The successful immunization of children with an orally administered attenuated type II poliovirus has been described by Koprowski, Norton, Jervis, Nelson, Chadwick, Nelsen, and Meyer (1956). The virus used was the TN type II rodent-adapted strain (Koprowski, Jervis, and Norton, 1952; Koprowski et al., 1956). One hundred and fifty children in institutions in the United States of America were vaccinated with this virus between 1950 and 1955. Prior to vaccination all children were without antibody to type II poliovirus; after vaccination nearly every child (in one series 86 out of 87) developed antibody. Reasonably high antibody titres were found and this antibody was shown to persist in a small number of children who were studied over a period of five years. No reactions or illnesses attributable to vaccination were observed in any subject vaccinated.

The TN vaccine virus which had been adapted to growth in mouse C.N.S. did not produce cytopathogenic changes when inoculated on to monkey-kidney-tissue cultures. Only when it was inoculated in large amounts intraspinally or intracerebrally into monkeys did TN vaccine virus cause paralysis or histological lesions. Virus was found in the faeces of about half the children vaccinated, and, like the vaccine virus, it was found to be non-cytopathogenic for tissue culture. For this reason it was detected by the intraspinal inoculation of mice. The last day after vaccination on which virus was found in the faeces was the 23rd day. The highest titre of virus found in any faecal specimen was 10 mouse-paralysing doses, (MPD, per gramme (Koprowski et al., 1956). No further information was available about the nature of the faecal virus, but, in view of the very small amounts of virus excreted, transmission from vaccinated subjects to their contacts seemed highly improbable. TN virus was never recovered from the throats or from the blood of vaccinated children.

These results were encouraging. The TN type II vaccine appeared, so far as could be judged from the laboratory data and the relatively small numbers vaccinated, to be both safe and immunogenic. With this background a small trial was planned in Northern Ireland to confirm Dr. Koprowski's findings. The possible value of an attenuated type II vaccine in reducing the rate of paralytic poliomyelitis caused by all three poliovirus types is quite unknown, but it seemed reasonable to conduct initial studies with type II virus on general grounds of safety both to the individuals vaccinated and to the community.

The trial was executed in the following manner. In the first place the laboratory characteristics of the vaccine virus as described by Dr. Koprowski were confirmed, then a small number of adults were fed with the vaccine. These adults were members of the laboratory staff and medical students. Later a few infants and the children of the investigators were vaccinated. Finally, the vaccine was fed to a larger group of children. The parents of most of these children were medical practitioners or members of the university staff. A total of 190 individuals were vaccinated between February and July, 1956. Many of the children were vaccinated without prior knowledge of their immune status, and among these there were some already immune to type II poliovirus; however, the majority of children and all the adults were without type II antibody at the time of vaccination. Laboratory investigations and clinical observations were planned in the light of available data. Due allowance had to be made for the fact that normal volunteer subjects leading normal lives were being studied. While in some ways this increased the value of our observations, it meant that the collection of specimens
lacked the precision that might have been attained in an institution or penitentiary. Also such studies as the detection of viramia, which involved daily bleeding, could not be carried out with volunteer children.

Materials and Methods

The vaccine was supplied by Dr. H. Koprowski. It had been prepared from a pool of mouse brain and cord suspension representing the 29th passage in PRI mice of TN type II poliovirus.

Tissue-culture Techniques.—Second-passage monkey-kidney-cell monolayers in roller tubes were used in the virus neutralization tests and for the isolation of cytopathogenic viruses. Lactalbumin medium described by Melnick (1955) was employed throughout.

Virus-neutralization Tests.—These were performed by the method described previously (Dane, Dick, Connolly, Briggs, and McLeod, 1956) using 100 TCD50 of virus. British Standard immune serum against type II poliovirus was used as a control. The titre of this serum was 1 in 640. In this study donors of sera which did not neutralize 100 TCD50 of virus at 1 in 4 dilution were regarded as being without antibody.

Virus Isolations.—The techniques employed for the isolation of virus from faeces, throat swabs, and blood specimens are described elsewhere (Dick, Dane, Fisher, Connolly, and McKeown, 1957).

Storage of all specimens was at −20°C. The vaccine was stored at −65°C.

Monkeys.—Rhesus monkeys of approximately 7 lb. (3.2 kg.) weight were employed. They were anaesthetized with thiopentone sodium. Intracerebral inoculations were made by the method described by Bodian, Morgan, and Schwerdt (1950). Intraspinal inoculations were made into the lumbar enlargement by placing the inoculum between the second and third lumbar spines. Monkeys were examined each day up to 28 days after intracerebral inoculation and for 14–18 days after intraspinal inoculation. On most days the monkeys were removed from the cages and tested manually for evidence of paralysis. The power of the muscles of the upper and lower limbs was recorded, following the method described by Bodian (1948).

Mice.—The mice were of the VSBS strain of Swiss mice. Intracerebral and intraspinal inoculations were done by the usual methods.

Histology.—Animals were perfused by the method described by Bodian (1948), using 10% formalin containing 1% acetic acid. The usual blocks from brain, brain stem, and cord were embedded in paraffin and stained with galloycyanin.

Laboratory Tests of the Vaccine

The batch of vaccine used in this trial was tested in the following manner by Dr. H. Koprowski at Lederle Laboratories, Pearl River, New York: (1) Monkeys were inoculated by the intraspinal and the intracerebral routes with the vaccine. (2) Guinea-pigs were inoculated subcutaneously and intracerebrally. (3) The vaccine was inoculated on to a variety of bacteriological media. These tests were repeated in our own laboratory. No evidence was obtained in either laboratory that any microorganism other than TN type II virus was present in the vaccine.

Results of the inoculation of monkeys with the vaccine are shown in Table I. Our results were similar to those of Dr. Koprowski; we found that large amounts of virus given by the intracerebral route caused no paralysis. But when given by the intraspinal route the vaccine virus did cause paralysis in some monkeys.

We found that the vaccine virus did not produce cytopathogenic changes in monkey-kidney-tissue culture; neither did it appear to interfere with the growth of small amounts of a cytopathogenic type II poliovirus in tissue culture.

<table>
<thead>
<tr>
<th>Source of Virus</th>
<th>Vol. of Inoculum and Route</th>
<th>Loga TCD50 Inoculated</th>
<th>Inoculated</th>
<th>With Paralysis</th>
<th>Lesions of Polio in CNS</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse brains and cords of 29th passage in PRI mice</td>
<td>0.5 ml i.c.</td>
<td>45</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td>Not examined for lesions</td>
</tr>
<tr>
<td>(Vaccine used for trial)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 ml i.c., 4°C</td>
<td>23</td>
<td>4</td>
<td>0</td>
<td>1</td>
<td></td>
<td>Not examined for lesions</td>
</tr>
<tr>
<td>0.5 ml i.c.</td>
<td>1.4</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td></td>
<td>Not examined for lesions</td>
</tr>
<tr>
<td>1 ml i.c.</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>Paralyzed, not examined for lesions</td>
</tr>
<tr>
<td>1 ml i.c., 0°C</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>Not examined for lesions</td>
</tr>
<tr>
<td>0.1 ml i.c., 0°C</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td></td>
<td>Not examined for lesions</td>
</tr>
<tr>
<td>0.1 ml i.c., 0°C</td>
<td>0.1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td>Not examined for lesions</td>
</tr>
<tr>
<td>0.1 ml i.c., 0°C</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
<td>Not examined for lesions</td>
</tr>
</tbody>
</table>

* Titration by Dr. H. Koprowski at New York. i.c. = intracerebral, i.s. = intraspinal. MPD50 = mouse intraspinal paralyzing dose 50.

Vaccination of Adults

Twenty-one adults were fed 500,000 MPD50 of TN vaccine diluted in 4 ml of milk. Tests had been completed for 18 (10 men and 8 women); their ages ranged from 20 to 50 years. None had type II antibody prior to feeding and nine had no antibody to any of the three poliovirus types. Only four subjects had developed type II antibody when their blood was tested two to three months after vaccination (see Fig. 1). Six of the non-responders were fed a second time with the same dose of virus contained in gelatin capsules. Once more none of these subjects developed antibody. Of the four subjects who did develop antibody two were men aged 21 and 22, and two were women aged 44 and 50 years. No common factor has yet been found which might explain the failure of vaccination in 14 out of the 18 adults.

Throat swabs and blood samples were collected daily for the first 10 days after vaccination from 10 of the adults, and faecal specimens were also obtained during the first two weeks (see Table I). No viramia was detected in any subject. A trace amount of virus was recovered from the throat of one subject from a pool of swabs taken during the first three days after vaccination. This virus was detected by mouse inoculation, but could not be detected in tissue culture. No virus was recovered in any faecal specimen either by mouse inoculation or in tissue culture, but in the light of later experience we consider that too few specimens were collected for too short a period for any conclusions
to be reached on the faecal excretion of TN virus by adults. In addition, it must be noted that only 3 of the 10 adults on whom these investigations were made developed antibody.

**Table II.—Summary of Results for Adults Vaccinated with TN Vaccine**

<table>
<thead>
<tr>
<th>Group</th>
<th>No. In Group</th>
<th>No. with Virus in Faeces</th>
<th>No. with Virus in Throat</th>
<th>No. with Virus in Blood</th>
<th>No. Who Developed Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested for virus excretion</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Not tested for virus excretion</td>
<td>8</td>
<td>N.T.</td>
<td>N.T.</td>
<td>N.T.</td>
<td>1</td>
</tr>
</tbody>
</table>

* Daily throat swabs and blood samples for 10 days after vaccination.
† Trace of virus in pool of throat swabs from days 1–3, not recovered from individual specimens days 1, 2, and 3.
N.T. = Not tested.

**Vaccination of Children**

One hundred and fifty-five children between the age of 10 months and 15 years were fed TN vaccine (500,000 MLD50 in 4 ml. milk) during May and June. Paired specimens of sera, the first taken at the time of vaccination and the second six or more weeks later (see Table III), were available for testing from 124 who had no type II antibody at the time of vaccination. The ages of the children in this group are shown in Table IV: there were 58 boys and 66 girls. Twenty-eight children (23%) failed to develop type II antibody after vaccination. The remaining 96 (77%) developed antibody titres ranging from a trace to 1 in 2,048 (see Fig. 2). The geometric mean antibody titre for these 96 children is 1 in 66. No correlation between the time when the second specimen of blood was taken and the antibody titre has been found. Sex did not appear to influence the response to vaccination. Among this group of 124 children there were 24 pairs of siblings. Siblings were vaccinated and bled on the same day. In 18 pairs both siblings developed antibody, in four pairs neither sibling developed antibody, and in two pairs only one sibling developed antibody. If all the individuals in this group of siblings had responded independently then it would have been expected that 13.5 of the pairs of siblings would have developed antibody, 1.5 pairs would have developed no antibody, and in 9 pairs one sibling would have developed antibody. The difference between the observed and expected antibody response of the siblings is highly significant ($x^2 = 8.7111; 0.01 > P > 0.001$).

Of the 20 pairs of siblings where either or both children developed antibody, the higher titre was found in the younger child in 15 cases. In one case the titres were equal and in the remaining four the elder child developed the higher titre. If the antibody titres had not varied with age we would not have expected this difference ($x^2 = 5.0; 0.05 > P > 0.02$).

