VACCINATION OF ADULTS WITH A BRITISH ORAL POLIOMYELITIS VACCINE PREPARED FROM SABIN STRAINS

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In 1956 Sabin described attenuated poliovirus strains of each serological type selected by passage in monkey-kidney cultures and segregated by plaque purification. Oral vaccines have been prepared from these strains in many countries and have been used extensively in a variety of epidemiological conditions (*Brit. med. J.*, 1961). There have, however, been few reports of the use in Britain of the Sabin vaccines. Vaccination of a small number of children with the type 3 vaccine was described by Clarke *et al.* (1958), and a Medical Research Council investigation among young children, using all three types, was begun in 1960 (*Brit. med. J.*, 1960). In both these investigations the original Sabin vaccines prepared in the U.S.A. were used. The present report is the first account of the use of oral poliomyelitis vaccines prepared in Britain.

Procedure

Plan of Investigation

The investigation was designed to study the frequency and duration of faecal excretion of viruses and the antibody responses after oral administration of the three different types of vaccine. It was limited to volunteers over the age of 21 years, who were all members of the staff of the Wellcome Research Laboratories, in normal health, and living at home. Only those whose young families had been vaccinated with inactivated poliomyelitis vaccine (Salk-type) were accepted. In all, 43 persons (34 males and 9 females) between 21 and 56 years of age took part; 21 had received one or more injections of inactivated vaccine and none had a history of paralytic or non-paralytic poliomyelitis.

The vaccines were given sequentially—type 1 followed by type 3, followed by type 2—and for each type the dose was 10^5 plaque-forming units of virus. It was originally planned to give the vaccines at four-weekly intervals, but owing to a delay in the laboratory testing of the type 3 vaccine the interval between the type 1 and type 3 was 17 weeks. Serum samples for antibody determinations were taken from participants one week before giving type 1 and four weeks after type 2. Faecal samples for virus isolation were collected before vaccination with type 1, and twice weekly after each vaccination, for four weeks after type 1 and type 3, and for three and a half weeks after type 2. Of the 43 volunteers, 33 completed the whole study and a further two the type 3 part only.

The Vaccines

The vaccines were prepared according to specifications laid down by Dr. Sabin. The original seed material consisted of aliquots of the large batches received from him and used extensively in field trials in many parts of the world (Sabin, 1959). Secondary seed and final vaccine batches were grown under rigorously controlled conditions in cultures of trypsinized kidney from cynomolgus monkeys, which had been kept in isolation and quarantine for at least six weeks before nephrectomy. Seed lots and vaccine batches were subjected to an identical and exhaustive series of tests. Modifications of the tests prescribed by Dr. Sabin and additional test procedures were introduced after discussion with the Biological Standards Control Laboratory of the Medical Research Council. The tests were specifically designed to detect contamination of the vaccines by viruses previously reported to occur latently in some batches of monkey-kidney cultures. Among the possible contaminants excluded in this way were measles virus, B virus, and the "simian viruses" of Hull *et al.* (1958). No evidence was obtained of the presence in the vaccines of any extraneous viruses.

On the day of vaccination an aliquot of the vaccine which had been stored frozen at −20° C. was thawed and diluted to a concentration of 10^5 P.F.U. per ml. The dilution was made either in tissue-culture maintenance medium containing added sugar and gelatin (types 1 and 3) or in a mixture of 0.1 M sucrose and 0.01 M phosphate buffer *p*H 7.2 (type 2). Each volunteer swallowed 1 ml. of diluted vaccine in about 20 ml. of distilled water. The virus content of the vaccines was determined on the day they were administered by titrating the dilution given to the volunteers.

Antibody Determinations

Neutralizing antibodies in the sera collected from the participants before and after vaccination were titrated simultaneously by the colour test (Shand, 1961), using a range of twofold dilutions from 1/4 to 1/2,048. Titres are expressed as the reciprocal of the final dilution of the serum in the virus–serum mixture. A titre of less than 4 was recorded as no antibody.

