VACCINATION AGAINST POLIOMYELITIS WITH LIVE VIRUS VACCINES

7. EFFECT OF PREVIOUS SALK VACCINATION ON VIRUS EXCRETION

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The problem of whether the large-scale use of formalin-inactivated poliomyelitis vaccine (Salk vaccine) can effect the circulation of polioviruses in a community is of considerable practical importance. Langmuir (1955) expressed a belief that after the wide acceptance of Salk vaccine in the United States which he anticipated polioviruses would virtually disappear from the country. The utilization of the vaccine has not yet been as great as had been hoped either in the United States or in the United Kingdom, and in many areas it has never reached levels at which one could expect any dramatic effect in reducing virus circulation *even* if three doses of vaccine did render a person unlikely to transmit the virus to others. More recently Alexander (1960) and Langmuir (1960) have drawn attention to the fact that outbreaks and epidemics of poliomyelitis in the United States are becoming limited to the poorly vaccinated areas and show little tendency to spread to contiguous better-vaccinated areas. In addition, the overall reduction in the incidence of poliomyelitis has been greater than can reasonably be accounted for simply on the basis of the individual protection afforded by inactivated virus vaccine. Too short a period has elapsed in the United Kingdom since the introduction of mass vaccination to determine whether an apparently similar trend is significant.

In contrast to these epidemiological observations the evidence from the majority of laboratory trials and small field investigations has indicated that previous Salk vaccination has no effect on virus excretion and dissemination during a subsequent poliovirus infection (Lipson *et al.*, 1956; Horstmann *et al.*, 1957; Paul *et al.*, 1957; Fox *et al.*, 1958; Davis *et al.*, 1958). Most of these studies were made in children who had not received a complete course of immunization with potent vaccine, and quantitative data on serum antibody levels and virus excretion are often incomplete. Nevertheless, in these various studies there is a considerable weight of evidence against the hypothesis that the use of Salk vaccine in a community will modify poliovirus transmission.

Experiments using chimpanzees have to some extent given results which conflict with those obtained in

human studies. Howe (1957) showed that the faecal excretion of a virulent type I poliovirus was significantly reduced in animals which had received three doses of Salk vaccine. These observations were extended by Bodian and Nathanson (1960), who showed that passively induced low levels of neutralizing antibody were associated with reduced excretion of virulent poliovirus from the throat of chimpanzees. They also found that in animals with antibody levels of 1 in 500 there was some suggestion of inhibition of faecal virus excretion, but they did not investigate the effects of higher antibody levels.

There are many difficulties in attempting to estimate field effects of vaccination indirectly from small laboratory trials. For example, large doses of attenuated vaccine viruses have to be used in human studies, whereas it is the effect on the transmission of very much smaller doses of virulent viruses that is of importance. In the case of chimpanzee experiments in which virulent viruses can be fed, some doubt has been expressed about the similarity of the infective process in these animals as compared with man. Another major difficulty is that a comparatively small quantitative reduction in the amount of virus excreted by infected Salk-vaccinated individuals (which would be difficult to measure) might have a profound epidemiological effect.

In the two trials described below we have attempted to throw further light on this problem.

Materials and Methods

Live Poliovirus Vaccines.—Sabin types I and II attenuated vaccine viruses were obtained through the Poliomyelitis Vaccines Committee of the Medical Research Council.

Inactivated Poliovirus Vaccines.—British commercial vaccines and "purivax" (kindly supplied by Merck Sharp and Dohme) were used.

Throat Swabs.—Both tonsils and the posterior pharyngeal wall were swabbed with a cotton-wool swab on a wooden stick. The swab was broken off into a bijou bottle containing tissue-culture medium.

Virus Titrations.—Four tissue-culture tubes were inoculated per $\log_{10}$ dilution.

Other details of techniques were as described in a previous paper (Dane *et al.*, 1961).

