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Introduction

Pertussis or whooping cough is still a major health hazard in developing countries especially for children below one year who are at the highest risk from the disease. The disease has been controlled in most of the developed countries with the use of killed whole cell pertussis vaccine. The hazards of vaccine range from minor local to systemic reactions with rare incidences of convulsions, infantile spasms and more serious neurological illnesses. Hence, the acceptability of pertussis vaccination by parents and physicians in some countries has been low.

A subcellular/acellular pertussis vaccine is highly desirable, but the problems to establish the clinical efficacy of such vaccine should not be underestimated. Recently, Japanese workers have developed a pertussis component vaccine with lower toxicity and lower side effects, but Preston cautioned in interpreting the preliminary trials reported by Japanese workers. Meanwhile the research on the whole cell vaccine that is available is continuing for reducing its toxicity and to make it more potent and stable. In a previous study, the effects of various inactivating agents used in the manufacture of pertussis vaccine were comparatively evaluated and it was observed that glutaraldehyde and formaldehyde were better inactivating agents for the preparation of a safer and potent pertussis vaccine. In this study only one method of inactivation by each
inactivating agent was used and vaccines thus prepared were evaluated by potency, mouse weight gain and histamine sensitization (HS) tests\textsuperscript{17,18}. Formaldehyde has been used as an inactivating agent in the preparation of pertussis vaccine\textsuperscript{19} and is also used in the preparation of acellular pertussis vaccine\textsuperscript{14,15}. The conditions for inactivation with formaldehyde are well established. There is hardly any work on the use of glutaraldehyde as an inactivating agent in the preparation of pertussis vaccine. Hence, there is a need for further studies to get optimal conditions for inactivation by glutaraldehyde with a view to detoxify the pertussis toxin, LPF activity or HSF activity. The vaccine thus prepared will be evaluated by leucocytosis promoting factor activity also in addition to potency, mouse weight gain and HS tests. Efforts would be made to develop an acellular pertussis vaccine. The acellular pertussis vaccine would be comparatively evaluated with the whole cell vaccine for various quality control tests. The effect of preservatives like benzethonium chloride or parabens on the potency of the pertussis vaccine would be studied with a view to producing adsorbed Diphtheria-Pertussis-Tetanus vaccine which can be combined with the inactivated polio vaccine for making Quadruple vaccine. At present, thiomersal is used as preservative for adsorbed DPT vaccine which is deleterious to the potency of the inactivated polio vaccine.

**Brief Review of Literature**

The use of vaccine against pertussis seems to have been started in 1909\textsuperscript{20}. In 1913, Nicolle and Conor\textsuperscript{21} used a live pertussis vaccine during an outbreak of the disease in Tunis. Killed vaccines have been used for the immunization of children by Huenekens\textsuperscript{22} and Hewlett\textsuperscript{23}.

The first trials with the vaccine indicated that the use of a vaccine
prepared from a freshly isolated strain of *B. pertussis* was chiefly of value in decreasing the severity and mortality of the disease\(^2\). In a number of trials in the United States a substantial lowering of the attack rate was observed in children injected with plain and alum-precipitated vaccines\(^2^5-^3^1\). Further favourable reports were made by a number of workers in other countries\(^3^1-^3^5\). But some workers in the United States\(^3^6,^3^7\) and in England\(^3^8\) failed to obtain any substantial degree of protection in controlled field trials among infants and children.

The British field trials conducted in 1950s by Medical Research Council concluded that it was possible by vaccination to produce a high degree of protection against the disease, as shown by substantial reduction in the attack rates among the home contacts and reduction in the severity and duration of the disease where the vaccination failed to give complete protection. The results also showed that different vaccines employed varied a great deal in their protective action. the low potency vaccines gave an attack rate in home contacts of 87% and the most effective gave an attack rate of 4% only\(^3^9\). The risks and benefits of pertussis immunization are still controversial\(^4^0,^4^1\). The disease remains too dangerous in relation to the known hazards of the vaccine and the continuation of vaccination has been recommended\(^2\).

Attempts to develop an improved pertussis vaccine started with the production of stromata-protective antigen (SPA) by Pillemer *et al.*\(^3,^4\). The SPA was prepared from extracts of sonicated *B. pertussis* cells adsorbed on human erythrocytes. The preparation was protective in the mouse potency assay and showed clinical efficacy in the British Medical Research Council trials\(^5,^6\). The extract adsorbed to erythrocytes and sensitized mice against histamine. So SPA was probably a mixture of cellular components including LPP.
Eli Lilly Company marketed DPT adsorbed vaccine containing an acellular pertussis vaccine prepared from a trisodium phosphate extract of B. pertussis cells from 1962 to 1977. Similarly Wyeth Laboratories, Inc. prepared experimental acellular pertussis vaccine which was potent in mouse potency assay and was antigenic in limited clinical studies, but the reaction rates did not differ markedly from whole cell vaccines.

