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Further studies with quadruple vaccine

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Since our description of a trial with a new quadruple vaccine (Dane *et al.* 1962) against poliomyelitis, diphtheria, tetanus and pertussis there have been changes in the composition of the vaccine and in its recommended method of use in N. Ireland. During the past 2 years we have been concerned with finding the optimum schedule for infant immunization using quadruple vaccine, taking into account the immunological, medical and administrative factors involved, and also with methods of reducing the reactions caused by the pertussis component of the vaccine.

In this report we describe the results of a recent trial in which the quadruple vaccine used had a modified pertussis component. Immunization was started at 6 or 7 months of age in the majority of infants and was given in three doses, separated by intervals of 6–9 weeks and 6 months. The vaccines used were similar to those which have been in general use in N. Ireland since October 1964.

MATERIALS AND METHODS

In co-operation with Medical Officers of Health, parents' permission was sought to obtain blood samples from infants immunized at clinics with quadruple vaccine.

The vaccine. This was commercial 'Quadrilin' vaccine prepared by Glaxo Laboratories for use in N. Ireland, and differs in its pertussis component from 'Quadrilin' available elsewhere. The poliovirus-D-antigen content (Beale & Mason, 1962) was: type 1, 75 units; type 2, 2 units; type 3, 4 units. It also contained 28 Lf of diphtheria formol toxoid (F.T.), 5 Lf of tetanus toxoid and $12{\cdot}5 \times 10^9$ inactivated *Bordetella pertussis* (serotypes 1, 2, 4 and 1, 3) per 1 ml. dose.

The vaccination programme. Fifty-eight infants who had received no previous immunization took part in this trial. The volume of serum obtained from some of them did not allow all specimens to be tested against all antigens. The number of infants whose serum was tested against any particular antigen is shown in the relevant table of results. The age in months of the infants at the time they received their first dose of vaccine is shown in Table 1. The intervals in weeks between first and second doses and the intervals in months between second and third doses are shown in Tables 2 and 3.

Poliovirus-neutralizing antibody. A standard cytopathic test with approximately 100 TCD50 of virus was used (Dane, Dick, Briggs & Nelson, 1961). Antibody levels are expressed in terms of British standard units (Perkins & Evans, 1959).

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Diphtheria and tetanus antitoxin. Assay was by the methods described in the British Pharmacopoeia (1963 ed., pp. 1107, 1118).

Pertussis agglutinins. Agglutinin titres were estimated using a killed suspension of *B. pertussis*, composed of several strains of serotypes 1, 2, 4 and 1, 3. The antigen–serum mixtures were placed in a 37° C. water bath for 16 hr. before the test was read. Control antisera were included in each test. The antigen and control antisera were kindly supplied by Dr P. W. Muggleton of Glaxo Laboratories.

Table 1. *Ages of the fifty-eight infants immunized*

Months	5	6	7	8	9	10	11	12	13–36
No. of infants	2	21	15	5	6	2	2	2	3

Table 2. *Interval in weeks between first and second doses*

Weeks	4	5	6	7	8	9	10	11	12
No. of infants	1	2	12	11	9	19	2	—	2

Table 3. *Interval in months between second and third doses*

Months	5	6	7
No. of infants	3	45	10

Reaction follow-up studies. A Health Visitor visited the home of each infant on the day after inoculation. She recorded any reactions or possible reactions to the vaccine on a special form. If the infant had had a severe reaction such as persistent screaming or shock and collapse, she reported this to the laboratory and one of us (M.H.) visited the home, obtained a more detailed history and made a clinical examination of the infant.

RESULTS

Poliovirus antibody response

Blood samples for serological tests were taken approximately 1 month after the third and last dose of vaccine (see Table 4). The possibility cannot be excluded that at some time in their lives some of the infants may have had natural infections with polioviruses; such infections are however likely to have been uncommon, because during the study period there was no evidence that 'wild' polioviruses had been circulating in N. Ireland (J. H. Connolly, personal communication). In addition, only limited amounts of oral poliomyelitis vaccine had been used in the areas in which the children lived. Therefore we consider that the post-immunization neutralizing antibody titres (Tables 5, 6, 7) developed, in the majority of infants, as a result of immunization with quadruple vaccine. All infants developed antibody to all three types of poliovirus. The majority of titres recorded were very high, only two being $< 1/100$. These lower titres were both against the least important type II poliovirus.

