Studies in Pertussis Immunity. III. Immunization of Children with Live and Killed Vaccine.* (22628)

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The immunity induced by killed pertussis vaccine is known to be less durable and less complete than that induced by an attack of the disease. It seemed reasonable to attribute this difference to some alteration of the antigen brought about by the procedures used to kill the organisms, and to attempt to secure a more complete and durable immunity by vaccinating with live organisms. The relative effectiveness of live and killed vaccines has previously been compared by means of the mouse intracerebral challenge test(1). It was found that live vaccine gave better protection to mice than did killed preparations ($p = 1\%—0.1\%$). In extending this study to the vaccination of children, a method of assaying immunity was needed. Since it was not possible to determine this by direct exposure, it was decided to compare the effectiveness of these two vaccination procedures by a test for passive protection in mice. Groups of subjects, injected with live and killed vaccine respectively, were bled at appropriate intervals. The serum was injected intraperitoneally into mice which were then challenged with live *H. pertussis* by the intranasal route (2). This test has shown good correlation with immunity developing after the disease. Known immune individuals and adults (who are presumably immune) respond to a booster injection with a sharp increase in mouse protective antibody; known susceptibles consistently fail to show any rise in protective power of their sera under these conditions(3).

Using this test we have not been able to demonstrate superiority of the live vaccine in children, but some evidence of a difference in response to the live and killed vaccine was found.

Observations have also been made of the development of antitoxin, and of antibacterial immunity as measured by agglutination, indices which may be regarded as less significant than mouse protection.

Methods and materials. A. Subjects. Half of 40 mentally defective children at the Willowbrook State School were given the live vaccine, the other half the killed vaccine. Age range was from 9 months to 11 years, for the most part between 18 months and 4 years. These children were selected after preliminary testing of their sera showed that they possessed neither circulating antibacterial agglutinins nor passive protection for mice in the intranasal challenge test.

B. Preparation of vaccines. H. pertussis strain #18323 was grown in Cohen and Wheeler fluid medium(4), and the 72 hr growth harvested and suspended in phosphate buffer (0.02 M—pH 7). The cells were washed once with the buffer, then resuspended to a concentration of 20 billion organisms/ml. The cells thus suspended in phosphate buffer were used as the live vaccine preparation. It was used for inoculation within 4 hours of harvesting of the cells.

The killed vaccine was prepared by adding 0.001% merthiolate to the above preparation and storing in the refrigerator; the cells were usually nonviable after 4 weeks and non-toxic after 2-3 months. One small group received the killed vaccine after one month, when the bacilli were no longer viable although the vaccine was still somewhat toxic, in order to use aliquots of the same cells for both the live and killed vaccine groups. As immunizations were spaced at monthly intervals, bacilli of the previous month's live vaccine were used the following month for inoculation of the killed vaccine group.

C. Scheme of immunization and bleeding. The vaccines were divided into 4 doses of 10 billion, 20 billion, 20 billion, and 20 billion

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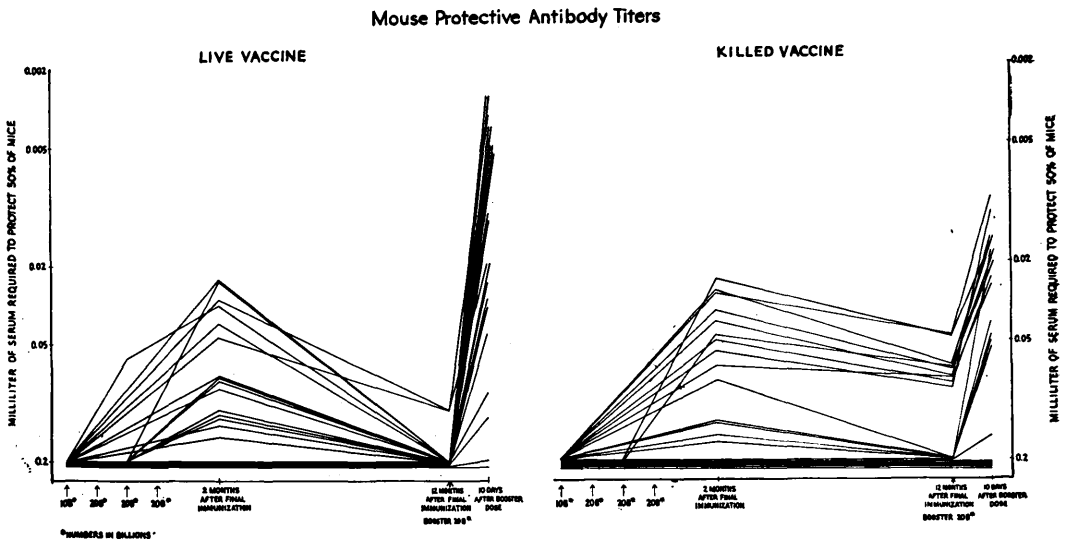