Results

Trial No. 1

Two groups, A and B, of 16 children each were selected to take part in this trial. Group A were given two 1-ml. doses of standard commercial Salk vaccine a month apart and then a fortnight after the second injection fed $10^{5.6}$ TCD₅₀ of Sabin type II attenuated poliovirus. Group B were fed the same dose of Sabin type II poliovirus on the same day, but had received no prior immunization with Salk vaccine. Blood samples were taken from the children in group A on the day the first dose of Salk vaccine was given, and from children in both groups on the day Sabin type II virus was fed. Faecal specimens were collected daily from the third day before feeding type II virus until the 20th day after. Nine children who were found to be excreting enteroviruses at the time of feeding, and one child from whom only a few specimens were obtained were excluded from the study. All faecal specimens from the remaining 22 children were assayed for virus content.

The ages of the 10 selected children in group A ranged from 5 to 15 months (average 7.7 months), and of the 12 selected children in Group B from 6 to 17 months (average 12 months). None of these children had active immunity to any poliovirus type, though five in group A and two in group B still had low-titre residual maternal immunity to type II poliovirus. The type II neutralizing antibody titres in the group A children after two doses of Salk vaccine ranged from 1 in 11 to 1 in 5,800 (geometric mean titre 1 in 525), better responses being obtained in children without detectable maternal antibody.

The geometric mean titre of virus excreted by children in the two groups on different days is shown in Fig. 1. In addition to comparing the faecal excretion of the two groups, which unfortunately could not be matched for age, the peak-titre faecal excretion of the five children in group A with type II antibody titres of <1 in 1,000 was compared with that of the five children in the same group with titres of >1 in 1,000 (see Table). There was some difference between the peak-titre faecal virus excretion by these two small groups ($0.05 < P < 0.02$).

FIG. 1.—Geometric mean titre of faecal virus excretion by children in groups A and B.

Proportion of Faecal Specimens With $\geq 10^5$ TCD₅₀ Virus/g. Collected from Children Having Pre-feeding Type II Poliovirus Antibody Titres of $<1:1,000$ and $>1:1,000$

Antibody before Feeding	Proportion of Faecal Specimens with $\geq 10^5$ TCD ₅₀ Virus/g.
$<1:1,000$	59/100
$>1:1,000$	38/90

Trial No. 2

This trial was conducted in a rather similar manner to the previous one, though more potent Salk vaccine was used and Sabin type I, and not type II, attenuated poliovirus was fed.

Seven children were given three 1-ml. injections of potent Salk vaccine (purivax) at fortnightly intervals and then a fortnight later fed 10^6 TCD₅₀ of Sabin type I virus. Three children were given two injections of standard commercial Salk vaccine a month apart and then fed with the 10^6 TCD₅₀ of Sabin type I virus, and nine children received no prior immunization with Salk vaccine, but were fed with the same dose of Sabin type I virus as the others. Faecal specimens were collected daily from all children from the third day before feeding vaccine virus until the 21st day after. Additional specimens were collected on the 28th and 35th days. Throat swabs were taken on the day of virus feeding

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and then daily for 14 days. All specimens were assayed for type I poliovirus content. At the start of the trial none of the children had any active or passive immunity to type I poliovirus, though 13 had been previously infected either directly or by contact with Sabin type II poliovirus and had type II antibody.

The seven children who received three doses of potent Salk vaccine had antibody titres to type I poliovirus ranging from 1 in 725 to 1 in 8,190 (geometric mean titre 1 in 2,750) at the time of feeding. Of the three children who received two doses of commercial Salk vaccine one had an antibody titre against type I virus of 1 in 512, and the other two had titres of <1 in 8. For the purpose of analysing the throat and faecal excretion of the attenuated type I virus the children were divided into two groups: group C, those with type I antibody following Salk vaccination; and group D, those without type I antibody. The geometric mean titre of type I antibody in the children of group C was 1 in 2,230 on the day Sabin type I poliovirus was fed. The two groups of children were living in the same institutions and were nearly comparable as regards age, sex, and exposure to naturally occurring enteroviruses. The ages of the children ranged from 6 to 18 months, the average age in group C being 10.5 months and in group D 11.6 months. One child in group C and two children in group D were excreting a non-polio enterovirus at the time of feeding, and a number of children excreted the same virus at some time during the trial. This virus may have interfered to some extent with the growth of type I virus in certain children, but it was unlikely to have affected one group more than the other.

