Prebooster titers in institutionalized and noninstitutionalized subjects are determined and compared with postbooster titers

Pertussis Agglutinins in Adults

PEARL L. KENDRICK, Sc.D., RUSSELL Y. GOTTSHALL, Ph.D., H.D. ANDERSON, Ph.D., V. K. VOLK, M.D., Dr.P.H., W. E. BUNNEY, Ph.D., and FRANKLIN H. TOP, M.D.

INFORMATION is scarce on pertussis agglutinin titers in adults in relation to their histories of disease and vaccine injections. Most of the published serologic studies have been concerned with infants. Provenzano and co-authors (*1*) recently referred to the literature on agglutinin titers following injection of pertussis vaccine in newborn and older infants.

Only two reports have come to our attention in which the serologic responses of adults to pertussis vaccine were studied. One of these was by Kendrick and co-authors (*2*), who determined the relative strength of opsonocytophagic reactions in pregnant women before and after a series of three injections of pertussis vaccine. Before vaccine, 50 percent of the women had weak reactions, 40 percent had moderately strong reactions, and 10 percent had strong reactions. After vaccine, the percentages were, respectively, 7, 25, and 68. Experience indicates that the curves representing the rise and fall of serum antibody levels as determined by agglutination and opsonocytophagic tests are roughly parallel.

In the other study, Volk and associates (*3*) measured pertussis agglutinins in 290 subjects in one institution before and after injection of a small dose of diphtheria and tetanus toxoids and pertussis vaccine combined, aluminum phosphate adsorbed (DTP). These 290 subjects were classed into three groups. Two groups had received a combined antigen containing pertussis vaccine 7 to 13 years before the administration of the small booster dose of DTP. The third group had received diphtheria and tetanus toxoids (DT) for a primary injection, but no pertussis vaccine. The prebooster agglutinin titers were low, and the responses to injection of the small booster dose of DTP vaccine were weak. The observations in this institution were part of a larger study concerned with the antibody responses following injection of a booster dose of DTP; DT; diphtheria toxoid, aluminum phosphate adsorbed (D); tetanus toxoid,

Dr. Kendrick is resident lecturer emeritus, department of epidemiology, School of Public Health, University of Michigan, Ann Arbor. Dr. Gottshall is chief, antigens and antisera unit, biologic products section, Michigan Department of Public Health, Lansing. Dr. Anderson is director of the biologics division of Parke, Davis and Co., Detroit, Mich. Dr. Volk is medical superintendent of Saginaw County Hospital, Saginaw, Mich. Dr. Bunney is retired vice president and director of manufacturing operations, E. R. Squibb & Sons, New York; he now lives in Wickenburg, Ariz. Dr. Top is head, department of hygiene and preventive medicine, State University of Iowa, Iowa City.

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aluminum phosphate adsorbed (T); or in subjects living in several institutions and also in noninstitutionalized subjects, a suspension of aluminum phosphate (*3*, *4*).

In most subjects in the larger study, a booster vaccine containing only the toxoids DT was used because in early tests DTP had given unacceptable local and systemic reactions (*5*); also, in the age groups studied, the need for boosting pertussis immunity was not then considered as pressing as for boosting diphtheria and tetanus immunity. The serum samples available from that study provided an unusual opportunity to investigate pertussis agglutinin levels in adults.

The present study includes (*a*) determination of resting or prebooster titers in institutionalized and noninstitutionalized subjects, and (*b*) comparison of these prebooster titers with the titers following a booster injection of DTP, DT, D, T, or a suspension of aluminum phosphate.

An added impetus to this study was provided by the observation of Lambert (*6*) that protection against whooping cough lessened as the interval between pertussis vaccination and exposure lengthened. Unfortunately, serologic data were lacking in his study.

Methods

As used throughout the study, the term "booster" refers to the injection of any material subsequent to previous vaccine injections for the purpose of testing its effect on existing antibody titers. Although the term is inadequate, it is used for ease of reference. It carries no assumption of an actual immunity-boosting effect. In addition to the antigens—diphtheria and tetanus toxoids and pertussis vaccine—the injected materials included the presumably nonantigenic mineral carrier aluminum phosphate.

