Papers and Originals

Advantages of Aluminium Hydroxide Adsorbed Combined Diphtheria, Tetanus, and Pertussis Vaccines for the Immunization of Infants

N. R. BUTLER,* M.D., F.R.C.P., D.C.H. ; M. A. VOYCE,† M.D., M.R.C.P.ED., D.C.H.
W. L. BURLAND,‡ M.B., CH.B., D.C.H. ; MARJORIE L. HILTON,§ B.SC., PH.D.

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Summary : Three combined triple antigen vaccines were used to inoculate infants receiving primary immunization at 3 to 6 months of age. Laboratory potency and toxicity tests and clinical evaluation again showed that the mouse weight gain test is able to predict which vaccines will give reactions in children. The addition of aluminium hydroxide to the vaccine both increased potency and reduced the tendency to cause reactions. Assays on sera showed that almost all children produced agglutinins to Bordetella pertussis types 1, 2, and 3 when the vaccine contained aluminium hydroxide.

Introduction

There has been much discussion in recent years about the toxicity and potency of the pertussis content of triple vaccines (whooping-cough, diphtheria, tetanus) used in combined immunization procedures in infancy and early childhood.

Hopper (1961) drew attention to illnesses following the administration of pertussis-containing vaccines in infancy, which ranged from local irritation through systemic disturbances to a state of shock or collapse. Haire *et al.* (1967) thought that reactions recorded after inoculations with triple vaccines, in infants under 6 months of age, were too severe and too frequent to be acceptable and suggested a reduction in the number of pertussis organisms/dose, thereby accepting a loss of potency which might be offset by greater acceptability. Perkins (1967) advised that by decreasing the bacterial content of pertussis these vaccines would be unlikely to retain sufficient immunizing potency, as measured by the mouse protection test, to satisfy the British Therapeutic Substances Requirements.

The appearance of infections due to predominantly type 1,3 strains of *Bordetella pertussis* in vaccinated and unvaccinated communities has thrown doubt on the effectiveness of pertussis vaccination (Preston, 1965 ; Wilson *et al.*, 1965). Holt (1967) calculated, from Medical Research Council committee reports (1956a, 1959), the expected frequency of whooping-cough in a partially vaccinated child community and the proportion of the total to be expected in the vaccinated component. The recent incidence of whooping-cough was much as had been predicted (Muggleton, 1967). Muggleton (1967) also suggested that the removal of adjuvants from British vaccines, following the M.R.C. Committee report (1956b) on the risk of provocation poliomyelitis, may have substantially reduced the effective potency of pertussis vaccines in children.

* Professor of Child Health, University of Bristol.
† Formerly Lecturer, Department of Child Health, University of Bristol. At present Consultant Paediatrician, West Cornwall Clinical Area.
‡ Clinical Research Unit, Glaxo Laboratories Ltd., Greenford, Middx.
§ Biological Division, Glaxo Research Ltd., Greenford, Middx.

In a preliminary report Burland et al. (1968) presented laboratory and clinical results indicating improved potency and reduced toxicity with a combined vaccine which included aluminium hydroxide as adjuvant. An investigation to compare the potency and toxicity of three combined vaccines is reported here. The investigation compared two vaccines containing aluminium hydroxide as adjuvant but differing in their pertussis content and a vaccine without adjuvant identical with diphtheria, tetanus, pertussis vaccine *B.P.*

Materials and Method

Vaccines.—Three triple vaccines, A, B, and C, were prepared from the same batches of antigens and contained equal numbers of 1,2,4 and 1,3 serotypes of *B. pertussis*. Vaccine A contained diphtheria and tetanus toxoids together with *B. pertussis* 20×10^9 organisms/0·5-ml. dose ; vaccine B had the same components with the addition of aluminium hydroxide adjuvant (Alhydrogel) ; and vaccine C was identical to vaccine B but contained only half the number of *B. pertussis* organisms/0·5-ml. dose. The precise composition of each vaccine is shown in Table I. They were tested for potency and toxicity in laboratory animals and then evaluated clinically for toxicity.

TABLE I.—*Contents of the Vaccines*

Vaccine	Content/0·5-ml. Dose			
	Diphtheria Toxoid	Tetanus Toxoid	Killed B Pertussis Organisms	Aluminium Hydroxide (Alhydrogel)
A	28 Lf	5 Lf	20×10^9	—
B	28 Lf	5 Lf	20×10^9	2·5 mg.
C	28 Lf	5 Lf	10×10^9	2·5 mg.

