findings in experimental models, the superiority of living vibrios as an antigenic agent in the prevention of experimental cholera (Panse et al. 1964, Panse & Dutta 1964, Basu et al. 1970).

Live Oral Vaccine

It would be ideal to have a live oral cholera vaccine like one available in the field of virus where oral polio vaccine is an example. No single strain, so far could satisfy all the basic requirements: that it should be able to multiply in the human bowel, it should not spread to non-immunised individuals by natural means, it should possess a high degree of genetic stability with no tendency to revert to virulence and that it should possess genetic marker by which it could be identified. One of the strains which has nearly approached to such basic criteria is Ogawa EW-6 (Mukherjee 1963). The strain is capable of inducing serum vibriocidal antibody level producing coproantibodies which persisted for some months and caused no untowards reaction in human volunteers when orally administered. But, this strain is capable of producing enterotoxin though to a lesser degree than Inaba 569 (Blachman et al. 1970). Recently, it has been shown that the live oral vaccine prepared from Ogawa EW-6 failed to colonise in the gut of human volunteers. (Ganguli et al. 1975).

Vaccines of the Future

Since cholera organisms produce diarrhoeal toxin, it would be possible to develop a toxoid which might be valuable agent to cause immunity. One of such studies (Dutta et al. 1971) was undertaken at the Haffkine Institute, a few years back in which a potent sycase toxin was prepared using *V. cholerae* Inaba 560 B. This toxin was formalised and the resulting toxoid did not produce diarrhoeal reaction in the baby rabbit nor elicited skin permeability reaction in the adult rabbits. The results of immunisation of rabbits showed that two doses of toxoid, injected intramuscularly at interval of two weeks was superior to conventional vaccines, as judged by agglutination test, inhibition of skin permeability and passive protection tests in infant rabbits. Perhaps, a combination of a whole cell vaccine and a toxoid may provide a better answer to the problem of prevention of cholera.

Further research is needed in the application of cholera vaccine adsorbed on to alluminium phosphate or hydroxide. Presently, clinical trials of two such vaccines are under way. The results are encouraging.

Investigations on liver oral, or live parenteral vaccine need to be pursued. This line of investigation, if successful, will prove most beneficial.

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