It was not practicable to collect throat swabs or daily blood samples from any of the children. Faecal specimens obtained from 18 of them during the first few weeks after vaccination were tested for the presence of virus both by the intraspinal inoculation of mice and in tissue culture. The results of these tests are shown in Fig. 3. The pattern of virus excretion was varied and cannot be assessed fully because specimens were collected over too short a period on the assumption that our findings would be broadly similar to those of Dr. Koprowski. Of the 18 children studied, 15 developed antibody in response to vaccination and faecal virus was detected in these 15 children both by mouse inoculation and in tissue culture.

No virus was found in the faeces of the three children who did not develop antibody. The titres of the faecal virus tended to be higher in tissue culture than when estimated by mouse inoculation. Comparative titrations of early and late faecal specimens from one child in tissue culture and in mice showed that the virus from the early specimen had a higher titre by mouse inoculation than in tissue culture, but that the virus from the late specimen had a higher titre in tissue culture. Peak titres of 10 TCD50 per gramme of faeces or higher were recorded for three children. One girl aged 10 months excreted 10 TCD50 of virus 10 months after feeding, and at this time her brother aged 2, who was vaccinated on the same day, was excreting 100 TCD50 per gramme of virus in his faeces. Unfortunately it was not possible to obtain further specimens from these children until the 55th day, when neither was found to be excreting virus. (It is possible, but improbable, that these siblings were infected naturally with a wild type II virus at about the time of vaccination.) Six other children were still excreting virus when collection of specimens ceased during the fourth or fifth week after vaccination. In Fig. 3 nine siblings have been grouped in the left-hand column. The pattern of their faecal excretion and their antibody response serve to illustrate the trend already mentioned that siblings responded to vaccination in a similar fashion, and that the younger sibling responded better.

**Vaccination of Infants**

Ten infants were fed TN vaccine. They received the same dose as that given to adults and children. The virus was administered in milk in a rubber-teated bottle. A blood sample from each infant was tested for type II antibody a few weeks prior to vaccination, on the day of vaccination, and again 6 to 11 weeks later. On the first occasion blood was also taken from the mother and tested for type II antibody. Six infants had antibody titres of greater than 1 in 16 when first tested. Antibody titres found in the blood of their respective mothers were of the order that might have been expected when allowance was made for the age of the infant. For this reason it was concluded that the antibody present in the blood of the six infants was passively acquired maternal antibody. At the time of vaccination three of these six infants still had antibody titres greater than 1 in 16. In response to vaccination antibody titres of 1 in 16 and 5 of the 10 developed antibody titres greater than 5 of the 10 developed antibody titres greater than 1 in 16 (see Fig. 4). A sixth infant (No. 287) had a rise in antibody from less than 1 in 4 on the day of vaccination to 1 in 6 seven weeks later. There was insufficient serum
from any of the remaining four infants to test for antibody below the level of 1 in 16. The antibody rise following vaccination which occurred in the three infants having moderate titres of maternal antibody at the time of vaccination is not high, but when the rate of decline of maternal passive immunity is taken into account it is significant.

Faecal excretion of virus was studied in all infants (see Fig. 5). Specimens were collected daily for periods ranging from 8 to 28 days. Six infants, all of whom developed antibody, excreted virus which was detected both by mouse inoculation and in tissue culture. One infant who did not develop demonstrable antibody excreted virus detectable.
by mouse inoculation but not in tissue culture. (In this case a tissue-culture-virus-isolation technique was employed as well as the usual screening method.) The remaining three infants from whose faeces no virus was recovered did not develop detectable antibody. All the six infants who excreted cytopathogenic virus were still excreting virus when the collection of specimens ceased. Peak titres for faecal virus of greater than 10^5 TCD_50 per gramme were recorded for three infants.

The Monkey Pathogenicity of Excreted Virus

At the time of this trial no information was available about the pathogenicity of faecally excreted TN virus for monkeys inoculated intracerebrally or intraspinally. For this reason we decided to compare the relative virulence of the vaccine virus with that of the excreted virus by the intracerebral inoculation of monkeys. In view of the very low titres of virus found in stools by Koprowski et al. (1956) we thought it would be advisable to investigate excreted virus in two ways: firstly, by the direct inoculation of stool suspensions; and, secondly, by the inoculation of first-mouse-passage faecal virus. The object of the second method was to raise the titre of virus inoculated to levels comparable with that of the virus used in the original monkey tests of the vaccine. At this time we were unaware that faecal TN virus was cytopathogenic and that tissue-culture-passage of faecal virus could have been used instead of mouse-passage virus. The results of the intracerebral inoculations of monkeys with faecal virus from two infants and one child are shown in Table V.

It will be seen that the mouse passage of faecal virus was unnecessary and could have been misleading. Mouse passage
did not in fact raise the titre of virus from subjects Nos. 265 and 341 to the desired level; and a comparison of the titles obtained in mice and in tissue culture for the original faecal suspensions and for first-mouse-passage material (subjects Nos. 254 and 341) suggest that the virus population may be altered by this procedure. The intracerebral pathogenicity of faecal TN virus may be compared with that of the original TN vaccine by reference to Tables V and VI. Twelve monkeys were inoculated intracerebrally with $10^{5.4}$ or $10^{5.6}$ MPD₉₀ of TN vaccine virus; none became paralysed. Twenty-four monkeys were inoculated intracerebrally with TN faecal virus; the amount of virus inoculated varied from $10^{4.8}$ to $10^{5.3}$ MPD₉₀. Eleven of 22 monkeys (excluding two non-paralytic deaths) became severely paralysed and two showed a moderate degree of paralysis. The low titre faecal suspensions from both subjects Nos. 254 and 341 produced severe paralysis in three out of five monkeys.

Clinical Observations Following Vaccination

Subjects were observed for a period of one month after vaccination, and a record was kept of all minor illnesses and ailments. Infants were visited daily by a nurse, and parents of children were asked to report to their own medical practitioner any signs or symptoms their children might have. Medical practitioners were informed by letter when children under their care were vaccinated. No serious illnesses occurred. The majority of children with minor illnesses were examined by one of us (O. D. F). Vaccinated adults were asked to report all signs and symptoms of ill-health, and the majority took their own temperatures twice a day for the first 10 days.

All minor illnesses reported, however unlikely their association with vaccination, are listed in Table VI. The number of minor illnesses reported during the first week was in excess of the number reported in subsequent weeks, but this may simply reflect the waning vigilance of parents during the period of observation. Sixteen minor illnesses were reported in the 10 infants and in 97* children whose parents were medical practitioners or members of the university staff (Table VII). No minor illnesses were reported in the other 51 children. There seems little doubt that with the first group of 107 infants and children the reporting was more careful. Examination of this group in detail showed that 13 out of 73 children who developed antibody in response to vaccination had had minor illnesses, whereas

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*This does not include seven vaccinated children on whom antibody tests were not possible.
only 3 out of 34 children who had either no response to vaccination or were already immune at the time of vaccination were recorded as having illnesses. This difference is not significant ($x^2 = 0.8507 ; 0.50 > P > 0.30$).

Though no serious illnesses developed in any of the vaccinated individuals, we are unable to say whether or not vaccination with TN virus may not have given rise to minor symptoms in some individuals. The fact that the vaccine might sometimes cause mild upsets for a day or two does not in our opinion constitute an argument against its use.

**Summary and Conclusion**

Twenty-one adults, 10 infants, and 159 children were fed TN type II attenuated poliovirus vaccine. No significant illness was observed in any individual during the month following vaccination.

Four of 18 adults (22%) and 96 of a group of 124 children (77%) developed type II antibody in response to vaccination. The geometric mean antibody titre for the 96 children was 1 in 66. There was a significant association in the way siblings responded.

Three infants who at the time of vaccination possessed moderate levels of passively acquired maternal antibody developed active immunity in response to vaccination.

The development of antibody in infants and in children was associated with the excretion of virus.

The pattern of faecal virus excretion showed wide variation. Some individuals had high titres of virus in their faeces and some excreted virus for more than four weeks.

TN vaccine virus was found to be non-cytopathogenic in monkey-kidney-tissue culture and to produce no paralysis when inoculated into monkeys by the intracerebral route. Excreted TN faecal virus was found to be cytopathogenic and to cause severe paralysis when inoculated intracerebrally into monkeys.

The laboratory characteristics of attenuation shown by TN type II virus which made it appear suitable for trial as a vaccine are not maintained after multiplication in human gut.

This investigation was supported by grants from the National Fund for Poliomyelitis Research, the Medical Research Council, the World Health Organization, and the Northern Ireland Hospitals Authority.

We wish to acknowledge the collaboration of all volunteers taking part in this trial and parents of children who were vaccinated. Particular thanks are due to Dr. W. Ritchie Russell, Dr. R. L. Volm, and their colleagues in Oxford and to many general practitioners. Thanks are due also to Dr. F. F. Main, Chief Medical Officer, Northern Ireland, and to the medical officers of health for their co-operation, and to Dr. E. A. Cheeseman for advice and help.

Numerous others assisted in this study, including Mrs. Doris Nelsen (Lederle Laboratories), Mr. Fred Burns (Senior technician), Miss Jane Foster (laboratory secretary, National Fund for Poliomyelitis Research), Mr. James Hagan (student technician), Mr. James Evans (animal technician), and Mr. P. Cranmer.
possessed the same pathogenicity for monkeys by the intracerebral route as the SM vaccine virus. A final reason for limiting the size of the trial was that we were dealing with type I virus.

Our trial was planned to answer two questions. Was SM virus likely to be transmitted within a normal family? And did SM faecal virus possess the laboratory characteristics of attenuation shown by SM vaccine virus? The vaccine was first tested in the laboratory and then fed to a small number of adults without type I antibody (the investigators, their colleagues, and medical students). The home contacts of these adults were studied to see whether or not virus transmission occurred. It did not. Then two infants possessing maternal type I antibody were fed and their contacts studied. No transmission was observed. Finally, one child in the family of one of us was fed SM vaccine to see whether transmission occurred in a normal family living under normal conditions. Originally it was planned to increase gradually the number of family contact studies, but in view of the results obtained with the first family further investigation along these lines seemed unnecessary.

Materials and Methods

The vaccine used in this trial was SM (N 90) type I attenuated poliomyelitis virus vaccine (Koprowski). The virus which had originally been adapted to PRI mice by the intraspinal route had subsequently been given 13 chick-embryo tissue-culture passages plus six alternating passages in monkey-kidney and chick-embryo tissue culture.

The tissue-culture techniques, virus neutralization tests, monkey inoculation experiments, and methods of storage were similar to those described for the trial of TN type II vaccine (Dane, Dick, Connolly, Fisher, and McKeown, 1957).

Methods Used for Isolation of Viruses

Faecal Specimens.—Twenty per cent. suspensions were prepared by shaking faeces with tissue-culture medium or distilled water in the cold and then centrifuging this mixture at 4,000 r.p.m. for one hour. These suspensions were diluted for inoculation into tissue-culture tubes to give a screening level of $10^{-1}$ TCD$_{50}$ per gramme of faeces. Selected specimens were titrated. Tubes were examined daily for six days for signs of degeneration. The direct method of virus isolation described by Dane and Briggs (1956) was also used for screening most of the specimens. Negative results by the latter method were checked by the quantitative screening method.

Throat swabs were broken off into bijou bottles containing 1 ml. of tissue-culture medium. 0.5-ml. amounts of this fluid were inoculated into roller tubes containing monkey-kidney monolayers from which the maintenance medium had been removed; the tubes were then incubated for 30 minutes, after which the throat-swab medium was removed and replaced by maintenance medium. Tubes were examined daily for 10 days.

Blood Specimens.—Heparinized blood was inoculated direct on to monkey-kidney monolayers in the manner described for the specimens of throat swabs. Tubes were examined daily for at least 10 days.