Immunization of Children.—Parental permission was sought before infants aged 3 months attending the clinic for primary immunization against diphtheria, tetanus, whooping-cough, and poliomyelitis were included in the trial. The vaccine used was determined by random selection at the time of the first inoculation. Each child returned twice at about four-weekly intervals to complete the schedule of inoculations with the same vaccine. Oral trivalent poliomyelitis vaccine was given at each visit. Vaccines were stored throughout at 4° C. No child with a history of convulsions or allergy was included in the trial. All inoculations were by deep subcutaneous injection into the upper arm or the thigh.

Follow-up.—The infants were visited at home by the same trained nurse on the day following each inoculation. She recorded any symptoms that had occurred since inoculation. Rectal temperature was recorded at each visit and particular attention was paid to generalized reactions—crying, fretfulness,

drowsiness, vomiting, anorexia, and rash. Reactions localized to the site of inoculation—erythema, induration, swelling, and tenderness—were also recorded.

Parental permission was granted for a number of babies to be bled before their third inoculation and again one to ten months after completing their primary course. All the sera were separated, stored at 4° C., and then submitted to in-vitro assays for pertussis antibodies. Diphtheria and tetanus responses to combined antigen in infants aged 3 months or over are known to be satisfactory (Barr *et al.*, 1955 ; Butler and Barr, 1960), therefore antitoxin titrations were not carried out. Similarly responses to three doses of trivalent oral poliomyelitis vaccine are known to be adequate (Perkins *et al.*, 1963).

Results

Laboratory Potency Tests

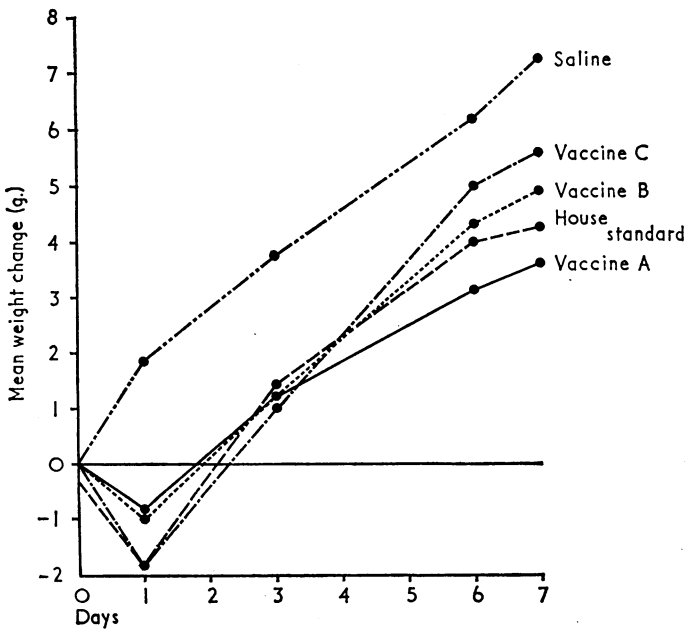
The vaccines were tested in guinea-pigs for diphtheria and tetanus potency by the methods required under the British Therapeutic Substances Regulations 1964, No. 1434, for unadsorbed vaccines and for pertussis by the mouse protection test (Kendrick *et al.*, 1947). The results are shown in Table II. The addition of aluminium hydroxide increased the antigenic response to both tetanus and pertussis components but did not significantly alter the diphtheria potency.

TABLE II.—*Laboratory Potency of Vaccines*

Vaccine	Minimum Geometric Mean Antitoxin (Units/ml. Serum)		Pertussis (Mouse Protection Test)	
	Diphtheria 1/20 Human Dose	Tetanus 1/10 Human Dose	ED_{50} (ml.)	Limits
A	2·26	1·72	0·0203	70–143%
B	3·59	18·09	0·0028	76–131%
C	5·20	7·52	0·01965	73–137%
House standard reference vaccine			0·0081	76–131%

Laboratory Toxicity Tests

One millilitre of each vaccine was injected subcutaneously into five guinea-pigs. No local or systemic reactions occurred during a period of 30 days' observation. The vaccines were tested for toxicity in mice by the weight gain test (Pittman and Cox, 1965). The test showed that vaccine A was more toxic than vaccine B, which in turn was more toxic than vaccine C.



Mouse weight gain tests on the vaccines.

