VACCINATION AGAINST POLIOMYELITIS WITH LIVE VIRUS VACCINES

1. A TRIAL OF TN TYPE II VACCINE

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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$_{50}$ (MPD$_{50}$) 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 vac-

cinated 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 viraemia, 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 TCD_{50} 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 TCD_{50} of virus at a 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 10% acetic acid. The usual blocks from brain, brain stem, and cord were embedded in paraffin and stained with gallocyanin.

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 micro-organism 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 I.—*Pathogenicity of TN Vaccine for Monkeys*

Source of Virus	Vol. of Inoculum and Route	Log₁₀ MPD₅₀ Inoculated	No. of Monkeys			Remarks
			Inoculated	With Paralysis	With Lesions of Polio in CNS	
	1·0 ml.i.c.	5·6	6	0		Not examined for lesions
Mouse brains and cords of 29th passage in PRI mice	0·5 ml.i.c.	4·5*	4	0	1	1 not examined for lesions
	0·5 „ „	3·5	4	0	1	
	0·5 „ „	2·5	4	0	0	
	0·5 „ „	1·5	4	0	0	1 not examined for lesions
(=Vaccine used for trial)	0·5 ml.i.s.	5·3	4	2		Not examined for lesions
	0·1 ml.i.s.	3·1*	3	1	1	Paralysed, not examined for lesions
	0·1 „ „	2·1	3	0	1	1 not examined for lesions
	0·1 „ „	1·1	3	1	1	
	0·1 „ „	0·1	3	0	0	1 not examined for lesions
29 passes in PRI and 1 pass in VSBS mice	1·0 ml.i.c.	5·4	6	0	1	
	0·5 ml.i.s.	5·1	6	2	4	

* Titration by Dr. H. Koprowski in New York.
i.c. = intracerebral. i.s. = intraspinal. MPD_{50} = mouse intraspinal paralysing dose ₅₀.

Vaccination of Adults

Twenty-one adults were fed 500,000 MPD50 of TN vaccine virus diluted in 4 ml. of milk. Tests have 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-reactors 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 II). No viraemia 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
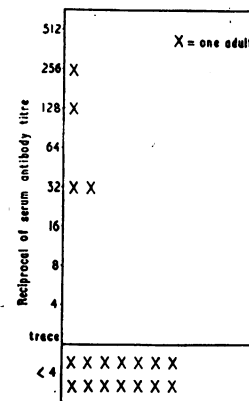

FIG. 1.—Type II antibody response in 18 adults fed with TN vaccine.

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*

Group	No. in Group	No. with Virus in Faeces	No. with Virus in Throat*	No. with Virus in Blood*	No. who Developed Antibody
Tested for virus excretion and viraemia ..	10	0	1†	0	3
Not tested for virus excretion	8	N.T.	N.T.	N.T.	1

* 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 MPD$_{50}$ 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 avail-

TABLE III.—*Time of Post-vaccination Bleeding for 124 Children Whose Antibody Response is Shown in Fig. 2*

Weeks after vaccination	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
No. of children ..	2	3	60	25	5	4	1	1	6	8	1	3	1	—	—	1	3

TABLE IV.—*Ages of 124 Children Whose Antibody Response is Shown in Fig. 2*

Age in years	<1	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
No. of children ..		3	7	13	16	12	30	11	4	4	12	4	2	2	2	1	—	1

able 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

FIG. 2.—Type II antibody response in 124 children fed with TN vaccine. All children had <1/4 type II antibody at time of vaccination.

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 ($\chi^2 = 8.7111$; $0.01 > P > 0.001$).

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 ($\chi^2 = 5.0$; $0.05 > P > 0.02$).