In 1960s Millman et al. developed a soluble pertussis vaccine by extracting cell envelopes of B. pertussis with sodium deoxycholate followed by adsorption on alum. At Behringwerke, a vaccine was prepared from urea salt extracts of B. pertussis adsorbed on aluminium carrier. The vaccine was potent in the mouse potency assay.

Recently a number of workers isolated LPF and filamentous haemagglutinin (FHA) by various techniques. The acellular vaccine manufactured in Japan mostly contains LPF and FHA.

For several years thimerosal has been used as a preservative in pertussis vaccine. The vaccine preserved with either formalin or phenol was less stable with regard to potency than with thimerosal. The merthiolate preserved vaccines were stable in potency with or without heating at 56°C. Even such vaccines were stable in comparison to vaccines without a preservative. Merthiolate is used in DPT vaccines as preservative but cannot be used with poliomyelitis vaccine because free mercury ions are deleterious on its potency. Benzethonium chloride, which is acceptable as a preservative in poliomyelitis vaccine is used in DPT polio (quadruple) vaccines. Studies on a number of lots of quadruple vaccine prepared in the USA disclosed that the pertussis component was unstable in the presence of Benzethonium chloride and the rate of loss was about 6% per month.
Benzethonium chloride and parabens have been found to be deleterious to potency of pertussis vaccine. Addition of cations prior to treatment with benzethonium chloride inhibited deleterious action. Relyveld stressed that the method of preparation of vaccine is an important point in the action of benzethonium chloride.

Aims and Objectives
1. The Bordetella pertussis organisms from one of the production strains (134 or 509) would be inactivated by different concentrations of glutaraldehyde for variable periods to get optimal conditions of inactivation. The quality of the vaccine will be comparatively evaluated with that of the heat inactivated vaccine.
2. The vaccine prepared under optimal conditions will be evaluated by potency, mouse weight gain, HS and LPF tests.
3. An acellular vaccine would be developed.
4. The effect of preservatives benzethonium chloride or parabens on the potency of pertussis vaccine will be studied with a view to produce the DPT vaccine which will be suitable to combine with inactivated polio vaccine for the production of Quadruple vaccine.

Materials and Methods (Brief Plan of Study)
Bordetella pertussis strains. The strains of Bordetella pertussis for vaccine production will be used in this study. In a previous study, it was observed that there was no difference in the effect of inactivating agents on various strains of B. pertussis organisms. So in the present study only one strain will be used to get the optimal conditions for inactivation by glutaraldehyde.

The freeze-dried culture will be opened on Bordet Gengou medium and the seed culture will be prepared in Verwey medium.
Cultivation of the organisms. The bacterial harvests will be prepared in Verwey medium. The bacterial mass will be separated by centrifugation and will be tested for opacity, identity and purity.

Inactivation of the bacterial harvest. One portion of the bacterial harvest will be inactivated by heat at 56°C for 10 minutes to be used as control. The other part will be divided into aliquots. Each aliquot will be inactivated by glutaraldehyde under different conditions (The concentration of glutaraldehyde, temperature and period of inactivation will be chosen on the basis of killing of B. pertussis organisms and inactivation of dermonecrotic toxin). After standardizing the conditions of inactivation, a few batches of pertussis vaccine will be prepared under optimal conditions of inactivation. The quality of the vaccine thus prepared will be evaluated by potency, mouse weight gain, HS and LPF tests.

Potency Test. The potency test will be performed as recommended by WHO.

Active Mouse Weight Gain Test. The mouse weight gain test described by Pittman and Cox will be performed according to the guidelines of WHO.

Test for Histamine Sensitization Factor. The test for HS activity will be performed as described by Pittman.

Test for LPF Activity. The LPF test described by Kurokawa et al will be performed as per the Japanese Government Minimum Requirement for Biological Products.

Production of Acellular Pertussis Vaccine. The Tohama or some other suitable strain of B. pertussis will be grown as a stationary culture in either Verwey medium or Stainer Scholte medium. The bacterial growth will be treated with 20-33% of ammonium sulphate. (The concentration will be standardized). The precipitates will be collected which will be dissolved in
phosphate buffered saline. After centrifugation the supernatant will again be precipitated with ammonium sulphate. The precipitates will be dissolved in phosphate buffered saline. This will be then dialysed. The preparation will be detoxified. The vaccine thus prepared will be evaluated by various tests described above.

Effect of benzethonium chloride or parabens on potency of pertussis vaccine.

A few lots of adsorbed DPT vaccine with benzethonium chloride (1 in 40,000) or parabens as preservative instead of thimerosal will be prepared and their effect on the potency of pertussis vaccine will be observed.

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