The Standard Reference Vaccine was less toxic than A but more toxic than B or C (see Chart). All the vaccines more than satisfied the minimum standard of non-toxicity prescribed in the minimum requirements of the U.S. Government Regulations (1953, 1961). The vaccines were tested for histamine-sensitizing potency (Pittman, 1951) and found to differ significantly ($P=0·01$). The most sensitizing vaccine was A, vaccine B was of equal sensitizing potency to that of the Standard Reference Vaccine, and vaccine C was the least sensitizing.

The mouse weight gain test is believed to be the most reliable test in laboratory animals for measuring likely reaction-provoking properties of pertussis-containing vaccines in children (Pittman and Cox, 1965 ; Muggleton, 1967). The trend in toxicity was also indicated from the results of the histamine-sensitizing tests in mice.

Clinical Investigation

One hundred and sixty-eight infants completed their primary immunization schedule with one or other of the vaccines. Thirty-one failed to return after the first inoculation (19 after vaccine A, 7 after B, 5 after C) and a further eight failed to return after the second inoculation (five after A, three after C). Therefore 84% completed their course (71% for vaccine A, 89% for B, and 93% for C). The incidence of reactions in the infants failing to attend for second or third injections was no greater than that seen in the corresponding group who completed their primary course. The higher incidence of reactions after first inoculations in infants receiving vaccine A may have accounted for the greater number of failures in this group.

Reactions following inoculations in those infants who completed their primary course are shown in Tables III, IV, and V. Where reactions were absent or so slight that they would not have been reported except on close questioning, these are grouped together and classified as "trivial or no reaction." There were no serious reactions in any of the three groups ; no convulsions were reported, no cases of collapse, and no case in which persistent and high-pitched screaming was a feature (Hopper, 1961 ; Haire *et al.*, 1967). The incidence of generalized reactions to vaccine A was significantly higher than with either vaccine B or C ($P<0·001$), there was no significant difference between vaccines B and C (Table III). There was a highly significant difference in the incidence of generalized reactions to the vaccines after the first injection ($P<0·001$), vaccine A producing far more than the other two, between which there was no significant difference. There was a similar though less significant finding after the second injections ($P<0·05$). Reactions were also more numerous after vaccine A at the third injection, but the figures are not in fact significant ($P>0·2$). The incidence of generalized reactions to vaccine A decreased with progression through the primary course, whereas the incidence with vaccines containing aluminium hydroxide adjuvant did not show any such trend or any significant difference between themselves despite their different pertussis content.

The 21 children with generalized reactions following their second injection of vaccine A had all suffered a generalized

TABLE III.—*Generalized Reactions*

Injection	Vaccine	None or Trivial Reaction	Reaction	Total Vaccinated
1st	A	9	38 (81%)	47
	B	43	13 (23%)	56
	C	52	13 (20%)	65
2nd	A	26	21 (45%)	47
	B	43	13 (23%)	56
	C	48	17 (26%)	65
3rd	A	28	19 (40%)	47
	B	39	17 (30%)	56
	C	47	18 (28%)	65
All injections	A	63	78 (55%)	141
	B	125	43 (26%)	168
	C	147	48 (25%)	195

TABLE IV.—*Localized Reactions*

Injection	Vaccine	None or Trivial Reaction	Reaction	Total Vaccinated
1st	A	32	15 (32%)	47
	B	45	11 (20%)	56
	C	51	14 (22%)	65
2nd	A	39	8 (27%)	47
	B	46	10 (18%)	56
	C	55	10 (15%)	65
3rd	A	42	5 (11%)	47
	B	36	20 (36%)	56
	C	49	16 (25%)	65
All injections	A	113	28 (20%)	141
	B	127	41 (24%)	168
	C	155	40 (20%)	195

TABLE V.—*Overall Reactions*

Vaccine	Children Reacting (General and/or Local)			No. with	
	1st Injection	2nd Injection	3rd Injection	0 Reactions in 3 Injections	3 Reactions in 3 Injections
A	38 (81%)	27 (57%)	20 (43%)	3 (6%)	10 (21%)
B	21 (37·5%)	22 (39%)	30 (43%)	10 (18%)	5 (9%)
C	26 (31%)	25 (38%)	28 (54%)	17 (26%)	8 (12%)

reaction to the first ($P<0·01$); nine children had generalized reactions after all three injections with this vaccine. This association of reactions to serial doses was not seen with vaccine B or C.

