SEROLOGICAL RESULTS OF ORAL POLIOMYELITIS VACCINE

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The potency of oral poliomyelitis vaccine can only be inferred from assays of virus content on tissue-culture cells. Cytopathic effect on monkey-kidney cells, however, does not measure an important characteristic of the vaccine virus, viz. the capacity to invade the cells of the alimentary tract, multiply, and induce the formation of humoral antibodies. The only reasonably certain way of measuring the potency of the vaccine is by testing its capacity to stimulate the production of neutralizing antibodies in human beings. To this end, a clinic was established at the Poliomyelitis Research Foundation. Staff and other parents were encouraged to bring their children for the 'Bleed and Feed' clinic. A number of doctors with their families volunteered and a number of children were referred by medical practitioners.

The following information on the vaccine manufactured at the Poliomyelitis Research Foundation from the Sabin attenuated virus strains was sought:

1. The dosage of virus necessary to elicit a measurable antibody response.

2. The efficacy of the administration of monovalent, divalent and trivalent preparations of the poliovirus fluids.

3. A vehicle and method of administration that would be acceptable by children and be compatible with good virus viability.

4. As an additional safety measure, the Poliomyelitis Research Foundation pioneered the use of chloroform in the vaccine manufacturing process. The effect of this treatment on the capacity of the virus to induce immunity was not known and had to be ascertained.

The safety of the Sabin virus vaccine at this stage had been shown to be of an order that was higher than several other commonly-used immunizing agents. Opportunity to test the safety of the Poliomyelitis Research Foundation vaccine came during epidemics of poliomyelitis that occurred on Mauritius and in East Africa. About $1\frac{1}{4}$ million doses of vaccine were used and no increase in the number of cases or pattern of untoward reactions were noted.

Vaccine was administered at monthly or longer intervals until immunity to all 3 types of poliovirus was demonstrated by serological tests. Specimens of blood were taken and tested at least 1 month after the last administration of vaccine, by the tube-tissue-culture neutralization technique on monkey-kidney cells using a 'screening' final serum dilution of 1/10 against a challenge dose of 100 $T.C.I.D._{.50}$ of virus. The virus fluids were administered diluted in syrupus simplex or in a raspberry-flavoured sucrose syrup. The virus dosage varied experimentally from 1,000,000 $T.C.I.D._{.50}$ to 100,000 $T.C.I.D._{.50}$ Owing to the limited number of subjects available no evaluation of the effect of virus dosage could be made and the tests had to be confined to ascertaining the efficacy of the administration of a trivalent vaccine with a virus content that was always over 100,000 $T.C.I.D._{.50}$ per poliovirus type, the average dosage being in excess of 300,000 $T.C.I.D._{.50}$. There was evidence that a trivalent vaccine could be effective, but that intertype interference, i.e. domination by, say type 2 virus to the exclusion of types 1 or 3 could limit this efficacy. However, since great advantages from an administrative point of view could be claimed for trivalent vaccine on the basis of a minimum of 3 exposures to each virus type at 3 vaccinations, it was essential to test this in practice.

902 specimens of blood were tested. Among these specimens the sera of 151 children under 6 years of age and of 67 adults were available for the evaluation of the

efficacy of the trivalent vaccine used. Attempts were made to gauge the effect of blending the vaccine so that type 1, as the important epidemic strain, and type 3 were favoured. Type 2 as a dominant invasive strain was restricted. Under the conditions of the test no advantage of this biased blending was apparent. The minimal dose appeared to be effective and no beneficial effect of much increased dosage or of varying the dosage of types could be ascertained. It very soon became clear that repeated exposures to the trivalent vaccine at monthly or longer intervals given in doses over 100,000 T.C.I.D.$_{50}$ gave excellent results by overcoming 'type interference' and interference by other enteroviruses.