After being fed type I virus five out of the eight children in group C became low-titre faecal excretors for periods of from one to four days during the subsequent 21 days. The remaining three did not excrete virus in the faeces and none of group C became throat carriers during the 14 days of sampling. In group D 9 out of 11 children became high-titre faecal virus excretors and four of them also became throat virus excretors. One child in group D failed to become infected, and one child excreted virus in the faeces at low titre for only a short period. On the 28th day and the 35th day three and two group D children respectively were found still to be excreting virus. No group C children were excreting virus on the 28th day, but two were excreting on the 35th day. It was not possible from these isolated positive specimens to determine the extent of intestinal multiplication during this late period.

The percentages of children in the two groups found to be excreting faecal virus at $\geq 10^2$ TCD₅₀ per g. and $\geq 10^4$ TCD₅₀ per g. are shown in Figs. 2 and 3 respectively. The numbers of children in the two groups excreting throat virus at these levels are shown in Figs. 4 and 5.

Discussion

In the first trial a group of children with a type II geometric mean antibody titre of 1 in 525 induced by Salk vaccination when fed Sabin type II attenuated virus excreted this virus in their faeces in the same fashion as a control group of children without active immunity.

In the second trial the faecal excretion of Sabin type I virus by a group of children with a type I geometric mean antibody titre of 1 in 2,230, also induced by Salk vaccination, was greatly reduced. The type I antibody titres in this group of children were higher than those

FIG. 2.—Percentage of children in groups C and D excreting faecal virus at levels $\geq 10^3$ TCD₅₀/g. X---X = Group C. O—O = Group D.

FIG. 3.—Percentage of children groups C and D excreting faecal virus at levels $\geq 10^4$ TCD₅₀/g. X---X = Group C. O—O = Group D.

FIG. 4.—Number of children in groups C and D excreting throat virus at levels $\geq 10^3$ TCD₅₀/g. X---X = Group C. O—O = Group D.

FIG. 5.—Number of children in groups C and D excreting throat virus at levels $\geq 10^4$ TCD₅₀/g. X---X = Group C. O—O = Group D.

reported in comparable studies conducted by others, and higher than those likely to be achieved, except with the most potent Salk vaccines; therefore it is not surprising that this type of reduction in faecal virus excretion has not previously been reported.

It is well known that immunity after either a natural or an attenuated poliovirus infection differs from that induced by Salk vaccination. Immunity after infections is generally accompanied by moderate or high levels of circulating antibody and a relative resistance to re-infection of the intestinal tract. In contrast, immunity induced by Salk vaccination may be accompanied by the same levels of circulating antibody as after natural infections, but without any detectable resistance to infection of the intestinal tract. (This latter point was demonstrated clearly in our first trial.)

To explain this phenomenon a concept of local intestinal immunity which develops only in response to intestinal infection has been put forward by Paul *et al.* (1959). Our finding that an apparently similar type of intestinal resistance to that which follows infections can be induced by the antigenic stimulus of a killed virus placed at a site distant from the intestinal tract is of interest as it conflicts with the hypothesis that a purely local immunity is the only mechanism producing intestinal resistance.

In the first trial it was not possible to take throat swabs, and thus we have no data on any inhibition of throat virus excretion which might have occurred among the children of group A. In the second trial we found no evidence of throat virus excretion in any of the nine Salk-vaccinated children, whereas 4 of the 11 non-immune children became throat carriers.

Though this finding confirms that immunity induced by Salk vaccine can prevent virus multiplication in the throat, it does not indicate what antibody level is necessary to achieve this. However, Bodian and Nathanson (1960) found that in chimpanzees relatively low levels of antibody may have a powerful inhibiting effect on viral excretion from the throat. Because of the general similarity of the infective process in chimpanzees and man we think that this is also likely to be true in man.

