J. Hyg., Camb. (1973), **71**, 193
Printed in Great Britain

Pertussis antibodies in the sera of children exposed to *Bordetella pertussis* by vaccination or infection

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(*Received* 10 *July* 1972)

SUMMARY

Low agglutinin titres to pertussis suspensions were found in 99% of sera from a group comprising healthy adults and non-vaccinated, non-infected infants of 1–6 months of age. These are attributable to agglutinins to heat-stable antigens and/or heat labile agglutinogen 1, and cross-absorption tests must be done on the sera in order to distinguish between the two. Agglutinins to agglutinogens 2 and 3 were found in only about 20% of adult sera. Bactericidal antibody was low in titre or absent in all sera from non-exposed individuals.

Raised bactericidal antibody titres and the presence of agglutinins 2 and 3 were attributed to exposure to *Bordetella pertussis* antigens, either as vaccine or as infection. The variation, amongst both vaccinated and infected children, was very great. A vaccinated child who became ill responded to the infection in much the same way as a non-vaccinated child. We were unable to relate the immunity of the child to the titres either of agglutinins or of the bactericidal antibody.

The protective ability of sera from vaccinated or infected children measured in mice against small, lethal brain infections was also unrelated to the state of immunity in the children, but this protective ability was correlated with the complement-mediated bactericidal antibody titres of the sera.

The distribution of agglutinins, bactericidal antibody, and anti-haemagglutinin in serum IgG and IgM was different in vaccinated and infected children.

INTRODUCTION

About 70 sera were sent to us from the Public Health Laboratory Service's investigation in 1967–68 from children ill with whooping cough whose vaccination history was known. Consecutive bleedings from a few of these children were available. The infecting strains had been isolated and typed for agglutinogens, and were reported by the PHLS committee (1969, 1972).

Sera were also collected from vaccinated children who had been exposed to pertussis in a practice in the Guildford area during the 1967 epidemic. Some of these children became ill while others remained well after exposure. Pre-epidemic sera from some of these children were held at the Wellcome Research Laboratories, having been collected as part of a measles vaccination survey 6 months to 3 years previously. Samples from about 60 children and a few adults were investigated.

Sera from vaccinated babies came from a group of 60 in Bristol (1968–70) and from a group of 14 surveyed earlier in Northern Ireland.

Base line titres were established on 20 sera from babies and on 100 from healthy adults.

The availability of these sera gave us the opportunity to study pertussis antibody titres in these various categories, particularly in response to exposure to pertussis antigens and their persistence afterwards. Workers in the past have made surveys for purposes of estimating the prevalence of disease in a community, for diagnosis, and for antigen response as a measure both of the effectiveness of a vaccine and, where this is known, of the detection of malfunction of the antibody-forming mechanisms in individual children who do not respond as expected.

Much of the work has, however, been done with very little background knowledge of the kind the results presented here may provide.

A further reason for our interest was to discover if any circulating antibody could be correlated with the state of immunity in the child.

METHODS

Measurement of agglutinins

Members of the genus *Bordetella* contain a number of heat-labile agglutinogens numbered 1–13 (Andersen, 1953; Eldering, Hornbeck & Baker, 1957), of which number 1 is common to all phase I *Bordetella pertussis* and specific to the species and numbers 2–6 are also pertussis-specific, but not necessarily all synthesized by all strains. Agglutinogens 2 and 4 tend to occur together, as do 3 and 6; agglutinogen 5 is relatively rare. The presence in antisera of agglutinins to these agglutinogens 2–6 has been measured by cross-absorption using selected, tested strains which were checked daily for agglutinogen content against our monospecific typing sera prepared by cross-absorption (see below).

Two hundred or so human sera from infected or vaccinated donors were analysed completely and in none was agglutinin 4 found without 2, nor 6 without 3. Out of 51 sera which contained agglutinin 2, agglutinin 4 was also present in only 35 %; out of 92 sera which contained agglutinin 3, agglutinin 6 was also present in only 25 %. Agglutinin 5 was found with 2 in only one child. Since response to agglutinogens 2 and 3 is stronger than to 4, 5 and 6, the measurement and recording of agglutinins to agglutinogens 2 and 3 only are given.

Agglutinations of unabsorbed sera against two suspensions of *B. pertussis* were also done.

The strains used for testing and absorptions were:

(*a*) Strain GL 353, originally from Glaxo Laboratories and containing only very small amounts of specific agglutinogens other than 1, so that in direct agglutinations using unabsorbed sera, titres are largely attributable to reactions with the heat-stable agglutinogens, and to agglutinogen 1.

(*b*) Strain B16, isolated in Northern Ireland, containing agglutinogens 1 and 3, but no demonstrable 6. This was used to absorb agglutinins 1 and 3, or to determine agglutinin 3 in absorbed sera.

