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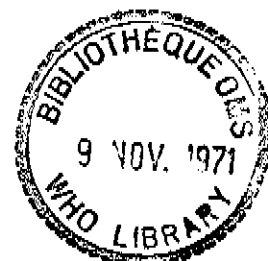
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EMERGENCY AND PREVENTION OF TYPHOID FEVER
AND CHOLERA

by

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Vaccines intended to control enteric infections have been employed since the end of the nineteenth century. Only in recent years have large controlled field trials and quantitative vaccine efficacy evaluations in volunteers with induced infections given us information on the relative effectiveness of vaccines to prevent cholera and typhoid fever. Both can be shown to be effective but reliability is lacking. Protection is incomplete and, in the case of cholera vaccine administration, of short duration. In some ways the currently available vaccines employed to prevent these two infections are similar. They are prepared from heat-killed, formalin-treated or phenol-treated whole organisms. Such treatment does not destroy the antigenicity of the cell wall antigens (O), but, as in the case of the typhoid bacillus, does render the envelope Vi antigen, which is very important in terms of human virulence of a strain, less antigenic. The utilization of acetone in order to preserve the antigenicity of Vi antigen has improved the old vaccine. Unfortunately no evidence of protection was noted in studies using Vi antigen alone as a vaccine. Thus the whole cell appears to be a better parenteral vaccine than a single antigen associated with "virulence". Similar "purified" cholera antigen vaccines have been tested in animals but no efficacy trial in man has been performed. A major difference between the typhoid and cholera vaccines is the inclusion of the two major serotypes, Ogawa and Inaba, in the latter. Monovalent cholera vaccines have been tested and are effective against the homologous infecting organism. Cross immunity of the monovalent vaccine, i.e. Inaba versus virulent Ogawa, has not been demonstrated.

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antibodies which are given parenterally, similar to the formation of antibodies in response to the cell wall, envelope antigens of typhoid bacilli (see in the case of *S. typhi*, of which the carrier state is a common occurrence). Voluntary studies have demonstrated that these antibodies are not related to resistance to the disease. The question remains whether these antibodies can react with the pathogen in the gut.

The cellular processes that are induced in man must interact with the mucosa of the gastrointestinal tract in order to initiate disease. Typhoid bacilli apparently can penetrate the mucosal barrier initially without causing irreversible changes to the living cells. The organisms are picked up by the lymphatic drainage, multiply in the draining lymph nodes and ultimately in the liver. It can perhaps be inferred from our present knowledge of vaccine efficacy that the circulating antibodies alone are not able to prevent the organisms from penetrating the epithelial barrier, to inhibit their spread to the liver or to prevent multiplication in the liver cells; therefore, such parenteral vaccine-stimulated antibodies do not induce complete protection. The cell wall antigens in vaccines have been implicated in the formation of antibodies which promote phagocytosis. In the presence of sufficient active phagocytic cells induced by vaccine administration, the antibodies acting as opsonins promote phagocytosis. Subsequently, the envelope antigens are removed allowing exposure of the vulnerable bacterial membrane. This is attacked by other antibodies and complement and bacterial death occurs (Rowley). There is little evidence to support this attractive concept in man. However, it may help to explain the immunity of typhoid carriers. These patients have millions of organisms passing down the lumen of the gastrointestinal tract daily without any evidence of penetration. Such individuals appear to have a very effective cellular immune mechanism which prevents typhoid bacilli from invading the host as outlined above. Just how typhoid vaccine does protect is unknown. Whether it does cause immune cells to confine the organisms in the gut, or initiate a multi-step defense response involving sensitized cells, phagocytes and antibodies is purely speculative at present.

Other views can be presented for cholera. In this disease, however, the penetration of the epithelial barrier is not necessary to induce disease. The arguments multiply in the lumen of the gut or near mucosal surfaces and require a potent enterotoxin which causes marked out-flowing of fluid from cells in the small intestine tract. This fluid is secreted primarily from the cells and not from cells higher up on the villi. Thus cells deep beneath the villi appear to be most affected by the enterotoxin which obviously has to reach these more remote areas of mucosa to cause disease. Therefore, to cause disease, the cholera vibrio injected by the host must pass through the gastric milieu where acid kills many, must overcome the antibacterial mechanisms of the lumen that block multiplication, and must diffuse to susceptible cells the enterotoxin neutralized by antitoxic substances. If antibody is primary to the latter mechanism, then it must either be secreted into the intestinal lumen or be closely associated with mucosal cells in order to effectively function. Carpenter's serum circulation studies in dogs suggest that the antitoxin from the donor animal enters the lumen of the toxin-contaminated loop of intestine of the recipient dog. Assuming this is IgG antibody, it is difficult to explain how this molecule can get into the lumen at the site of toxin activity and how it can resist enzymatic degradation. It is of interest to note that partial protection from this disease occurs in recipients of killed vaccines living in endemic areas but that this protection persists for only 3-6 months. Again the mechanisms involved in this induced resistance are unknown. In addition to the mucosal mechanisms mentioned above, there is one reason to suspect that phagocytosis is not important; vibrios do not penetrate. However, it cannot be summarily dismissed as an effective defence mechanism since mobile phagocytes may be involved. As with typhoid fever one cannot correlate the level of circulating agglutinating or vibriocidal antibodies in volunteers with their response to challenge with virulent vibrios. The enterotoxin appears to be all important in the pathogenesis of cholera therefore, induced antitoxin should be protective in tetanus antitoxin. Volunteers given a non-purified toxoid preparation, when challenged, demonstrated some protection against induced disease. However, this protection was also not complete. Again the titres of the circulating antitoxin could not