Laboratory Tests of SM Vaccine Virus

The SM (N 90) type I vaccine was supplied by Dr. H. Koprowski, Lederle Laboratories, Pearl River, New York. Dr. Koprowski tested the pathogenicity of the vaccine virus for monkeys inoculated by the intracerebral and the intraspinal routes. He also conducted appropriate tests to exclude the presence in the vaccine of micro-organisms other than type I poliomyelitis virus. These were similar to the tests described elsewhere for TN vaccine (Dane, Dick, Connolly, et al., 1957).

Similar laboratory studies were carried out with SM vaccine in Belfast. The results of the monkey pathogenicity tests done in New York and in Belfast are shown in Table I. They are comparable, and demonstrate that, while the SM vaccine virus did not cause paralysis of monkeys when inoculated in large amounts by the intracerebral route, it was able to cause paralysis when moderate amounts were inoculated by the intraspinal route.

Vaccination of Adults

During February, 1956, three adults were fed 54 TCD$_{50}$ of SM type I vaccine virus in capsules. Only one (No. 235) (see Tables II and III) excreted the virus and developed antibody; the other two (Nos. 193 and 218) did not on this occasion become infected.

During March and April a further seven adults were vaccinated and the two who had failed to respond on the first occasion were revaccinated. Another adult was vaccinated in June. All these subjects received 3,500 TCD$_{50}$ of vaccine virus in capsules given by mouth. Faecal specimens were examined (except in the case of No. 314), and all subjects were found to be excreting virus (see Table II). Intestinal carriage lasted for up to 36 days and some specimens contained moderately high titres of virus. These results are in general similar to those reported for children by Koprowski et al. (1956). The homotypic antibody responses in vaccinated adults are shown in Table III. Every subject produced antibody, but the post-vaccination antibody levels showed wide variation. Tests on sera taken from seven of these individuals, six to nine months after vaccination, showed that the antibody titres had in most cases fallen. No estimate of the future rate of decline of antibody can be made at present.

An analysis of the relationship between height and duration of virus excretion with antibody production is not possible with such a small number of observations. However, we may speculate that there is some relationship. For example, the two poorest virus excreters (Nos. 326 and 330) produced very low levels of antibody, and the four subjects (Nos. 235, 193, 212, and 218) who excreted for the longest periods had late peak antibody titres (see Tables II and III).
TABLE II.—Excretion of Fecal Virus by Adults Fed SM Vaccine

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>235</th>
<th>193</th>
<th>212</th>
<th>218</th>
<th>353</th>
<th>207</th>
<th>326</th>
<th>330</th>
<th>334</th>
<th>284</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
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<td>M</td>
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<td>M</td>
</tr>
<tr>
<td>Age in years</td>
<td>25</td>
<td>41</td>
<td>20</td>
<td>29</td>
<td>24</td>
<td>22</td>
<td>53</td>
<td>46</td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td>Antibody before feeding* Type</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>I</td>
</tr>
<tr>
<td>Amount of virus fed†</td>
<td>54</td>
<td>5,000</td>
<td>3,500</td>
<td>3,500</td>
<td>3,500</td>
<td>3,500</td>
<td>3,500</td>
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</tr>
<tr>
<td>Date fed</td>
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<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
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</tr>
<tr>
<td>Days after feeding</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Antibody before feeding: + = titre of >1 in 4; – = titre of <1 in 4.
† Dose of virus expressed as TCD₃₀
| Each fecal specimen tested is represented by + or –.
| + = titre per gramme faeces >10⁻⁸ TCD₃₀
| – = titre per gramme faeces ≤10⁻⁸ TCD₃₀
| Where individual specimens have been titrated the result is shown as log to the base of 10, TCD₃₀ per gramme.

Daily throat swabs and blood samples were taken from 10 of the 11 adults for the first 10 days after vaccination. No throat virus was isolated, but one individual (No. 212) had a trace amount of virus in his blood on the eighth day. This isolation was repeated for confirmation. In connexion with his viraemia it is of interest to note that subject No. 212, unlike the other nine subjects, had no type II antibody and that he also produced the highest type I antibody titre. Studies of heterotypic antibody responses are incomplete, but it is worth mentioning that No. 212 developed low-titre type II antibody as well as the high-titre type I antibody after vaccination.

Vaccination of Infants with Maternal Antibody

Two infants (aged 2 and 3 months) who had maternal passive immunity were fed 6,400 TCD₃₀ of SM vaccine in milk. Three specimens of serum were obtained from each infant, one a few weeks before vaccination, one at the time of vaccination, and one five weeks later. Their mothers were also bled on the first occasion and a comparison of infant and maternal type I antibody titres demonstrated that the infants' immunity was passively acquired. Both infants became faecal virus carriers and both developed active immunity to type I poliovirus (see Table IV). The rises in antibody titre between the time of vaccination and five weeks later were small, but significant when allowance is made for the rate of decline of passive immunity. One infant (No. 242) excreted virus for at least 35 days (the negative specimen obtained on the 40th day is not by itself sufficient evidence of the cessation of virus excretion), and the other infant (No. 349) excreted faecal virus for at least 50 days. Peak titres of 10⁻⁶ (No. 242) and 10⁻⁵ TCD₃₀ (No. 349) of virus per gramme of faeces were recorded.

Vaccination of Family

Family and household contacts of the vaccinated adults and infants were investigated for signs of infection with SM virus. No evidence of transmission was found. However, we considered the crucial test of whether SM virus could spread within a family would come only when we vaccinated one young child in a family having other non-immune siblings. The family chosen for this study consisted of a father aged 41 who had been successfully vaccinated three months previously with SM type I vaccine, a mother aged 38, a son aged 2, and two daughters aged 4 and 6. Another son aged 8 was away at boarding school. All the family had naturally acquired immunity to type II poliovirus, but only the father had antibody to type I. When the father had been vaccinated he had failed to transmit SM virus to any other members of the family.

The daughter aged 4 was fed 6,400 TCD₃₀ SM virus in milk on May 3, 1956. She became a faecal virus carrier for about the next eight days (see Table V). A peak titre of 10⁻⁴ TCD₃₀ virus per gramme of faeces was found on the fifth day after vaccination. No poliovirus was isolated from

TABLE III.—Antibody Response in Adults Fed with SM Vaccine

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>235</th>
<th>193</th>
<th>212</th>
<th>218</th>
<th>353</th>
<th>207</th>
<th>326</th>
<th>330</th>
<th>331</th>
<th>314</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>25</td>
<td>41</td>
<td>20</td>
<td>29</td>
<td>24</td>
<td>22</td>
<td>53</td>
<td>46</td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td>Sex</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Antibody Prior to Vaccination</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>I</td>
</tr>
<tr>
<td>Days</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Weeks</td>
<td>Trace</td>
<td>&gt;4</td>
<td>128</td>
<td>256</td>
<td>89</td>
<td>199</td>
<td>128</td>
<td>Trace</td>
<td>&gt;4</td>
<td>6</td>
</tr>
<tr>
<td>Months</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
<td>Trace</td>
</tr>
</tbody>
</table>

her throat, and by the sixth week she had developed a type I antibody titre of 1 in 256. Her younger brother aged 2, who had not been vaccinated, became a faecal carrier of type I virus on the fifth day after his sister had received the vaccine. He continued to excrete virus in his faeces for about a week. The peak titre recorded for virus in his faeces was $10^{-4} \text{TCD}_{60}$ per grammme on the fifth day of excretion. No throat swabs were collected from this child. He developed type I antibody with a titre of 1 in 32 six weeks after his sister had been vaccinated. Insufficient specimens of faeces were collected from the mother to determine whether or not she became a faecal carrier of type I virus, but during the six-weeks study period she did develop low titre (1 in 6) antibody to type I. Her paired sera were tested on two separate occasions with the same result, and we consider that she became infected as a result of contact with one of the children. In view of the low titre of antibody which she developed she may have excreted faecal virus for only a short period, like some of the vaccinated adults. The other child living at home, a daughter aged 6, did not develop detectable type I antibody. In our opinion SM virus spread within the family by the faecal–oral route. The children of this family had fairly close contact with children from two other families. The infection did not spread to these other children.

When considering the significance of this study we should remember that it was conducted during the time of year when notified cases of poliomyelitis were at a low level. We do not know whether the chain of infection would break as readily during the later months of the year.

**Monkey Pathogenicity of SM Faecal and SM Blood Viruses**

Monkeys were inoculated intracerebrally with faecal SM virus from an infant (No. 242), an adult (No. 212), the

---

**TABLE IV.**—Faecal Excretion of Virus and Antibody Response in Two Infants Possessing Maternal Antibody who were fed SM Vaccine

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Age</th>
<th>Sex</th>
<th>Virus in faeces</th>
<th>Days After Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>242</td>
<td>3 months</td>
<td>M</td>
<td>++ + + + + + + +</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- - - - - - - - -</td>
<td>4-0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Antibody*</td>
<td>68</td>
</tr>
<tr>
<td>349</td>
<td>2 months</td>
<td>F</td>
<td>++ + + + + + + + +</td>
<td>4-5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Antibody*</td>
<td>48</td>
</tr>
</tbody>
</table>

+ = $10^{-3.5}$ TCD$_{60}$ per grammme of faeces, - = $10^{-4}$ TCD$_{60}$ per grammme of faeces. Where specimens have been titrated log$_{10}$ TCD$_{60}$ per grammme of faeces is given. Reciprocal of serum antibody titre. Where specimens have been titrated log$_{10}$ TCD$_{60}$ per grammme of faeces. * Reciprocal of serum antibody titre. + = $10^{-3.5}$ TCD$_{60}$ per grammme of faeces, - = $10^{-4}$ TCD$_{60}$ per grammme of faeces. Where specimens have been titrated log$_{10}$ TCD$_{60}$ per grammme of faeces is given. § Taken 11 weeks after.

**TABLE V.**—Spread of SM Vaccine Virus within a Family

<table>
<thead>
<tr>
<th>Days After No. 337 Received SM Type I Vaccine</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>6 Weeks After</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 337 female aged 4 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>256</td>
</tr>
<tr>
<td>Fed 6,400 TCD$_{60}$ SM Type I vaccine on 3/5/56</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody*</td>
<td>&lt;4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Throat virus†</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal virus‡</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 338 male aged 2 years, Contact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Antibody</td>
<td>&lt;4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal virus‡</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 347 female aged 38 years, Contact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Antibody</td>
<td>&lt;4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fecal virus‡</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 280 female aged 6 years, Contact</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;48</td>
</tr>
<tr>
<td>Antibody</td>
<td>&lt;4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Reciprocal of serum antibody titre. † Sensitivity of test described in methods. § = $10^{-3.5}$ TCD$_{60}$ per grammme of faeces, - = $10^{-4}$ TCD$_{60}$ per grammme of faeces. Where specimens have been titrated log$_{10}$ TCD$_{60}$ per grammme of faeces. Where specimens have been titrated log$_{10}$ TCD$_{60}$ per grammme of faeces is given. § Taken 11 weeks after.