(*c*) Strain 3865, first typed in Denmark in 1950, containing agglutinogens 1, 2 and 4. This was used to absorb agglutinins 1, 2 and 4 or to determine agglutinins 2 (plus 4) in absorbed sera.

Agglutinins were titrated in two ways: (i) in 0·3 ml. of serum dilution with 0·3 ml. of suspension of organisms, incubated at 37° C. for 4 hr. in Dreyer tubes, or (ii) in 0·02 ml. of serum dilution with 0·02 ml. of suspension of organisms, incubated at 56° C. for 1 hr. in covered, plastic trays (Disposo trays, Linbro Chemical Co., Inc., 681 Dixwell Avenue, New Haven, Connecticut 06511, U.S.A.). Both tests were held overnight at room temperature before reading.

Method (i) was used in tests for unabsorbed sera; serial doubling serum dilutions were made from 1/5 in saline and incubated with suspension harvested from 20 hr. Bordet–Gengou plates into saline and adjusted to an opacity of 5×10^9 organisms per ml. using the International Opacity Standard. Agglutination end-points were read to the dilution giving a marked agglutination as seen by a hand lens, but just below visibility to the naked eye.

Method (ii) was used to economize on the absorbed sera used for the routine titrations for the determination of agglutinins to heat-labile agglutinogens 2 and 3. Serial doubling dilutions from 1/2 in saline were used in 0·02 ml. volumes measured in '50 dropping pipettes' onto the plastic trays. An equal volume of the suspension, harvested as before, but at 10^{10} organisms per ml., was added. Results were read with a Greenough plate microscope at a twenty-fold magnification. The suspensions used were checked daily for agglutinogens against monospecific pertussis typing sera (prepared by a method to be published by C. J. Shanbury and others). A titre of less than 1/4 was regarded as negative and of 1/4 or more as positive.

Serum absorptions

These were done using (i) suspensions harvested from Bordet–Gengou plates into 0·25 % formol saline, left 24 hr. at room temperature or 2–3 days at 4° C. at kill the cells, and then centrifuged to remove the formol saline. Absorption with B 16 suspension and testing the absorbed serum against 3865 gave the '2,4' agglutinin titre, and absorption with 3865 and testing the absorbed serum against B 16 gave the '3' agglutinin titre; (ii) suspensions similarly harvested, but into saline, the suspensions then being autoclaved at 15 lb. for 30 min. to destroy the heat-labile agglutinogens, and centrifuged.

All suspensions were used wet, $0{\cdot}5 \times 10^{12}$ organisms to 0·3–0·5 ml. undiluted serum. Sera were absorbed on a turntable rotating at 26 rev./min. (Matburn blood cell suspension mixer, Baird & Tatlock, Chadwell Heath, Romford, Essex) for 3 hr. at 37° C. with each lot of organisms. Three absorptions were used routinely.

Bactericidal antibody

The complement-mediated antibody, killing *B. pertussis in vitro* (Dolby & Vincent, 1965), was tested for by mixing 0·2 ml. of serial fourfold dilutions of antiserum in 1 % Casamino acids with 0·2 ml. of a suspension of living organisms of the antiserum-sensitive *B. pertussis* strain 18–323 and 0·2 ml. of fresh guinea-pig serum (stored at − 15° C.) 1/15 as the complement source, making final serum dilutions of 1/30, 1/120, 1/480 and 1/1920. The organisms of 18–323 were harvested from a 20-hr. Bordet–Gengou plate into 1 % Casamino acids and diluted to contain 5×10^6 organisms per ml., about one-tenth of which were viable.

Table 1. *Definition of the bactericidal activity of a serum according to the percentage of organisms killed*

Percentage of organisms killed	Serum dilution		
	1/30	1/120	1/480*
75	−	−	+
85	−	+	
100	+		

* And higher dilution(s).

The mixtures were incubated together in glass tubes on a rotating turntable at 26 rev./min. (see above) for 40 min. at 37° C., and then diluted out for three ten-fold dilutions in 7 % sodium chloride made up in 1 % Casamino acids to stop the bactericidal reaction. Dilutions in 0·02 ml. volumes were pipetted onto Cohen & Wheeler agar plates made with liquid medium (Cohen & Wheeler, 1946) modified by using 0·5 % glutamate and 5 % blood. The colonies were counted after incubating the plates for 4 days at 35° C., and the number of organisms in 0·02 ml. of each final serum dilution was calculated. The maximum count in the 'serum' control and 'complement' control tubes was usually 1500–2500 organisms; the percentage kill at each of the four dilutions of antiserum was estimated. Sera were reported as negative (−) or positive (+) according to the scheme in Table 1, that is for a serum to be considered as positive at 1/30, there must be a 100 % bactericidal effect; positive at 1/120, an 85 % kill, etc. Those positive at 1/1920 were reported as strongly positive (++). The titres where given are the highest positive dilution.

