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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TABLE I. Agglutinin Response of Adults to a Booster Dose of Various Pertussis Antigens.

	Agglutinogen (Smolens and Mudd)	Soluble antigen (Pennell and Thiele)	Intracerebral protective antigen (Pillemer)	Whole cell vaccine	Hemagglutinin (Masry)
No. of individuals showing response	19	18	13	12	7
Total No. of individuals	20	20	20	20	20
% of response	95	90	65	60	35

ration contained <2 E.D./50 ml when tested by the intracerebral potency test(7). *Pillemer's protective antigen*: Lot #134L32, supplied through the courtesy of Dr. H. D. Piersma of the Lederle Laboratories, did not absorb agglutinins (<25 units/ml) and assayed at 91 E.D./50/ml in this laboratory when tested for intracerebral potency. The product was labeled as containing 26 protective units/ml(8). Approximately 23 E.D./50 or 6.5 protective units in 0.5 ml was used to test for booster action. Protective Antigen was also prepared in this laboratory from strain #18323 by a method similar to that described by Pillemer(3). It was not as potent as Pillemer's preparation containing only 25 E.D./50/ml. It differed from Pillemer's own antigen in that it showed agglutinin absorbing activity. The product was concentrated by centrifuging down the stroma and resuspending it in a smaller volume of saline; 20 E.D./50 in 0.5 ml was used for the booster tests.

Hemagglutinin Antigen was prepared by extraction #18323 cells with molar sodium acetate for 24 hours at 37°C(9). The hemagglutinin present in the crude extracts was then adsorbed on to stroma by incubating at 37°C for one hour in the waterbath; the stroma was washed several times with saline and finally resuspended in saline. The final product did not absorb agglutinins (<25 units/ml), and did not protect against intracerebral infection (<5 E.D./50 ml). It contained 40 hemagglutinin units/ml; 20 units in 0.5 ml served as the booster dose.

Soluble Antigen was obtained through the courtesy of Dr. W. F. Verwey of Sharp and Dohme Laboratories. Lot #1148-4B was used in these experiments. It had been prepared by the method of Pennell and Thiele (5) and was an alum precipitated product. This preparation contained 1000 agglutinin absorbing units/ml and 71 E.D./50/ml by the intracerebral potency test when assayed in this laboratory; 250 or 500 units of agglutinogen and 17 or 35 E.D./50 in 0.5 ml were used as the booster stimuli. Whole cell vaccine was prepared from *H. pertussis* strain #18323 and suspended in phosphate buffer (0.02 M—pH7) after once washing the cells with the buffer Two different lots were used. They contained 200 agglutinin absorbing units/ml each and assayed at 50 E.D./50/ml and 81 E.D./50/ml respectively. 100 units of agglutinogen and 25 or 40 E.D./50 in 0.5 ml were used as the booster dose.

B. *Assay of the response to the booster dose.* Determination of the antibacterial agglutinin titers and the intranasal passive protection tests were performed by methods previously described(1).

Results. A. *Booster response in adults as measured by rises in antibacterial agglutinin titers.* Table I shows numbers of individuals in each of the 5 groups developing increases in agglutinins. The best responses were obtained with agglutinogen and soluble antigen complex. Hemagglutinin gave the poorest response.

B. *Booster response in adults as measured by mouse protection tests.* Mouse protective antibody was determined in 10 of the 20 subjects studied in each group. The labor involved in these tests precluded studying the entire group. The sera tested were those which had shown the greatest response in antibacterial agglutinins. Considerable discrepancies exist between the results of agglutination and mouse protection tests (Fig. 1). The soluble antigen gave an excellent response in both tests. However, the agglutinogen which gave