Seven groups of subjects were studied (table 1). One group was noninstitutionalized, four were from three homes for the mentally retarded, and two included mentally ill subjects within one institution. From 1943 to 1950 all the subjects, except those in group B of institution 1, had been injected with pertussis vaccine in different combinations with diphtheria toxoid, tetanus toxoid, typhoid vaccine, or scarlet fever toxoid. The group 1–B subjects had received only DT antigens. The original study had been conducted to measure the serologic response and the severity of clinical reactions following injections of various combinations of the five antigens. For the present study, which was conducted 7 to 13 years later, a 0.2 ml. dose of DTP, DT, D, T, or a suspension of aluminum phosphate was injected intramuscularly to measure any possible boosting effect on the pertussis agglutinin levels. In institution 4, fewer than 20 group B subjects received either D or T or aluminum phosphate, and the results from those subjects were combined when the data were assembled.

All of the substances described were products routinely distributed by the Michigan Department of Public Health except the aluminum phosphate suspension which was included for control. The booster dose of 0.2 ml. of DTP vaccine contained 4.8 thousand million pertussis organisms (1.6 mouse protective units), 4 Lf (limes flocculation) units of diphtheria toxoid, 2 Lf units of tetanus toxoid, and 0.112 mg. of aluminum phosphate. Blood samples were

Table 1. Grouping of 711 subjects by immunization history, age, and sex

Subject groups	Primary pertussis vaccination [1]	Booster	Mean age (years)	Number	
				Male	Female
In institutions:					
Group 1–A (133 subjects)	P	DTP	58.5	55	78
Group 1–B (125 subjects)[2]		DTP	57.3	67	58
Group 2 (97 subjects)	P	DT	33.0	82	15
Group 3 (216 subjects)	P	DT	44.3	65	151
Group 4–A (17 subjects)	P	DTP	41.2	9	8
Group 4–B (47 subjects)	P	D, T, or $AlPO_4$	48.4	31	16
Noninstitutionalized (76 subjects)	P	DT	16.1	36	40

[1] P=pertussis vaccine.
[2] None of these subjects had received primary pertussis vaccination.

Table 2. Prebooster pertussis agglutinin titers 7–13 years after primary vaccination

Subject groups	Titers of less than 20		Titers of 20 or 40		Titers of 80 or 160		Titers of 320 or more		Total number
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	
In institutions:									
Group 1–A	96	72	36	29	1	<1	0	0	133
Group 1–B [1]	90	72	34	27	1	<1	0	0	125
Group 2	58	60	36	37	3	3	0	0	97
Group 3	34	16	98	45	83	38	1	<1	216
Group 4–A	13	76	4	24	0	0	0	0	17
Group 4–B	28	60	17	36	2	4	0	0	47
Noninstitutionalized	8	11	16	21	36	47	16	21	76
Total	327	46	241	34	126	18	17	2	711

[1] Subjects in this group had not received primary pertussis vaccination (see table 1).

taken before injection, then at 1 and 2 weeks and 2, 6, 12, and 24 months after injection. The immunization histories of the seven groups of subjects in relation to injection of pertussis vaccine, their age and sex, and the booster material administered in this study are shown in table 1. Information on previous booster injections was not always complete in the institutions' records. However, we know that repeated DT injections had been given previously in institution 2.

Pertussis agglutinins were determined by mixing 0.1 ml. each of agglutinating antigen containing 20 opacity units per ml. (determined photometrically in comparison with NIH standard) and twofold dilutions of the serum to be tested. These mixtures were shaken for 3 minutes and incubated at 37°–40° C. for 1 hour. After the addition of 0.5 ml. of 0.85 percent sodium chloride solution to each tube, the mixtures were left overnight at room temperature before final reading. The titer was the highest final serum dilution in which definite agglutination (read as 2+) was observed. Two lots of antigen were used; both were made on the same kind of medium and from the same strain of *Bordetella pertussis*. In the tables, the titers are expressed as reciprocals of the final dilutions. The procedure was the same as used in a previous study (*3*), except that in the analysis of results, any reactions in final serum dilutions of less than 1:20 were not included as positive; in the calculation of geometric means, any such weak reactions were arbitrarily given the value of 5. All tests were done and read by the same two technicians. Preinjection and 1 and 2 week postinjection serum samples were tested concurrently. Antigen and antiserum control tests were always included.