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 10^4 TCD$_{50}$ per gramme of faeces or higher were recorded for three children. One girl aged 10 months excreted 10$^{6\cdot4}$ TCD$_{50}$ of virus on the fifth day after feeding, and at this time her brother aged 2, who was vaccinated on the same day, was excreting 10$^{5\cdot1}$ TCD$_{50}$ 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 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
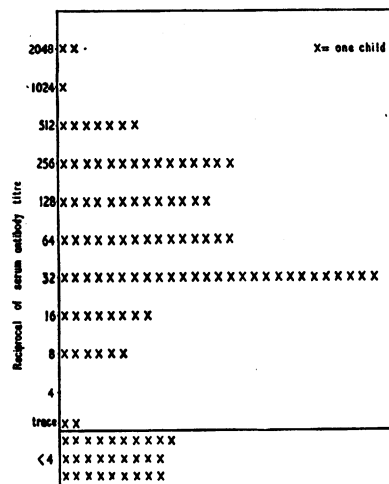
from any of the remaining four infants to test for antibody below the level of 1 in 16. The antibody rise following vaccination which occurred in the three infants having moderate titres of maternal antibody at the time of vaccination is not high, but when the rate of decline of maternal passive immunity 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
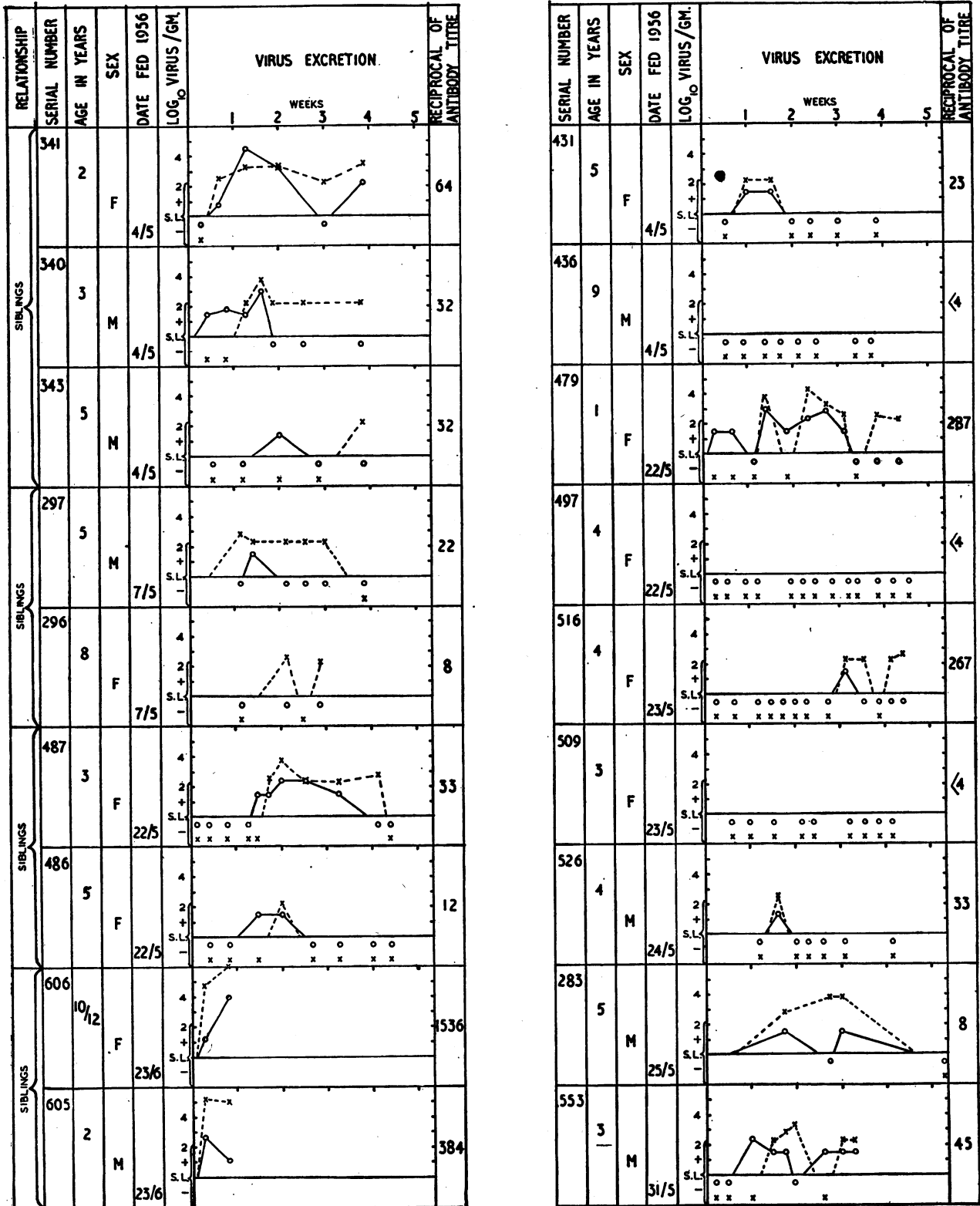
FIG. 3.—Faecal excretion of virus in children fed with TN vaccine. At time of vaccination all subjects had type II antibody <1/4. Each O or X represents one specimen or pools of two or three specimens. O————O=level of excreted virus detected in mice. X - - - X =level of excreted virus detected in tissue culture. S.L.—Screening level is $10^{2 \cdot 5}$ TCD$_{50}$ for tissue culture and $10^{3 \cdot 4}$ PD$_{50}$ for mice.

