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A study of poliovaccination in infancy: excretion following challenge with live virus by children given killed or living poliovaccine

By J. L. HENRY and E. S. JAIKARAN
London County Council

JOAN R. DAVIES and A. J. H. TOMLINSON
Public Health Laboratory Service, County Hall, London, S.E. 1

and P. J. MASON, JOAN M. BARNES and A. J. BEALE*
Glaxo Research Ltd, Sefton Park, Stoke Poges, Bucks.

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INTRODUCTION

Infants in this country are usually vaccinated against diphtheria, tetanus, pertussis and poliomyelitis in the first year of life, and the resultant immunity is subsequently reinforced by booster doses at intervals.

It was the policy in the London County Council Clinics to offer immunization with a triple antigen (diphtheria, tetanus, pertussis) at age 2, 3 and 4 months, with a booster dose at 15 months. Immunization against poliomyelitis was carried out by three doses of trivalent oral poliovaccine at age 7, 8 and 9 months. When a quadruple vaccine, incorporating killed poliovaccine and triple antigen, became available (Beale & Ungar, 1962), it was decided to assess its value under the routine L.C.C. conditions. It was appreciated that the children might be too young for an optimal response to the poliovaccine component, since Perkins, Yetts & Gaisford (1958, 1959) have shown that the response, particularly to the type 1 component of an inactivated vaccine, was poor in infants under 6 months of age. They showed that the response was improved by increasing the strength of the type 1 component. We therefore used a vaccine containing an increased amount of type 1 poliovirus antigen.

The trial was designed with two main objectives. First, the serological response of infants to the poliovaccine component of quadruple vaccine was compared with the response of infants to oral poliovaccine as routinely administered. Second, the effect of administering graded doses of type 1 attenuated poliovirus was studied to assess the degree of gut immunity conferred by poliovaccination.

In order to keep the trial small enough to be feasible only a single type of poliovirus could be used for the challenge experiments. Type 1 virus was chosen since it is the most important type causing paralysis and because it has been more difficult to produce adequate immunity to this type with killed virus vaccine.

* Requests for reprints should be addrssed to Dr A. J. Beale.

PLAN OF THE TRIAL

The children were enlisted at the age of 2 months from two areas of London, each of which contributed about twenty-five children to each of the four groups, A, B, C and D. The groups received vaccine, and specimens of blood and faeces were examined in accordance with the schedules shown in Table 1.

Table 1. *Plan of trial*

Group	Vaccine	Surveillance	Blood sample	Challenge
A	Quadruple vaccine at 2, 3, 4 months	Monthly faeces specimens 2–6 months	At 2 and 6 months	At 6 months. Faeces twice weekly for 3 weeks after challenge
B	Triple antigen at 2, 3, 4 months	Monthly faeces specimens 2–6 months	At 6 months	At 6 months. Faeces twice weekly for 3 weeks after challenge
C	Primary quadruple vaccine 2, 3, 4 months. Booster at 15 months	Monthly faeces specimens 2–16 months	At 15 and 16 months (before and 1 month after booster)	At 16 months. Faeces twice weekly for 3 weeks after challenge
D	Primary triple antigen at 2, 3, 4 months. Booster at 15 months. Trivalent oral poliovaccine 7, 8, 9 months	Monthly faeces specimens 2–16 months	At 16 months (7 months after oral poliovaccine)	At 16 months. Faeces twice weekly for 3 weeks after challenge

The children in group A received a primary course of killed poliovaccine, those in group B constituted a control group who had no immunization against poliomyelitis. The children in group C received a primary course and a booster dose of killed poliovaccine, those in group D were immunized with oral poliovaccine. No detailed study of reactions to the vaccines was made, but reactions to quadruple vaccine did not seem to be more severe than those following triple antigen.

The children were randomly allocated to their vaccine groups and to five subgroups for challenge. The challenge doses consisted of five serial tenfold dilutions of attenuated type 1 poliovirus, LSc.2ab, the highest dose containing $10^{5\cdot7}$ TCD_{50}. Specimens of faeces were examined at monthly intervals from the start of the trial to challenge, so as to detect natural poliovirus infections, and twice weekly after challenge to estimate the excretion of the poliovirus type 1 challenge virus.

METHOD AND MATERIALS

Vaccines

The quadruple and triple vaccines and the attenuated oral poliovaccine were prepared by Glaxo Laboratories Ltd.; the quadruple vaccine was 'Quadrilin' batch 12, containing at least 75, 1 and 1 D antigen units of poliovirus antigen for types 1, 2 and 3 respectively, 28 Lf of diphtheria toxoid, 10 Lf of tetanus toxoid and 200 million killed *Bordetella pertussis* organisms in each 1 ml. dose. The triple antigen (batch 431) contained the same quantity of diphtheria, tetanus and pertussis antigens as the quadruple vaccine in each 0·5 ml. dose.