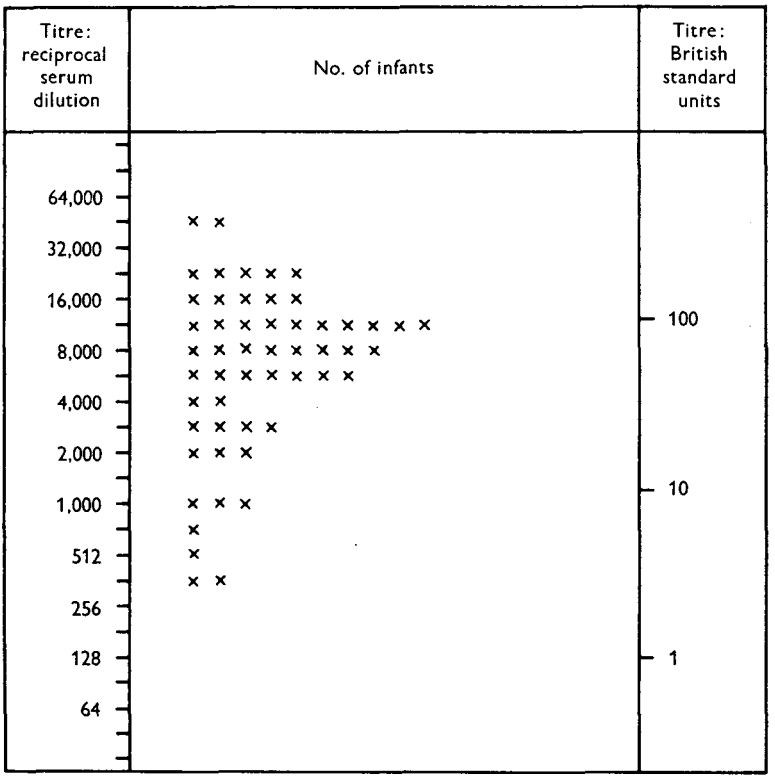
Diphtheria antitoxin response

The antitoxin titres after immunization in the serum of fifty infants are shown in Table 8. No clinical diphtheria has been recorded in N. Ireland since 1959; therefore these levels can be presumed to have resulted from immunization with quadruple vaccine.

Table 4. *Interval in weeks between third dose and obtaining serum sample*

Weeks	2	3	4	5	6	7	8	9
No. of infants	1	5	7	21	6	7	9	2

Table 5. *Type-I poliovirus-neutralizing antibody levels in fifty-three infants one month after the third dose of modified quadruple vaccine*



Tetanus antitoxin response

The antitoxin titres in the serum of fifty-eight infants after immunization are shown in Table 9. A few children had rather low titres, but these were well above the protective level of 0·01 units of antitoxin per millilitre of serum (Eckmann, 1963).

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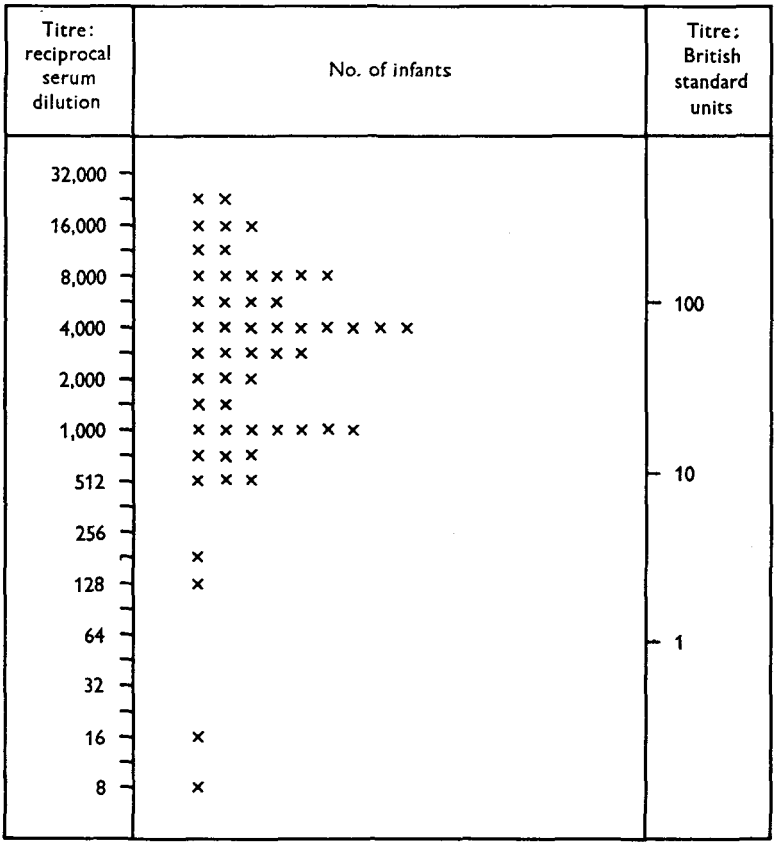
Pertussis agglutinins

