Pertussis vaccine

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Report on an informal Working Group on the Production and Testing of Pertussis Vaccines

Geneva, 13 and 14 October 1977

The Secretary (Dr F. T. Perkins, Chief, Biologicals) welcomed the participants to Geneva and thanked them for sparing the time to discuss a problem that was most important to WHO especially in its Expanding Programme on Immunization. A list of participants is included in Annex I and the Agenda in Annex 2.

The Secretary was pleased to acknowledge the most generous support that the UNDP had given to WHO concerning the quality control of vaccines. They had now given additional support in order that the participants of the Working Group could be assembled.

In the developing countries it is apparent that help is needed in the improvement of both vaccine production and the cold chain for the storage and their distribution.

The vaccine causing the greatest concern is the pertussis component of the DPT vaccines because there are not sufficient data concerning its stability especially when the vaccines may be involved in a refrigeration breakdown in a country with a high ambient temperature. Furthermore, the information concerning severe reactions has reached these countries and, although illfounded, such data are having an adverse effect upon the rates of immunization. It seems timely, therefore, to have a detailed discussion concerning the gaps in knowledge as well as to consider the investigations that may be initiated in order to improve the potency of pertussis vaccine and its stability.

1. The testing of pertussis vaccines for toxicity

The test being applied to the measurement of toxicity of the vaccine is the weight gain test in mice. It was generally agreed that the factors causing the initial loss in weight with recovery are the thermolabile toxin (which can be eliminated by heating) as well as the thermostable endotoxin. The endotoxin is the major contributor to the initial loss in weight, but there is no conclusive evidence that the mouse weight gain test is measuring the factors responsible for the reactions in children. In addition, the test was not a good biological assay system in that reproducibility, parallelism and linearity were poor. It was emphasized that in measuring the toxicity in children the neurological complications, because of their low incidence, could not be included in these considerations.

In Denmark the age of pertussis vaccination had been moved from 5, 6 and 7 months to 5 and 9 weeks, and comparisons of the data had shown that infantile spasms were not associated with pertussis vaccine because these continued to occur in the older age group. This was probably the best evidence that we had to show that the cause of such rare events was not pertussis vaccine.

However, most manufacturers were including this test either because it was mandatory or because it was a useful "in process" control for the detection of toxic substances. Although other tests, such as the subcutaneous inoculation of vaccine in the suckling mouse for dermonecrosis, were under investigation, none was known to correlate with the reactions shown in infants.

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2. Factors affecting toxicity

There was general agreement that vaccines prepared on solid media were less toxic than those grown in liquid media. Although this was true the problems of growing large quantities of vaccine on solid media were considerable and many producers, therefore, had studied the problem of detoxifying vaccines grown in fermenter tanks.

Studies in Sweden had shown that the conditions of production of vaccines in fermenter tanks had a profound effect upon toxicity. A high rate of aeration (1 litre/minute per 4 litres of culture medium) gave rise to less toxic vaccine than a low rate (0.2 litres/minute) under similar conditions. The size of the inoculum, the growth conditions of the inocula, the media as well as the time of harvest were all important factors. Furthermore, some antigens such as type 3 appeared to be more easily dispersed into the medium than the other antigens.

The treatment of the harvest was also important since the bacteria separated from the supernatant fluid by acid precipitation were more toxic than those separated by centrifugation. It was a common finding that holding bacterial suspensions in the cold (4°C) for 3 to 6 months caused a marked decrease in the toxicity of the bacterial suspension. It was usual, therefore, to hold such suspensions in the refrigerator until they passed the mouse weight gain test.

The method of killing the organisms also affected the toxicity; heat alone, formaldehyde, heat with merthiolate, and merthiolate alone at refrigerated temperatures (+4°C) are used. Although all these methods are successful in killing the organisms they give marked differences, especially on storage, in the preservation of the antigenic composition and potency. Formaldehyde is particularly deleterious to the stability of the potency of the organisms.