The trivalent oral attenuated polio vaccine was T.V. 101 prepared from the Sabin strains of attenuated poliovirus, containing $10^{5\cdot7}$ TCD_{50} of type 1 (LSc.2ab), $10^{5\cdot0}$ TCD_{50} of type 2 (Ch.2ab) and $10^{5\cdot5}TCD_{50}$ of type 3 (Leon.12ab) strains.

Challenge doses

The attenuated type 1 challenge virus was prepared from monovalent Sabin attenuated poliovaccine, Glaxo batch A.V. 5 type 1, and was preserved in 50% sucrose solution. Virus was diluted to the chosen strength and dispensed into dropper bottles, one for each child. Each bottle contained a dropper into which one dose could be drawn. The highest dose contained $10^{5\cdot7}TCD_{50}$, and there were four serial tenfold dilutions of this dose. The challenge doses at each dilution were checked for virus content before dispensing and were stored at $-25°$ C. until the day before they were required. After transport at ambient temperature, they were stored at 4° C. overnight. After a dose had been given, the bottle containing residual fluid was returned to the laboratory and stored at $-25°$ C. until it was retitrated. A random selection of each of the doses given at any clinic session was titrated, and the results are shown in Table 2. There was no difference between the titre of the challenge doses used early in the trial, i.e. for groups A and B, and those used 15–18 months later for groups C and D. The virus proved stable even in a diluted form when stored in 50% sucrose.

Neutralizing antibody titrations

These were performed against all three types of poliovirus by a micromethod as only small quantities of blood were obtained from the children by heel prick. The technique was based on that described by Durand (1962). Serial twofold dilutions of serum were made and mixed with an equal volume of poliovirus containing approximately 100 TCD_{50}. Virus-serum mixtures were held at 37° C. for 3 hr. before addition of cells. Virus-serum and cells were drawn up into capillary tubes, which were sealed with liquid paraffin and incubated at 37° C. The British standard poliovirus antisera were put up with each test so that the results could be expressed in terms of international units of antibody per ml. (Perkins & Evans, 1959; Lyng & Bentzon, 1963). The micromethod of performing the antibody titrations gave results similar to those obtained at Glaxo Laboratories with the cytopathic test used in the collaborative assay of the British Standard poliomyelitis antisera (Perkins & Evans, 1959).

Virus isolation

Surveillance and pre-challenge specimens of faeces were examined in secondary monkey kidney cells (Mair & Tobin, 1960) and in a continuous cell line (HeLa or HEp 2). Pre-challenge specimens from children who failed to excrete poliovirus type 1 after challenge were also examined in unweaned mice. Post-challenge specimens were examined only in monkey kidney cells.

Table 2. *The challenge doses of attenuated poliovirus type* 1

Dose	Dose at time of dispensing (TCD_{50})	After challenge		
		No. of titrations	Mean TCD_{50}/dose	Range
1	$10^{5.7}$	19	$10^{5.55}$	$10^{4.86-5.98}$
2	$10^{4.7}$	19	$10^{4.58}$	$10^{4.0-5.04}$
3	$10^{3.7}$	19	$10^{3.55}$	$10^{3.0-3.98}$
4	$10^{2.7}$	13	$10^{2.56}$	$10^{2.2-2.78}$
5	$10^{1.7}$	16	$10^{1.61}$	$10^{1.2-1.81}$

RESULTS

A total of 236 children entered the trial. Most of them were between 2 and 3 months of age, but thirty-six were just over 3 months old.

Twenty children failed to complete the appropriate course for the following reasons: moved away, 8; intercurrent illness, 5; irregular attendance, 5; given wrong vaccine, 1; severe reaction to vaccine, 1. Of those who completed the course 55 were in group A, 52 in group B, 52 in group C and 57 in group D.

Poliovirus antibody response

The antibody titres for the children in the four groups are shown in Fig. 1. (The presentation of the results has been simplified by assigning intermediate readings to the next lowest value in the twofold dilution series.) Owing to the small quantities of serum available for examination, many titrations did not give an end-point and an arrow shows that the titre was more or less than the indicated titre. The number of sera examined against each type of poliovirus is less than the number of children completing the course because some children could not be bled at the correct time, some specimens of serum were so small that they could only be tested against two virus types and some children experienced natural poliovirus infections. These last children were only excluded so far as the antibodies homotypic to their infecting virus were concerned since the response of these children to the other virus types was not different from that of uninfected children.