is correlated with the severity of clinical infection induced. Very similar intestinal antibodies if only to the cholera vibrios can be demonstrated in individuals who have recovered from overt infection and are challenged with a virulent dose. The nature of this protective reaction is unknown at present but is readily demonstrable by failure to isolate vibrios from the stools of this group of rechallenged volunteers. This level of immunity is obviously very high and should serve as the goal to be achieved by vaccines. Conceivably, some antibodies may be involved. Hunter has shown in rabbits that local intestinal antibodies prevent adhesion of pathogens to the mucosal surface thereby preventing initiation of disease. No antibody of this type has been identified in man.

Several epidemiological factors are common to all enteric infections, especially cholera and typhoid fever. In endemic areas of the world these infections occur most commonly in children; adults appear to acquire the disease almost as an accident. This would suggest that the repeated small exposures during childhood induce intestinal (and perhaps humoral, acting in a synergistic fashion) immune mechanisms which protect during adult years. This is probably a dynamic type of immune process which is continually boosted by repeated exposure, i.e. the intestinal tract becomes sophisticated. Infection in adults could occur when a large number of organisms are swallowed or when, for obscure reasons, immune mechanisms in the intestinal tract are temporarily diminished.

From the evidence that we now have regarding the efficacy of killed vaccines for these enteric infections and the knowledge regarding the pathogenesis of these infections, one would hope that the administration of live, attenuated organisms by mouth would result in immunity. The portal of entry would be closed by induced specific and non-specific resistance mechanisms. Studies with shigella, typhoid and cholera organisms have been under study in volunteers. The results with the oral shigella vaccines utilizing several serotypes have been encouraging. Immunity to a virulent, moderate challenge can be accomplished in volunteers.

Oral typhoid vaccines have been obtained using both killed and streptomycin-resistant attenuated strains. The administration of large doses of the killed vaccine (Taboral) resulted in no clear-cut resistance to disease. However, there was suggestive evidence that some intestinal immunity was generated by these killed oral antigens. The vaccinated volunteers had fewer positive stool cultures for typhoid bacilli following the virulent challenge.

Administration by mouth of 10 billion SM-dependent typhoid plus 1 gram of SM once weekly for four weeks was arbitrarily employed as a means of trying to immunize the gastrointestinal tract. Very little evidence of humoral antibody production was documented. H antibody was found in about 20% and O and Vi antibodies in about 10% of participants. When these volunteers were challenged 4-6 weeks after the last dose of vaccine, effective local immunity was demonstrated. Disease was detected in 13% of vaccinees and 46% of controls. Of great interest was the very marked suppression of typhoid multiplication in the gut. Only 3% of vaccinees had positive stool cultures following ingestion of the virulent strain. And these men usually had only 1 or 2 positive cultures. Controls, on the other hand, had an incidence of positive stools of 46%; furthermore, the number of positive stools per man was usually greater than 10. This experiment was repeated in the past few months using a lyophilized culture as the inoculum in the hope that lyophilized organisms may eventually be given as a dry vaccine powder. In addition, the bicarbonate and streptomycin were given simultaneously rather than preceding the vaccine. These details are presented because there was no evidence of vaccine-induced resistance in this year's experiment compared with the previous one. Other explanations are under study.

Several types of typhoid bacilli have been selected in various laboratories that lack portions of the cell wall. One of these has had a preliminary test as an oral vaccine and no evidence of immunity was apparent after a single dose of the epimeraseless mutant was administered.

These early studies have been provocative and should be continued. However, some means of evaluating immunity to enteric infections is badly needed. This would allow for simpler determination of resistance to infections than inducing disease in volunteers or undertaking large-scale field trials. Oral polio vaccine stimulates circulating neutralizing antibodies which are a definite measure of the host's immune status. Circulating tetanus antitoxin is an obvious indication of protection against tetanus. No similar simple serological method correlates to immunity in enteric infection.

We have begun analysis of saccus entericus to determine if anti-bacterial activity is induced by oral vaccines. This is somewhat cumbersome since of necessity a tube must be swallowed to obtain the test material from the intestinal tract. Nevertheless, if excellent activity could be identified and quantitated in the small bowel of convalescent cholera patients or from fecal fluid from oral typhoid vaccinees one could anticipate that even better activity would be apparent in partial immune patients in endemic areas. By identifying the means to then stimulate these and other intestinal defence mechanisms, methods of inducing them could be formulated and effective control achieved. In the oral vaccine trials to date a limited dosage schedule was employed; more multiple doses might be necessary or a different schedule of administration may be more effective. These variations need exploration because of the exciting leads that have already been observed in the initial investigations of oral enteric vaccines.