The incidence of local reactions tended to be low, and not significant when taken together (Table IV). However, there was a significant fall in the incidence for vaccine A with progression through the schedule ($P<0·01$) and a significant rise with vaccine B at the third injection ($P<0·02$). Vaccine A produced significantly more reactions at the first injection than B or C, and B more than A or C at the third. However, when children returned for second or third injections in the series 24 subcutaneous nodules at the site of the previous injection were found after 112 inoculations with vaccine B and 22 after 130 with vaccine C. None occurred after vaccine A. One infant developed a "sterile abscess" after vaccine C, otherwise the nodules were small and uncomplicated.

Table V compares the incidence of reactions, whether generalized and/or local, for the three vaccines. The unfavourable figures for the first two injections with vaccine A are confirmed. Children suffered reactions at all three injections more commonly with vaccine A than with B or C, and childen found to have been free from any reaction throughout the course were much more likely to have received vaccine B or C ($P<0·01$). Further examination of the results showed that the incidence of reactions remained unaltered with season and with storage of vaccines.

The response to pertussis antigens was estimated by measuring the agglutinins and the bactericidal antibody response of a number of children.

Agglutinins were measured by two methods. It has been found (Dolby, to be published) that the sera of infants with no history of exposure to *B. pertussis* or of vaccination with pertussis vaccines nevertheless have measurable quantities of antibody to agglutinogen 1; of 20 such sera analysed so far, only one had antibodies to agglutinogen 2, 3, 4, 5, or 6. The average titre in the 19 babies was taken as a baseline, and in Table VI the numbers of vaccinated children with titres raised above this baseline is given. In this table only antibody levels against the standard "1" suspension or the standard "1–6" suspension which are raised above the "normal" baseline have been recorded as caused by vaccination.

TABLE VI.—*Agglutinin and Bactericidal Antibody Response of 62 Children Bled 1–10 Months After Vaccination With A, B, or C. The Figures Show the Number of Sera in Each Group With "Raised" Titres—That is, Those With Antibody Levels Higher than the Average for 20 Babies Without Known Exposure to B. pertussis Antigens*

Vaccine:	A	B	C
No. of sera tested	14	23	21
Agglutinin To "1" only ..	1	6	5
Agglutinin To "1 – 6" ..	0	7	3
No. of sera tested	11	17	14
Bactericidal activity Moderate ..	4	6	5
Bactericidal activity High	0 (4)	4 (10)	7 (12)

The bactericidal test was carried out by incubating serum dilutions with a serum-sensitive strain of *B. pertussis* in the presence of guinea-pig complement for 45 minutes at 37° C. and then doing viable counts (Dolby and Vincent, 1965). The average low level of bactericidal activity (against *B. pertussis*) found in nearly all normal human sera was used as the baseline and the numbers of enhanced titres are recorded (Table VI).

The second method of measuring agglutinins is by scoring the presence or absence of agglutinins to agglutinogens 2 to 6. Table VII shows that many of the children tested had responded to agglutinogen 3 when bled between one and ten months after vaccination. The response was most marked in children vaccinated with the adjuvant-containing vaccines.

TABLE VII.—*Specific Agglutinins Found in Sera From 62 Children Bled 1–10 Months After Vaccination with A, B, or C. Sera Grouped By Presence or Absence of Agglutinin*

Sera Containing Agglutinins	A	B	C
1 only	3/14	0/24	2/22
1 and 2*	2/14	1/24	0/22
1 and 3*	1/14	1/24	1/22
1, 2, and 3*	8/14	22/24	19/22
*With 4 and/or 5 and/or 6 ..	2/8	5/11	3/10

Discussion

The investigation confirms the finding of Burland *et al.* (1968) that the vaccines adsorbed on aluminium hydroxide as adjuvant were less toxic in laboratory tests, were more potent in laboratory tests, and were less reaction-provoking in children. The significance of the weight gain test in mice was further emphasized. The difference in reactions to the various vaccines in children was most marked for the first injection, less for the second, and not significant at all for the third. The incidence of generalized reactions after plain vaccine was highest following first injections and decreased with progress through the primary course. This result was not influenced by those infants who failed to attend after their first inoculation, since the incidence of generalized reactions in that group was similar to that of the group who completed their schedule. This feature did not apply to injections with the adsorbed vaccines in this trial, where the incidence of generalized reactions was unaltered with progression through the course.