FIG. 1. Response of children to immunization with *H. pertussis* vaccine.

organisms (0.5 ml, 1 ml, 1 ml and 1 ml) and injected at monthly intervals. Injections were given deep into the deltoid muscle of older children or the buttock of the younger ones, alternate sides being used. Blood was drawn before the first inoculation, 4 weeks after the second, and 2 months after the final injection. After 12 months had elapsed, a booster dose of 20 billion organisms in 1 ml volume of a heterologous killed vaccine was administered to both groups of children. Blood was drawn at this time and again 10 days later.

D. Methods of assay. The mouse protection tests and antibacterial agglutination titers were performed by methods previously described(5). Two methods of titration of antitoxin were used. The sera of individuals in each group were tested against 2-4 S.D./50 of pertussal toxin intradermally in rabbits (6). Pools were made of sera from each vaccine group and tested against 2-4 L.D./50 of toxin intraperitoneally in mice(7). Whenever possible the sera of individuals from both groups were assayed together, all sera of each individual being tested at one time.

E. Reactions to Live and Killed Vaccine. Children given the killed vaccine preparation developed neither local nor systemic reactions of any note; there was no evidence of local tenderness, erythema, or induration, and there were no temperatures recorded above 101°F. Among the 20 children given the live preparation, 5 developed fever between 102-103°F. which could be attributed to the inoculation of the vaccine. Three of these children showed a similar rise of temperature after the second inoculation; thereafter, temperature rises were less marked. Local reactions developed in 7 of the 20 children, 3 moderately severe and 4 severe. The remaining 13 children either did not develop local reactions or developed only a very small hard nodule which could be palpated deep in the muscle. The 3 reactions classified as moderately severe were those showing erythema and induration up to 2-4 cm in diameter; erythema faded rapidly and induration disappeared within 7 days. Severe reactions were characterized by the presence of erythema and induration up to 4-6 cm in diameter. These reactions often persisted for longer than one week and occasionally for 3 weeks. These children appeared to develop areas of sub-surface necrosis, but none of the areas showed actual ulceration. One child developed this type of reaction after each of the 4 injections. Another developed a severe reaction after each of the first 3 injections. The remaining 2 children showed such reactions only after the first injection.

Results. A. *Mouse protective antibody*

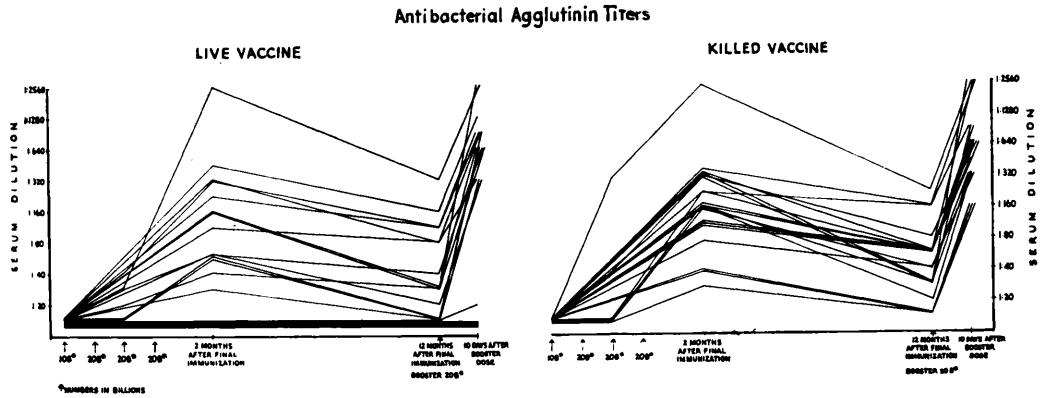

FIG. 2. Response of children to immunization with *H. pertussis* vaccine.