Of the three postbooster blood samples, the one at 2 weeks generally had the highest agglutinin titer and was used in the tabulation of results or in calculating the means. When, however, the titer at 1 week, or rarely at 2 months, was higher than at 2 weeks, it was used. This procedure resulted in use of the highest postbooster titer observed for each subject. Whenever titers before and at 1 and 2 weeks after the booster were obtained on the same subject, whether or not the diphtheria and tetanus titrations had been completed, the results were included; this practice accounts for a few differences in the numbers of subjects between this and previous reports concerning the larger study (*3*, *4*).

Results

All the subjects except those in group B of institution 1 had received a primary vaccination with pertussis vaccine (table 1). As for the prebooster or resting titers shown in table 2, in these group 1–B subjects with no record of pertussis vaccination, the agglutinin levels were essentially the same as in group 1–A subjects who were known to have received primary pertussis vaccination. Most of the subjects in institutions 1, 2, and 4 had resting agglutinin titers of less than 1:20; that is, the results were essentially negative, while in institution 3 the majority had titers of 1:20 to 1:160. Practically all the subjects with titers of 1:320 or higher were in the noninstitutionalized group.

In table 3 the effect of a booster injection with

the product indicated is expressed in terms of an increased pertussis agglutinin titer for each of the subject groups according to their prebooster titers. Of the institutionalized subjects who received a booster dose of pertussis vaccine, 69–88 percent responded with at least a fourfold increase in agglutinin titers.

As shown in both tables 3 and 4, in institutions 3 and 4–B a small rise in the postbooster titer was also observed in some of the subjects who had received only DT, D, T, or aluminum phosphate as a booster injection. This rise in titer was so small as to be considered unimportant. In institution 2, however, the increase in titer observed in similarly treated subjects was sufficiently great to demand consideration; 60 percent showed some rise in titer after injection of a booster dose of DT (table 3). However, the fold increase in titer, that is, the ratio of postbooster to prebooster titer, as shown in table 4, was only 3.9 in contrast to 6.4 or more in those subjects who had received DTP.

An effort was made to check this observation of an apparent increase in titer following the injection of a vaccine which did not contain pertussis antigen. An assumption was made that if the subjects' titers had shown a nonspecific response to a booster injection of DT, as indicated by an increase in pertussis agglutinins, they could be expected to do so again. Accordingly, 30 of the subjects in institution 2 whose titers had shown the largest increases were tested again 4 years later. Agglutinin titers were determined before and after another booster injection of the same lot of DT. In these repeated tests, 25 of the 30 subjects showed no increases in titers; in four, the readings were

Table 4. Geometric mean pertussis agglutinin titers before and after booster injections

Subject groups	Booster	Geometric mean titers		
		Prebooster	Postbooster	Fold increase
In institutions:				
Group 1–A	DTP	8	80	10.0
Group 1–B	DTP	8	51	6.4
Group 2	DT	10	39	3.9
Group 3	DT	37	41	1.1
Group 4–A	DTP	7	47	6.7
Group 4–B	D, T, or $AlPO_4$	10	16	1.6
Noninstitutionalized.	DT	79	143	1.8

NOTE: Subjects in all groups except 1–B had received primary pertussis vaccination (see table 1).

Table 3. Number and percent of subjects showing at least a fourfold increase in pertussis agglutinin titers after booster injections, by prebooster titers, subject groups, and type of booster

Prebooster titers	In institutions						Noninstitutionalized, DT
	Group 1–A, DTP	Group 1–B, DTP	Group 2, DT	Group 3, DT	Group 4–A, DTP	Group 4–B, D, T, or $AlPO_4$	
Total subjects with <20	96	90	58	34	13	28	8
Number showing increase	75	58	35	0	12	1	2
Percent showing increase	78	64	60	0	92	3	25
Total subjects with 20 or 40	36	34	36	98	4	17	16
Number showing increase	25	28	21	1	3	0	2
Percent showing increase	70	82	58	1	75	0	13
Total subjects with 80 or 160	1	1	3	83	0	2	36
Number showing increase	0	0	2	0	0	0	6
Percent showing increase	0	0	67	0	0	0	17
Total subjects with 320 or more	0	0	0	1	0	0	16
Number showing increase	0	0	0	0	0	0	2
Percent showing increase	0	0	0	0	0	0	13
Total with prebooster titers indicated	133	125	97	216	17	47	76
Total number showing increase	100	86	58	1	15	1	12
Total percent showing increase	75	69	60	0.5	88	2	16

NOTE: Subjects in all groups except 1–B had received primary pertussis vaccination (see table 1).

