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Pertussis agglutinins in vaccinated children: better response with adjuvant

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SUMMARY

Children were immunized with a single batch of pertussis vaccine, either adsorbed on aluminium hydroxide or plain. With a primary course of three injections, adsorbed vaccine produced higher titres of pertussis agglutinins in the serum than did plain vaccine. There was no obvious difference in response between those who received the three doses at intervals of 1–2 months, starting at 3–4 months of age, and those in whom the third dose was delayed until about 6 months after the second, but the number of children in each group was small.

INTRODUCTION

A previous investigation of pertussis agglutinins in vaccinated children (Abbott, Preston & Mackay, 1971) showed a rather poor response to three doses of plain vaccine given at intervals of 4–6 weeks, starting at 3–4 months of age. The authors recommended a further study to assess the relative efficacies of different immunization schedules and to compare plain vaccine with that containing adjuvant. We are reporting here on the results of such a study.

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MATERIALS AND METHODS

Pertussis vaccine

A single batch of Glaxo vaccine, manufactured in 1970, was used. It was in the form of triple antigen (diphtheria and tetanus toxoids together with 20,000 million cells of *Bordetella pertussis* per 0·5-ml. dose). It had been divided into two portions, one remaining as a plain saline suspension and the other being adsorbed on aluminium hydroxide as adjuvant. Both were stored at 4° C. before the vaccination of children between May 1971 and expiry of the vaccine in October 1972.

Immunization of children

The mothers of children attending the neonatal assessment clinic in Hope Hospital, Salford, were offered a course of injections for their children, to be followed by a laboratory check on the response. The children had no obvious physical disorder, with two exceptions they were not gestationally immature, and they had no known pertussis contact. At the first visit samples of blood were taken from mother and child, and the child received an intramuscular injection of vaccine. Six to ten weeks after the third dose of vaccine, a second sample of blood was taken from the child. On the basis of the laboratory findings, parents were informed of the child's response and, if this was poor, a booster dose was offered.

According to age at the first visit, the children were given a course of injections on either of two schedules: in Schedule I, commonly used in this country for many years, three injections were given at intervals of 1–2 months, starting at 3–4 months of age; in Schedule II, recommended by the Central Health Services Council (1968), the first dose was given at 4–6 months of age, followed by a second dose after about 6 weeks and a third dose about 6 months later. For each schedule, the children were assigned alternately to two groups for immunization with either plain or adsorbed vaccine. At subsequent visits, each child received the same vaccine as previously. In addition, oral polio vaccine was given at each visit; and the Local Authority was notified on the completion of these standard courses of immunization for each child.

Similar conditions applied to children vaccinated in the infant clinic at St Mary's Hospital, Manchester, though a higher proportion was 3–4 months of age at the first visit.

Immunization of rabbits

Ten rabbits were immunized by intravenous injection, five with plain vaccine and five with adsorbed. Each animal, previously shown to have bordetella agglutinin titres of less than 4, received a total of 50,000 million organisms in four doses over a period of 10 days, and was bled again 3 weeks after the last injection.

Four other rabbits were immunized intramuscularly, each with a total of 50,000 million organisms in four doses over a period of 10 days, and they were bled 2 weeks after the last injection. Because of the relatively poor response, they were given a single injection of 20,000 million organisms 2 weeks later, and were bled again after a further 3 weeks. Two months later, they were given a total of 60,000

million organisms in three injections over a period of 7 days, and were bled once more 2 weeks after the last injection.

Estimation of pertussis agglutinins in sera

The content of agglutinin 1 was estimated by titration of serum against the type 1 strain of *Bord. pertussis*, GL353. In the samples which failed to agglutinate this strain the titres of the sera against strains 360E (type 1, 2) and H36 (type 1, 3) were taken to indicate the content of agglutinins 2 and 3 respectively. Sera that contained agglutinin 1 were absorbed with strain GL353 (type 1) until they no longer agglutinated it, and their content of agglutinins 2 and 3 were then estimated by titration of the absorbed serum against strains 360E and H36. The details of these techniques have been recorded by Preston (1966, 1970).

RESULTS

Pertussis agglutinins in normal sera

Table 1 shows that the sera of most of the mothers contained agglutinin 1 but only a minority had agglutinin 2 or 3. Pertussis agglutinin was detected in only nine of the 38 children before vaccination, and the clear relation between the agglutinin titres of the children's sera and those of their mothers' sera suggested that these were residual maternal antibodies. In each case this residual antibody, in children of 3–6 months of age, was only of a low titre.