titers. Fig. 1 shows the results of the passive protection tests in mice challenged intranasally. *Initial response*: In those who responded to primary immunization the titers that developed ranged from a P.D.$_{50}$ of 0.15 ml to 0.024 ml in the live vaccine group and from 0.16 ml to 0.024 ml in the killed vaccine group. Five of the 20 children who were given live vaccine did not develop circulating mouse protective antibodies. The sera of 6 of the 20 children given killed vaccine also showed no rise in this antibody. Over a 12 month period the levels of circulating mouse protective antibody among the children given live vaccine dropped markedly and were still measurable in only 2 individuals. The same was not true of those receiving the killed vaccine. The children in this group who had developed substantial protective titers after immunization retained these titers at moderate levels at the end of the 12 month period. *Booster response*: A booster injection was given one year after the primary immunization had been completed. In response to this booster injection, 10 of the 20 children given the live vaccine developed a 20 to 50-fold or greater increase in mouse protective antibody titer. Six of the children showed a 4 to 10-fold increase. The remaining 4 children showing little or no increase after the booster injection were those who had failed to respond to primary vaccination; the fifth child who had not developed measurable antibodies did show a significant booster response. Fourteen out of 20 children given the killed vaccine responded with increases in titers of roughly 4 to 10-fold; the remaining 6 children who failed to respond had originally failed to produce mouse protective antibody after primary immunization.

B. Antibacterial agglutinin and antitoxin titers. Fig. 2 shows antibacterial agglutinin titers developed by the children immunized with the 2 vaccines. Essentially there were no significant differences in the results. After primary immunization 6 of the children given the live vaccine did not develop agglutinins and only one of these children showed a slight response to the booster injection. Although all 20 individuals given killed vaccine showed increases in titer after immunization, responses in a few instances were low. On the whole, both groups showed a comparable response to the booster injection. The majority of titers after primary immunization were in the range of 1:160-1:320 and from 1:640-1:1280 after the booster injection. Neutralization of pertussal toxin was not demonstrated with sera of either vaccine group.

Discussion. The results of this study give no clear-cut answer as to the merits of the 2 types of vaccine. The number of subjects who responded poorly by any criterion is more than would have been anticipated and raises some question as to whether mental defectives as a group are as readily immunized as the population at large. We also cannot explain the apparently different response to the live and killed vaccine—the more rapid loss of mouse protective antibody titer and the

greater booster response seen in the case of the live vaccine. It is unfortunate that an opportunity to challenge the immunity of the two groups did not present itself. To date—some 2 years after immunization was completed—no exposure to whooping cough has occurred.

Summary. 1. The relative effectiveness of live and killed *H. pertussis* vaccine has been studied in children. 2. By means of a passive mouse protection test, using the intranasal route of challenge, a more rapid loss of antibody after primary immunization and a greater booster response was observed with live vaccine. 3. Severe local reactions occurred in some children given the live vaccine.

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Studies in Pertussis Immunity: IV. Booster Response in Man Induced by Various Fractions of *H. pertussis*.* (22629)

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It has previously been reported(1) that sera of children convalescing from whooping cough show a consistent rise in mouse protective antibody. Similarly it has been found that a high percentage of adults respond to a booster injection of killed pertussis organisms with a sharp rise in this same antibody. The present report deals with the ability of various fractions of the *H. pertussis* bacillus to induce this recall response. Four antigens or fractions have been studied: 1) the agglutinogen of Smolens and Mudd(2), 2) the protective antigen of Pillemer(3), 3) the hemagglutinin of Keogh and North(4), and 4) the soluble fraction of Pennell and Thiele(5). Whole cell vaccine was used as the control booster stimulus.

The study was carried out in a group of 100 adults at the Willowbrook State School. Each fraction was tested in 20 individuals and 20 were injected with the control killed vaccine. Blood was drawn prior to intramuscular inoculation of the test booster dose and again 10 days later. The sera were tested for the presence of antibacterial agglutinins as well as for their ability to protect mice against intranasal inoculation with lethal doses of *H. pertussis*.

Methods and materials. A. *Preparation of the fractions. H. pertussis* strain #18323 (Kendrick) was used for the fractions prepared in this laboratory. The organisms were grown in Cohen and Wheeler fluid media(6) and collected after 72 hrs incubation. *Agglutinogen* was prepared by the acid extraction method of Smolens and Mudd(2). The final preparation assayed at 1000 agglutinin absorbing units/ml; 250 or 500 units in 0.5 ml was used as the booster dose. This prepa-

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