Antihaemagglutinin

Serum was diluted serially in two-fold dilutions in 0·02 ml. volumes in plastic trays, as for tray agglutination. Haemagglutinin, contained in whole, freshly harvested (20 hr. Bordet–Gengou plates) cells of *B. pertussis* strain Gl 353, was added in 0·02 ml. volumes. The suspension of Gl 353 was titrated daily in the system and adjusted so as to contain about 2·5 minimal haemagglutinating units against the red cell suspension (see below), usually about 2×10^9 organisms per ml. The mixture of antiserum and suspension was left for 5 min. at room temperature and then a washed, sheep red cell suspension (red cells from 0·1 ml. blood resuspended in 8 ml. buffered saline) added, also in 0·02 ml. The trays were incubated for 2 hr. at 37° C. and read at once by eye, from above, taking complete agglutination as the end-point.

Passive protection tests against a 5000 challenge

Serum dilutions were incubated for 30 min. at 37° C. with the mouse-virulent strain of *B. pertussis*, 18–323. The mixtures, 0·03 ml. of which contained 5000 total organisms of which a tenth were viable (the count was unaffected by incubation with serum), were injected intracerebrally into lightly anaesthetized mice of strain TF1 or Theiler's Original. The organisms were harvested from 20 hr. Bordet–Gengou plates into 1 % Casamino acids.

The effect of the antiserum was determined by estimating the number of live bacteria in the brain at 2 and 5 days after infection and comparing with the number of mice given organisms and normal serum. A 1/10 dilution of antiserum and normal serum was used. A standard rabbit antiserum (6660) at a 1/100 dilution was used as the positive control. Mice were killed with coal gas in groups of five and each brain removed aseptically into 9 ml. of 1% Casamino acids containing glass beads. The bottles were shaken for 3 min. on a vertical shaker, 325 rev/min., throw $2\frac{3}{4}$ in., and the contents diluted ten-fold and pipetted in 0·02 ml. volumes on to Cohen & Wheeler blood plates (p. 196). Numerical values were given to each antiserum based on the log viable count per brain at 2 days + log viable count per brain at 5 days, abbreviated to 'v.c. 2+5'. The value was calculated:

$$\frac{(\text{v.c. } 2+5 \text{ for normal serum}) - (\text{v.c. } 2+5 \text{ for } x)}{(\text{v.c. } 2+5 \text{ for normal serum}) - (\text{v.c. } 2+5 \text{ for standard serum})}$$

where x is the serum under test. A figure of less than 0·2 was graded as negative (−), 0·2–0·7 as positive (+), and greater than 0·7 as strongly positive (++).

Passive protection tests against a 50,000 *challenge*

Undiluted sera were mixed with a ten-times stronger challenge than used for the previous test, to give 50,000 total organisms in a mouse dose of 0·03 ml. Organisms of 18–323 were grown and harvested as previously and 0·03 ml. of the incubated serum/challenge mixture (30 min. at 37° C.) injected into Theiler's Original female mice. There was no decrease in viable count due to incubation.

Results were expressed as the percentage of survivors at 14 days and graded as negative (−) for less than 50% and as positive (+) at greater than 50%.

Separation of 7S *and* 19S *globulins*

This was done as described by Dolby & Dolby (1969). The serum pools were made from individual child sera as follows:

Group A. Eight sera from 5-month-old babies 1 month after two doses of adsorbed triple vaccine. All the sera were positive to both 2 and 3 agglutinogens with titres ranging from 1/16 − > 1/640. Antihaemagglutinin was negative in five out of the eight tested; bactericidal antibody was in moderate or high titre.

Group B. Five sera from 7- to 8-year-olds vaccinated 2–8 years previously with unadsorbed triple vaccine. All the sera were positive to agglutinogen 2, but only with low titres of 1/8 and 1/16, and were negative to agglutinogen 3. Antihaemagglutinations were not done on the individual sera; bactericidal titres were low (1/30) on two out of five tested.

Group C. Five sera from 3- to 6-month-old babies infected 2–9 weeks previously. All were positive to agglutinogen 3 with moderate or high titres; two out of five also had agglutinin 2. Antihaemagglutinin was positive in two and negative in two, and bactercidal titres were moderate.

Group D. Five sera from $1\frac{1}{2}$- to 5-year-old children infected 5–14 weeks previously. All had agglutinin 3 and two out of five also had agglutinin 2. Antihaemagglutinin was positive in one and negative in one; bactericidal titres were high.