Agglutinin response to vaccination

Twenty children completed the course of three injections in accordance with Schedule I, and the attendances of eight others could be classed as Schedule II, but the visits of a further five did not conform to either schedule (Table 2). From a study of the combined results with Schedules I and II, it appears that adsorbed vaccine gave a better response than plain. This applied to each of the three pertussis agglutinins but was most significant for agglutinin 2: with adsorbed vaccine all fourteen children achieved a titre of at least 20, but plain vaccine gave a poorer response ($P = 0{\cdot}005$) in which seven out of thirteen children failed to achieve this titre. Schedule II was not obviously better than Schedule I, but the number of children in each group was small. In general, the response to antigen 3 was weaker than that to antigen 2.

The presence of all three pertussis antigens in the vaccine was readily demonstrated in the laboratory by the production of agglutinins in the sera of rabbits injected either intravenously or intramuscularly (Table 3). Perhaps not surprisingly, adjuvant did not enhance agglutinin production after intravenous vaccination. But after intramuscular vaccine, the response in rabbits had similar features to those found in children – a tendency for adsorbed vaccine to give a better response than plain (after injection of 50,000 – 70,000 million organisms), and a weaker response to antigen 3 than to antigen 2. It is worth noting also that the presence of all three antigens, and a suspicion of a lower content of antigen 3, had been detected by the simple procedure of slide-agglutination of the plain

Table 1. *Pertussis agglutinins in normal children and their mothers*

Child		Titres of three pertussis agglutinins in serum of Mother			Child		
Age (months)	Code no.	1	2	3	1	2	3
3	HH2	0	0	0	0	0	0
	SM4	16	10	0	4	0	0
	SM5	32	40	10	4	(10)	0
	HH12	16	0	0	0	0	0
	SM9	4	0	0	0	0	0
	HH9	8	0	0	0	0	0
	SM13	0	0	16	0	0	0
	SM6	32	10	0	8	(10)	0
	SM19	16	0	0	4	0	0
	HH3	32	0	0	0	0	0
3½	SM10	0	0	0	0	0	0
	SM3	16	0	0	(4)	0	0
	SM11	32	0	0	0	0	0
	SM1	16	0	10	0	0	0
	SM7	8	0	10	0	0	0
4	SM8	8	0	0	0	0	0
	SM2	16	0	0	0	0	0
	SM15	4	10	(10)	0	0	0
	SM18	4	0	0	0	0	0
	SM12	32	40	0	0	8	0
	HH14	32	0	0	0	0	0
	SM16	16	0	20	0	0	0
	SM17	(4)	0	0	0	0	0
	SM22	128	20	0	8	0	0
5	SM20	32	20	0	8	0	0
	SM14	0	16	0	0	0	0
6	HH5	8	0	0	0	0	0
	HH6	64	0	0	0	0	0
	HH11	16	0	0	0	0	0
	HH10	16	0	0	0	0	0
	SM21	128	0	0	8	0	0
	SM24	4	0	0	0	0	0
6½	HH1	16	20	0	0	0	0
7	HH13	0	16	0	0	0	0
	SM23	16	0	0	0	0	0
7½	HH4	0	0	0	0	0	0
	HH8	64	10	0	0	0	0
9	HH7	16	0	0	0	0	0

( ), weak reaction.
0, less than 4 (less than 10 for agglutinin 2 or 3 if agglutinin 1 present).

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Table 2. *Pertussis agglutinins in sera of vaccinated children*

	Plain vaccine							Adsorbed vaccine						
Course of injections	Child	Ages when injected (months)			Titres of three pertussis agglutinins			Child	Ages when injected (months)			Titres of three pertussis agglutinins		
					1	2	3					1	2	3
Schedule I (intervals of 1–2 months, starting at 3–4 months of age)	HH2	3	4	5	0	0	0	SM5	3	4	5	8	20	0
	SM4	3	4	5	0	0	0	HH12*	3	4	5	8	40	0
	SM10	3½	4½	5½	(8)	10	0	SM9	3	4	5	8	40	10
	SM8	4	5	6	16	10	10	HH9	3	4	5	16	160	40
	SM2	4	5	6	16	80	10	SM13	3	4	5	64	40	10
	SM15	4	5	6	4	80	20	SM3	3½	4½	5½	32	NT	NT
	SM18	4	5	7	32	10	0	SM11	3½	4½	5½	128	160	40
	SM12	4	5½	7	64	320	40	SM1	3½	4½	6	32	320	10
	HH14	4	5½	7½	16	0	0	SM7	3½	5	7	32	80	10
	—	—	—	—	—	—	—	SM16	4	5	6	64	80	20
	—	—	—	—	—	—	—	SM17	4	5	6	128	320	160
Schedule II (later start; delayed third dose)	SM22	4	5½	12½	16	40	0	SM20	5	6½	12½	16	20	20
	HH5	6	7	11	64	160	20	HH10	6	7½	12½	64	40	20
	HH6	6	7	12	64	20	0	SM21	6	7	13	128	160	40
	HH11	6	7½	12½	16	0	0	SM24	6	7½	13½	128	320	40
Other	SM14	5	6½	8	128	320	320	HH1	6½	9	12½	32	160	80
	—	—	—	—	—	—	—	HH4	7½	10	16	64	160	20
	—	—	—	—	—	—	—	HH7†	9	10	21½	32	80	0
	—	—	—	—	—	—	—	HH3‡	10	11	16½	16	160	20