As this was partly a pilot trial of the use of the vaccine in a mass vaccination campaign, monovalent vaccination was only done in the few cases where failure to convert negative antibody tests to positive occurred after the fourth or fifth feeds. In a few resistant adult cases large doses of the appropriate monovalent vaccine did not always prove to be immediately effective.

The ideal subjects for testing trivalent vaccine are the 'triple negatives'. Owing to extensive use of Salk vaccine in the Republic of South Africa and the relatively early acquisition of naturally induced antibodies, very few completely negative sera were available for the test. However, as the vaccine was due to be used in a population that was known to be partially immune by natural and artificial means, its performance in individuals lacking only one or two antibodies was of value.

RESULTS

1. *Children*

Of 150 sera tested before vaccination, 70 showed lack of type 1 antibodies; 79 showed lack of type 2 antibodies; 97 showed lack of type 3 antibodies. These had received inactivated poliomyelitis vaccine (Salk type) at an average of 2·3 injections per child. The ages ranged from 6 months to 16 years, with an average age of 6 years 10 months.

After one feed the results were as follows:

Type 1: Conversion from negative to positive for antibodies occurred in 36 out of 70 sera (51·4%).

Type 2: 69 out of 79 (87·3%).

Type 3: 83 of 97 (85·5%).

After the first feed 100 out of 150 (66·6%) had become immune to all three types of poliovirus.

After the second feed. Type 1: A further conversion of 29 negative antibodies (85%) took place out of the 34 failures at the first feed. The total conversions for type 1 became 93%. Type 2: A further 9 conversions took place out of the 10 first-feed failures (90%) and the total conversions after 2 feeds were 78 out of 79 (98%). Type 3: Out of 14 failures after the first feed, 13 converted (93%), bringing the total type 3 immunity up to 96 out of 97 (99%). Triple immunity, 143 out of 150 (95·3%).

A *third vaccination* was given to the second-feed failures for type 1 and all 5 converted from negative to positive, bringing the total conversion to 100% after 1 - 3 feeds. In types 2 and 3, the one failure in each type after the second feed responded to the third feed and the total conversion was 100%. The results are summarized in Table I.

TABLE I. POLIOMYELITIS RESEARCH FOUNDATION CLINIC VACCINE POTENCY TEST: 151 WHITE CHILDREN UNDER 16 YEARS OF AGE, AVERAGE AGE 6 YEARS 10 MONTHS

Vaccination history	*Type 1*	*Type 2*	*Type 3*	*Triple immunity acquired*
Pre-vaccination missing antibodies	70	79	97	—
After 1st feed conversions	$\frac{36}{70}=51{\cdot}4\%$	$\frac{69}{79}=87{\cdot}3\%$	$\frac{83}{97}=85{\cdot}4\%$	$\frac{100}{150}=66{\cdot}6\%$
After 2nd feed conversions of 1st feed failures	$\frac{29}{34}=85\%$	$\frac{9}{10}=90\%$	$\frac{13}{14}=93\%$	—
Total conversions	$\frac{65}{70}=93\%$	$\frac{78}{79}=98\%$	$\frac{96}{97}=99\%$	$\frac{143}{150}=95{\cdot}3\%$
After 3rd feed conversion of 2nd feed failures	$\frac{5}{5}=100\%$	$\frac{1}{1}=100\%$	$\frac{1}{1}=100\%$	—
Total conversions	$\frac{70}{70}=100\%$	$\frac{79}{79}=100\%$	$\frac{97}{97}=100\%$	$\frac{150}{150}=100\%$

2. *Adults*

The sera of 67 adults (16 years of age and over) were available for antibody studies. After one feed 16 of the 24 with negative type 1 antibodies (66%) changed to positive. In type 2, 25 negatives out of 29 became positive (86%). In type 3, 20 out of 29 negatives became positive (69%). After the second feed, 5 out of the remaining 8 type 1 negatives converted (62%), all 4 type 2 negatives converted (100%), and 8 out of 9 type 3 negatives converted (89%). The third feed failed to convert the remaining 3 type 1 and 1 type 3 failures. One more type 1 conversion took place at the fourth feed, and at the fifth feed 1 type 1 negative and the remaining type 3 negative converted.