Table 2. *Pertussis agglutinins in human sera*

	Agglutinins to							
	B. pertussis suspension GL 353				Mixed *B. pertussis* suspension			
Titres	< 1/10	1/10–1/40	1/80–1/160	> 1/160	< 1/10	1/10–1/40	1/80–1/160	> 1/160
Babies 1–6 months	1/20	8/20 40%	9/20 45%	2/20 10%	1/20	13/20 65%	5/20 25%	1/20
Adults 18–60 years	0/50	50/50 100%	0/50	0/50	0/100	6/100	58/100	36/100

Table 3. *Pertussis agglutinins in absorbed human sera*

		Numbers of sera with agglutinins to		
	Number tested	2 only	3 only	Both 2 and 3
Babies 1–6 months	19	0	0	0
Adults 18–60 years	93	14	3	0

RESULTS

Antibody titres in non-vaccinated, non-infected individuals

Agglutinins

Sera from 100 healthy adults between 18 and 60 years of age (history of vaccination or exposure to *B. pertussis* unknown, but only the youngest, born in 1950, were likely to have received vaccine in childhood) and from 20 babies 1–6 months of age were titrated against two *B. pertussis* suspensions: (i) a suspension of *B. pertussis* strain Gl 353 which contains mostly heat-labile agglutinogen 1 with only traces of 2 and 3, and (ii) a mixed suspension of B 16 and 3865 which contains heat-labile agglutinogens 1, 2 and 3; both suspensions contain the heat-stable agglutinogens. Table 2 shows that 100% of adult sera were positive and 19 out of 20 baby sera.

Ninety-three adult sera and the nineteen positive baby sera were then tested for agglutinins to the heat-labile agglutinogens 2 and 3. The sera were absorbed by 1, 2, 0 or by 1, 0, 3 suspensions and then tested for the presence of agglutinin 3 or 2. (As all strains of *B. pertussis* contain agglutinogen 1, agglutinins to 1 are removed from all sera on absorption.) Table 3 shows that only 17/93 adult sera contained agglutinins to 2 or 3 and none of the 19 baby sera were positive. All adult sera, before absorption, agglutinated with *B. pertussis* suspensions; after absorption with organisms containing heat-stable agglutinogens, agglutinogen 1 and either 2 or 3, only 17/93 agglutinated (14 were positive for agglutinin 2 and three for 3), therefore the other 76 sera must have agglutinated with either agglutinogen 1 or the heat-stable agglutinogens.

Agglutinogen 1 and the heat-stable agglutinogens were separated in five adult sera and for comparison seven convalescent child sera all selected as they did not contain agglutinins 2 or 3. The five adult sera were absorbed with autoclaved

Table 4. *Antibody response after pertussis vaccination*

		Babies. Unadsorbed vaccine (see Haire *et al.* 1967)	Schoolchildren. Various vaccines. Bled between 1965 and March 1967	Babies. Unadsorbed vaccine (see Butler *et al.* 1969)	Babies. Adsorbed vaccine (see Butler *et al.* 1969)
Agglutinins*	Sera tested	14	49	14	44
	−	12	31	3	0
	+ for 2	2	15	2	1
	+ for 3	0	2	1	2
	+ for 2, 3	0	1	8	41
Bactericidal antibody*	Sera tested	5	17	11	31
	−	2	5	7	9
	+	1	10	4	11
	+ +	2	2	0	11
Passive protection (5000)*	Sera tested	n.t.	10	3	11
	−	n.t.	4	0	1
	+	n.t.	3	3	5
	+ +	n.t.	3	0	5
Passive protection (50,000)*	Sera tested	n.t.	11	2	4
	−	n.t.	10	1	1
	+	n.t.	1	1	3
Interval between last vaccine dose and bleeding		1–2 months	6 months–10 years†	1–4 months	1–10 months

* For gradings of response see Methods: agglutinins 2 and 3, page 195; bactericidal antibody, page 196; passive protection (5000), page 197; passive protection (50,000), page 197.
† See text. n.t. = not tested.

organisms, after which they were unable to agglutinate pertussis, therefore the cross agglutination must have been due to heat-stable agglutinogen and its antibody. Of the seven child sera similarly absorbed with autoclaved suspension, three behaved like the adult sera, were not agglutinating and must, therefore, have contained agglutinin only to the heat-stable antigen; four out of the seven were still positive and must have contained agglutinin 1.

To summarize this, human sera fall into three classes: (i) sera with agglutinins to heat-labile 1, 2 and/or 3; (ii) sera with agglutinins to heat-labile 1; and (iii) sera with agglutinins to heat-stable antigens only. In the rest of the paper the response to agglutinogens 2 and 3 only is given.

Bactericidal antibody

None of the nine adults nor the eight babies tested had bactericidal antibodies in their blood according to the definitions of positive and negative set out in Table 1.