During the discussions it was pointed out that the toxicity of vaccines was related to the number of organisms contained in the vaccine. There are two opacity standards in use; the International Standard and the US Standard whose opacity had not been calibrated in International Units. It is essential that in reporting data International Units of opacity are used, and that the appropriate action concerning the US Standard should be taken without further delay.

Many data were presented which showed that by using different methods of preparation there was no correlation between toxicity and potency. Such a relationship was obtained only when a single preparation was diluted. Other data showed that for acid precipitated bacilli there was a correlation between mouse weight gain and mouse protection (I.U.) but the same could not be observed for centrifuged bacilli.

3. The correlation of toxicity and reactions in children

There is a need for a test for toxicity that will correlate with the local reactions observed in children. Attempts should be made to obtain such data. A cytotoxic or cytopathic test in cell cultures was suggested, and it was agreed that such a test may be most useful. Tests in tissue culture had shown promise some years ago but the presence of methiolate in the vaccine had stopped further progress. It was suggested that the toxicity of the bacterial harvest could be measured before the addition of merthiolate and this may provide useful data.

In a recent international laboratory study carried out by WHO, 15 vaccines had been shown to have different toxicities. It was important to observe the reactions to these vaccines in children in an attempt to obtain some correlation between the laboratory tests and field experiences with regard to toxicity.

A request was made for an International Toxicity Standard. Such a Standard had been in use in the USA for some years, but because of non-linearity with some vaccines it had not been shown to be useful for inclusion in each test. It was considered, therefore, that such a reference preparation would not serve a useful purpose.

Finally, in view of the dilemma concerning the significance of the mouse weight gain test it was emphasized that the working group should make a statement about its use. It was agreed that:

"The results obtained with the mouse weight gain test have not been adequately correlated with the clinical reactogenicity experienced in the use of pertussis vaccine. It does not seem appropriate at this time, therefore, to recommend the use of this test as a measure of freedom from toxicity of the vaccine.

In order to study the toxicity of vaccines during their production, however, the test may have some value as an "in process" control. Further work should be done in order to identify the toxicity giving rise to reactions in children and investigating a laboratory method to measure its activity in animals."

4. Studies with non-toxic vaccines

Dr Zakharova had no new data to report about her cell-free preparations. Some difficulties had been encountered when an adsorbed combined vaccine was prepared using the extract vaccine because the extract flocculated with $Al(OH)_3$, but further studies are underway to resolve this problem.

Dr Sato presented his data on the isolation and characterization of different cell components (LPF, HA, HSP, PA). HA and LPF were found to be antigenically and physico chemically distinct. A haemagglutinin had been prepared from bacterial surface fimbria and it was found to be protective in mice. The preparation contained no endotoxin and, furthermore, HSF and LPF were not detectable by the usual methods. Preparations on an industrial scale had been made, and when 0.2 mg $Al(OH)_3$/ml was added they had: (1) a protective activity of 9 - 13 PU/ml, (2) HSF of 0.1 - 0.2 U/ml, and (3) LPF of 0.2 U/ml. There was a marked similarity between this material and that isolated by Pillemer. The recovery of the protective activity was reported to be approximately 10%; the protective material was about 1% of the whole organism.

There was general agreement that the mechanism of immunity to whooping cough in man is not known. Reliable laboratory tests for measuring the specific protective efficacy are not available. The separation of all antigenic fractions was a first step, and the study of these components was long term.

Dr Sato reported that in Japan whooping cough had returned after vaccination had been reduced to a low level and this was clear proof that immunization must continue in order to reduce the incidence of this disease. Similar events had occurred in Europe and more were likely to occur in view of the reduction in immunization rate currently experienced.

In Denmark an increase in whooping cough had occurred when vaccines giving a lower immunity had been used.

It was suggested that the Japanese non-toxic extract vaccine might be tested in countries where an increase in whooping cough had been observed due to a decreased immunization rate. Such vaccines that passed all the WHO requirements could be used in the areas in which there was a high incidence of whooping cough. WHO would be prepared to look for areas in which this vaccine might be used.