TABLE II. POLIOMYELITIS RESEARCH FOUNDATION CLINIC VACCINE POTENCY TEST: 67 ADULTS

Vaccination history	*Type 1*	*Type 2*	*Type 3*	*Triple immunity acquired*
Pre-vaccination missing antibodies	24	29	29	—
After 1st feed conversions	$\frac{16}{24}=66\%$	$\frac{25}{29}=86\%$	$\frac{20}{29}=69\%$	$\frac{48}{67}=71\%$
After 2nd feed conversions of 1st feed failures	$\frac{5}{8}=62{\cdot}5\%$	$\frac{4}{4}=100\%$	$\frac{8}{9}=89\%$	—
Total conversions	$\frac{21}{24}=87{\cdot}5\%$	$\frac{29}{29}=100\%$	$\frac{28}{29}=96\%$	$\frac{64}{67}=95{\cdot}5\%$
After 3rd feed conversion of 2nd feed failures	$\frac{0}{3}$	—	$\frac{0}{1}$	—

THE BOKSBURG VACCINE TRIAL

A group of young White children who attended clinics of the Boksburg Municipal Health Service were selected for a study of the efficacy of a trivalent vaccine syrup. (A similar group of non-White children were selected for a similar investigation, but after the first vaccination the attendance was so poor that it was abandoned.) The study was designed to test the immunogenic capacity of the vaccine as diluted in a raspberry-flavoured, red-coloured sucrose syrup, in anticipation of the national poliomyelitis campaign. The test began in November 1960 and the final bleeding took place in June 1961.

Blood specimens of 85 children, ranging in age from 7 months to 14 years, with an average age of 4 years 8 months, were available for study. Of these children 58 (68%) had received inactivated (Salk-type) poliomyelitis

vaccine at an average of 2·4 injections per child. They all lacked one or more antibodies to the 3 types of poliovirus.

The vaccine was administered at intervals of 6 weeks in a 20% sucrose syrup coloured red and flavoured with a synthetic raspberry essence. The pH was adjusted to 7·2 and the 4-ml. dose (one average-sized teaspoonful) contained approximately $2{\cdot}7\times10^5$ T.C.I.D.$_{50}$ of type 1 virus, $1{\cdot}9\times10^5$ of type 2 and $5{\cdot}3\times10^5$ of type 3. Blood was taken 1 month or more after each feed and vaccination was discontinued in all cases where triple immunity was demonstrated. The results are summarized in Table III.

TABLE III. BOKSBURG VACCINE TRIAL: ANTIBODIES PRESENT AFTER 1, 2, 3 OR 4 VACCINATIONS IN 85 WHITE CHILDREN (AVERAGE AGE 4 YEARS 8 MONTHS)*

Number of vaccinations	*Type 1*	*Type 2*	*Type 3*	*Complete immunity acquired*
After 1 feed	$\frac{25}{46}=54{\cdot}3\%$	$\frac{49}{54}=90{\cdot}7\%$	$\frac{65}{75}=86{\cdot}7\%$	$\frac{56}{85}=65{\cdot}9\%$
After 2 feeds	$\frac{37}{46}=80{\cdot}4\%$	$\frac{54}{54}=100\%$	$\frac{73}{75}=97{\cdot}3\%$	$\frac{75}{85}=88{\cdot}2\%$
After 3 feeds	$\frac{45}{46}=97{\cdot}8\%$	—	$\frac{75}{75}=100\%$	$\frac{84}{85}=98{\cdot}8\%$
After 4 feeds	$\frac{46}{46}=100\%$	—	—	$\frac{85}{85}=100\%$

* Vaccination discontinued when triple immunity was demonstrated.