Antihaemagglutinin

Twelve out of 12 unvaccinated babies were negative for antihaemagglutinin at a 1/3 dilution.

https://doi.org/10.1017/S0022172400046362 Published online by Cambridge University Press

Table 5. *Antibody titres* of twenty 6-month-old babies bled one month after two injections of one of three vaccines*

	Agglutinins		Bactericidal antibody	Antihaem-agglutinin
	2	3		
Children vaccinated	64	32	480	3
with an unadsorbed	< 4	4	30	< 2
vaccine, dose 20×10^9	20	640	480	n.t.
(group 3, Table 4)	4	< 4	480	n.t.
	16	8	120	n.t.
	40	40	480	> 27
Children vaccinated	4	4	120	< 2
with an adsorbed	128	64	120	< 3
vaccine, dose 20×10^9	512	512	120	> 27
(group 4, Table 4)	> 512	> 512	480	n.t.
	> 512	> 512	> 1920	n.t.
	640	40	120	> 27
	16	320	480	n.t.
Children vaccinated	4	8	120	< 2
with an adsorbed	8	8	1920	3
vaccine, dose 10×10^9	128	32	< 30	n.t.
(group 4, Table 4)	32	128	480	n.t.
	64	32	> 1920	< 3
	40	160	> 1920	< 2
	160	> 640	480	< 3

* Reciprocal. n.t. = not tested.

The effect of vaccination on pertussis antibody titres in children

Table 4 shows the results of tests on sera from four groups of children given various pertussis vaccines. There were adequate amounts of agglutinogen 2 in all the vaccines, but 3 was low in the vaccines used in group 1, of unknown amount in group 2, and equal to agglutinogen 2 in the vaccines used in groups 3 and 4.

The best response in all the tests was found in babies of group 4 with a maximum interval between the last dose of adsorbed vaccine and bleeding of 10 months. The schoolchildren (group 2) were bled at various times up to 10 years after the last dose of vaccine; a breakdown of these 49 schoolchildren into small groups to show the relationship between the time interval between vaccination and bleeding and agglutinin 2, gave a 30–50% response from 2 months to 2 years, falling to a 20–30% response afterwards. Their response of 16/49 in the group as a whole to agglutinogen 2, although less good than in groups 3 and 4, was higher than in the 'normal' population with a figure of 14/93 positive for agglutinin 2 (Table 3). The difference is significant (with a probability of 0·01) and could be attributable to vaccination.

Table 5 shows, however, the enormous variation in the response of individual children of groups 3 and 4 of Table 4 to vaccination with one of three vaccines and also emphasizes again the better antibody response obtained with adsorbed vaccine. Table 5 also demonstrates that high antibody titre to one antigen does not necessarily go hand in hand with high antibody titres to other antigens.

Table 6. *Pertussis antibodies in sera collected at intervals after infection in 3-month to 6-year-old unvaccinated children*

Time from appearance of symptoms to bleed	Serotype of infecting strain	Number of sera	Number of sera possessing: Agglutinin* 2	Agglutinin* 3	Bactericidal antibody†
0–1 month	1, 0, 3	16	0	5	8
	1, 2, 3	3	1	0	0
1–2 months	1, 0, 3	13	0	8	6
	1, 2, 3	10	3	4	5
2–6 months	1, 0, 3	3	0	3	2
	1, 2, 3	3	2	3	2

* At titre of 1/4 or more.
† At titre of 1/30 or more (see Table 1).

The consecutive bleedings of a few vaccinated babies were available; individual responses to repeated doses of vaccine varied enormously and in some instances another dose caused a *decrease* of antibody titre. In a group of ten babies bled one month after two doses and one month after three doses of vaccine, six showed increased titres to agglutinins and bactericidal antibody, one showed no change, and three showed decreases.

Antibodies resulting from infection

Table 6 shows the antibody response of 48 Manchester children aged 3 months to 6 years who became infected with strains of *B. pertussis* containing either 1 and 3, or 1, 2 and 3 agglutinogens. Not all the children showed a positive agglutinin response up to a period of 2 months after symptoms. Vaccinated children who became infected behaved similarly to the non-vaccinated ones. Unfortunately, the number of children bled 2–6 months after the appearance of symptoms is too small to show whether the agglutinin response is 100% by this time, but the proportion having agglutinins at least is certainly increased over those bled before 2 months after onset.

Breaking down the group providing data for Table 6 into age of child reduced the subgroups to very small numbers, but of nine 1- to 3-month-old babies, two out of four had bactericidal antibody within 1 month of infection but not agglutinin 3, whereas by 2 months, three out of four had agglutinin. Three 9-month-old babies were no quicker at antibody production and, in fact, only three out of eight produced agglutinin 3 by 2 months after infection. In 1- to 6-year-old children, three out of five produced agglutinin 3 at 1 month and five out of ten at 2 months.

Without frequent consecutive bleedings, it cannot be determined how much the time of optimum antibody titres following infection varies. Five Manchester children aged 3–6 months were available for this. The results shown in Table 7 indicate wide variation in the time and degree of response to pertussis antigens.