In a Medical Research Council trial of pertussis vaccines (Report, 1956) it was shown that the agglutinin response of children was related to the field protective effect of the vaccines which had been prepared in the same way as the pertussis components of the various quadruple vaccines which we have studied. For this

Table 6. *Type-II poliovirus-neutralizing antibody levels in fifty-three infants one month after the third dose of modified quadruple vaccine*



reason the likely effectiveness of the vaccine was measured by means of the agglutinin response and comparisons were also made between the agglutinin responses recorded in this trial and those from previous trials. The agglutinin responses of the infants in the present trial are shown together with results from two previous trials in Table 10. It will be seen that, despite the fact that the total number of organisms given in the present schedule (trial C) was less than half that given in trial A, in which the three doses of quadruple vaccine were spaced at monthly intervals, the geometric mean titre (GMT) was considerably higher in trial C. The GMT for the infants in trial B was somewhat higher than for those in trial C, but four doses had been given and a total of three times as many pertussis organisms.

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Reactions

After the introduction of quadruple vaccine in N. Ireland in 1963 reports were received that reactions, similar to those which are known to follow the administration of vaccines containing inactivated *B. pertussis* (Hopper, 1961), were occurring with sufficient frequency to influence the acceptance of the vaccine. In order to obtain a clear picture of the type, severity and frequency of the reactions a follow-

Table 7. *Type-III poliovirus-neutralizing antibody levels in fifty-three infants one month after the third dose of modified quadruple vaccine*

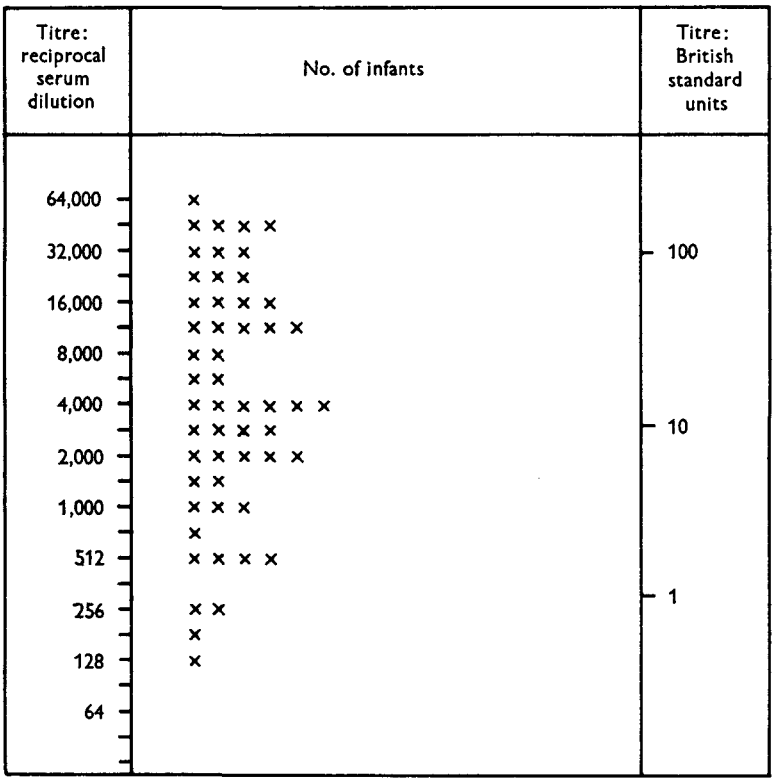

Table 8. *Diphtheria antitoxin levels in fifty infants one month after the third dose of modified quadruple vaccine*

Diphtheria antitoxin units per ml. serum	No. of infants
< 0·1	0
0·1–1·0	6
1·0–10·0	15
> 10·0	29

up study was arranged in co-operation with Medical Officers of Health and Health Visitors. The manufacturers were asked to produce a trial batch of quadruple vaccine in which the pertussis component was prepared in a different manner and