**TABLE VI.**—Pathogenicity of SM Faecal Virus for Monkeys

<table>
<thead>
<tr>
<th>Sample of Faeces from</th>
<th>Day After Vaccination</th>
<th>Treatment of Faeces for Inoculation</th>
<th>Log$<em>{10}$ TCD$</em>{60}$ Inoculum</th>
<th>No. of Monkeys Inoculated</th>
<th>Incubation Periods (Day)</th>
<th>Monkey Died With No Paralysis (Day)</th>
<th>No. of Monkeys with Paralysis as Grade of Severe</th>
<th>Paralytic Ratio of Survivors</th>
<th>Lesion Ratio</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>242</td>
<td>3/2</td>
<td>1 tissue culture passage</td>
<td>6-6</td>
<td>4</td>
<td>6, 9, 13, 20</td>
<td>1</td>
<td>0</td>
<td>2, 1</td>
<td>3/4 (24/4)</td>
<td>4/4</td>
</tr>
<tr>
<td>337</td>
<td>4</td>
<td>45% faecal suspension</td>
<td>5-1</td>
<td>5</td>
<td>7</td>
<td>8, 17</td>
<td>1</td>
<td>0</td>
<td>1/3</td>
<td>3/5</td>
</tr>
<tr>
<td>212</td>
<td>20</td>
<td>1 tissue culture passage</td>
<td>6-4</td>
<td>5</td>
<td>20, 21</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>1/4 (72/4)</td>
<td>4/5</td>
</tr>
<tr>
<td>338</td>
<td>2</td>
<td>Contact, infection, see text</td>
<td>20% faecal suspension 1 tissue culture passage</td>
<td>4-9</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>1/5</td>
<td>3/5</td>
</tr>
</tbody>
</table>

All monkeys inoculated intracerebrally with 1 ml. of virus suspension. Non-specific deaths within 72 hours of inoculation have been excluded from the table. Monkeys showing mild transient weakness and their incubation periods are shown in bold type.
vaccinated child (No. 337), and her brother, whom she infected (No. 338) (see Table VI). The infant's faecal virus was from a late specimen taken on the 35th day after vaccination. The adult's faecal virus was from the specimen collected the day following his viramia. The specimens from the two children were both taken on the fifth day after the commencement of virus excretion. Three specimens were given a single passage in monkey-kidney-tissue culture to enhance their titres before being tested. Twenty-two monkeys were inoculated and seven became paralysed (Table VI). Typical lesions of poliomyelitis were found in the central nervous system of 18 of the 22 monkeys. The amounts of virus inoculated into the five groups of monkeys varied from $10^{5.0}$ to $10^{5.6}$ TCD$_{50}$, but there was no difference in the number of monkeys paralysed or showing typical histological lesions between the five groups. There was no apparent difference between the SM faecal virus recovered from the child (No. 337) who was vaccinated and that recovered from the child (No. 338) who became infected after contact.

These results of the intracerebral inoculation of monkeys with faecal virus can be compared with those obtained with the vaccine virus (see Table I). Of 12 monkeys inoculated intracerebrally with large amounts ($10^{5.8}$ and $10^{5.6}$ TCD$_{50}$) of SM vaccine virus none showed paralysis, but two had histological lesions. A direct numerical comparison between the results obtained with the vaccine and faecal SM viruses is not possible because of the variations in titre of the different inocula. However, if these differences are ignored we may express the results of the intracerebral inoculations in the following manner: SM vaccine virus paralysed 0 out of 12 monkeys, and SM faecal virus paralysed 22 monkeys. Histological lesions were found in 2 of the 12 monkeys inoculated with SM vaccine virus, and in 17 out of the 22 inoculated with SM faecal virus. The interpretation of these data may be open to argument, but we conclude from them that SM faecal virus is more paralytogenic than SM vaccine virus when inoculated intracerebrally.

Another comparison of SM vaccine and faecal viruses was made by the intraspinal inoculation of monkeys. The faecal virus used in this experiment was from the child infected by his vaccinated sister and thus represented the second human passage of SM virus. The two viruses were inoculated in titres ranging from $10^{4.6}$ to $10^{4.6}$ (see Table VII). The results of these two titrations are broadly similar and show that the intraspinal pathogenicity of SM virus had not increased after two human passages.

**Table VII.**—Comparative Intraspinal Monkey Pathogenicity of SM Vaccine Virus and Second Human Passage SM Faecal Virus

<table>
<thead>
<tr>
<th>Source of Virus</th>
<th>$\log_{10}$ TCD$_{50}$ Inoculated</th>
<th>No. of Monkeys Inoculated</th>
<th>Paralytic Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM N-90 Type I poliomyelitis virus vaccine</td>
<td>4.6</td>
<td>6</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>3.6</td>
<td>4</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>4</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>4</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>4</td>
<td>0.4</td>
</tr>
<tr>
<td>Second human passage</td>
<td>4.6</td>
<td>5</td>
<td>4.6</td>
</tr>
<tr>
<td>SM faecal virus</td>
<td>3.6</td>
<td>4</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>2.6</td>
<td>4</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>4</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Only a trace amount of virus was recovered from the blood of one subject (No. 212), but because of the possible importance of viramia in the pathogenesis of paralytic poliomyelitis this virus, or more correctly its progeny, was inoculated into monkeys. Sufficient virus for the inoculations could be obtained only by using second-monkey-kidney-tissue-culture passage of the blood virus. Eleven monkeys were inoculated intraspinally with amounts of virus ranging in titre from $10^{5.0}$ to $10^{5.9}$ TCD$_{50}$ (see Table VIII). None of these monkeys became paralysed. Assuming that two tissue-culture passages did not greatly alter the nature of the virus, we may conclude that the virus isolated from the blood of subject No. 212 on the eighth day after vaccination was not of high intracerebral virulence for monkeys.

**Table VIII.**—Pathogenicity for Monkeys of Second Tissue-culture Passage SM Virus from Blood of Subject No. 212

<table>
<thead>
<tr>
<th>Dilution of Tissue Culture Fluid</th>
<th>$\log_{10}$ TCD$_{50}$ Inoculated</th>
<th>No. of Monkeys Inoculated</th>
<th>Paralytic Ratio</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated</td>
<td>5.9</td>
<td>3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>10$^{-3}$</td>
<td>4.9</td>
<td>4</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>10$^{-3}$</td>
<td>2.9</td>
<td>4</td>
<td>0.4</td>
<td></td>
</tr>
</tbody>
</table>

All monkeys inoculated intraspinally with 1 ml of virus suspension.

**Reactions**

All subjects vaccinated were under continual medical observation during the month following vaccination. No serious illnesses were reported, but minor illnesses or symptoms were recorded in 9 of the 16 persons who were successfully vaccinated or who were infected by contact (Table IX). A cytopathogenic virus was recovered from the throat of one child (No. 337) who had a fever following vaccination, but this proved not to be a poliovirus.

**Table IX.**—Clinical Observations in Individuals who had Minor Upsets after Vaccination with SM Vaccine

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>*Temp. (°F.) Rise on Day ()</th>
<th>Symptoms and Signs on Day ()</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>312</td>
<td>25</td>
<td>M</td>
<td>None</td>
<td>None</td>
<td>Contact of 337 (see text)</td>
</tr>
<tr>
<td>303</td>
<td>24</td>
<td>F</td>
<td>Backache (2-4)</td>
<td>Occasional loose stool (3-5)</td>
<td>No virus in throat</td>
</tr>
<tr>
<td>303</td>
<td>31</td>
<td>M</td>
<td>Off feed (2)</td>
<td>Feeding unwell (0-3)</td>
<td>Anxiety</td>
</tr>
<tr>
<td>315</td>
<td>39</td>
<td>M</td>
<td>Off colour for 8 weeks</td>
<td>Fever</td>
<td></td>
</tr>
<tr>
<td>336</td>
<td>53</td>
<td>M</td>
<td>Off colour for 8 weeks</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Twice-daily temperature records for 10-14 days after vaccination.

It is quite impossible to draw any conclusions from the small numbers observed. In our opinion the question of whether minor signs and symptoms may follow the administration of SM vaccine has still to be answered.

**Summary and Conclusion**

Eleven adults, two infants, and one child were fed SM type I poliomyelitis virus vaccine. In addition one adult and one child became infected in a study of the transmissibility of the vaccine virus.

None of these 16 subjects developed any serious illness. It was impossible to be certain whether or not some of the minor illnesses recorded were due to vaccination.

All subjects were without type I antibody at the time of vaccination and all developed antibody after vaccination. The two infants possessed moderate titres of maternal antibody at the time of vaccination, but this did not prevent them becoming faecal virus carriers and developing an active immunity. A decline in circulating antibody from the highest titre reached was observed in the majority of adults during the months following vaccination.

All subjects became faecal carriers of virus. The duration of faecal excretion varied from one week to
more than seven weeks, and high titers of virus were found in early faecal specimens from some individuals.

A trace amount of virus was recovered from the blood of one adult on the eighth day after vaccination. No throat virus was recovered from any of the vaccinated individuals.

SM virus was shown to spread within a normal family living under normal conditions of hygiene following the vaccination of one child in the family.

SM *faecal* virus was found to cause paralysis in some monkeys inoculated intracerebrally, whereas monkeys inoculated by this route with SM *vaccine* virus were not paralysed.

SM type I poliomyelitis virus was considered suitable for trial as a vaccine because it did not cause paralysis in monkeys when inoculated by the intracerebral route. However, the virus underwent a change after multiplication in the human gut, and SM *faecal* virus caused paralysis in a proportion of the monkeys inoculated by this route. In addition SM virus can spread from the vaccinated to the unvaccinated.

We thank the medical student volunteers who took part in this trial and also Dr. Ritchie Russell and his colleagues. This trial was financed by grants from the National Fund for Poliomyelitis Research, the Medical Research Council, the World Health Organization, and the Northern Ireland Hospitals Authority.

**REFERENCES**


3. THE EVALUATION OF TN AND SM VIRUS VACCINES*

BY

G. W. A. DICK, M.D., D.Sc., F.R.C.P.Ed.
Professor of Microbiology

AND

Lecturer in Microbiology

*Awaided by grants from the National Fund for Poliomyelitis Research, the Medical Research Council, the World Health Organization, and the Northern Ireland Hospitals Authority.

The Queen's University of Belfast

There are three approaches to active immunization against micro-organisms. The first of these is to inactivate the micro-organisms by chemical or physical means and to infect the host with measured amounts of the inactivated organism. Formalized tissue-culture poliovirus vaccines, which are now commonly referred to as Salk-type vaccines, are of this type. The second method is to immunize with living micro-organisms which have been rendered avirulent for their original host. This type of vaccine is exemplified by B.C.G. or the 17D strain of yellow fever. Attempts to produce attenuated strains of poliovirus have been undertaken by several investigators, and trials of attenuated living poliovirus vaccines in human beings have been described by Koprowski, Norton, Jervis, Nelson, Chadwick, Nelson, and Meyer (1956), by Sabin (1955a), and in the preceding papers by Dane, Dick, Connolly, Fisher, and McKeown (1957) and Dick, Dane, Fisher, Connolly, and McKeown (1957). The third method of immunization is to employ micro-organisms from a different species which are immunogenically related to the micro-organisms against which protection is required. The Jennerian method of vaccination with cowpox to protect against smallpox employed this approach, but it has not yet been investigated in the poliomyelitis field.

The reasons for using attenuated viruses for immunization against poliomyelitis are based primarily on the hope that they will produce long-lasting immunity, for there is as yet no evidence that the immunity which will follow immunization with inactivated (Salk-type) vaccines will be durable. Attenuated poliovirus vaccines can be given by mouth and thus present a considerable administrative advantage and dispense with the use of syringes. Furthermore, the cost of attenuated poliovirus vaccines should be very much less than that of inactivated vaccines.

Before attempting to evaluate TN and SM attenuated poliomyelitis virus vaccines some epidemiological aspects of a trial of these vaccines (Dane et al., 1957; Dick et al., 1957) are discussed: in conclusion we shall present the criteria which we, at present, consider are required for attenuated poliovirus vaccines.