At the age of 2 months (group A, 1st serum) many children had high titres of maternal antibody, particularly to type 2 virus, and these antibodies had not entirely disappeared when the children were bled at 6 months of age (group B). In one area where records were available, 80 % of the mothers had been immunized —60 % with inactivated vaccine and 20 % with oral poliovaccine—either before or during the pregnancy resulting in the study child.

The primary course of quadruple vaccine (group A) stimulated the production of

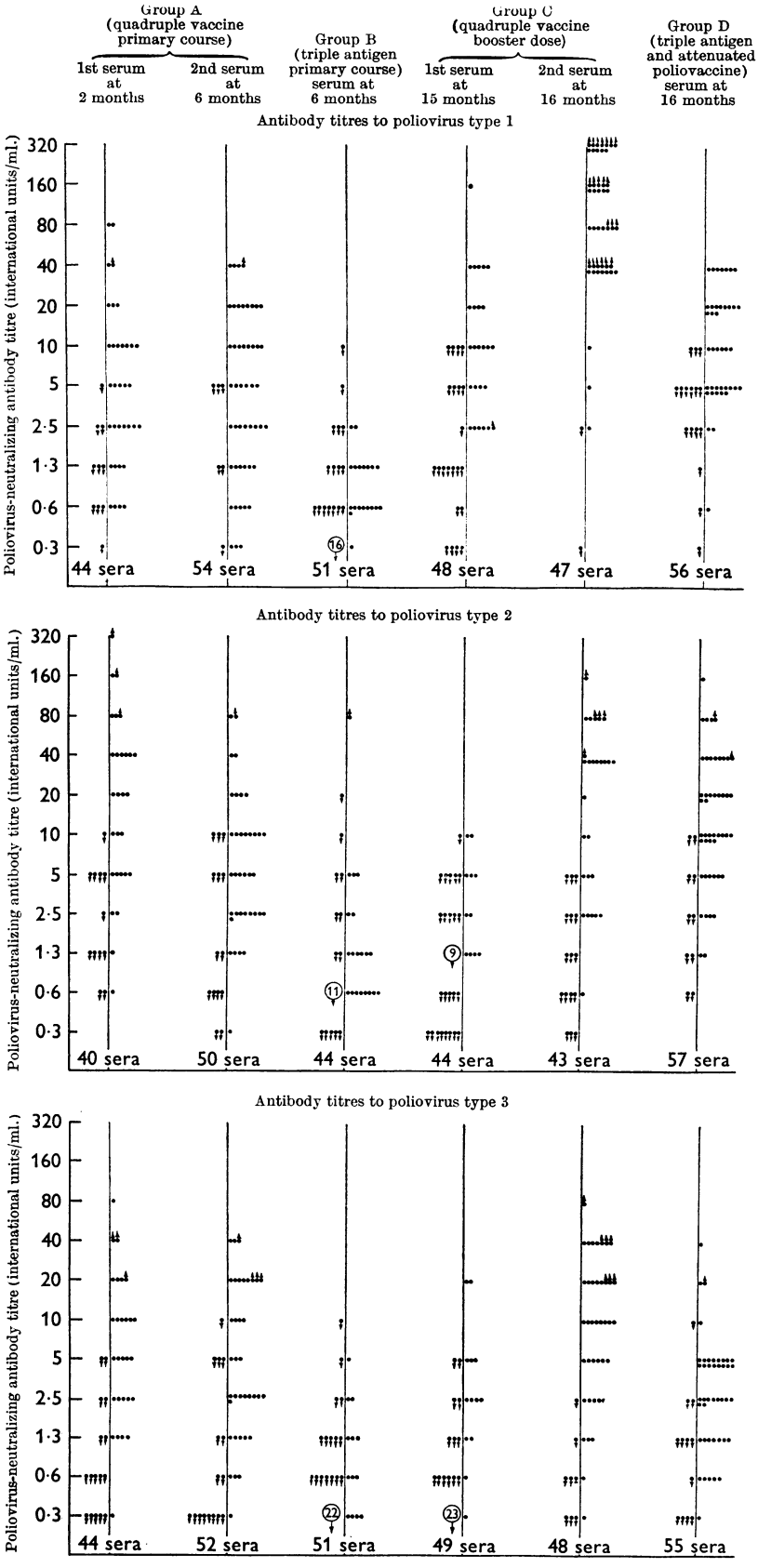
Group A (quadruple vaccine primary course)
1st serum at 2 months
2nd serum at 6 months
Group B (triple antigen primary course) serum at 6 months
Group C (quadruple vaccine booster dose)
1st serum at 15 months
2nd serum at 16 months
Group D (triple antigen and attenuated poliovaccine) serum at 16 months
Antibody titres to poliovirus type 1
Poliovirus-neutralizing antibody titre (international units/ml.)
320
160
80
40
20
10
5
2·5
1·3
0·6
0·3
44 sera
54 sera
51 sera
48 sera
47 sera
56 sera
Antibody titres to poliovirus type 2
40 sera
50 sera
44 sera
44 sera
43 sera
57 sera
Antibody titres to poliovirus type 3
44 sera
52 sera
51 sera
49 sera
48 sera
55 sera