Virus Isolations

Faecal samples were brought to the laboratory by each participant within 12 hours of collection. Within two hours of receipt, extracts of the samples (approximately 10%) were made in tissue-culture medium containing extra antibiotics by homogenization and low-speed centrifugation. Two tubes of monkey-kidney cultures were each inoculated with 0.5 ml. of extract. These cultures, already containing 1.5 ml. of medium, were left at room temperature for one hour; they were then emptied, washed once, and, after the addition of 2 ml. of fresh medium, incubated at 36.5° C. in a roller apparatus. The liquid phase of the cultures was harvested after seven days in the case of negative specimens, for passage to other cultures, or earlier in the event of the cytopathic effect of poliovirus, for serological typing. All strains isolated were serologically identified, either by complement-fixation test (Le Bouvier *et al.*, 1954) or, when the test proved unsuccessful, by neutralization.

The type 1 strains were also tested by a modification of the neutralization test capable of detecting minor antigenic differences between type 1 strains and of distinguishing the vaccine strain (LSc) as a subtype (Goffe and Shand, 1961).

Results

Pre-vaccination Antibodies.—Thirty-five sera taken before vaccination were examined; six had no antibody to type 1 virus, seven had none to type 2, and four had none to type 3. Twenty-four had antibodies to all three virus types and two were triple-negative. The distribution of antibody according to the history of vaccination with inactivated vaccine is given in Table I.

Antibody Response after Vaccination.—The antibody responses are shown in Fig. 1. All five persons with no immunity to type 1 virus before vaccination formed antibody after oral vaccination; the corresponding results for types 2 and 3 were 5/7 and 4/4 respectively. The geometric mean titres of these groups were: type 1, 34; type 2, 58; type 3, 49. The two who failed to form type 2 antibody did not excrete type 2 virus; they were the only triple-negative subjects in the study. Of those with previously acquired antibody approximately 50% showed a rise in titre.

Excretion of Polioviruses.—Of a total of 744 stools examined virus was isolated from 101; in two samples both type 2 and type 3 viruses were isolated. All the strains isolated were identified serologically either by complement fixation, using the first or second passage in tissue culture, or by neutralization (Table II). In addition, all 21 strains of type 1 were shown to belong to the LSc subtype. No enteroviruses were isolated from the pre-vaccination faecal samples of any of the participants.

Oral vaccination resulted in the excretion of type 1 virus by seven persons, of type 2 by nine, and of type 3 by 10. All had at least one positive faecal specimen during the first eight days after vaccination. With some, excreted virus was detected on one occasion only, while with others excretion was intermittent or continuous for several weeks. Neither type 1 nor type 2 virus was found after the third weeks, but type 3 virus was detected up to the seventh week (Table III, Fig. 2). In three participants the duration of excretion may have been underestimated, since final faecal samples were not available.

TABLE I.—*Distribution of Poliomyelitis Antibodies Before Oral Vaccination*

Group	Total No.	Type 1		Type 2		Type 3		All Types	No Type
		+	−	+	−	+	−		
A	21	20	1	21	0	20	1	19	0
B	14	9	5	7	7	11	3	5	2
Totals	35	29	6	28	7	31	4	24	2

Group A had previously received inactivated polio vaccine and Group B had not.

Relation of Excretion of Virus to Antibody Levels

Virus excretion was more frequent among those without the corresponding antibody before vaccination than among those with antibody. In the latter group virus excretion occurred more often in those with low antibody levels than in those with high levels. The

TABLE II.—*Identification of Viruses Isolated*

Type	No. Isolated	Identified by C.F.T.		Identified by Neutralization
		1st Passage	2nd Passage	
1	21	16	0	5
2	18	12	2	4
3	64	36	14	14
Totals	103	64	16	23

TABLE III.—*Frequency of Virus Excretion According to the Period Elapsing Since Vaccination*

Period after Vaccination with Each Virus Type (Weeks)	Ratio of Virus Isolations to Samples Collected after Vaccination with Each Virus Type						
	Type 1		Type 3		Type 2		
	Isolations (Type 1)	Samples Examined	Isolations (Type 3)	Samples Examined	Isolations (Type 3)	Isolations (Type 2)	Samples Examined
½	6	39	9	38	3	6	35
1	4	39	10	35	5	7	30
1½	4	33	9	32	3	1	29
2	2	33	5	32	1	2	26
2½	2	30	6	32	1	1	22
3	3	31	4	30	0	1	21
3½	0	32	6	29	0	0	19
4	0	30	2	27			

Interval of 17 weeks between type 1 and type 3. Interval of 4 weeks between type 3 and type 2.