Some Epidemiological Aspects of Trials of TN and SM Virus Vaccines

Antibody Production

While comparing the response of children and adults to vaccination it was found that 28 of 124 children (23%) and 14 of 18 adults (78%) who were devoid of type II antibody before vaccination failed to develop antibody after feeding with TN (type II) vaccine (diff./S.E. diff. = 4.708%). Part of the explanation of this significantly different response might be thought to be due to a higher proportion of the adults having had previous experience with heterotypic polioviruses which prevented infection with the TN strain. This does not, however, seem likely from tests of the heterotypic antibodies in the vaccinated adults.

All individuals who were fed with 3,500 or more TCD50 of SM (type I) vaccine developed antibody, but only one out of three adults who were fed 54 TCD50 developed antibody.† On the other hand, Kopprowski et al. (1956) found that they were able to infect two out of three children with two plaque-forming particles (PFP) of SM virus and that 20 PFP were adequate to infect four out of four children.

This difference in the response of adults as compared with children to infection with the TN and SM viruses suggests that in certain population groups some adults may develop barriers other than homotypic circulating antibody to infection with some strains of poliovirus. We stress certain population groups, for this did not appear to be so in adults of the Inbred Eskimos of Chesterfield Inlet, in the Canadian Arctic (Pearl, 1949), who may have been genetically highly susceptible to poliomyelitis.

There is a suggestion from our studies that in families where one sibling failed to develop antibody after vaccination with TN vaccine the other sibling also failed to respond (Dane et al., 1957). We have already indicated in antibody surveys in Northern Ireland (Dane, Dick, Connolly, Briggs and McLeod, 1956) that siblings usually show the same pattern of antibody to the three types of poliovirus: thus if one sibling had either antibody or no antibody to one or other of the types, it was usual to find a

†Before vaccination each of these three adults had type II antibody and one of them who failed to become infected had also type III antibody.

‡Comparative titrations have shown that one plaque-forming particle by the technique of Koprowski and his associates = 1 TCD50 by our methods.
similar picture in the younger siblings. It may be, then, that some families are less susceptible to infection with poliovirus than others, and if such families are the least susceptible members of the community then the vaccination failures which we found with TN live virus vaccine may be of little consequence.

We do not know how far it is possible to draw an analogy between the response of an individual to an attenuated strain as compared with a wild strain. If some families are genetically relatively resistant to infection with wild strains of poliovirus then it may be that in serological surveys a proportion of the individuals devoid of antibody may nevertheless be resistant to infection. Thus a population group in which 80% of the individuals have antibody to type II virus could represent a group who are all resistant to infection with that virus.

Koprowski (1955) has claimed that the circulating antibody which follows vaccination with TN vaccine is durable for at least five years. After vaccination with SM vaccine he showed that none of seven subjects who were tested had any decline in the level of antibody induced by vaccination over a period of 12 to 15 months. These individuals (who were presumably children) had antibody titres at three weeks and at 12-15 months after vaccination as shown in Fig. 1.

In seven adults whom we vaccinated with SM virus and whose sera were tested at varying intervals up to nine months after vaccination, it was found that, with one exception, the antibody titres had fallen to a lower level than the peak titres observed within the first few weeks after vaccination (Fig. 2). It may be that after the initial drop which we have observed in the antibody levels the subsequent rate of decline will be slow.

**Fig. 1.—Antibody levels months after vaccination with SM type I virus.** (Drawn from results presented by Koprowski, 1955).

**Fig. 2.—Antibody levels months after vaccination with SM type I virus.** (Dick et al., 1957).

The most important disagreement between our results and those of Koprowski et al. (1956) is in the character of the virus which is excreted after vaccination and also, in the case of TN virus, in the duration of virus excretion. Koprowski et al. were of the opinion that TN faecal virus was, like the TN vaccine virus, non-cytopathogenic for tissue-culture-grown fibroblasts or epithelium of either simian or human (normal or neoplastic) origin. Their tests for faecal virus were accordingly made by inoculating faecal extracts intraspinaly in mice. We have shown that TN faecal virus is also cytopathogenic for monkey-kidney-tissue culture (Dane et al., 1957). In all individuals whom we tested (with one exception) the virus which was excreted after vaccination was not only paralytogenic for mice inoculated intraspinally but also cytopathogenic for tissue cultures. Since Koprowski et al. had stated that TN vaccine was never excreted for more than 23 days we did not collect many specimens for longer than that period. It is clear, however, from our results that virus continues to be excreted for periods in excess of 23 days after TN vaccination, and in many individuals for at least a month. Koprowski et al. were of the opinion that the excretion of TN faecal virus was sporadic and of low titre (never exceeding 10 mouse PD50 per gramme of faecal material) and that it occurred in only about half of the successfully vaccinated individuals.

Quite apart from the tissue-culture tests we were able, by mouse inoculation, to detect TN faecal virus in all but one of the individuals who were successfully vaccinated, and titres of 10^5 or more mouse PD50 per gramme were found in some faecal specimens. We do not consider that the difference in our results compared with those of Koprowski et al. is due to minor differences in the techniques used in preparing stool samples, for we have found no significant differences in the titre of virus in stools prepared by the method of Dr. Koprowski (personal communication) and the method we employed. It may be, however, that the strain of mice which we used (VSBS) were more susceptible than the PR1 strain used by Koprowski et al. Although the majority of individuals whom we tested excreted TN virus which was of relatively low titre in mice and in tissue cultures, there were individuals who were excreting from 10^6 to more than 10^8 TCD50 of virus within five days of vaccination. The TN virus which is excreted by vaccinated individuals differs from the TN vaccine virus in its cytopathogenicity, and in some individuals this change from non-cytopathogenic to cytopathogenic virus occurs very rapidly. In addition, our results have clearly demonstrated that after multiplication in the alimentary tract the TN faecal virus is paralytogenic for monkeys inoculated intracerebrally (Dane et al., 1957).

The majority of individuals whom we vaccinated with SM virus were adults (Dick et al., 1957), and therefore our results on the excretion of SM virus are not strictly comparable with those of Koprowski et al., whose studies were made mainly in children. It appears that, after vaccination with SM virus, excretion of virus in adults is usually of shorter duration than in infants or children. In our studies, SM faecal virus was present in highest titre during the first week after vaccination. By analogy this may explain why the natural disease is most infectious during the early stages of infection.

SM faecal virus is different from SM vaccine virus in its ability to paralyse monkeys after intracerebral inoculation (Dick et al., 1957). The change found with SM virus after multiplication in the human gut is not so marked as that found with TN virus. The examination of stools taken on a single day may not, however, be sufficient to show any marked change, as it is possible that there are variations in the proportion of paralytogenic particles at different times.

**Viraemia and Throat Virus**

Koprowski (1955) found no viraemia or throat virus in any subject vaccinated with SM or TN viruses. Among
the individuals whom we vaccinated with TN virus we found no viraemia in the few individuals tested. We recovered a trace of virus from the throat of one volunteer within the first three days after vaccination, but do not consider this was definite evidence of multiplication of TN virus in the throat. Our failure to demonstrate virus multiplication in the throat does not, however, exclude this possibility, because had we suspected that TN virus was going to revert in the alimentary tract to a cytopathogenic virus which was intracerebrally virulent for monkeys we would have made more extensive investigations of the throats of vaccinated individuals. Although TN vaccine virus might not multiply in the throat, TN faecal virus might do so, and individuals might infect their own throats with the cytopathogenic faecal virus.

We found no evidence of multiplication of SM virus in the throats of vaccinated individuals or their contacts. On the other hand, virus was demonstrated in the blood of one individual eight days after vaccination with SM vaccine. This individual (a man aged 20) was devoid of antibody to all three types of poliovirus before vaccination; all the other volunteers whom we tested for viraemia had type II antibody before feeding with SM (type I) vaccine. While we do not wish to draw any conclusions from one case it is of interest to speculate that the presence of type I antibody might have prevented type I virus entering the bloodstream in the majority of the subjects vaccinated. If virus, or SM virus, is important in the pathogenesis of paralytic poliomyelitis, it may be that circulating type II antibody reduces the chance of paralytic type I infections.

Contagion of Vaccine Viruses

Koprowski et al. (1956) note that there are “formidable odds” against the contagiousness of the TN strain because of the character of the virus. It seems to us that the character of TN faecal virus is similar in many respects to a wild type III. Although Koprowski et al. refer to experiments which show that TN virus would only be sporadically excreted in very small amounts by about 50% of vaccinated individuals, we considered that person-to-person transmission of TN virus was unlikely, and accordingly we did not plan experiments to test this. In the few contacts of TN vaccinated individuals whom we have observed there was no evidence of transmission, but, on theoretical grounds, we see no reason why TN faecal virus may not on occasion be transmissible from person to person. Of great importance, so far as the use of SM virus vaccine was concerned, was the observation of Koprowski et al. that transmission of the virus from child to child occurred in 5 of our 15 contacts. Their contact experiment was made in an institute for mentally defective children under abnormal conditions of hygiene, and they concluded that the attenuated type I virus is not very contagious and that “when principles of simple personal hygiene are practised, the attenuated SM virus, after its administration in a capsule, may be completely prevented from passing from one subject to another.” The object of feeding the virus in capsules was to prevent virus multiplying in the mouth or throat. If, however, SM virus had an affinity for the throat, it would be difficult to see how this would prevent it getting to the throat by faecal-oral contamination.

As already described (Dick et al., 1957), it was found that SM virus was transmitted by a vaccinated child to her brother and mother living under normal family conditions. The vaccinated child was given the virus in a milk drink, but no poliovirus was recovered from her throat. Although, from the available evidence, we think that transmission of SM virus was by the faecal-oral route, it does not really matter by what mechanism it occurred. The important fact is that the virus was passed from a vaccinated individual to family contacts. There was no evidence of extra-familial contact infections, and one of the three siblings in the family escaped infection.

Satellite Cases

We found no evidence of contact infections from the vaccinated subjects, except within the single family mentioned above. There is also no evidence that our studies contributed to the viruses in the community. Throughout the period of our trials and during the following four months no case of poliomyelitis was notified in Belfast.

Stability of TN Strains

We have no evidence that TN vaccine virus is a mixed population of tissue-culture-cytopathogenic and mouse-pathogenic particles, of which the cytopathogenic particles are able to infect human gut and the mouse particles are unable to do so. In a small infectivity titration Koprowski et al. (1956) were able to infect children with as little as 320 mouse PD₅₀ of TN virus. This suggests that if the vaccine was a mixture of cytopathogenic and mouse-pathogenic virus the proportion of cytopathogenic to mouse-pathogenic virus was 1:320 or more. We have failed to detect any cytopathogenic effect when TN vaccine was left in contact for 30 minutes with monkey-tissue monolayers which were then incubated for seven days. Subsequently, two blind passes were made with the tissue culture fluids, but no evidence of cytopathogenicity was found.

Secondly, when comparative titrations of TN faecal cytopathogenic virus were made with a diluent consisting of (a) tissue-culture maintenance fluid, or (b) tissue-culture maintenance fluid containing 5,000 mouse PD₅₀ of TN virus per ml there was no significant difference in the titres. Thus we could find no evidence that there was any interference of tissue-culture cytopathogenic particles by non-cytopathogenic particles, even when the former were present in minute quantities compared with the latter. Indeed, the titre of TN cytopathogenic faecal virus in the presence of the non-cytopathogenic vaccine virus was 10⁴ as compared with a titre of 10⁶ TCD₅₀ in the absence of the vaccine.