( ), weak reaction.
0, less than 4 (less than 10 for agglutinin 2 or 3 if agglutinin 1 present).
NT, not tested (sample of serum inadequate for absorption).
* three weeks premature.
†, three months premature.
‡, received single dose at 5 months; course re-started at 10 months.

vaccine: agglutination by antibody 1 and by antibody 2 was complete within 3 min. whereas antibody 3 gave complete agglutination within 5 min. but not at 3 min. (For details of technique, see Preston, 1970.)

DISCUSSION

This investigation has confirmed and extended the results of an earlier study with pertussis vaccines manufactured in 1967 (Abbott *et al.* 1971) which showed that three doses of plain vaccine, given to children at intervals of 4–6 weeks, produced a rather poor agglutinin-response. The present findings, with vaccine made in 1970, show a slightly better response even with plain vaccine. But in several cases, agglutinin (especially agglutinin 3) was not detected either in children vaccinated at intervals of 1–2 months, starting at 3–4 months of age, or in those with a later start and a delayed third dose. In the earlier study, a better response was obtained by the injection of a fourth (booster) dose. The present findings show a similar improvement, with only three doses, by the

Table 3. *Pertussis agglutinins in sera of vaccinated rabbits*

Injection			Titres of three pertussis agglutinins					
			Plain vaccine			Adsorbed vaccine		
Route	Total dose of bacteria (millions)	Rabbit	1	2	3	1	2	3
Intravenous	50,000	1	320	1600	800	—	—	—
		2	640	3200	800	—	—	—
		3	320	800	400	—	—	—
		4	320	800	400	—	—	—
		5	320	1600	400	—	—	—
		6	—	—	—	160	800	200
		7	—	—	—	160	800	400
		8	—	—	—	320	1600	400
		9	—	—	—	160	1600	200
		10	—	—	—	160	3200	400
Intramuscular	50,000	11	32	80	10	—	—	—
		12	32	40	< 10	—	—	—
		13	—	—	—	64	160	10
		14	—	—	—	32	320	10
	70,000	11	32	160	40	—	—	—
		12	64	80	10	—	—	—
		13	—	—	—	128	320	80
		14	—	—	—	32	640	80
	130,000	11	256	640	80	—	—	—
		12	512	1280	160	—	—	—
		13	—	—	—	256	320	160
		14	—	—	—	128	1280	320

incorporation of adjuvant in the vaccine. Thus they confirm the conclusions of Feldman (1957) and Butler, Voyce, Burland & Hilton (1969) that adjuvant improves the agglutinin-response to pertussis vaccination, and they show also the extent to which this improvement applies to each of the three major pertussis antigens.

Once again, laboratory tests have been shown to be capable of predicting the agglutinin-response in the child. By agglutinin-production in rabbits, or even by the simple technique of slide-agglutination of the vaccine, it was possible to indicate that all three pertussis antigens were present but that antigen 3 was weaker than antigen 2 in this batch.

Most of the children had no detectable pertussis agglutinin before vaccination (Table 1), but a low titre of agglutinin 1 or 2 or both 1 and 2 was detected in nine of them and was probably residual maternal antibody. However, three of these (SM12, SM20, SM21) gave a good agglutinin response to vaccination (Table 2), and so it seems unlikely that the trace of maternal antibody in three others (SM4, SM5, SM22) was the cause of their poor response. We can provide no evidence, therefore, that residual maternal antibody may seriously interfere with the response to pertussis vaccine, if the course of injections starts at 3 months of age or later.

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