The importance of pharyngeal virus in the spread of polioviruses has often been disputed. Many investigators, including ourselves, have shown that virus transmission to close contacts can occur in the absence of throat virus. This in no way diminishes the likelihood that pharyngeal virus may at times be of importance, for example, in extrafamilial spread, as suggested by Fox (1959). The attenuated poliovirus vaccines, unless fed in very large doses, seldom colonize the pharynx (Paul *et al.*, 1959), and this may perhaps also be true of the rather similar naturally occurring avirulent strains. In contrast, epidemic strains of poliovirus do colonize the pharynx, even though the infecting dose of virus must be very small. We believe that the apparent community-protective effect of Salk vaccine described by Langmuir (1960) and Alexander (1960) may well be due to a reduction in pharyngeal transmission of virulent polioviruses after the widespread use of Salk vaccine in some areas of the United States.

In conclusion it should be remembered that with this problem it is quite impossible in small trials to parallel exactly the natural state of affairs under investigation. Epidemiologists may in the end produce the most precise answer from a broader study of events.

Summary

Two trials were made to measure the effect of previous Salk vaccination on virus excretion by children fed with oral poliomyelitis vaccine viruses.

In the first trial a group of children received two doses of Salk vaccine and were then fed Sabin type II vaccine virus. A control group of children received only Sabin type II vaccine virus. Faecal virus excretion by the two groups of children was similar. No investigation of throat virus excretion was done.

In the second trial a group of children received either two or three doses of Salk-type vaccine, and were then fed Sabin type I vaccine virus. A control group of children received only Sabin type I vaccine virus. Faecal virus and throat virus were excreted in smaller amounts for a shorter time by the Salk-vaccinated group than by the control group.

The possibility that widespread use of Salk vaccine might influence the spread of naturally occurring virulent polioviruses is discussed.

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The outlines and objectives of this investigation were approved by the Medical Research Council Poliomyelitis Vaccines Committee.

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"In Ceylon the average expectation of life at birth last year was 62.5 years which is the highest so far recorded. That the child born in Ceylon to-day can reasonably expect to reach this age is in heartening contrast to the position about 40 years ago when a child born could have expected normally to live up to only 30 or 31 years." (Mr. A. P. Jayasuriya, Ceylon Minister of Health.)

VACCINATION AGAINST POLIOMYELITIS WITH LIVE VIRUS VACCINES

8. CHANGES IN SABIN TYPE I ORAL VACCINE VIRUS AFTER MULTIPLICATION IN THE INTESTINAL TRACT

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During the course of a trial of Sabin type I attenuated poliovirus (Dick *et al.*, 1961) some of the changes which occurred in the virus after human passage were investigated, and these are described below. The reason why changes in the characteristics of infectious vaccine viruses after human passage are of importance is discussed elsewhere (Dane *et al.*, 1961).

Materials and Methods.—These were as described in previous papers (Dane *et al.*, 1961; Dick *et al.*, 1961).

Results

Eleven children aged 7 to 17 months (average 11.6 months) who were without antibody—that is, <1:8—to type I poliovirus were fed with 10^6 TCD₅₀ of Sabin type I attenuated poliovirus. After feeding, daily faecal specimens were collected for 21 days and again on the 28th and 35th days, and throat swabs were taken for the first 14 days. In addition, faecal specimens were collected daily from 23 children in contact with those who had been fed. All specimens were tested for the presence of type I poliovirus, and, if positive, the amount of virus was estimated by titration. Eight of the children fed became good faecal virus excretors and four of them also excreted virus from the throat. The immune status of the contacts was unknown, but three became infected during the study period.

Faecal and throat virus strains isolated from children fed the vaccine virus and from their contacts were tested for neurovirulence in monkeys and for the *t* and *d* marker character (Dane *et al.*, 1961).

Monkey Neurovirulence Tests.—In all, 10 strains of first- and second-human-passage type I vaccine viruses were tested in rhesus monkeys by the intracerebral inoculation of large amounts of virus ($10^{5.8}$ to $10^{6.8}$ TCD₅₀). The results of these tests are shown in Table I.