5. Stability of vaccines

Dr Higy Mandic reported that vaccines made from certain B. pertussis strains had been found to be much more stable than those prepared from other strains. A fluid vaccine from one strain, for example, retained its activity for 18 months, whereas vaccines from three other strains lost potency completely under identical circumstances. It was concluded that the stability of the potency of strains would be a useful test for the selection of strains for vaccine production.

Dr Joo prepared freeze-dried vaccines - pertussis as well as DPT - and these were found to be stable for five years for DPT vaccine, and for more than ten years for the pertussis vaccine alone. For tropical countries this may be an ideal solution, but the cost of such vaccines was considerably higher than that of fluid vaccine. It was interesting to note that the presence of merthiolate during freeze drying caused no loss of potency. There were some preliminary data to show that freeze-dried adsorbed DPT vaccine was stable for 4 weeks when held at $37^{\circ}C$.

Dr Beale presented some data about the stability of liquid DPT vaccines held at different temperatures. These showed that at 50° there was a rapid loss in potency, whereas the stability was maintained at 40° for 2 weeks, at 35° for two months, at 25° for 9 months, and at 4° it was stable for very long periods.

It was reported that frequent temperature fluctuations may be much more detrimental than a high temperature provided that it was steady. There was a warning too, that during the storage of vaccines under adverse conditions the reactivity may increase even though the potency may remain acceptable.

It was agreed that it is most important that data on stability at 30° were needed especially for those vaccines to be used in tropical countries.

6. Tests for the potency of vaccines

The Secretary was most anxious to have the views of the Working Group concerning the potency assay of pertussis vaccine, particularly the shortcomings of the active mouse protection test and alternative methods that might supersede it.

Dr Sheffield presented a paper in which he reminded the meeting of a report in which it had been stated that pertussis vaccine made and used in the United Kingdom prior to 1968 was largely ineffective. He described a series of potency assays of vaccines obtained from various countries and showed that, with the single exception of a UK vaccine, all had provided estimates of potency at the National Institute of Biological Standards and Control (NIBSC), London, of less than 4 iu per human dose. The low results were discussed in statistical terms and it was shown that almost all were inhomogeneous with the potency values obtained by the various manufacturers. In addition, it was shown that the British-made vaccine, which had provided an acceptable estimate of potency at the NIBSC, provided low or very low estimates of potency when tested by two laboratories from which two vaccines which behaved very poorly in the NIBSC tests had emanated. No convincing explanation of these "between laboratory" discrepancies was adduced and it was assumed that there were factors which militated against the acquisition of homogenous results when different vaccines were tested in different laboratories.

The Secretary reminded the members of the Working Group of the correlation between the efficacy of the vaccines in children and the mouse protective potency as observed in the MRC Trial of 1953-56 and also of the less publicised and better correlation of the efficacy with the agglutinin production in mice. He suggested, however, that it now seemed apposite for workers in the field to look for an alternative and more reliable assay procedure. He encouraged the members to discuss the present situation freely, and the salient points that emerged were as follows :

(a) Any new potency assay procedure must be correlated with efficacy in children, and this was extremely difficult to demonstrate because of the low pertussis attack rates in industrialized countries and the difficulties of conducting trials in the developing countries.

(b) Agglutinin production in mice and in guinea-pigs provided an indication of the antigenicity of pertussis vaccines, and in the hands of some workers good dose-response lines had been obtained. Agglutinins were not, however, themselves considered to be protective but merely indicators of the, as yet unidentified, protective antigen or antigens.

(c) Adsorption of agglutinins and titration of monotypic agglutinating potency was considered feasible but passive protection tests were rejected as indices of the presence in an adsorbed serum of protective antibody on the grounds that adsorbing suspensions may introduce into serum substances capable of inducing "immediate" protection or substances capable of inducing the protection effective in the active mouse protection test. Agglutination tests for antibody detection in sera from children and animals had been used in Czechoslovakia using both Bordetella pertussis and Bordetella parapertussis. The need for all workers using these tests to use the same adsorbing and agglutinating strains was emphasized.