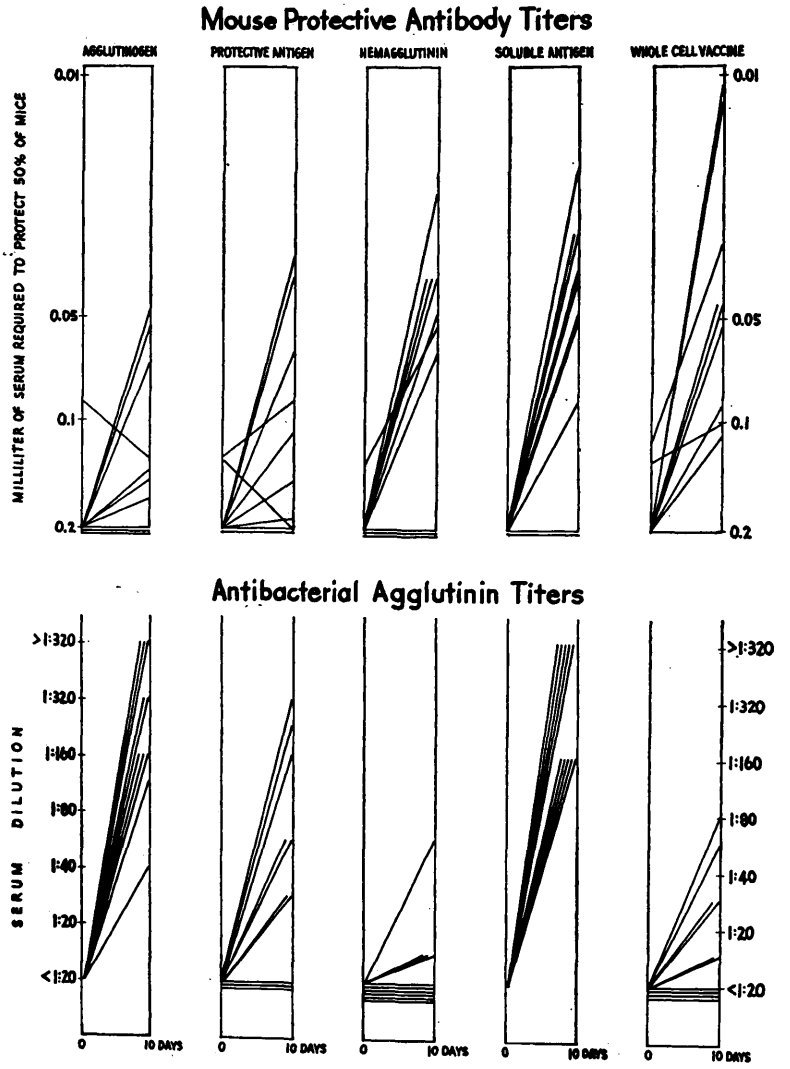

FIG. 1. Booster response of man to fractions of *H. pertussis*.

excellent agglutinin titers performed poorly when tested for mouse protection. Conversely, the hemagglutinin, a poor agglutinin producer induced a satisfactory mouse protective antibody response. Results with the intracerebral protective antigen were not striking when measured by either test. Whole cell vaccine which served as the control showed greater increases in mouse protection than in agglutinins. Where 2 different levels or 2 different preparations of a given antigen were used, there was no difference in the response observed.

Discussion. When passive protection in mice against intranasal challenge was used as the index of booster response, only the soluble antigen and hemagglutinin gave results comparable to whole cell vaccine. The complex nature of the soluble antigen fraction, believed to contain all the immunogenic activity of the intact organism(5), does not permit any conclusion as to the specific antigen responsible for the protection observed. The hemagglutinin on the other hand did not produce a good agglutinin response, nor did it protect against intracerebral challenge in active immunization experiments. It was somewhat surprising therefore to find that this

fraction was capable of stimulating a booster response in mouse protective antibody against intranasal challenge. The further finding that the intracerebral protective antigen did not perform well in this study suggests that protection against intracerebral and intranasal manifestations of pertussis infection in mice depend on different mechanisms. Keogh and North(4) had originally postulated hemagglutinin as the protective antigen against intranasal challenge. Except for the present study, subsequent evidence(1,9), has not supported this view. Since in our experiments as well as those of Keogh and North, hemagglutinin was adsorbed on to stroma from crude extracts of the *H. pertussis* organism, it is possible that antigens other than hemagglutinin may have played a role in the mouse protective antibody response observed. The question arises where deductions can be made in regard to the relative value of these different antigenic agents in protecting against the disease. Admittedly extensive field trials are needed to answer the question definitely, but it would appear that the multiple antigens such as whole organisms or the soluble antigen have more to offer than single antigens for their greater ability to develop antibodies associated with protection against both intracerebral and intranasal challenge of mice. The value of antigens which fail to perform well in mouse protection tests, although they are good producers of other antibodies remains questionable.

Summary. A comparison has been made of the ability of 4 different pertussis antigens to produce a booster antibody response. Using increase of mouse protective antibody as measured by intranasal challenge as the criterion, excellent results were obtained with both soluble antigen and hemagglutinin. An explanation of the findings with the latter antigen is discussed.

We gratefully acknowledge the cooperation of Dr. Harold H. Berman and Dr. Martin Lazar of the Willowbrook State School in planning and carrying out this study. We also wish to thank Dr. Robert Ward and Dr. L. Emmett Holt, Jr., of this department, for their help and encouragement. Loretta Brown gave excellent technical assistance.

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