Discussion

The results of these vaccine potency tests showed that type 2 is the dominant virus strain and causes a distinct suppression of type 1 virus activity and to a lesser extent of type 3 at the first feed. In spite of this interference one vaccination could achieve from 51% to 71% protection against the important type 1 poliovirus strain in individuals who were susceptible. Two vaccinations were sufficient to overcome type interference and other enterovirus interference to achieve a very high degree of immunity to all 3 types. From an epidemiological point of view the third feed does not seem necessary. However, it is inexpensive and easy to administer, and a virtual 100% immunity can be obtained by giving it. This is a unique achievement for an immunizing agent used on such a scale and stimulates the hope that the disease will be eradicated. In administrative practice three vaccinations should always be offered because 100% attendance on all occasions is very seldom achieved under average field or clinic conditions.

These studies showed clearly that the Sabin-type oral vaccine as manufactured at the Poliomyelitis Research Foundation and the method of field administration were highly effective in achieving a near-perfect immunization after 2 - 3 feeds, under the prevailing urban conditions, which were favourable for the routine application of anti-poliomyelitis immunizing procedures.

THE NATIONAL CAMPAIGN: EFFICACY CONTROL AT BOKSBURG

For the purposes of serological evaluation during the national campaign, blood specimens taken at Boksburg Municipal Health Centres 1 month after each phase of the poliomyelitis campaign were tested on monkey-kidney tube-tissue cultures for the presence of neutralizing antibodies. A final serum dilution of 1/10 was used against a 100 T.C.I.D.$_{50}$ challenge dose of virus. No pre-vaccination specimens were available and results were evaluated as complete immunity achieved after each feeding. Failures to convert were noted.

1. *White Children*

Blood specimens of 98 White children ranging in age from 5 months to 10 years (average age 2 years 3 months) were available for antibody determinations. Of these children 69 (70·4%) had received inactivated poliomyelitis vaccine (Salk type) at an average of 3·4 injections per child. During November 1960, when a campaign was conducted to immunize against type 1 poliovirus, 89 of this group (90·8%) had received a dose of type 1 oral vaccine. The bleeding was done about 1 month after each of the 3 trivalent vaccinations during the national campaign in May, July and September 1961. The results are shown in Table IV.

TABLE IV. BOKSBURG CAMPAIGN CONTROL: ANTIBODIES PRESENT AFTER 1, 2 OR 3 VACCINATIONS IN 98 WHITE CHILDREN (AVERAGE AGE 2 YEARS 3 MONTHS)*

Number of vaccinations	*Type 1*	*Type 2*	*Type 3*	*Complete immunity acquired*
After 1 feed	$\frac{94}{98}=95{\cdot}9\%$	$\frac{94}{98}=95{\cdot}9\%$	$\frac{89}{98}=90{\cdot}8\%$	$\frac{86}{98}=87{\cdot}8\%$
After 2 feeds	$\frac{96}{98}=98\%$	$\frac{98}{98}=100\%$	$\frac{98}{98}=100\%$	$\frac{96}{98}=98\%$
After 3 feeds	$\frac{97}{98}=99\%$	—	—	$\frac{97}{98}=99\%$

* Vaccination discontinued when triple immunity was demonstrated.

After the first feed 87·8% of children were immune to all 3 types of poliovirus. This figure was increased to 98% after the second vaccination. After the third feed 99% of children had developed antibodies against all 3 types of poliovirus, leaving a single failure of type 1 to convert after 3 vaccinations.

2. *Non-White Group*

Blood specimens of 136 non-White children at an average age of 4 years 1 month were available for antibody determinations. None of these had received injections of inactivated Salk-type vaccine and no previous oral vaccine had been given them. The results are shown in Table V.