<1:10 before and 1:10 after; in one, 1:10 before and <1:10 after, that is, in the same subjects, the earlier finding of increased titers was not observed.

In the noninstitutionalized subjects, the prebooster titers in general were higher than the titers in the institutionalized groups, and a rise in titer following injection of DT was also observed in some; the rise, however, was not as great as that seen in institution 2.

Discussion

The resting pertussis agglutinin titers observed 7 to 13 years after primary vaccination with pertussis vaccine showed considerable variation between institutions and also between several of the instutionalized and the noninstutionalized groups. This variation could have been predicted if agglutinin levels can be taken as an index of whooping cough prevalence or of pertussis immunization. There are known differences among the study groups that surely would influence the antibody levels; for example, the age range and the wide differences that must have existed in opportunities for exposure to recognized and unrecognized pertussis. The noninstutionalized subjects may have had higher titers than institutionalized subjects because of their contact with living pertussis organisms through children in their families or in the community; thus they periodically received a natural booster which was reflected in an increased antibody level.

The increased titer of pertussis agglutinin after a booster injection of DT toxoids in group 2 subjects is not as easily explained. In relation to this increase, the findings of Ashcroft and associates (7) are of interest. They observed a small rise in agglutinin titer toward typhoid H antigen among 23 of 283 paired serums from human subjects before and after injections of tetanus toxoid and a substantial rise among four serums. They were unable to explain this rise in titer following injection of the unrelated antigen.

The increases were comparatively small (table 4). The fold increases were 1.1, 1.6, 1.8, and 3.9 for the groups receiving DT, in contrast to a range of 6.4 to 10.0 for those who received a booster injection containing pertussis vaccine. According to the histories of the subjects in institution 2, all had had a primary stimulus with a vaccine containing pertussis antigen. They would have been expected to respond to a pertussis booster injection by agglutinin production; a relatively high level then would have fallen gradually to a lower resting level; restimulation to higher levels would have depended either on a booster injection of pertussis antigen or contact with living *B. pertussis* during clinical or subclinical infection. Booster injections of pertussis vaccine had not been given. A plausible explanation, therefore, could be found in exposure to living organisms associated with pertussis infection. However, there is no record of the occurrence of whooping cough in this institution near the time when the booster injections were given. If exposure to whooping cough occurred, it was unrecognized. This disease is not likely to run a typical course in adults and can go unrecognized more easily than in children. The fact that the rise in titer following injection of DT toxoids occurred only in one institution and, to a lesser degree, among the noninstitutionalized subjects is in keeping with the hypothesis of subclinical infection following unrecognized exposure. (Exhaustive tests were performed in the Michigan Department of Public Health Laboratories to confirm the fact that the DT lots contained no pertussis antigen.)

In an attempt to test the observation that a rise in pertussis agglutinins followed injection of DT, Kendrick, with the technical assistance of M. M. Cook and H. S. Kim (unpublished data) gave guinea pigs a primary stimulus with DTP followed by booster injections of DT. In no instance was a rise in pertussis agglutinin observed following injection of DT.

Although the present study was limited to serologic response, one cannot resist trying to understand the meaning of the results in terms of actual protection against whooping cough. While we avoid taking the agglutinin response as a direct measure of protection, it is reasonable to assume, at least when an active whole culture vaccine is used, that the protective and measurable serologic responses do develop in parallel and that the chances for good protection are better in a group of vaccinated children or adults with relatively high levels of aggluti-

nin than in a group with low levels. Miller and associates (*8*) made periodic tests in 554 vaccinated children, recorded all indoor exposures, and then correlated attacks with the last observed titers. They concluded that "whereas immunity may exist in the absence of demonstrable agglutinins, susceptibility does not occur in the presence of agglutinins in high titers." Munoz (*9*) recognized that while agglutinogen is not concerned with protection, it can be expected that agglutinin formation will be correlated with immunity in children immunized with whole culture vaccine.