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 10^4 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 that 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
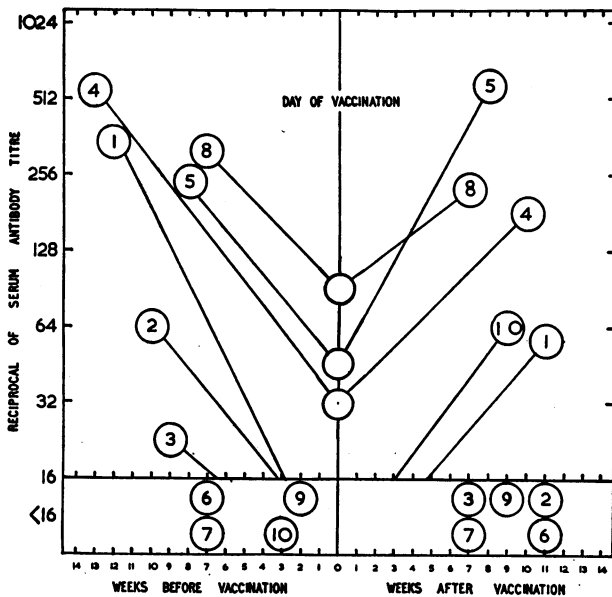
FIG. 4.—Type II antibody response in infants fed with TN vaccine. Antibody prior to vaccination was passively acquired maternal antibody. Serial numbers and age at time of vaccination:

No.	Age in months	No.	Age in months
One—254	3	Six—286	2
Two—258	5	Seven—287	2
Three—264	3	Eight—288	2
Four—265	3	Nine—381	5
Five—272	2	Ten—439	6

Three specimens of serum from each infant were tested : one was taken a few weeks before vaccination, one on the day of vaccination, and one a few weeks later.

FIG. 5.—Faecal excretion of virus in infants fed with TN vaccine. Each O or X represents one specimen or pools of two or three specimens. O——O=level of excreted virus detected in mice. X - - - X =level of excreted virus detected in tissue culture. S.L.=Screening level is $10^{2 \cdot 3}$ TCD$_{50}$ for tissue culture and $10^{2 \cdot 4}$ PD$_{50}$ for mice.

TABLE V.—*Pathogenicity of TN Faecal Virus for Monkeys*

No.	Age, Months	Day After Vaccination	Treatment of Faeces for Inoculation	Titre of Inoculum Log$_{10}$ MPD$_{50}$	Titre of Inoculum Log$_{10}$ TCD$_{50}$	No. of Monkeys Inoculated	Incubation (Days)	Monkey Died with No Paralysis, Day	Paralysis of Grade 0-1 Severe	Paralysis of Grade 2-3 Moderate	Paralysis of Grade 4 Mild	Paralytic Ratio (Excludes Deaths)	Lesion Ratio
254	3	24 and 25	1 mouse pass 10% suspension of mouse cords	5·3	4·6	5	10, 10, 10, 17	17	4	0	0	4/4	5 5. Day 17 death histo. pos. for polio
			20% faecal suspension	1·8 or <	2·5	5	10, 10, 11, 11	—	3	1	0	4/5	5/5
265	3	21 and 22	1 mouse pass 10% suspension of mouse cords	3·5 or <	4·6	5	11	18	0	0	1	0 4 (?1/4)	4/5. Day 18 death histo. pos. for polio
341	31	27	1 mouse pass 10% suspension of mouse cords	3·4	2·8	4	10, 24	—	1	0	1	1 4 (?2,4)	4/4
			20% faecal suspension	2·8 or <	3·5	5	6, 11, 13, 13, 15	—	3	1, 1	0	4/5 (?5 5)	5/5

All monkeys inoculated intracerebrally with 1 ml. of virus suspension.   Non-specific deaths within 48 hours of inoculation have been excluded from the Table.   Monkeys showing mild transient weakness and their incubation periods are shown in bold type.

did not in fact raise the titre of virus from subjects Nos. 265 and 341 to the desired level; and a comparison of the titres 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* virus by reference to Tables V and I. Twelve monkeys were inoculated intracerebrally with $10^{5·4}$ or $10^{5·6}$ MPD$_{50}$ of TN *vaccine* virus; none became paralysed. Twenty-four monkeys were inoculated intracerebrally with TN *faecal* virus; the amount of virus inoculated varied from $10^{3·3}$ to $10^{1·8}$ or less MPD$_{50}$. 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

*This does not include seven vaccinated children on whom antibody tests were not possible.