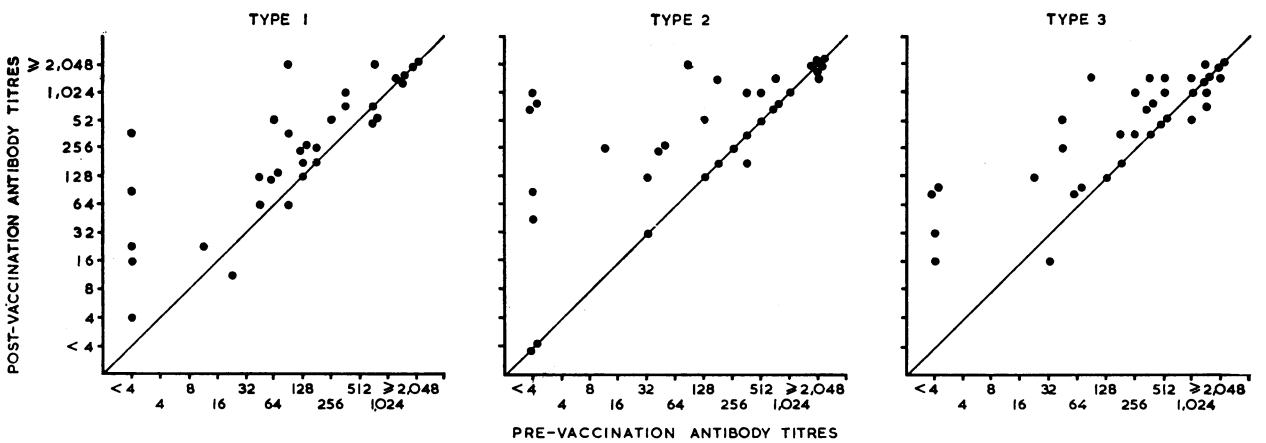

FIG. 1.—Poliovirus neutralizing antibody levels before and after oral vaccination.

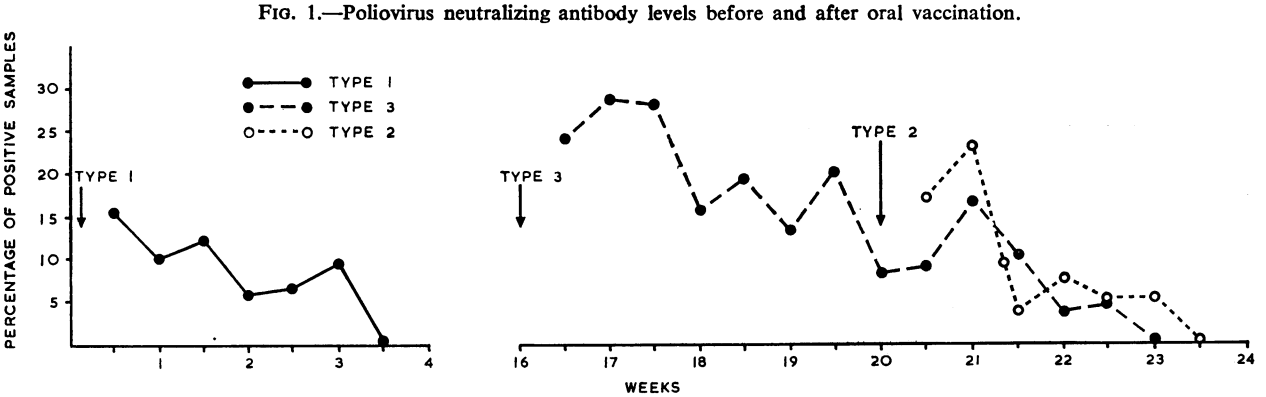

FIG. 2.—Frequency of virus excretion after oral vaccination.

number of persons who excreted virus, in relation to their antibody status before vaccination, is shown in Table IV.