TABLE V. BOKSBURG CAMPAIGN CONTROL: ANTIBODIES PRESENT AFTER 1, 2 OR 3 VACCINATIONS* IN 136 NON-WHITE CHILDREN (AVERAGE AGE 4 YEARS 1 MONTH

Number of vaccinations	*Type 1*	*Type 2*	*Type 3*	*Complete immunity acquired*
After 1 feed	$\frac{127}{136}=93{\cdot}4\%$	$\frac{129}{136}=94{\cdot}8\%$	$\frac{122}{136}=89{\cdot}7\%$	$\frac{111}{136}=87{\cdot}6\%$
After 2 feeds	$\frac{134}{136}=98{\cdot}5\%$	$\frac{134}{136}=98{\cdot}5\%$	$\frac{131}{136}=96{\cdot}3\%$	$\frac{127}{136}=93{\cdot}4\%$
After 3 feeds	$\frac{136}{136}=100\%$	$\frac{136}{136}=100\%$	$\frac{134}{136}=98{\cdot}5\%$	$\frac{134}{136}=98{\cdot}5\%$

* No previous vaccination. Vaccination was discontinued when triple immunity was demonstrated.

Complete immunity was present in 87·6% after the first vaccination. This increased to 93·4% at the second and 98·5% at the third feed.

DISCUSSION

No cases of paralytic poliomyelitis were reported from these vaccinated groups, comprising more than 470 children and 67 adults.

In the two trial experiments where pre- and post-vaccination sera were available a definite suppression of type 1 at the first vaccination was demonstrated. This was overcome to a remarkable degree at the second vaccination, where interference by type 2 and type 3 was no longer highly active. The figure of about 90% for type 1 after 2 vaccinations, and its increase to near 100% after 3 vaccinations, clearly justifies the use of trivalent vaccine. Type 2 shows up as an excellent antigen, and there is the added advantage that the high conversion rate of 86 - 87% after one feed confers some cross-protection against type 1 poliovirus. The response to type 3 was good and remained so throughout all the tests.

The occasional 'resistance' to vaccination that occurs, mainly to type 1 and type 3 poliovirus, particularly in adults, is being investigated — in some cases conversion could only be achieved after 6 vaccinations. Minimal quantities of antibodies were demonstrated in some instances, and it may be that this prevents a 'take' by the poliovirus concerned. The cross-immunity conferred by type 2 virus is also a possible factor.

The tests done before and during the national campaign showed that good results were being achieved under field conditions by the vaccine as made by the Poliomyelitis Research Foundation, and that the 3 methods of application—automatic syringe (spray) method, drop-on-sweet method, and 'teaspoon and syrup' method—were effective, at least under urban conditions where good attention is paid to routine immunization procedures. This finding has been confirmed by the marked reduction of cases of paralytic poliomyelitis reported throughout the country since the completion of the campaign. In the test groups the response of the White and non-White groups after one feed was about the same and no doubt reflects the effects of previous partial artificial immunization in the White group and the relatively greater natural immunity possessed by the non-Whites. If the prevalence of other enteroviruses was greater among the non-White groups, with their lower standard of living, the vaccine overcame any possible 'interference' action.

SUMMARY

1. Serological tests on the administration of a trivalent oral poliovirus vaccine showed that 66·6% of partially immunized individuals became triply immune after one trivalent vaccination, 95·3% after two vaccinations, and 100% after three vaccinations. Suppression of type 1 at the first feed is corrected at the second and third feeds.

2. Similar results were obtained in a group of 85 White children at the Boksburg Municipal Health Centres, when 65·9%, 88·2%, 98·8% and 100% conversion after 1, 2, 3 and 4 feeds were achieved.

3. Adults show occasional resistance, but 95·5% immunity was achieved after 3 vaccinations.

4. Sera obtained during the national campaign showed good response in both White and non-White groups of children tested, and 99% and 98·5% complete immunity, respectively, was achieved after 3 trivalent vaccinations.

5. No paralytic cases or serious reactions were encountered in the 470 children and 67 adults during the study.

6. The vaccine as produced by the Poliomyelitis Research Foundation was shown to be efficacious by seroconversion studies. This finding was confirmed by the unprecedented reduction in the number of paralytic poliomyelitis cases reported in the Republic of South Africa. The safety of the vaccine also was confirmed by experience in the vaccination campaign.

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