TABLE VI.—*All Minor Illnesses Observed in 190 Subjects During the 30 Days Following Feeding with TN Vaccine*

Group	No.	Age	Signs and Symptoms On Day After Vaccination	Signs and Symptoms Description (Temperature)
Individuals with no homologous antibody before vaccination and no antibody response to vaccination	92	5	1, 2	Pharyngitis
	508	5	7, 8	Diarrhoea, catarrh
	536	7	25–28	Sore throat
	346	21	1, 4, 5	Sore throat, headache
	345	33	7	Fever (99·4° F.; 37·4° C.) only
	274	35	1–2	? cold
Individuals with no homologous antibody before vaccination and antibody response to vaccination	265	3/12	3, 4, 6	Fretful, did not finish feed
	254	3/12	2, 9, 11	" " "
	606	10/12	4	" Off colour " "
	464	1	8, 9	Coryza, otitis media (101·8° F.; 38·8° C.)
	605	2	4	Listless
	527	2	22	Chicken-pox
	340	3	2, 3	Coryza (101·4° F.; 38 6° C.)
	479	3	20	Fractured clavicle
	526	4	22	Chicken-pox
	516	4	5	? Rubella (99·4° F.; 37·4' C.)
	486	5	14, 30	Cold Sore throat (100° F.; 37 8° C.)
	534	5	4	Pain back of neck, enlarged post-occipital glands
	431	5	3	Pain in thighs
	468	6	10	" Off colour "
	494	8	6, 7	Pain in abdomen (100 8°F.; 38·2° C.)
	296	8	21	? Rubella
	211	22	2	Sore throat
	333	50	19–24	Sore throat, glands in neck
Immune prior to vaccination†	493	5	14–16	Sore throat, headache (101° F.; 38·3° C.)
Post-vaccination serum not tested	465	2	8, 13, 14	Anorexia Coryza (100° F.; 37 8° C.)

† This child developed no antibody rise following vaccination.

TABLE VII.—*A Carefully Observed Group of 107 Infants and Children who Were Fed TN Vaccine*

Group	Description	No. in Group	No. with Minor Illness up to 1 Month After Vaccination
A	Children with no antibody before vaccination and antibody response to vaccination	73	13
B	Children with no antibody before vaccination and no antibody response to vaccination	22	2
	Children immune before vaccination	12	1

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 ($\chi^2 = 0.8507$; $0.50 > P > 0.30$).

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.

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Reader in Morbid Anatomy

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Koprowski and his associates (1956) have reported the successful immunization of 75 children by the oral administration of SM type I live poliomyelitis virus vaccine. All children whom they vaccinated developed homotypic antibody in response to vaccination. No signs or symptoms referable to the feeding of the vaccine were observed. All the children became intestinal carriers of virus, but virus was never recovered from their throats or their blood. Faecal virus was excreted for periods up to 171 days after vaccination and frequently reached high titres (Koprowski, 1955). The SM vaccine virus did not cause paralysis in monkeys when inoculated by the intracerebral route. However, it did paralyse monkeys when inoculated intraspinally (see Table I). The significance of paralysis in monkeys inoculated by these two routes and the reasons for considering lack of intracerebral pathogenicity as a sign of attenuation are discussed elsewhere (Dick and Dane, 1957). Koprowski and his associates (personal communication) tested the monkey pathogenicity of faecally excreted SM virus and concluded that it had not changed significantly from the SM vaccine virus.