Fig. 1.

some poliovirus antibodies which can be judged by comparing, in Fig. 1, the results for group A second sera with the group B children who had had no poliovaccine. A more precise analysis can be attempted by comparing the titres obtained from the sera of individual children before and after the primary course. The decline of maternal antibody has to be offset against active antibody production by the infant. Assuming that maternal antibody has a half-life of 28 days, its titre might be expected to fall 16-fold between the 2nd and 6th months of life. The following arbitrary categories have been defined:

Good response. Second titre equal to or greater than the first.

Doubtful response. Second titre half to one-eighth of first titre.

No detectable response. Second titre one-sixteenth or less of the first titre. A few children were included in this group when the second serum showed no antibodies when tested at a low dilution.

Unassessed. Insufficient data to assess response: few, if any, in this group made a good response.

Table 3. *Response to the primary course of inactivated vaccine*

	Number of children				
Poliovirus type	Good response	Doubtful response	No detectable response	Unassessed	Total
1	21	10	8	4	43
2	10	9	14	2	35
3	17	5	15	2	39

The response of the children to the primary course of vaccine is given in Table 3. Examination of the individual protocols showed clearly that it was those children with the lowest titres of maternal antibody who made the best response. Technically, of course, it is easier to demonstrate the active production of antibody in a child whose initial titre is low but the striking response of children with no initial antibody could not be explained in this way.

When the children in group C were 15 months of age, they were bled immediately before the booster dose. The antibody titres in the serum at this time were for the most part low. Some children, with no detectable antibody, reacted to one or more of the poliovirus antigens in the booster dose by producing antibodies to a high titre. In these children the antibody-forming mechanism had been sensitized by the primary course. The response to any one of the three poliovirus antigens was not related to the response to the other types.

Most children responded well to the booster dose of vaccine. A consideration of the titres reached after the booster dose offers a more realistic assessment of the immunization procedure than a comparison of the titre obtained from individual children before and after the booster dose. In assessing the response of the children to the complete course of vaccine, the following arbitrary definitions have been made:

Good response. Final serum titre equal to or greater than 10 I.U./ml.

Some response. Final titre 2·5 or 5·0 I.U./ml.

Minimal or no response. Final titre less than 2·5 I.U./ml.

For type 3 vaccine, half the above-mentioned units of antibody have been used.

The results obtained from the children in group C are given in Table 4. The response of a child to a course of poliovaccine is essentially individual and will depend, among many other things, on the amount of antigen injected and the titre of maternal antibody at the start of the course. The antibody titre after the booster dose is the most reliable indication of the effectiveness of the immunization procedure and a close study of the results obtained with the different virus types reveals interesting discrepancies when the assessment of the effectiveness of immunization is made at other times as shown in Fig. 1.

Table 4. *Serological response to the complete course of inactivated vaccine*

(Results assessed 1 month after booster.)

	Number of children				
Poliovirus type	Good response (≥ 10 I.U.)	Some response (2·5 or 5 I.U.)	Minimal or no response (< 2·5 I.U.)	No detectable antibody*	Total
1	43	2	2	0	47
2	18	8	14	3	43
3	30†	8†	9†	1	48

* Sera not tested at low dilution.
† Antibody levels one half those for types 1 and 2.

Table 5. *Serological response to the complete course of attenuated poliovaccine*

(Results assessed 7 months after third dose.)

	No. of children with antibody titre				
Poliovirus type	Good response (≥ 10 I.U.)	Some response (2·5 or 5 I.U.)	Minimal or no response (< 2·5 I.U.)	No detectable antibody*	Total
1	24	15	8	9	56
2	35	10	8	4	57
3	20†	17†	15†	3	55

* Sera not tested at low dilution.
† Antibody levels one half those for types 1 and 2.

The serological results from the children in group D who were bled 7 months after their last dose of live vaccine are given in Fig. 1 and Table 5. It will be seen that the circulating antibody titres, especially for type 1, were not as high as they were 1 month after a booster dose of killed poliovaccine, but it may be that the titre of antibody is not the most important criterion of immunity after oral poliovaccine.

Infection after challenge

Challenge doses were given to 216 children. In determining whether or not a child became infected as a result of the challenge dose, twenty-six children had to be excluded: natural infection with poliovirus type 1 during surveillance, 7;

concurrent infection with another virus at challenge, 15; too few faeces specimens examined after challenge, 4.