(d) Complement fixing antibody production was considered as a potency assay method but there was little support for this method.

(e) Immunofluorescence and fluorimetry were suggested as techniques for the measurement of cellular antigens and of antibody titre.

(f) Cell mediated immunity was agreed to have been studied very inadequately. Phagocytosis of Bordetella pertussis by polymorphonuclear leucocytes and by macrophages in various in vitro systems was considered as was the use of the macrophage inhibition technique.

(g) Changes in the growth conditions of Bordetella pertussis as well as in the harvesting time had been shown to result in organisms of different character, although often in uneconomic yield, and a suitable combination might give a highly effective non-toxic vaccine. There was evidence that growth on solid media provided vaccines which, organism for organism, were more potent in the mouse protection test than vaccines grown in liquid media. This area had not been studied with anything like the thoroughness that had been expended on the preparative conditions for diphtheria and tetanus toxoids.

(h) Adjuvants in many vaccines increased the statistical invalidities as all standard vaccines were unadsorbed freeze-dried preparations. There was evidence, from different sources, that adjuvants increased, decreased and were without effect on, the potency of pertussis vaccines.

(i) Homogeneity of the mouse strain may be an important factor in the potency assay. A carefully regulated colony at the Bureau of Biologics, Bethesda, USA (B.O.B.) had given very consistent ImD50 values for the standard vaccine and might thus contribute greatly to an international agreement of the assay of potency of the vaccines. The mice were not syngeneic. In Britain a syngeneic strain of mice had given rather variable ImD50 values with the standard vaccine.

The Secretary thanked members of the Working Group for their participation in the discussion and expressed the views of many members when he described the active mouse potency test as an unsatisfactory procedure but one which would remain indispensible until such time as an alternative method of assay could be proven and introduced. He asked members of the Group if they would collaborate one with another towards an alternative and all agreed so to do. Various members offered to participate in specific ways, as follows:

1. Co-ordination of studies by discussion and dissemination of information (WHO Secretariat).

2. Distribution to interested members of an authentic strain of 18323 (F. Sheffield).

3. Distribution of pairs of mice from the B.O.B. colony for establishing new breeding colonies together with instructions (Manclark).

4. Preparation of monospecific antisera of high titre (Zakharova). Professor Zakharova offered to prepare a multispecific antiserum to be used as a working reference serum in the agglutination reaction for determination of agglutinin production.

5. Preparation of standard suspensions of *Bordetella pertussis* for agglutination tests (Nagel - WHO contract).

6. Preparation of reference vaccines for agglutination tests (A. J. Beale).

7. Laboratory studies to correlate the production of haemagglutinin, inhibiting antibodies, agglutinins, and C.F. antibodies in guinea-pigs, particularly with respect to vaccines made from cell fractions (Sato and others).

The Secretary believed that the only way in which progress would be made was by testing a number of batches of vaccine for their ability to induce protection and antibody responses in animals, and to compare these data with the ability of these vaccines to protect children against whooping cough. This would involve a long-term study of laboratory tests with epidemiological data. There was money to support such studies and participants were asked to contact the Secretary and declare their interests in such participation.

The meeting concluded with the expression by Dr Joo of the members' appreciation of the determined way in which the Secretariat had pursued the purpose of the meeting and the efficiency of the arrangements, both scientific and social, that had been made for members of the Working Group.

ENCS. Annex 1 and Annex 2

2 February 1978

ANNEX 1

LIST OF PARTICIPANTS ATTENDING THE MEETING FOR DISCUSSIONS ON THE PERTUSSIS VACCINE

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ANNEX 2

AGENDA

For the Pertussis Meeting, to be held in Sall A,
on 13 and 14 October, at 9.00am

1. Welcome to participants.

2. The testing of pertussis vaccines for toxicity.

3. The current situation with respect to the production of an extract and non-toxic vaccine.

4. The thermo stability of the pertussis strains used in vaccine production.

5. Tests for the measurement of potency other than the mouse protection test.

6. Any other business.