More experience had been gained with SM vaccine than with any other oral type I vaccine, and we thought that it was suitable for a further trial. For several reasons we considered that only a very small trial of this vaccine was desirable. The large amounts of virus excreted in the faeces suggested that transmission of virus from vaccinated to unvaccinated individuals might happen quite readily. Koprowski *et al.* (1956) had in fact shown that transmission could sometimes occur, but only among mentally defective children with low standards of hygienic behaviour. They concluded: " It is quite clear, however, when the 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." Another reason for caution was that from the evidence available we could not conclude that SM *faecal* virus

TABLE I.—*Pathogenicity of SM Vaccine for Monkeys*

Source of Virus	Vol. of Inoc. and Route	Log₁₀ TCD₅₀ Inocu-lated†	No. of Monkeys			Remarks
			Inocu-lated	With Para-lysis	With Lesions of Polio in C.N.S.	
SM (N-90). Type I vaccine = chick-embryo-tissue culture. SM virus (SM virus→ 13 chick-embryo-tissue culture plus 6 alternating monkey-kidney chick-embryo tissue culture passes)	*1 ml. i.c.	6·8	8	0	1	
	0·5 ml. i.c.	5·0	4	0	1	
		4·0	4	0	0	
		3·0	4	1	3	Transient weakness
		2·0	4	0	2	
		1·0	4	0	0	
	*0·5 ml. i.s.	6·5	6	4	4	
	0·1 ml. i.s.	3·6	3	1	2	1 paralytic not examined for lesions
		2·6	3	1	1	
		1·6	3	1	2	1 paralytic possible bacterial infection on histological examination
		0·6	3	0	0	
		0·06	3	0	0	

* Vaccine passed one additional time in monkey-kidney-tissue culture to enhance titre.

† TCD₅₀ = tissue-culture dose₅₀.

‡ Titration by Dr. H. Koprowski in New York.

i.c. = intracerebral. i.s. = intraspinal.

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 10²·³ TCD₅₀ 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 TCD₅₀ 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 TCD₅₀ 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 Faecal Virus by Adults Fed SM Vaccine*

Subject No.	235	193	212	218	353	207	326	330	331	284
Sex	M	M	M	M	F	M	M	M	M	M
Age in years	25	41	21	39	24	21	53	46	31	20
Antibody before feeding* Type I	−	−	−	−	−	−	−	−	−	−
„ II	+	+	−	+	+	+	+	+	+	+
„ III	−	−	−	+	+	−	+	+	−	−
Amount of virus fed†	54	3,500	3,500	3,500	3,500	3,500	3,500	3,500	3,500	3,500
Date fed ..	14/2	19/3	19/3	4/4	4/4	4/4	9/4	9/4	9/4	11/4

Days after feeding										
	−	−	−	−	−	−	−	−	−	−
7	+ + +3·3 + + +3·3	+ + +5·3 + + +	+ + + +4·8 + +4·8 +	+ + + +5·3 + + +2·3 −	+ +4·8 + + +2·3	− +2·8 + +4·3 +	+ + + +2·8 +	+ + + +2·8	+ + + +3·8 + −	+ + +5·3 + + −
14	+	+		+ +2·3		−		−	−	−
21	+ +	+2·8	+ +2·8	+		−	−	−		−
28	+									−
35	+	+2·3								
42	−	−								
49	−	−								
	−	−								

* Antibody before feeding: += titre of >1 in 4; − = titre of <1 in 4.
† Dose of virus expressed as TCD₅₀.
Each faecal specimen tested is represented by + or −.
+= titre per gramme faeces >10²·³ TCD₅₀.
−= titre per gramme faeces < 10²·³ TCD₅₀.
Where individual specimens have been titrated the result is shown as log to the base of 10, TCD₅₀ 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 TCD₅₀ 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 10⁶·⁵ (No. 242) and 10⁴·⁵ TCD₅₀ (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 TCD₅₀ 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 10⁶·⁵ TCD₅₀ 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*

Serial No.	Age	Sex	Antibody Prior to Vaccination I	II	III	Time After Vaccination Days 7	14	Weeks 3	4	5	6	7	8	Months 3	4	5	6	7	8	9
235	25	M	−	+	−	4*			11	22		11	32	32						4
193	41	M	−	+	−	<4	6				32	45			6				8	
218	39	M	−	+	+	<4	>16	32	22		32							32		
353	24	F	−	+	+	<4	8	8	16		6							4		
207	22	M	−	+	−	<4	>16	89			33							45		
284	20	M	−	+	−	<4	128	256	89	179	128							89		
212	20	M	−	−	−	<4	Trace		16	359							89			
326	53	M	−	+	+	<4	6	6	4											
330	46	M	−	+	+			<4		4										
331	31	M	−	+	−	<4	Trace		6	24										
314	28	M	−	−	−									Trace						

+ = >1 in 4.   − = <1 in 4.   * Reciprocal of serum antibody titre.