The results of feeding graded doses of Sabin's type 1 vaccine virus to 190 children are shown in Table 6.

It will be seen that the smallest challenge dose (about 50 TCD_{50}) was capable of infecting about half the children in groups A and B and that a primary course of killed poliovaccine did not influence the size of dose required to infect. The children in group D, who had had attenuated oral vaccine, required a much higher dose to initiate infection. The children in group C seemed a little more resistant to infection than those in groups A and B, but a proportion could still be infected by the lowest challenge dose. Infection after challenge was not influenced by the titre of neutralizing antibody at the time, nor was it affected by natural infection with poliovirus types 2 or 3 during surveillance.

Table 6. *Excretion of poliovirus type* 1 *after challenge*

		Vaccine group			
Dose sub-group	Dose (TCD_{50})	A (quadruple vaccine, primary course)	B (triple antigen)	C (quadruple vaccine, after booster)	D (triple antigen, attenuated poliovaccine)
1	$10^{5\cdot7}$	9/10	9/9	8/8	8/11
2	$10^{4\cdot7}$	9/9	9/10	9/9	7/12
3	$10^{3\cdot7}$	10/11	11/11	4/8	1/10
4	$10^{2\cdot7}$	7/9	6/8	4/10	0/8
5	$10^{1\cdot7}$	7/10	5/10	3/8	0/9
Total		42/49	40/48	28/43	16/50

Numerator = no. of excretors; denominator = no. challenged.

Duration of excretion

It was possible to assess whether or not challenge led to infection in 190 children. In considering the duration of excretion of poliovirus type 1 after challenge, it was necessary to exclude a further 13 children; 2 of these became infected with another virus during the observation period and 11 (4 in group A, 4 in group B and 3 in group C) did not submit a specimen after the 16th day, poliovirus having been isolated from their last specimen.

The duration of excretion of the remaining 177 children is given in Table 7. Specimens of faeces were examined twice weekly, and the duration of infection has been calculated as half-way between the last positive and the first negative specimen.

It will be seen that almost all the children in group B who became infected excreted poliovirus type 1 for more than 18 days after challenge. About two-thirds of the infected children in group A and one half of those in group C excreted for this period. None of the infected children in group D excreted for more than 14 days. Provided that infection was initiated, the size of the challenge dose had no effect on the duration of excretion.

Poliovirus type 1 was isolated on more than one occasion from all the children in groups A, B and C who were infected after challenge, but from seven of the children in group D it was isolated from a single specimen only. However, the timing of these positive specimens and the titre of virus they contained indicated that virus multiplication had occurred in the gut.

Table 7. *Duration of excretion of poliovirus type* 1 *after challenge*

	Number of children in vaccine group			
Duration of excretion (days)	A (quadruple vaccine, primary course)	B (triple antigen)	C (quadruple vaccine, after-booster)	D (triple antigen, attenuated poliovaccine)
No excretion	7	8	15	34
1–9	1	0	0	5
10–18	13	2	12	10
> 18	23	34	13	0
Total children	44	44	40	49

Table 8. *Effect of pre-challenge antibody titres on duration of excretion after challenge*

	No. of children with indicated serum titre after primary course quadruple vaccine (A)		No. of children with indicated serum titre after booster dose of quadruple vaccine (C)	
Duration of excretion (days)	< 10 I.U.	⩾ 10 I.U.	< 100 I.U.	⩾ 100 I.U.
No excretion	3	4	3	12
1–9	0	1	0	0
10–18	6	7	2	10
> 18	18	5	7	5
Total	27	17	12	27

Effect of antibody titre on duration of excretion

In order to determine whether the child's antibody titre at the time of challenge had any effect on the duration of the subsequent poliovirus excretion, the children in groups A and C were divided according to their antibody titre, with the results shown in Table 8. One child in group C has been excluded since no serum was obtained.

It will be seen that in group A those children with the higher antibody titres tended to excrete poliovirus for a limited period only. A similar trend can be discerned among the group C children, although the actual titres of antibody were much higher. In this group there appears to be an excess of children with high antibody titres who were not infected; it is difficult to assess the significance of this finding, since by chance these children all received the smaller doses of challenge virus.

In group D, fifteen children excreted poliovirus after challenge. The duration of this excretion did not appear to be related to the child's antibody titre at the time of challenge.

Amount of virus excreted after challenge

Poliovirus type 1 was excreted by 126 children after challenge. All the faecal extracts from which poliovirus had been isolated were titrated using tenfold dilutions and two tubes per dilution. The 50% end-point was calculated and plotted graphically against the day after challenge on which the specimen had been collected. The area under the curve for each child was measured with a planimeter; from the result the mean virus content of the faeces was calculated from day 6 to day 15 after challenge and expressed as log TCD_{50} per gramme faeces per day.