The frequent absence of the agglutinin and bactericidal antibody response after infection was confirmed in a small group of London children with clinical whooping

Table 7. *Pertussis antibodies in sera from consecutive bleeding in non-vaccinated, infected babies*

Sera	Age of child	Serotype of infecting strain	Weeks after infection	Agglutinin* 2	Agglutinin* 3	Bactericidal antibody*
M3/20	3/12	1, 2, 3	4	−	−	−
			8	+	+	+
M24/31	5/12	1, 0, 3	2	−	−	+
			8	−	−	+ +
M32/47	6/12	1, 0, 3	3	−	−	+
			8	−	+	+
M38/44	5/12	1, 2, 3	3	−	−	+
			7	−	−	+
M55/56/89	3/12	1, 0, 3	1	−	−	−
			2	−	+	−
			8	−	+	−

* For gradings of response, see Methods under test for each antibody: agglutinins 2 and 3, page 195; bactericidal antibody, page 196.

cough who were investigated in the first half of 1971. Their ages ranged from 1 month to 5 years, and bleeding times after onset from 2 weeks to $4\frac{1}{2}$ months. Only one, a baby of $5\frac{1}{2}$ months, who had had pertussis for 4 months before being bled, responded with high titres. All the sera had antihaemagglutinin.

Location in the different serum globulins of antibodies elicited by vaccination and infection

Pooled sera from children (five to eight per pool) were separated into 19S and 7S globulins and *in vitro* antibody tests were done to determine the relative efficiency of the fractions from different pools (Table 8).

A group of babies given unadsorbed vaccine produced lower titre sera than a group given adsorbed vaccine but the ratio of activities in the 19S and 7S globulins was similar for both. Results for only the adsorbed vaccine group (A) are given in the table.

A comparison of the 5-month-old vaccinated children bled 1 month after vaccination (group A) with older vaccinated children bled after a longer interval (group B) shows that the antihaemagglutinin titres were similar, but that more bactericidal antibody and agglutinins were in the 7S than the 19S fraction of the baby serum collected soon after vaccination, whilst in older children with a longer vaccination-bleed interval, the 19S was the more active for bactericidal antibodies. Time intervals between vaccination and bleed were more likely to be responsible for these differences than age of child.

The infected babies had higher antihaemagglutinin titres in the 19S than the 7S globulins (group C), while in the older children (group D) infection produced similar antihaemagglutinin levels in both globulins. Levels for other antibodies following infection are similar in both groups in the 7S and 19S fractions.

The type of vaccine, plain or adsorbed, therefore seems to influence only the

Table 8. *Pertussis antibodies in the* 19S *and* 7S *globulin fractions of pooled child sera collected after vaccination or infection*

Donors of serum	Interval between exposure and bleeding and number of samples in pool	Globulin fraction	Total serum protein (%)	Minimal effective concentrations of serum protein: Agglutinins (mg./ml.) 2	Agglutinins (mg./ml.) 3	Bacteri-cidal antibody (μg./ml.)	Antihaem-agglutinin (μg./ml.)
Group A							
5-month-olds, vaccinated with 2 doses of adsorbed triple vaccine	1 month (8)	19S	17·2	> 5·0	1·25	68	200
		7S	11·8	1·25	0·3	14	> 1700
Group B							
7 to 8-year-olds, vaccinated with unadsorbed triple vaccine	2–8 years (5)	19S	18·8	> 5·0	n.t.	340	300
		7S	24·0	> 5·0		1700	> 1700
Group C							
3 to 6-month-olds, infected with 1, 3 strains	2–9 weeks (5)	19S	15·6	n.t.	1·25	340	170
		7S	12·9		2·5	340	850
Group D							
1½ to 5-year-olds, infected with 1, 3 strains	5–14 weeks (5)	19S	18·0	n.t.	1·25	13·4	42
		7S	18·7		2·5	13·4	42

n.t. = not tested.

Table 9. *Pertussis antibody titres* of six children from whom blood samples were available before and after known exposure to a* 1, 0, 3 *strain of* Bordetella pertussis

Serum number	Time from vaccination to 1st bleeding	1st bleeding titres*: Agglutinin 2	Agglutinin 3	Bacteri-cidal antibody	Time from 1st bleed to exposure	Clinical pertussis	Time from exposure to 2nd bleed	2nd bleeding titres*: Agglutinin 2	Agglutinin 3	Bacteri-cidal antibody
WP 138/G 134	10 months	< 4	< 4	> 1920	1 year	+	6 weeks	< 4	< 4	480
WP 50/G 39	7½ years	< 4	< 4	30	5 months	+	4 weeks	< 4	< 4	480
G 128/G 58	7 months	256	< 4	120	3 years	−	1 week	> 8	4	120
WP 67*a*/*b*	1½ years	16	< 4	480	2 months	−	2 years	16	< 4	120
WP 101/G 109	3 years	< 4	< 4	> 1920	2 years	−	3 months	< 4	< 4	480
WP 28/G133	5½ years	< 4	< 4	120	6 months	−	6 months	512	< 4	n.t.