The use of the term "protective titer" is discussed by Provenzano and co-authors (*1*). Here the problem of standardization of procedure is encountered. For example, a titer of 1:160 by the 1 ml. volume test would be equivalent to a considerably lower titer in the small volume test. In the latter, a fifth of the amount of serum used in each tube of the serum dilution series of the larger volume method is tested with approximately the same amount of antigen. If expressed in units of agglutinin per milliliter, as suggested by Raffel (*10*), 1:160 in the 1 ml. test would mean 160 units per ml.; in the 0.2 ml. test with antigen concentrated five times, the same titer would mean 160 units per 0.2 ml., or 800 units per ml. Another technical source for marked variation in titers between laboratories is the culture of *B. pertussis* used for the test suspension.

Even if standardization could assure that a particular titer would mean the same thing in different laboratories, the designation of "protective titer"—a tacit acceptance of a quantitative relationship between circulating pertussis agglutinins and protection against the disease—is hardly justified by present knowledge. Current studies on the serotypes of *B. pertussis*, for example, by Eldering and associates (*11*), and investigations in progress of antigenic components, such as those by Munoz and Bergman (*12*), give promise of providing a better basis than is now available for understanding pertussis serologic findings. Data on the relation between laboratory results and protection against whooping cough in children appear in a report of the Medical Research Council of Great Britain (*13*).

In the meantime, tests for pertussis agglutinins with due attention to standardization of technique and careful interpretation can provide guidance in the study of pertussis immunity.

Summary

Pertussis agglutinin levels were determined in 711 adults before and several times after injection of a booster dose of either aluminum phosphate-adsorbed diphtheria and tetanus toxoids and pertussis vaccine combined, diphtheria and tetanus toxoids, diphtheria toxoid, tetanus toxoid, or a suspension of aluminum phosphate. Seventy-six of the 711 subjects were noninstitutionalized and the remaining 635 were in six groups in four institutions. All the subjects except those in one institutional group had received primary injections of pertussis vaccine 7 to 13 years previously.

Wide differences in agglutinin levels were observed in prebooster tests both within and between groups; the highest titers were in the noninstitutionalized subjects. The resting or prebooster agglutinin titers in the majority of subjects in five of the six institutional groups were less than 1:20 and considered essentially negative.

Three of the six institutional groups received a booster dose of diphtheria and tetanus toxoids and pertussis vaccine combined, aluminum phosphate adsorbed. A mean sixfold to tenfold increase in pertussis agglutinin titer was observed in these groups of subjects.

In the other three institutional groups and the one noninstitutional group, the subjects received no pertussis vaccine in the booster injections; they received aluminum phosphate adsorbed diphtheria toxoid, tetanus toxoid, or diphtheria and tetanus toxoids combined, or a control suspension of aluminum phosphate. A small increase in the pertussis agglutinin titer occurred among the subjects of the noninstitutional group and two of the institutional groups; a mean 3.9 fold increase in titer was observed in the other institutional group.

The results of the study suggest that the selective use of pertussis agglutinin determinations has a place in the study of levels of immunity to whooping cough in population groups.

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Designing Socially Acceptable Cities

A training program to help professional city planners learn to design more socially acceptable cities has been established at the Florida State University in Tallahassee by the award of a grant from the National Institute of Mental Health, Public Health Service.

The Florida State graduate school will supplement its urban planning curriculum with a master's specialty in social planning management. The present curriculum includes specialty in urban design, regional planning, transportation planning, or administration and policies planning.

In the new specialty, additional courses will teach planners how to design cities that will better meet people's social and psychological needs. Students will take courses on social planning, social psychology, social welfare and policies, and other subjects.

During the first year in the urban planning curriculum, students take courses on urbanism and planning theory, methods, and practice. The following summer they receive field training in a metropolitan planning agency or the office of a mayor or city manager. The second year they specialize in one of the areas mentioned before.

The new program will be supported for the first year by a NIMH $54,009 grant. An additional 2 years of support is planned by NIMH, subject to annual review. The funds will be used to add faculty members, provide student fellowships, and hire consultants.