So far as we know, cytopathogenic particles arise only after the multiplication of the TN vaccine virus in the alimentary tract. There is some evidence that when TN faecal virus is adapted back to mice it rapidly loses its tissue-culture cytopathogenicity, but we have not yet completed these studies.

Infection in the Presence of Antibody

Our results have shown that babies with maternal antibody can be infected with TN and SM viruses; the infection, as in other successfully vaccinated individuals, being associated with excretion of virus and the development of antibody. Three of 10 babies vaccinated with TN (type II) virus failed to excrete virus and one excreted virus which was demonstrable only by mouse inoculation. None of these four babies developed antibody titres as great as 1:16 after vaccination. The failure to infect these babies was not due to the fact that they had high levels of maternal antibody. In fact, the maternal antibody in these failures was lower than in those babies who developed antibody after vaccination. In two babies vaccinated with SM (type I) virus there was no evidence that the presence of homotypic maternal antibody at titres of 1:48 and 1:68 on the day of vaccination interfered with infection.

Seasonal Factors in Virus Multiplication

As already mentioned, all our vaccinations were done outside the “poliomyelitis season” and we have no knowledge how TN or SM virus might behave at other times of the year. It is important to know this before any sizable trials of attenuated poliovirus vaccines are done in tropical countries where epidemics may not be seasonal. Some of our observations suggest that there might be variations in the duration and degree of excretion of poliovirus at different times of the year. Perhaps a seasonal change in the way in which wild polioviruses are excreted may explain the seasonal incidence of paralytic poliomyelitis.
Evaluation of the TN and SM Strains

In evaluating an attenuated virus vaccine we must consider what properties the vaccine should possess. Is it sufficient to establish that the vaccine virus has the properties of naturally occurring avirulent strains or must it possess some properties which distinguish it from naturally occurring strains? It seems to us that it must have some marker which distinguishes it from naturally occurring strains. At the moment we must assume that all naturally occurring strains or their mutants may at times cause paralysis in man. This assumption may not be correct, but it seems justifiable to assume its correctness until it is proved incorrect.

The initial laboratory tests of the vaccines, which are a necessary first step, are not really of as much value in assessing the safety of the vaccine for the individual or the community as are tests on the virus excreted after vaccination. Excreted virus should obviously fulfill the same criteria of safety as the vaccine virus.

In testing the safety of the vaccines some standard must be laid down. We used as a standard that the vaccine viruses should be as avirulent or less virulent for monkey C.N.S. than any known naturally occurring viruses. We considered that the most important test of the safety of the vaccines was that they should not cause paralysis in monkeys inoculated with large amounts intracerebrally. This is based on the assumption that the pathogenicity for monkey C.N.S. gives an indication of the virulence for human neurones, for it seems probable that, with polioviruses, high virulence by intracerebral inoculation of monkeys and the ability to cause paralysis in man go hand in hand. There is a certain amount of evidence to support this assumption. Although not paralytogenic by Intracerebral inoculation, the SM and TN strains can produce paralysis in rhesus monkeys by intraspinal inoculation, for the lower motor neurones of monkey spinal cord are more susceptible to polioviruses than their brain-stem neurones (Sabin, 1955b).

It should be stressed that we do not know what cerebral virulence for monkeys means in relation to potential paralytogenicity in man, other than by comparison with naturally occurring strains. Ramos-Alvarez and Sabin (1954) and Sabin (1955b) have described the recovery of intracerebrally avirulent types II and III poliovirus strains from healthy children who had no recent contact with recognized cases of poliomyelitis. These strains, although intracerebrally avirulent, produced paralysis in monkeys on intraspinal inoculation. So far as we know, no intracerebrally avirulent type I strain has been recovered in nature, and all strains recovered from paralytic cases have shown intracerebral pathogenicity. It appeared to us, from monkey pathogenicity tests, that the TN and SM vaccine viruses were as avirulent if not more avirulent than naturally occurring strains.

Now if we establish criteria of safety for the vaccines we must use the same criteria for the virus which is excreted. Our results show that the TN faecal virus is not avirulent for monkeys inoculated intracerebrally; indeed, after multiplication in the alimentary tract TN virus is as virulent for monkey C.N.S. as many naturally occurring paralytic strains. Although the SM faecal virus is paralytogenic in a proportion of intracerebrally inoculated monkeys, it is not highly virulent for monkeys by this route, and there are naturally occurring strains which are more virulent and there may be naturally occurring strains which are less virulent.

If, then, the excreted vaccine viruses are more virulent or as virulent as naturally occurring strains, are there any other properties which, in spite of their intracerebral avirulent property, would make the TN and SM viruses acceptable? Other properties which can be tested are (a) the duration and quantity of virus excretion, (b) the presence or absence of viraemia, (c) the presence or absence of multiplication of virus in the throat, and (d) transmission by contact.

It is possible to compare the duration and quantity of virus excretion found after vaccination with that found in natural infections. It might be argued that the more nearly the faecal excretion of a vaccine virus paralleled the faecal excretion in natural infections, the more likely would it be that the vaccine virus resembled some of the naturally occurring viruses. The description by Koprowski et al. (1956) of the pattern of excretion of the TN vaccine was very different from what occurs in natural infections, but our findings suggest that the level and duration of TN virus excretion in some children is not dissimilar to that which occurs in infections with wild viruses. We have no evidence that the excretion pattern of SM virus is different from that found in some natural infections.

So far as viraemia is concerned it is known that viraemia occurs in family associates of paralytic cases and in chimpanzees fed virulent strains of poliomyelitis (Bodian, 1952). We do not know whether viraemia is absent or present in infections with wild avirulent strains; therefore we cannot use the apparent absence of viraemia in individuals infected with vaccines as a marker which differentiates the vaccine viruses from naturally occurring strains. The occasional transient viraemia observed after feeding SM virus may be similar to that which occurs after infection with wild avirulent strains.

We have no information on the presence or absence of throat virus in individuals experiencing natural inapparent infections during non-epidemic periods. Therefore, although we demonstrated no significant multiplication of TN or SM viruses in the throats of vaccinated individuals, this does not necessarily make these strains different from all wild strains. Our studies were done outside the “poliomyelitis season,” and we do not know whether wild strains have a greater tendency to colonize the throat during the epidemic season than in non-epidemic times. It is known that poliovirus often can be found in the throats of paralytic cases and their contacts, and in non-paralytic infections during epidemics. Absence or demonstrable virus multiplication in the throat would be advantageous in a vaccine, for it would mean that the virus is lacking in a characteristic associated with epidemic strains of poliomyelitis.

We have shown that SM virus can be transmitted to family contacts, and this is obviously a characteristic shared with naturally occurring strains. We did not plan our studies to demonstrate the spread of TN virus by contact, but since the faecal TN virus is at times excreted like wild virus there is no reason to assume that it may not spread.

In the light of our present knowledge it does not seem that SM and TN faecal viruses differ in any measurable way from naturally occurring strains, and therefore we do not consider that the SM and TN vaccines should be used at the moment on a large scale. It might well be that TN virus could be given to thousands of individuals without producing a clinical case of poliomyelitis, for it is known that the paralytic infection rate with some strains of type II virus is very low.

Further controlled trials of SM virus in gradually increasing numbers of people in normal communities are not practicable, since the virus may spread. Furthermore, the use of a vaccine which spreads readily by contact raises, in a normal community, the problem of involving persons other than volunteers.

In our trials with the SM and TN strains we advised against injections, fatigue, and tonsillectomy. Most of Kopprowski’s subjects were mentally defective children. In interpreting the absence of serious untoward reactions in the individuals so far fed with SM vaccine, these two facts should be kept in mind.

We have not yet considered the antibody response which is the primary object in evaluating a poliomyelitis vaccination procedure. The proportion of individuals developing antibody after TN vaccine is less than after Salk-type vaccine, but we have already noted that the failure to respond may be in those individuals who are relatively
insusceptible to infection with polioviruses. The fact that all individuals who were vaccinated with SM virus developed antibody is probably a reflection of the large dose of cytopathogenic virus which was fed to them; the wide range in their antibody titres may indicate the variation in their susceptibility.

Acceptable Attenuated Strains

Although we consider, with our present knowledge, that the TN and SM strains are not at the moment acceptable for mass immunization, this does not mean that suitable strains will not be developed. We have formulated what we believe are the minimum requirements for attenuated poliovirus vaccines. These are as follows:

1. The vaccine virus and any excreted virus should be intracerebrally avirulent for monkeys and perhaps also intraspinally avirulent. Intracerebral avirulence alone may be acceptable provided the other requirements are fulfilled.

2. The virus should not be transmissible from vaccinated to non-vaccinated individuals. This may be difficult to achieve with viruses which are given by mouth and excreted. Sabin (1955a) showed that after intramuscular injection of the KP34 type III strain there was no immunogenic response unless the virus localized in the alimentary tract, where it multiplied and was excreted. This does not mean that strains of poliovirus might not be found which would multiply in tissues other than the alimentary tract but not in the C.N.S. Lack of transmissibility is a characteristic of all established attenuated vaccines. Thus 17D yellow fever virus is not transmissible by mosquitoes, the spread of vaccinia is rare and limited, B.C.G. does not spread like naturally occurring Myco. tuberculosis. If oral vaccines are used, then the duration of faecal excretion of virus should be at such a low level and for such a limited duration (as was claimed for TN virus by Dr. Koprowski) that transmission would be unlikely to occur.

3. The vaccine virus and any of its progeny which are excreted should have some marker which differentiates them from naturally occurring wild strains of virus.

4. The vaccine virus should preferably not multiply in the throat.

5. The vaccine should prevent paralytic poliomyelitis in those who are susceptible. This may not require 100% antibody response in vaccinated individuals.

A live virus vaccine with these properties should be safe to the individual vaccinated and to the community.

Future of Attenuated Poliovirus Vaccines

The future for the types of attenuated viruses developed by Koprowski and by Sabin is not clear. Although Sabin (1955a) states that his KP34 type III strain which he fed to volunteers came out as avirulent as it went in, the KP33 (type I) and KP51 (type II) strains after multiplication in the alimentary tract "yielded a certain proportion of virus particles with increased virulence for brain-stem neurones of monkeys." Sabin indicated that he was continuing his studies to find better attenuated strains. In the evaluation of his published trials, which were done in adults, it should be remembered that the alimentary tract of the small child is probably a better milieu for polioviruses than that of the adult.

As already noted, it would seem to us that if strains are found which fulfil laboratory criteria for safety, then they must also be shown to have properties which make them different from wild strains. If these vaccine strains differ from wild strains only in their monkey or chimpanzee pathogenicity, and if they are transmissible, then trials in increasing numbers of people are rendered impracticable because of lack of control. While preliminary trials may be done in institutions, their final evaluation must be in children living in normal environments. There can be no assurance of how the vaccines will behave in a community, for it would seem that mutants of the less virulent naturally occurring strains may occasionally cause paralysis. How else can one explain the sporadic cases of poliomyelitis which occur in communities where extensive inapparent infections are occurring? We have no knowledge of how often mutants arise from naturally occurring avirulent strains, and whether or not they can cause epidemics. No one can predict what may happen if a community is heavily seeded with a transmissible avirulent virus.

The risks involved in large-scale trials of avirulent poliovirus vaccines may be justifiable in communities where severe epidemics are common. Such trials should only be undertaken step by step, and never without careful laboratory control, however difficult this might be.