* Reciprocal.

antibody titres, not the ratio in 19S and 7S globulins; the time interval between vaccination and bleeding, and the age of the child, and whether exposure to pertussis antigens is in the form of vaccine or infection, do influence the ratio.

Antibodies in sera of children exposed to infection

Table 9 gives the details of six children in the Guildford series for whom pre-exposure and post-exposure serum samples were available; unfortunately, these were the only children from whom both pre- and post-samples were obtained. Table 10 gives details of 27 children and six adults from whom only one serum

https://doi.org/10.1017/S0022172400046362 Published online by Cambridge University Press

Table 10. *Pertussis antibody titres* of 33 children and adults from whom blood samples were available either before or after known exposure to a 1, 0, 3 strain of* Bordetella pertussis

	Serum number	Time between: Vaccination and bleed	Time between: Bleed and exposure	Time between: Exposure and bleed	Clinical pertussis	Agglutinin: 2	Agglutinin: 3	Bactericidal antibody
Pre-exposure samples	WP 140	6 months	1 year	.	+	32	< 4	30
	WP 167	1½ years	5 months	.	+	< 4	< 4	n.t.
	WP 27	4½ years	6 months	.	+	4	< 4	120
	WP 127	2 years	1 year	.	+	64	< 4	120
	WP 175	3 years	3 months	.	+	4	< 4	480
	WP 128	1 year	1 year	.	+	64	4	120
	WP 65	4 years	4 months	.	−	< 4	< 4	30
	WP 29	5½ years	6 months	.	−	< 4	< 4	480
	WP 30	5½ years	6 months	.	−	< 4	< 4	480
	WP 148	2½ years	1 year	.	−	< 4	< 4	120
Post-exposure samples	G 92	3 years	.	2 days	+	< 4	< 4	120
	G 91	2 years	.	2 days	+	< 4	< 4	n.t.
	G 51†	.	.	1 week	+	8	< 4	n.t.
	G 93†	.	.	3 days	+	8	< 4	30
	G 101	10 years	.	1 week	+	< 4	< 4	120
	G 95	2 years	.	1 week	+	128	< 4	480
	G 98	2 months	.	2 weeks	+	< 4	16	> 1920
	G 102	3 weeks	.	2 weeks	+	> 512	< 4	> 1920
	G 108	1 year	.	1 month	+	128	< 4	480
	G 106	5 weeks	.	1 month	+	< 4	< 4	480
	G 107†	.	.	3 months	+	8	< 4	120
	G 116	10 years	.	3 months	+	< 4	< 4	> 1920
	G 113	3 years	.	3 months	+	< 4	< 4	480
	G 114	2 years	.	3 months	+	< 4	< 4	n.t.
	G 126	3½ years	.	3 months	+	< 4	< 4	480
	G 127	1 year	.	3 months	+	16	< 4	480
	G 96	5 years	.	2 weeks	−	4	< 4	120
	G 105†	.	.	2 weeks	−	32	4	480
	G 94	3 years	.	4 weeks	−	< 4	< 4	480
	G 130†	.	.	4 weeks	−	< 4	< 4	120
	G 110	3 years	.	3 months	−	< 4	< 4	480
	G 132	1½ years	.	3 months	−	64	< 4	120
	G 129†	.	.	3 months	−	< 4	< 4	480

* Reciprocal. † Adults. n.t. = not tested.

sample was available, ten children (of whom six developed clinical pertussis) with pre-exposure samples and the rest (16 developed clinical pertussis, seven though exposed did not) with post-exposure samples only. These were children in a suburban practice and there was great variation in age and in details of time between vaccination and exposure and in the relative times the bleedings were taken.

It will be seen from these tables that there is no consistent difference between pre-exposure titres and post-exposure titres, nor in the titres of infected or non-infected children. It is impossible to tell from the results the difference between those resisting infection and those not, either before or after exposure. Passive protection tests against a 5000 challenge done in the ten 'pre-exposure only' sera could not distinguish between them.

The passive protection tests and correlation with bactericidal complement-mediated antibody, acting in vitro, *and the immune state of the child*

Some of the results for sera tested for ability to protect against a 5000 challenge when introduced into the brain with the infection, as measured by a reduction in bacterial numbers, are shown in Table 4. The antibody is usually present soon after vaccination (groups 3 and 4) and is still present in some vaccinated children (group 2). On the small number of tests done on sera described in the last section, ability to protect against this small challenge does not distinguish, however, between protected and non-protected children.