At least fourfold rises in antibody titre were shown by all of the seven excreting type 1 virus, by five of the

TABLE IV.—*Virus Excretion After Receiving Oral Vaccines in Relation to Antibody Status Before Vaccination*

Virus Type	Antibody before Vaccination	No. of Persons	No. of Persons Excreting Virus at Least Once
1	Present	28 } 33	3 } 7
	Absent	5 }	4 }
3	Present	31 } 35	6 } 10
	Absent	4 }	4 }
2	Present	26 } 33	7 } 9
	Absent	7 }	2 }

nine excreting type 2, and by 8 of the 10 excreting type 3. The remainder, comprising four excreting type 2 and two excreting type 3, showed unchanged antibody titres of moderate or high levels (32 to 2,048), and each excreted virus on one occasion only.

The geometric mean antibody titres of the 26 participants who did not excrete type 1 virus rose from 181 to 556, of the 24 who did not excrete type 2 from 98 to 416, and of the 25 who did not excrete type 3 from 389 to 592.

Discussion

The advantages of oral vaccination against poliomyelitis using attenuated polioviruses have been stated by many workers. Manufacture of vaccine from the Sabin strains was started in Britain in 1960, and this is the first account of the use of a British preparation. It is also the first account of oral vaccination of adults in this country.

The study population consisted of normal healthy adults living at home. Two-thirds had already received a course of inactivated poliomyelitis vaccine, and almost all of these had antibodies to the three types of virus before oral vaccination. Of those who had not been vaccinated previously, however, about one-third lacked type 1 antibody, half had no type 2, and one-fifth had no type 3. This level of potential susceptibility was surprising even in a group predominantly professional in social composition, and leaves no room for complacency about the immunity to poliomyelitis of adults in this country. The object of giving oral vaccine to this group was primarily to obtain some experience of its use in individuals who were conveniently situated for close clinical and laboratory observation. As the trial progressed, however, sufficient information became available to compare our results of antibody responses and virus excretion with those reported after using the original Sabin vaccines.

After a dose of 10^5 P.F.U. of each virus type in sequence the response in terms of antibody formation appeared to be as good with the British preparation as with original vaccine (Gelfand *et al.*, 1959). Increases in antibody titre were observed in approximately half those already possessing antibody, and those with lower initial antibody levels showed greater and more frequent antibody rises than those with high initial levels. The results of the primary responses, although few in number owing to the restricted size of the study, were encouraging. All those without initial antibodies to types 1 and 3 developed antibodies after vaccination, while five of the seven without initial antibody to type 2 also formed antibody after vaccination. Viral interference probably accounted for the failure of the two persons to respond to type 2. The antibody levels for types 1 and 3 were lower than after natural immunization as judged by the findings on the pre-vaccination sera, and repeated doses of vaccine might be required in order to achieve higher antibody levels for these types.

Each virus type was isolated most often during the first week after vaccination, and isolations then tended to decline. After type 1 and type 2 vaccines, virus was detected up to the third week, and type 3 up to the seventh week. The number excreting vaccine virus in this series was too small to estimate the duration of excretion for individual types, but the tendency for type 3 to be excreted for longer than types 1 and 2 was in agreement with the finding of Gelfand *et al.* (1959). They found, using the original Sabin vaccines in a study which included infants, that types 1, 2, and 3 were excreted for average periods of 25, 25, and 35 days respectively. Decline in frequency of virus isolation with increasing time is also characteristic of infection with wild poliomyelitis virus strains (Horstmann *et al.*, 1946).

It is interesting to note that primary antibody stimulation in the case of type 2 and secondary antibody stimulation for all three types often occurred without evidence of virus excretion. On the other hand, the excretion of virus was almost invariably followed by the appearance of antibody or an increase in the level of existing antibody.

There were two cases which appeared to be instances of viral interference. Both were originally triple-negative and did not form type 2 antibody, and both continued to excrete type 3 virus after receiving type 2 vaccine. Since type 2 virus was not found in any of the faecal samples the vaccine virus probably failed to establish itself in the gut.

No adverse clinical reactions were observed. One participant noticed a slight soreness of the throat on the fourth day after taking type 1 vaccine; type 1 virus was isolated from his throat.

Summary

Oral poliomyelitis vaccines prepared in Britain from the Sabin attenuated poliovirus strains were given to a group of 43 normal adults using a dose of 10^5 P.F.U. of each type in sequence.

The antibody responses and virus excretion rates were similar to those reported elsewhere after the use of the original Sabin vaccine. No adverse clinical reactions occurred.

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