Reactions have rightly been attributed to the pertussis content (Hopper, 1961) and have been shown to be directly proportional to the number of pertussis organisms present (McFarlan *et al.*, 1945; Bousfield, 1952). However, a reduction in numbers of organisms/dose of plain vaccine might lead to an unacceptable loss of potency (Perkins, 1967). A reduction by half in the number of pertussis organisms in the adjuvant-containing vaccine C did not significantly alter the incidence of reactions found when compared with vaccine B, but the potency was reduced, though not beyond acceptable limits. It would seem that the addition of adjuvant in the form of aluminium hydroxide is a much more significant step in terms of both potency and toxicity than is a reduction in pertussis content. Poliomyelitis was thought to have been an additional risk following inoculation with alum-containing vaccines (M.R.C. Committee 1956b), but with its almost complete eradication and the simultaneous inoculation with poliomyelitis vaccine this is no longer a hazard.

Immediate local reactions were not a problem with any of the vaccines, though reactions to vaccines B and C increased through the course as those with A decreased. The appearance of small symptomless nodules at the site of subcutaneous injec-

tion of alum-containing vaccines serves to emphasize the need for deep injection, preferably by the intramuscular route for these vaccines (Sauer and Tucker, 1954 ; Burland *et al.*, 1968). Few would disagree with the advisability of discontinuing pertussis vaccination in children who have suffered a severe generalized reaction to a previous dose of the vaccine. In the case of plain vaccine the wisdom of this may have been justified by the finding of a high incidence of generalized reactions to a second dose of antigens in children who reacted to the first. However, with the adsorbed vaccines generalized reactions were mild and infrequent, and recurrent reactions were not common in those infants who had reacted to a first dose of vaccine. The use of such a vaccine could result in fewer children losing the benefits of a full course of pertussis immunization.

Vaccination in almost all the children in this survey caused the appearance of specific agglutinins ; in this respect vaccines B and C were superior to A. There was considerable individual variation in amounts of specific agglutinins and of bactericidal antibody produced.

Conclusions

The value of the mouse weight gain test as an indicator of reaction-provoking tendencies in children is again shown, and it is suggested that it should continue to be applied to all vaccines containing pertussis antigen. The addition of aluminium hydroxide as adjuvant to diphtheria, tetanus, and pertussis combined vaccine served to increase antigen potency and significantly reduce the incidence of generalized reactions in children. A reduction by half in the number of pertussis organisms in the adsorbed combined vaccine did not further reduce reactions and therefore served no useful purpose. Vaccines containing aluminium hydroxide are in widespread use for immunization against diphtheria and tetanus, and it is suggested that protection in infancy can best be obtained by using an adsorbed diphtheria, tetanus, pertussis vaccine.

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Cyclophosphamide Therapy in the Nephrotic Syndrome in Childhood*

M. W. MONCRIEFF,† B.M., M.R.C.P., D.OBST.R.C.O.G. ; R. H. R. WHITE,‡ M.B., M.R.C.P., D.C.H.
C. S. OGG§ M.D., B.SC., M.R.C.P. ; J. S. CAMERON,|| M.D., B.SC., M.R.C.P.

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Summary: Forty-six children with the nephrotic syndrome whose renal biopsy specimens showed minimal changes and whose response to corticosteroid therapy was unsatisfactory were treated with cyclophosphamide. Three patients were completely steroid-resistant from the outset and the remainder were steroid-dependent. In several patients steroids controlled the condition less effectively with time. Most patients showed signs of steroid toxicity, and growth retardation was striking.

A moderate leucopenia was induced with cyclophosphamide, and treatment was maintained for three to four months in the majority of cases. Thirty-eight children (83%) have remained in complete remission off all treatment for periods of 3 to 23 months, 33 after one course of cyclophosphamide and five after a second course. Two other patients who remitted but relapsed later are still on treatment. In only six patients was full remission not obtained, and three of these were steroid-resistant from the start. Two died from pneumonia and adrenal failure and four continued to have proteinuria, though in one an impressive reduction occurred.

The results indicate that cyclophosphamide therapy is an effective alternative for nephrotic children with normal glomeruli on light microscopy who develop steroid dependence or resistance, and who exhibit toxic effects of steroid therapy.

Introduction

It is nearly 20 years since cytotoxic drugs were first used in the treatment of the nephrotic syndrome (Chasis *et al.*, 1949, 1950) but they were soon to be replaced by corticosteroids. Recent

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† Lecturer in Paediatrics and Child Health, Birmingham University.
‡ Senior Lecturer in Paediatrics and Child Health, Birmingham University ; Honorary Consultant Paediatrician, United Birmingham Hospitals.
§ Physician in Renal Diseases, Guy's Hospital, London S.E.1.
|| Physician in Renal Diseases and Senior Lecturer in Medicine, Guy's Hospital Medical School, London S.E.1.