Table 9. *Excretion of poliovirus* 6–15 *days after challenge*

Vaccine group	Number of children with mean log TCD_{50}/day/g. faeces				Total
	< 3·0	3·1–4·0	4·1–5·0	> 5·0	
Primary quadruple vaccine (A)	5	11	20	4	40
Primary triple antigen (B)	0	2	20	16	38
Booster quadruple vaccine (C)	2	8	11	2	23
Attenuated poliovaccine (D)	12	2	0	0	14
					115

The results obtained from eleven children have had to be excluded for the following reasons: intercurrent infection with another virus, 2 children; faeces extracts toxic, 3 children; too few specimens during the relevant period, 5 children; excretion for less than 6 days, 1 child. The results for the remaining 115 children are given in Table 9.

From the 6th to the 15th day after challenge, most of the unimmunized children in group B excreted more than 10^4 TCD_{50} poliovirus/g. faeces per day, and nearly half of them excreted more than 10^5 TCD_{50}. Few of the children in groups A and C excreted 10^5 TCD_{50}/g. faeces per day and about 40% of the children in these two groups excreted less than 10^4 TCD_{50}. Twelve of the fourteen infected children in group D excreted less than 10^3 TCD_{50}/g. faeces per day.

The period 6–15 days after challenge was chosen for the calculation, since the largest number of observations was available for plotting the excretion curve for each child. Similar results were obtained when the virus excreted for the period 10–19 days after challenge was calculated, but naturally there was a marked shift to the left; 36% of the children in group A, half of those in group C and all of those in group D excreted 10^3 TCD_{50}/g. faeces per day or less. Only one child in group B came into this category.

If infected faeces are the means whereby poliovirus spreads in a community, and if a child is to pass on his infection to others, this must happen while the virus titre in the faeces is reasonably high. If it is assumed that children ingest milligrams

rather than grams of faeces then a virus titre in the faeces of the order of 10^4 TCD_{50}/g. is necessary for a child to be infective. Clearly, the amount of faeces passing from child to child will depend on the domestic hygiene of the family; under present-day conditions the above assumption appears justified.

Figure 2 shows how the different vaccine schedules affected the potential infectivity of the children. All children have been included, whether infected or not, who received the four highest challenge doses of poliovirus and who had not

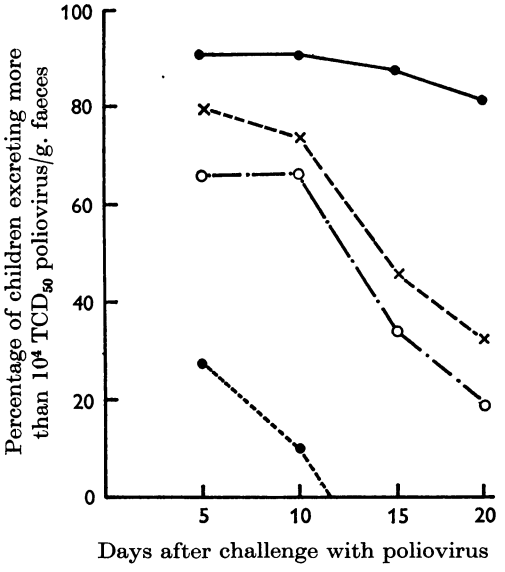

Fig. 2. Effect of vaccination schedules on potential infectivity of children. ●—●, Group B, triple antigen. ×— — ×, Group A, primary course, quadruple vaccine. ○—·—○, Group C, primary and booster dose, quadruple vaccine. ●- - - - -●, Group D, attenuated poliovaccine.

Table 10. *Effect of pre-challenge serum titre on amount of virus excreted 6–15 days after challenge*

Vaccine group	Serum titre (I.U.)	No. of children with mean log TCD_{50}/day/g. faeces				
		< 3·0	3·1–4·0	4·1–5·0	> 5·0	Total
Primary course quadruple vaccine (A)	< 10	1	7	15	3	26
	⩾ 10	4	4	4	1	13
Booster dose quadruple vaccine (C)	< 100	0	1	4	2	7
	⩾ 100	2	7	6	0	15

been excluded from any previous analysis. The proportion excreting 10^4 TCD_{50}/g. faeces at different times after the challenge dose has been plotted. The area under the curves gives a measure of the infectivity of groups of children vaccinated in different ways and exposed to infection with at least 500 TCD_{50} of attenuated virus. When the areas were measured, it was found that, taking the infectivity

of the unvaccinated as 100, the infectivity of the children who had had killed vaccine was 60 while that of the children immunized with oral vaccine was about 10. These observations were confined to the first 20 days after infection; it seems probable that had the observations been continued, the differences between the groups would have been greater.