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the total number of organisms reduced. The reactions to this modified vaccine and to the standard vaccine were then compared in a double-blind trial. The modified vaccine, which had passed the usual potency tests (British Pharmacopoeia, 1963 ed., p. 1122) was found to produce significantly fewer and milder reactions than the standard vaccine. Production batches of this modified vaccine have been in general use in N. Ireland since October 1964, and the infants immunized in the present study received only modified vaccine. A recent follow-up study of 400 infants immunized at routine clinics has shown that reactions are less severe and less

Table 9. *Tetanus antitoxin levels in fifty-eight infants one month after the third dose of modified quadruple vaccine*

Tetanus international antitoxin units per ml. serum	No. of infants
0·1	0
0·1–0·2	2
0·2–0·4	1
0·4–0·8	3
0·8–1·6	7
1·6–3·2	6
3·2–6·4	26
6·4–12·8	6
12·8	7

Table10. *Pertussis agglutinin titres (reciprocal of serum dilution) for infants in three quadruple vaccine trials*

	Trial A	Trial B	Trial C*
No. of doses given . . .	3	4	3
Approximate age for each dose (months) . . .	6, 7, 8	3, 4, 5, 12	6, 7½, 14
No. of organisms per dose ...	29×10^9	29×10^9	$12{\cdot}5 \times 10^9$
No. of infants in trial ...	31	60	58
		No. of infants	
Pertussis agglutinin titres			
<4	3	—	—
4	1	—	—
8	1	—	—
16	1	—	—
32	2	—	—
64	3	2	1
128	2	3	14
256	11	30	22
512	5	15	15
1024	2	10	1
2048	—	—	—
4096	—	—	5
Geometric mean titre ...	96	354	259†

* The present trial.

† The five infants with titres of 1/4096 have been omitted from the GMT calculation because they possibly had natural infections.

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frequent than in the past. This was shown clearly when the reactions recorded for 250 infants who had received former production batches of quadruple vaccine were compared with those for 250 infants of the same age and sex who had received the modified vaccine (Table 11). Satisfactory reports have also reached us from the M.Os.H. using the modified vaccine indicating that reactions have been greatly reduced and that vaccine acceptance has been good.

Table 11. *Comparison of incidence of reactions following the original 'Quadrilin' vaccine and the modified 'Quadrilin' vaccine now used in Northern Ireland*

(Total numbers are given. Percentage figures are given in parentheses.)

	Original quadrilin	Modified quadrilin
No. observed	250	250
Mild reactions		
Fretful	176 (70·4)	78 (31·2)
Flushed and feverish	75 (30·0)	22 (8·8)
Drowsy	28 (11·2)	13 (5·2)
Skin rash	8 (3·2)	0
Painful arm	140 (56·0)	39 (15·6)
Swollen arm	83 (33·2)	16 (6·4)
Off food	38 (15·2)	10 (4·0)
Severe reactions		
Persistent screaming	12 (4·8)	1 (0·4)
Shocked or collapsed	3 (1·2)	0
No reported reactions	41 (16·4)	149 (59·6)

Table 12. *The age of infants in all quadruple vaccine follow-up studies who had severe reactions*

Age	No. of severe reactions*	Total observed
Under 6 months	23	498
Over 6 months	1	471

* Persistent screaming, and shock and collapse.

Though no direct comparison was made between the reactions following triple (diphtheria, tetanus, pertussis) vaccine and those following the quadruple vaccines, similar follow-up studies have been done. Two triple vaccines made by different manufacturers had quite different reaction rates. One was comparable to the modified quadruple vaccine, but the other gave rise to reactions of much the same order of frequency and severity as the original quadruple vaccine.

The severe type of reaction such as persistent screaming and shock and collapse (Hopper, 1961) was found to be more common amongst younger infants, and the large majority of the severe reactions were in infants under the age of 6 months (Table 12).

A detailed account of these follow-up studies of reactions to quadruple and triple vaccines will be published shortly.

DISCUSSION

The serological response to the poliovirus antigen in the modified quadruple vaccine was highly satisfactory, particularly against the important type I poliovirus component. The type I antibody titres were rather higher than those for types II and III and as a consequence the manufacturers have recently approximately doubled the 'D' antigen content for types II and III. It seems probable that, if the first dose of quadruple vaccine was given at 4 or 5 months of age, instead of at about 6 months of age as in the present trial, a satisfactory serological response would be obtained with three *suitably spaced* doses of the vaccine despite the relative immunological immaturity and higher levels of passive maternal antibody present at this earlier age. Our own reservation about starting immunization before the age of 6 months with vaccines containing a pertussis component are based in part on the greater toxicity of that component of the vaccine for younger infants.