To determine whether the bactericidal *in vitro* antibody (which also did not seem to be correlated with child protection) was the one responsible for the reduction in numbers in the mouse brain, the results on the 41 sera tested for their ability to reduce a 5000 challenge in the mouse brain were compared with their *in vitro* performance in the presence of complement; when each individual passive protection index (see Methods for formula) was compared with the $\log_{10}$ of the inverse of each bactericidal titre, a correlation coefficient of 0·57 was obtained. If constants are used for both the normal and standard sera, calculated by averaging these two values for all the tests done, then the correlation coefficient is increased slightly to 0·67; the correlation is only moderate.

Passive protection tests were done on a small number of sera with a challenge of 50,000 organisms, mixing challenge and serum and measuring protection as the percentage of mice surviving 14 days. This antibody was present in 4/6 children soon after vaccination (Table 4). It was absent in the sera of 17 infected children, each bled once, at 1–12 weeks after infection, but present in two out of three children who had been exposed but remained well (sera G 109 and G 96 positive, G 94 negative, Tables 9 and 10). In the few sera tested, this antibody was unrelated to any other measured.

DISCUSSION

The finding that 99% of unabsorbed human sera had agglutinins to pertussis and that low bactericidal activity was common made us wonder if there may not be a heterophile antigen involved. The sera used included adult sera and sera from babies. Without absorption, it was impossible to tell whether the positive agglutination was due to the presence of agglutinins to heat-stable antigen(s) of pertussis or to the heat-labile agglutinogen 1 common to all *B. pertussis* (Andersen, 1953). Our results with a few sera suggest that agglutinin 1 is present only after fairly recent contact with pertussis, and that the widely distributed agglutinin was to heat-stable antigen(s).

These agglutination titres of 1/40–1/320 with even normal sera are stronger than those reported by Kendrick *et al.* (1969) and different from the uniform negatives reported by others (Abbott, Preston & MacKay, 1971; Raška, 1971). Our tests were done in several ways using both tubes and trays, and the low positive titres were independent of technique. These agglutinations, however, were not of the strong type associated with agglutinogens 2 and 3, so the difference may be one of degree of reaction, a point emphasized in a general discussion on agglutination

end-points by G. Eldering (personal communication). The other consideration is that only four strains of pertussis were used in these tests: Gl 353, 18–323, B 16 and 3865; recently, Dr Holt (personal communication) has found that some strains of pertussis are easily agglutinated by 'normal' serum and others are not, so we may have selected strains with a lot of heterophile, heat-stable antigen. The finding of Muschel & Osawa (1959) that *Escherichia coli* 'bactericidal antigen' and human group B red cells cross-react, and that of Ackers & Dolby (1972) that pertussis 'bactericidal antigen' and sheep red cells cross-react, may not be irrelevant.

The above observations, and those of the great variations in the response of children to both vaccination and infection, should be taken into account in attempting to assess the value of vaccination (Butler *et al.* 1969; Abbott *et al.* 1971) and in the diagnosis of disease (Cruickshank *et al.* 1970) by the assay of antibodies.

Attention had previously been drawn to variation in response to vaccine by Dane *et al.* (1966). Dr C. B. Wood (personal communication) has pointed out that the antibody titres of the sera of vaccinated babies, of which the data given in Table 5 are an example, do not fall on a Poisson distribution curve, but into groups of good and bad responders.

There is a tendency, pointed out by the Scottish group (Cruickshank *et al.* 1970), which we have also observed, that older children do, on the whole, respond better than young ones, but this cannot be applied to individuals; a 4-week-old baby may respond better, for example, to the agglutinogen of his infecting strain than a 12-year-old.

Whether our failure to detect an antibody on which the immune state depends is a basic one, simply because this is not the mechanism of child immunity to pertussis, only future work can show. That the failure was because we were measuring the wrong antibodies, or measuring them at the wrong time, is a possibility. It is hoped that the passive protection test against a large infection can be done on more samples available in the future.

The response to infection may be quite different from that to vaccination – the distribution of antibody activities amongst the serum globulins suggests this. The extension of such tests and the inclusion of 11S antibody may be worth while. Bactericidal antibody, for instance, is present in much higher amounts in the 7S than the 19S fractions in rabbits, mice and children injected parenterally, but children infected have high equal amounts of 7S and 19S, and antihaemagglutinin is present in both globulins.

We thank all those in the PHLS who sent us sera from infected children, particularly Dr J. D. Abbott for the large Manchester group. Sera were also gratefully received from Drs W. L. Burland, A. H. Griffith, M. Haire, L. B. Holt, W. d'A. Maycock, G. I. Watson, and others.

Strain B 16 was received from Dr Haire, strain 3865 from Dr E. K. Andersen, and strain Gl 353 from Dr J. Ungar.

We thank Dr D. E. Dolby of this Institute for carrying out the globulin fractionation of the pools of child sera; and Mr D. H. Simpson for the statistical calculations.

Part of this work has been submitted by one of us, S. S., as a Thesis for membership of the Institute of Biology.

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