References

In a report recently published by the World Health Organization (1958), the Expert Committee on Poliomyelitis strongly recommended that controlled field trials of live attenuated poliovirus vaccines should be carried out. The committee suggested suitable circumstances and populations for the trials and specified the strains of virus which it considered should be used. Trials are now in progress in many parts of the world, and a preliminary report of one of them has already been published (Courtois, Flack, Jervis, Koprowski, and Ninane, 1958). The purpose of this review is to give the reader who is unfamiliar with the subject some idea of the progress that has been made in the development and testing of attenuated poliomyelitis vaccines.

The possible advantages of living attenuated poliovirus vaccines over formalinized vaccines (Salk type) are that they can be given by mouth, that they are cheaper to produce, and that they may give a better immunity. They certainly give a broader type of immunity which will for a time prevent or modify subsequent infection of the alimentary tract with poliovirus. This is in contrast to the immunity at present produced by vaccination with formalinized vaccines which does not modify subsequent alimentary infection, at any rate to the same degree. The difference in the immunity produced by the currently available formalinized vaccines and attenuated virus vaccines is important because the use of attenuated virus vaccines in a community might interfere with the natural spread of virus, and so far there is no evidence
that formalinized vaccine is capable of doing this (Lam-
muir, 1957; Fox, 1957a, 1957b).

In the last few years there have been a number of small laboratory-studied trials of attenuated poliovirus vaccines, and there has been much progress in their development (Sabin, 1957a, 1957b), but the rapid emergence of a successful formalinized vaccine in 1955 removed some of the urgency which might have led to their earlier trial in the field. This delay allowed time for cautious progress, which may have been a good thing because attenuated poliovirus vaccines have certain novel features not previously encountered in human vaccines.

The poliovirus strains incorporated in current living vaccines developed by Sabin (1957b), by Cox and his associates (see da Silva, McKelvey, Bauer, Prem, Cooney, and Johnson, 1957), and by Koprowski (1957) have all been grown in monkey-kidney-tissue culture and selected for their comparative lack of neurotropism when inoculated into the C.N.S. of monkeys in large amounts. The vaccine viruses are given orally and cause an inapparent infection. They multiply in the alimentary tract like natural polioviruses, and the vaccinated subjects develop an immunity which is probably similar to that following an infection with one of the less invasive naturally occurring strains.

Attenuated poliovirus vaccines, like any other vaccines, require testing in the field for safety and effectiveness, both to the individuals vaccinated and to the community as a whole. For reasons of clarity these different aspects are considered separately.

Safety to the Vaccinee

The safety of attenuated poliovirus vaccines to vaccinees has been assessed both by direct clinical observation and, indirectly, by studying alterations which may occur in the character of the vaccine virus after multiplication in the human host.

To the best of our knowledge no illness definitely attributable to the use of attenuated poliovirus vaccines has so far been reported among thousands of persons who were observed carefully after vaccination in different trials with several different attenuated virus vaccines. This is a most encouraging fact, but we must bear in mind that nearly all natural poliomyelitis infections are mild or inapparent, especially with types II and III poliovirus, and that many of the people vaccinated in these trials have either been immune before vaccination or have been selected in a manner which has been far from ideal.
The indirect assessment of safety is made by testing the properties of the virus excreted by vaccinees. While there is a wide spectrum of monkey neuropathogenicity among naturally occurring or wild polioviruses, markedly neurotropic strains have always been recovered from the faeces of paralysed patients. If the excreted vaccine virus remains considerably less neurotropic for monkeys than wild polioviruses, which have been isolated from the faeces of paralytic cases, then it is usual to presume that the vaccine virus will do no harm to the vaccinee.

So far only one vaccine virus has been shown to change greatly in its neurotropism after growth in the human gut (Dane, Dick, Connolly, Fisher, and McKeown, 1957). This was the rodent-adapted TN type II poliovirus (Koprowski, Norton, Jervis, Nelson, Chadwick, Nelson, and Meyer, 1956), and there is general agreement that it is unsuitable for further trial (Dick and Dane, 1957; World Health Organization, 1958). All the other vaccine viruses which have been grown in monkey-kidney tissue culture and have been tested have shown some change towards greater neurotropism, but these changes have been insufficient to cause anxiety for the safety of vaccinees. If safety for the individuals receiving vaccine were all that was required of a vaccine, then there would be few problems in planning progressively larger trials of the currently available attenuated strains, though opinions would vary on the relative importance of direct clinical assessment and the indirect laboratory study of excreted virus; and they would also vary on the degree of neurotropism theoretically permissible in the attenuated virus.

Unfortunately the vaccine viruses now undergoing trials have been shown on numerous occasions to spread from vaccinees to their associates. Spread has not always occurred, but neither does it always occur with natural poliovirus infections. Because of this ability to spread by contact infection we have to consider carefully the safety of the vaccine viruses to the community in which they are used.

**Safety to the Community**

The spread of a poliomyelitis attenuated vaccine virus from vaccinees to their contacts was first demonstrated by Koprowski et al. (1956) with SM type I virus. His subjects in this trial were incontinent institutional children. Later we demonstrated that SM virus could spread in a normal household (Dick, Dane, Fisher, Connolly, and McKeown, 1957). With the more recently developed attenuated polioviruses spread of virus from vaccinees to their contacts has been shown (Paul, Horstmann, Melnick, Niederman, and Deutsch, 1957; da Silva et al., 1957; Smorodintsev, Drobitshevskaya, Gorev, Ilienko, Kluchareva, and Kurnosova, 1958; Smorodintsev, personal communication). To date no significantly greater change towards neurotropism has been
reported for virus recovered from infected contacts than has been observed in the excreted virus of some vaccinees. This finding is satisfactory, but the number of observations has necessarily been limited by the number of monkeys which are required for this type of test.

The basic problem we have to consider is the safety of the virus which is excreted by vaccinees and which will probably spread to other members of the community. As stated earlier, there is a broad spectrum of neurotropism among wild polioviruses. From the evidence so far published we do not consider that the virus excreted by some vaccinated subjects in recent trials can be differentiated from the less neurotropic strains of wild poliovirus. This opinion is based on our own interpretation of results from other laboratories, and we realize that not everyone will agree with it. Further work may show that there has been an irreversible change in the vaccine virus and that the excreted viruses are in fact unlike wild polioviruses and are never likely to change sufficiently to become dangerous. At the moment we consider that the irreversibility of the change is not proved.

If we accept that excreted vaccine polioviruses are similar to wild avirulent polioviruses, then how safe are they? Because neurotropic strains of virus have always been isolated from paralytic cases we may presume that the avirulent wild strains are probably safe provided that they are stable and cannot change to a more neurotropic form. Unfortunately there is little information on the stability of wild polioviruses, and for this reason we have been attempting to discover more about the problem by comparing the monkey neurotropism of polioviruses isolated from the faeces of paralytic patients and their close contacts. This method of approaching the problem is open to the criticism that, however carefully one may select a case and a contact, there is never absolute proof that both were infected with the same virus strain. The results of tests on three pairs of viruses

Comparison of the Monkey Neurotropism of Viruses Excreted by Paralysed Children and their Contacts

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<tr>
<th>Serial Letter of Pair</th>
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<th>Monkeys Paralysed when Inoculated with the Following TCD_{50} of Virus</th>
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<td>—</td>
</tr>
<tr>
<td>C</td>
<td>W.N. K.S.</td>
<td>Paralytic</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minor Illness</td>
<td>—</td>
</tr>
</tbody>
</table>

* Denominator = number of monkeys inoculated. Numerator = number of monkeys paralysed.

— Indicates not tested.

The techniques used in these tests are fully described elsewhere (Dane, et al., 1957).
are shown in the Table. Faecal virus was given one passage in monkey-kidney-tissue culture and then inoculated intracerebrally into rhesus monkeys in various amounts.

With two pairs (B and C) the viruses isolated from both the contact and the paralytic case were probably equally neurotropic. But in pair A, though the virus from the paralytic case (D.D.) was highly neurotropic, the virus from the contact (P.B.) was only slightly neurotropic and fell into the group of naturally occurring avirulent strains. The virus from P. B. resembled in monkey neurotropism (as judged by intracerebral inoculation) the virus excreted by subjects vaccinated with SM 100 type I poliovirus vaccine (Dick et al., 1957; Koprowski et al., 1956) and by subjects vaccinated with more highly attenuated poliovirus strains in a recent Minnesota trial (da Silva et al., 1957). If other currently used vaccine viruses had been subjected to the same type of trial that was conducted at Minnesota similar results might have been obtained.

We do not consider that the small piece of circumstantial evidence we have given above is in any way conclusive proof that wild polioviruses are unstable in their neurotropism, but we do think that these preliminary results might serve as a reminder to those planning attenuated vaccine trials of the sort of problems they have to face in evaluating vaccine safety.

The W.H.O. Expert Committee on Poliomyelitis (World Health Organization, 1958), in its review of present knowledge about live poliovirus vaccines and in its recommendations for trials, virtually ignores the problem of spread. It points out "that preliminary tests on attenuated polioviruses in the hands of several investigators have failed to reveal signs of illness or other harmful effects in the vaccinees or their associates" (our italics). Apart from this one oblique reference to spread there is no indication to the reader that it might be important. Whether the committee considered the spread of these vaccine viruses potentially dangerous or not, it was odd that it dismissed the whole problem with such brevity. The uncontrolled spread of poliomyelitis vaccine virus in a community must either be beneficial or potentially dangerous. If spread is dangerous this will be discovered only if field trials are planned with the object of answering this most important question. If spread is beneficial then it would be useful to know this. A new concept in the use of attenuated virus vaccines might be evolved which could be of great value in combating such diseases as influenza, mumps, and measles.

**Effectiveness in the Vaccinee**

The ultimate test of the effectiveness of attenuated poliovirus vaccines must await controlled field trials if these are in fact possible. In the meantime it seems reasonable to
judge likely effectiveness by measuring the levels of neutralizing antibody in vaccinated subjects.

In the trials where virus grown in monkey-kidney-tissue culture has been used the proportion of vaccinees developing neutralizing antibody has usually been over 90%, and the levels of antibody appear to have been satisfactory. With the TN type II rodent-adapted virus we found that a smaller proportion of children developed antibody (77% of 124), but the levels of antibody in children who did so were broadly similar to those reported following vaccination with viruses grown in tissue culture. Information about the persistence of neutralizing antibody comes mainly from trials with strains of vaccine virus no longer in use. Our own follow-up study on children vaccinated with TN type II virus (Dane, Dick, Briggs, and Nelson, 1958) is open to this criticism, but we consider that the results obtained with TN type II virus are likely to be at least as good as, or better than, those which will be found with the less virulent current vaccine viruses that do not undergo such a marked change after growth in the human gut. We found that about two months after vaccination 77% of children had developed neutralizing antibody at levels comparable to that found by others following two injections of formalinized virus (Report, 1957a), and that in a proportion of the children there was a marked decline in antibody level over the next year, which again was like that reported after two injections of formalinized virus (Report, 1957b).

Very high levels of neutralizing antibody have been shown to develop following a third booster injection of formalinized vaccine (Report, 1957b), and it is doubtful whether refeeding with live virus vaccines would have such a marked effect. Furthermore, in view of the alimentary resistance to reinfection which follows vaccination with live virus vaccine, it would be difficult to predict whether or not, or when, reinfection would occur. This does not mean that refeeding would not be a possible way of maintaining immunity induced by live virus vaccines, but only that certain problems would arise which have not arisen with formalinized vaccine.

The alimentary resistance to reinfection which follows vaccination with live virus is often presumed to be some sort of local gut immunity. Little is known about the nature of this immunity or how long it will last, and, although it is of obvious benefit to the individual vaccinee, it may have more far-reaching consequences to the community as a whole.