The antitoxin responses to diphtheria and tetanus toxoids were satisfactory. Several infants had rather low levels of circulating tetanus antitoxin and the general level of response could be raised by increasing the amount of toxoid (5 Lf) present in the vaccine. However, if the present amount is adequate there seems little point in increasing it, as even tetanus toxoid may occasionally cause reactions (Brindle & Twyman, 1962; Eisen, Cohen & Rose, 1963).

Though the agglutinin response to *B. pertussis* is a less satisfactory way of measuring protection indirectly than the methods used for assessing the effectiveness of the other components it is of some value (Report, 1956) and comparisons made between results following different immunization schedules are probably valid. The fact that better results were obtained with modified quadruple vaccine given in three doses spaced 6–9 weeks and 6 months apart than with the original quadruple vaccine given in three doses at monthly intervals, though the original vaccine contained more than twice the number of pertussis organisms, is almost certainly a reflexion of superior dosage spacing. When sufficient time is allowed before the third dose is given there is a good secondary antibody response. In fact immunization with pertussis antigen seems no different from immunization with many other antigens such as poliovirus or tetanus toxoid where this type of dosage spacing has been accepted for some time. The majority of children, in our experience, receive triple vaccine (diphtheria, tetanus, pertussis) in three doses at monthly intervals, though in fact in both schedules P and Q of the Ministry of Health (Ministry of Health, 1961) it was recommended that there should be an interval of about 6–12 months before the last dose was given. We believe therefore that quadruple vaccine given in the present schedule could do much to raise the level of artificially induced pertussis immunity in young children at the age of maximum incidence of the disease.

For those who prefer giving three doses of triple vaccine and at the same time oral poliovaccine as an alternative to quadruple vaccine we would recommend that serious consideration be given to a similar type of dosage spacing to that used in the present trial, even if immunization is started at 4 or 5 months of age. Admini-

stratively it is somewhat easier to give three doses at monthly intervals, but in this instance administrative factors should probably not outweigh immunological factors. We have found that a three-dose schedule is much more acceptable than a four-dose schedule and is more likely to be completed.

We were surprised to find the frequency of reactions such as persistent screaming and shock and collapse that occurred not only after the original quadruple vaccine but also after some commercial triple vaccines. Hopper (1961) noted these reactions but we think that it was not generally appreciated how common they were. It has been obvious from our recent follow-up studies of quadruple vaccine that infants under 6 months of age are much more frequently subject to severe reactions than those over 6 months (Table 12). The fact that the majority of infants in our original trial of quadruple vaccine and also those in the Medical Research Council whooping-cough vaccine trials (Medical Research Council, 1951; Report, 1956; Report, 1959) were over 6 months of age may have been partly responsible for this ignorance.

The methods adopted to overcome the problem of too frequent and severe reactions were firstly to alter the technique of preparing pertussis antigen to one that was thought to produce a less reactogenic but equally protective vaccine, and secondly to reduce the number of pertussis organisms in each dose.

The reduction in the number of pertussis organisms did not in fact appear to reduce the infants' agglutinin response appreciably (Table 10), though unfortunately no direct comparison was possible between the original and the modified quadruple vaccines using the same dosage schedule. In our opinion any slight loss of antigenic potency which has followed the reduction in the number of pertussis organisms present in quadruple vaccine has been fully offset by the reduction in the severity and frequency of reactions and by the increased acceptability of the vaccine.

SUMMARY

Reactions to the pertussis component of the original commercial batches of quadruple vaccine against poliomyelitis, diphtheria, tetanus and pertussis (Quadrilin, Glaxo Laboratories) gave some cause for concern. Severe reactions were found to be more common in infants under 6 months of age than in older infants.

A modified quadruple vaccine, which has been used in N. Ireland since October 1964, was found to give rise to significantly fewer and milder reactions. This vaccine when given in three doses separated by intervals of 6–9 weeks and 6 months to fifty-eight infants most of whom were 6–7 months of age at the start of immunization was found to give a satisfactory immunological response.

We wish to thank the Medical Officers of Health and Health Visitors of Belfast, County Down and County Antrim for their co-operation; the children and their parents; and Mr J. J. McAlister, Miss Ffiona Wells, Mr K. Johnston, Mr R. J. Cummings and Mr A. W. P. Stevens for technical assistance.

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