Influence of antibody on amount of virus excreted

If the results obtained from the children in groups A and C are divided according to the antibody titre in the pre-challenge specimen of serum, Table 10 is obtained. Two children have been excluded, since no specimens of their sera were examined. In both groups the children with the higher antibody titres tended to excrete less poliovirus than those who had responded less well to the vaccine.

DISCUSSION

Serological results

Killed poliovaccine

The striking feature of the results when antibody titres at 16 months are studied is the excellent response to the type 1 component of the quadruple vaccine compared with that to types 2 and 3. This was presumably due to the high D-antigen content of the type 1 component (Beale & Ungar, 1962); similar good results have been obtained in older children by Dane *et al.* (1962). The inhibitory effect of maternal antibody, first reported by Perkins *et al.* (1958, 1959), was overcome by the potent type 1 antigen.

The results at 15 months (Fig. 1 group C, first serum) show in general the expected fall in antibodies after the primary course and titres were disappointingly low in some children, nevertheless the response to the booster dose showed that the children without detectable antibodies fell into two groups. One group failed to respond to the primary course, probably because of inhibition by maternal antibody, and the other group had been sensitized and made a booster response. Some children in group C (10 out of 48) had antibody titres to type 1 virus of 20 units or more at this time—a figure not expected from the results given by the children in group A after the primary course. These high titres might have been caused by missed natural poliovirus infections but this suggestion was not supported by the results of challenge with attenuated virus. Butler, Barr & Glenny (1954) using diphtheria toxoid found that the response of infants who lacked maternal antibody was excellent. They also observed that, in those with maternal antibody, the antibody response was delayed so that when infants were immunized at 1 and 6 weeks of age and bled at 3 months and again at 6 months, titres of diphtheria antitoxin were higher at the second bleeding. A similar delay in poliovirus antibody production may have operated in this trial.

The response to poliovirus types 2 and 3 was less good than that to type 1. Quadruple vaccine of the same composition as used in this trial was found to give excellent antibody titres for types 2 and 3 in children over 6 months of age (Dane *et al.* 1962) and also in children whose immunization was started at age 4 months

(Dane, 1964). The discrepancy between these results and our findings could well be due to the earlier start of immunization in this study and to the high titres of maternal antibody due to the policy of active immunization of the mothers. A delay of 2 months will be associated with a fourfold fall in the titre of maternal antibody, assuming as we have done a half-life of 28 days, based on the work of Orlandini *et al.* (1955). The antibody titres were assessed only 1 month after the booster dose, and the duration of the high titres has not been ascertained. We have found however (A. J. Beale, unpublished) that the antibodies produced by this course of quadruple vaccine are 7S immunoglobulins (1 gG) and are therefore likely to be associated with a long-lasting immunity.

Attenuated oral poliovaccine

The serological results for the children who had attenuated poliovaccine are at first sight disappointing; some children had little or no detectable antibody even though they had received a course of immunization generally accepted as adequate. The children in group D were bled 7 months after their third dose of poliovaccine. It was found in the Public Health Laboratory Service trial of live poliovaccine (Report, 1961) that, except possibly for type 3, antibody titres did not change much between 1 and 6 months after the third dose of a course of oral poliovaccine. The distribution of antibody titres of all three types reported here did not differ greatly from the results obtained from the sera examined at Colindale in the P.H.L.S. trial; comparative titrations of antibody carried out by Glaxo Laboratories and the Virus Reference Laboratory, Colindale, have in the past given similar results (Perkins & Evans, 1959).

The titres observed in the children who responded to the killed vaccine (group C) were much higher for types 1 and 3 than those in the children who had had attenuated vaccine. The possibility that this might represent a strain-specific response was considered. Some sera were retested using attenuated viruses rather than our stock strains (Brunenders type 1, MEF 1 type 2 and Saukett type 3) but the difference between groups C and D could not be explained in this way.

The serological response may not be the most important criterion of immunity after oral poliovaccine. The excretion of poliovirus by the children in group D after challenge showed that there was a substantial degree of intestinal immunity which was unrelated to the titre of circulating antibody. Moreover almost all the children with no detectable antibody (whose sera could be tested at low dilutions) were known from the monthly surveillance specimens to have excreted virus of the appropriate type during the time that they were receiving attenuated vaccine.

Infection following the challenge dose of poliovirus

It was not surprising that it was possible to infect unimmunized infants with a dose of poliovirus of about thirty virus particles (equivalent to 50 TCD_{50}) since the virus dose involved in the transmission of the natural infection is probably of this order. The children who had received a primary course of killed poliovaccine were just as susceptible to infection with small doses of challenge virus as the

unimmunized controls. Children who had been given a primary course of killed vaccine were also susceptible to natural infection with poliovirus; the fifty-two children in group C experienced thirteen natural infections with poliovirus between the end of their primary course and their booster dose 11 months later. Four of these infections were with type 1, seven with type 2 and two with type 3.