Effectiveness to the Community

The resistance or comparative resistance of people vaccinated with live attenuated polioviruses to reinfection with these viruses gives hope that they will be similarly resistant to infection with wild polioviruses. If this proves to be the case, then there is a real chance that wild polioviruses might
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disappear from a community where sufficient numbers had been vaccinated with live virus vaccines. This does not appear to have happened in the U.S.A., following the use of formalinized vaccine, but until a high proportion of the susceptibles in a country have been adequately vaccinated with potent formalinized vaccine the possibility that the elimination of polioviruses from a community can be achieved with this type of vaccine cannot be excluded. Experimentally, Howe (1957) has shown that there was a significant reduction in the amount of virus excreted by chimpanzees fed a virulent strain of poliovirus if they had previously been vaccinated with formalinized virus. Poliovirus infection in chimpanzees is characterized by a greater pharyngeal multiplication than occurs in man, and for this reason a direct comparison may not be valid.

Use and Testing of Current Attenuated Strains

There appear to be four possible uses of attenuated poliovirus vaccines. They could be used (a) instead of formalinized vaccines; (b) to supplement formalinized vaccines; (c) to halt epidemics; and (d) in countries whose financial resources do not permit the use of formalinized vaccines.

In the first case, it seems unlikely that any country with the finances available to undertake a vaccination programme using formalinized vaccines would use, or switch to, attenuated virus vaccines until it has been shown that they are as safe as, or safer than, formalinized vaccines and produce as good or better levels of immunity.

In the second case, attenuated virus vaccines might be used to supplement formalinized vaccines in the hope of eliminating wild viruses from the community. Before undertaking such a programme one would like to know whether virulent polioviruses may not in fact be largely eliminated from communities which are efficiently immunized with potent formalinized vaccines. Enough time has not elapsed from the introduction of formalinized vaccines, and sufficiently thorough immunization has not yet been achieved to answer this question.

The use of attenuated vaccines to control epidemics at the present time involves the employment of a contagious virus whose safety and effectiveness during non-epidemic periods has been inadequately studied. It cannot be predicted how avirulent viruses would behave if they were introduced into a community in which conditions are ripe for an epidemic, and in many areas it would be difficult to organize voluntary immunization of a whole population as was done recently in Africa (Courtois et al., 1958). The problems involved in attempting to evaluate the results of vaccinating a community during an epidemic are great enough to deter some authorities from introducing a largely unproved vaccine in this manner.
There remains the possibility that live virus vaccines might be used in countries in which formalinized vaccines cannot be afforded. In our opinion carefully conducted trials of attenuated virus vaccines should be undertaken in such countries if there is a genuine need for vaccination, and they should be done during non-epidemic times of the year. The previous epidemic history of poliomyelitis should be available, and should be carefully considered before initiating the trials. Such trials would be the most valuable first step in gaining information in the field, but it must be appreciated that there are great difficulties in planning trials of a vaccine which spreads from vaccinees to their contacts. Any trial of safety must take this factor into account and must attempt to evaluate the harm or good which has been achieved by the uncontrolled spread of a living virus. The information so far published about the currently available attenuated vaccines does not suggest that they will necessarily be either dangerous or completely safe. It is important that trials should aim at defining the limits of safety of these vaccines, rather than at sidestepping the possible dangers which accompany the use of a contagious vaccine.

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VACCINATION AGAINST POLIOMYELITIS WITH LIVE VIRUS VACCINES

5. NEUTRALIZING ANTIBODY LEVELS ONE YEAR AFTER VACCINATION

BY

Lecturer in Microbiology

G. W. A. DICK, M.D., D.Sc., F.R.C.P.Ed.
Professor of Microbiology

MOYA BRIGGS,* B.Sc.

AND

ROBERT NELSON*

From the Department of Microbiology, The Queen's University, Belfast

During the first half of 1956 small laboratory-studied trials of TN type II and SM type I oral attenuated poliomyelitis vaccines (Koprowski, Norton, Jervis, Nelson, Chadwick, Nelsen, and Meyer, 1956) were made in Northern Ireland (Dane, Dick, Connolly, Fisher, and McKeown, 1957; Dick, Dane, Fisher, Connolly, and McKeown, 1957). Neither of these vaccines fulfilled what we considered to be the necessary theoretical requirements of safety (Dick and Dane, 1957), but the results of investigations into the duration of immunity following their administration are reported here because they may be of some value in predicting the sort of immunity which is likely to follow the use of the more attenuated virus vaccines now available.

Materials and Methods.—The materials and methods used were as described previously (Dane et al., 1957). Comparative results for levels of neutralizing antibody in any individual are given only when paired sera were titrated in the same test.

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Results

Persistence of Neutralizing Antibody After Vaccination with SM type I Poliovirus

A number of volunteers who were fed SM type I virus in 1956 were bled at frequent intervals after vaccination in order to estimate the highest titre of antibody which they would develop. The serum with the highest titre from each of six adult volunteers was tested again with another serum taken about a year later. The results of these tests are shown in Table I.

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Age</th>
<th>Sex</th>
<th>Pre-vaccination Titre</th>
<th>Weeks After Vaccination</th>
<th>Titre</th>
<th>Weeks After Vaccination</th>
<th>Titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>193</td>
<td>41</td>
<td>M</td>
<td>&lt;4*</td>
<td>7</td>
<td>22</td>
<td>67</td>
<td>&lt;4</td>
</tr>
<tr>
<td>218</td>
<td>39</td>
<td>M</td>
<td>&lt;4</td>
<td>6</td>
<td>22</td>
<td>68</td>
<td>11</td>
</tr>
<tr>
<td>353</td>
<td>24</td>
<td>F</td>
<td>&lt;4</td>
<td>4</td>
<td>16</td>
<td>68</td>
<td>4</td>
</tr>
<tr>
<td>284</td>
<td>20</td>
<td>M</td>
<td>&lt;4</td>
<td>3</td>
<td>179</td>
<td>99</td>
<td>64</td>
</tr>
<tr>
<td>212</td>
<td>20</td>
<td>M</td>
<td>&lt;4</td>
<td>5</td>
<td>359</td>
<td>62</td>
<td>39</td>
</tr>
<tr>
<td>235</td>
<td>25</td>
<td>M</td>
<td>&lt;4</td>
<td>9</td>
<td>8</td>
<td>72</td>
<td>256</td>
</tr>
</tbody>
</table>

* Reciprocal of serum antibody titre.

The antibody titre had fallen in five of the individuals, but had risen significantly in the other (No. 235). A number of serum specimens taken from No. 235 over the study period were tested in an attempt to explain this rise in antibody. It was found that between the ninth and the fifteenth month after vaccination he had developed a significant rise in neutralizing antibody to both type I (from 1:4 to 1:256) and type II poliovirus (from <1:4 to 1:96). It appears, therefore, that during this time he had had at least one natural poliovirus infection.

Persistence of Neutralizing Antibody After Vaccination with TN type II Poliovirus

Blood samples from 60 children vaccinated orally with TN type II poliovirus in 1956 were taken approximately one year later. The comparative levels of neutralizing antibody in this specimen and one taken about two months after vaccination were estimated by testing the two sera at the same time. The variation in antibody levels during this period is shown in the Chart.

In a proportion of the children a significant antibody rise occurred after the first post-vaccination blood specimen had been taken. It is impossible to prove that the antibody rises in these children were not the result of infections with naturally occurring type II polioviruses, but the most satisfactory explanation is that vaccine virus multiplication in the gut (and therefore the antigenic stimulus) continued in these children beyond the date when the first post-vaccination blood sample was taken (approximately the ninth week after
Individual variation of type II neutralizing antibody titre in 60 children following vaccination with TN type II poliovirus. All the children were without type II antibody at the time of vaccination.

We have no laboratory data to support this hypothesis, because the trial was originally planned on the basis of observations by Koprowski et al. (1956) that TN type II vaccine virus was excreted in scarcely detectable amounts over a short period, and for this reason collection of faecal specimens was not continued long enough. However, a number of children were excreting faecal virus during the fourth week after vaccination, when specimen collection ceased, and there seems to be no reason why they should not have continued doing so for several months, as was the case in children vaccinated with SM type I virus (Koprowski et al., 1956).

The possibility that some of the rises in type II antibody might have been due to natural infections with heterologous poliovirus types was also considered. Serum specimens taken at the time of vaccination and one year later were tested at a 1:4 dilution against types I and III poliovirus to find out whether any children had developed antibody against these poliovirus types. Titrations of the paired sera from children found to have developed antibody showed that six natural infections had occurred—three with type I poliovirus
and three with type III poliovirus. In the course of another investigation type I poliovirus had in fact been isolated from two of the children who showed serological evidence of a type I infection.

We conclude that heterotypic poliovirus infections could only have contributed to the late rise in type II antibody in these six instances.

After testing the first post-vaccination blood sample (taken after about two months) we reported that only 77% of 124 children had developed neutralizing antibody following vaccination with TN type II virus (Dane et al., 1957). We were able to obtain blood samples one year later from 15 of the 28 children who did not develop antibody. None were found to have developed type II antibody during this interval, thus confirming our original conclusion that the vaccine had failed to "take" in these individuals.

**Influence of Heterotypic Antibody on Vaccination with TN Type II Virus**

Because of the possibility that previous natural heterotypic poliovirus infections might have influenced the response of children to vaccination with TN type II virus, pre-vaccination samples of blood from 110 children who had been vaccinated with TN type II virus were tested for neutralizing antibody at a 1:4 dilution against types I and III poliovirus. The pre-vaccination antibody patterns found are shown in Table II. Children possessing different pre-vaccination heterotypic antibody patterns showed no significant difference in their response to vaccination.

**Table II.**—Poliomyelitis Neutralizing Antibody Patterns at the Time of Vaccination in 110 Children Fed TN Type II Virus in 1956

<table>
<thead>
<tr>
<th>No. of Children</th>
<th>Type I Antibody</th>
<th>Type II Antibody</th>
<th>Type III Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>63</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

—Titre of < 1 in 4.  +—Titre of > 1 in 4.

**Discussion and Conclusions**

The neutralizing antibody levels which we found in children one year after vaccination with TN type II virus were of the same order as those reported in non-immune children who had received two injections of formalinized vaccine a year before (Report, 1957). A number of the TN-vaccinated children were found to have had a marked decline in their neutralizing antibody level over the year, and our previous finding that 23% of children failed to develop antibody after vaccination was confirmed.
One of the advantages claimed for oral attenuated poliomyelitis vaccines is that they will produce a more durable immunity than formalinized vaccines. We can find no evidence that this was true in a proportion of children vaccinated with TN type II virus. Admittedly our yardstick for the measurement of immunity has been circulating neutralizing antibody. Some sort of local gut immunity follows infection with oral poliovirus vaccines, but in the absence of more precise information about this local immunity it would be unwise to assume that it is more durable than circulating antibody.

Further trials of Koprowski’s TN type II vaccine have been abandoned because the virus is liable to change its character and become virulent after growth in the human gut. If it can be shown that the recently developed more highly attenuated poliovirus vaccines do not suffer from this drawback, then it would seem desirable to show also that they can produce a reasonably durable immunity in the majority of vaccinated subjects before they are used on a large scale. On the basis of the results reported here we do not think that durability of immunity should be considered as an automatic benefit associated with living vaccines.

We wish to acknowledge grants from the National Fund for Poliomyelitis Research, the Medical Research Council, and the Northern Ireland Hospitals Authority.

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