The children in group C, challenged 1 month after their booster dose of quadruple vaccine, were somewhat more resistant than the children in groups A and B to infection with challenge doses of 5000 TCD_{50} or less. Calculation, from Table 6, shows that, in order to infect 50 % of the children in group C, a dose of virus 8–10 times larger was needed compared with groups A and B. These children were older and although age by itself may not increase the dose required to infect, it implies that the children had been exposed to the risk of natural infection for longer and this might not have been detected by the examination of the monthly surveillance specimen. Any failure to detect natural infection would, of course, make the children in group C appear more resistant to infection. The serological results and the excretion of virus after challenge did not suggest that we failed to detect natural infection in many children. It is not possible to say whether the increased resistance to infection was a function of the increased age of the children or the result of their active immunization.

As was expected, the children in group D were considerably more resistant to infection with the challenge virus, requiring about 1000-fold higher doses to infect them compared with the unimmunized controls.

Duration of excretion and amount of virus excreted

The quantitative study of virus excretion after infection showed some interesting differences between the various groups. Most of the unimmunized children in group B excreted large amounts of virus for the whole of the 3-weeks observation period. The children who had had the killed vaccine, groups A and C, showed a tendency to excrete less virus than the unimmunized controls. This tendency was most obvious in the children who had responded best to the killed vaccine by producing the higher antibody titres.

The effect of the serum antibody titre on the duration and amount of poliovirus excreted was clearly not a direct one. As can be seen from Tables 8 and 10 the children in group A with antibody titres of 10 I.U./ml. or more showed a limitation of virus excretion similar to that of the children in group C with titres of 100 I.U./ml. Those children in group D who were infected by the challenge dose excreted only small amounts of virus for short periods, yet their serum antibody titres at the time of challenge were only about one-tenth of those in group C. It is not known how a natural poliovirus infection of the intestine is brought to an end; possibly antibody is produced in the gut in response to the presence of virus there. This antibody-producing mechanism might be partially sensitized by parenteral injections of killed vaccine and come into play more rapidly after infection.

A study by the P.H.L.S. (Report 1965) also showed some reduction in poliovirus excretion by children who had received a primary course of killed vaccine, but the

conditions of this study were so different from ours that detailed comparison is not possible.

In agreement with these findings Galbraith (1964) observed that fewer Salk-vaccinated children were excreting poliovirus in a random survey compared with unvaccinated children of the same age. This phenomenon may afford an explanation for the greater protection of communities by killed poliovaccine than would be expected from the number vaccinated. This appears to have occurred in Sweden, where only killed poliovaccine has been used (Gard, 1964), and also in Holland according to a report by Hofman in a paper presented at a meeting of the Society for General Microbiology in 1964. Gard reports that in Sweden no cases of clinical poliomyelitis have occurred since 1963 in either the vaccinated or unvaccinated section of the population. He also reported that no isolations of poliovirus had been made from individuals admitted to infectious disease wards of hospitals from 1963 onwards although other enteroviruses remained prevalent.

The community effect following the widespread use of attenuated poliovaccine is well known. It is difficult to infect children immunized in this way except with large doses of virus, and their infectivity, compared with unvaccinated controls, is reduced by 90 %. Children immunized with killed vaccine can still be infected with quite small doses of virus and their infectivity is reduced only by 40 %. A smaller community effect may be expected from the use of killed vaccines.

SUMMARY

Quadruple vaccine containing 75 D antigen units of killed type 1 poliovirus was given to children at ages 2, 3 and 4 months followed by a booster dose at 15 months.

The serological response to the primary course was difficult to assess owing to maternal antibody. Antibody titres to the type 1 component after the booster dose were very satisfactory and about 10 times higher than those observed in a similar group of children given attenuated vaccine. Response to the poliovirus types 2 and 3 in the quadruple vaccine was less satisfactory.

Graded doses of attenuated poliovirus type 1 were fed to the children 2 months after the primary course and 1 month after the booster dose. Children who had received no poliovaccine and children immunized with attenuated vaccine were included for comparison.

Immunization with killed vaccine did not greatly affect the size of the minimal infecting dose of live virus but reduced both the duration of the subsequent infection and the titre of virus in the faeces.

The epidemiological significance of these findings is discussed.

A trial of this type which involved the collection of nearly 3000 specimens of faeces could not have been completed without the help of a large number of people. We are particularly indebted to Dr W. G. Harding and to Dr F. R. Waldron in whose Divisions the trial was carried out, to Miss Conway and to Mrs Watson who were responsible for the record-keeping and co-ordination in the two

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