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 $10^{5.6}$ TCD$_{50}$ per gramme 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*

Serial No.	Age	Sex		Days After Vaccination						
				7	14	21	28	35	42	49
242	3 months	M	Virus in faeces	+ +++++++ ++++ 4·0 5·5 3·5 5·0 5·0 4·0				+ + 4·0 + + 4·5	−	
			Antibody*	68				96		

349	2 months	F	Virus in faeces	+++++++++++++ +
				4·5 + +
				4·5 +
					+	+ +
						  + +
						3·5
							+ +
							3·0 3·5
								+ + + +
								   + +
								3·5		

			Antibody	48				96		

+ = >$10^{2.3}$ TCD$_{50}$ per gramme of faeces.   − = <$10^{2.3}$ TCD$_{50}$ per gramme of faeces.   Where specimens have been titrated log$_{10}$ TCD$_{50}$ per gramme of faeces is given.   * Reciprocal of serum antibody titre.
Note.—No. 242 had antibody titre of 1 in 512 twelve weeks before vaccination.   No. 349 had antibody titre of 1 in 89 two weeks before vaccination.

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

		Days After No. 337 Received SM Type I Vaccine				6 Weeks After
		7	14	21	28	
No. 337 female aged 4 years	Antibody*	<4				256
Fed 6,400 TCD$_{50}$ SM Type I vaccine on 3/5/56	Throat virus†	− − − − − −				
	Faecal virus‡	+++ + 6·5 + + 2·3 − · − −				
No. 338 male aged 2 years. Contact	Antibody	<4				32
	Faecal virus‡	− − − − + + + 4·8 5·6 − − − −				
No. 347 female aged 38 years. Contact	Antibody	<4				6
	Faecal virus‡	− − − − − − −				
No. 289 female aged 6 years. Contact	Antibody	<4				>4§

* Reciprocal of serum antibody titre. † Sensitivity of test described in methods. ‡ + = >$10^{2.3}$ TCD$_{50}$ per gramme of faeces. − = <$10^{2.3}$ TCD$_{50}$ per gramme of faeces. Where specimen has been titrated log$_{10}$ of titre per gramme faeces is given. § Taken 11 weeks after.

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

Sample of Faeces from			Treatment of Faeces for Inoculation	Log$_{10}$ TCD$_{50}$ Inoculum	No. of Monkeys Inoculated	Incubation Periods (Day)	Monkey Died With No Paralysis (Day)	No. of Monkeys with Paralysis of Grade			Paralytic Ratio of Survivors	Lesion Ratio	Notes
No.	Age	Day After Vaccination						0–1 Severe	2–3 Moderate	4 Mild			
242	3/12	35	1 tissue culture passage	6·6	4	6, 9, 13, **20**	—	1	0	2, 1	3/4 (?4/4)	4/4	In 3 monkeys paralysis graded as 4 only
337	4	5	4% faecal suspension	5·1	5	7	8, 17	1	0	0	1/3	3, 5	Day 8 death histology neg.; day 17 death histology pos. for polio
212	20	9	1 tissue culture passage	6·4	5	**20, 21**	6	0	1	1	1/4 (?2/4)	4, 5	Day 6 death histology neg.
338	2	Contact, infection, see text	20% faecal suspension	4·9	5	6	—	1	0	0	1/5	3/5	
			1 tissue culture passage	7·3	3	**12, 13**	—	0	1	1	1/3 (?2, 3)	3/3	

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 viraemia. 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 $10^{4 \cdot 9}$ to $10^{7 \cdot 3}$ TCD50, but there was no difference in the numbers 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 ($10^{6 \cdot 8}$ and $10^{5 \cdot 0}$ TCD50) 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 7 out of 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 into monkeys.

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 $10^{4 \cdot 6}$ to $10^{0 \cdot 6}$ (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*

Source of Virus	Log10 TCD50 Inoculated	No. of Monkeys Inoculated	Paralytic Ratio
SM N-90 Type I poliomyelitis virus vaccine	4·6	6	4/6
	3·6	4	3/4
	2·6	4	3/4
	1·6	3	2/3
	0·6	4	0·4
Second human passage SM faecal virus	4·6	5	3/5
	3·6	6	4·6
	2·6	4	2/4
	1·6	4	2/4
	0·6	4	0/4

Only a trace amount of virus was recovered from the blood of one subject (No. 212), but because of the possible importance of viraemia 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 $10^{5 \cdot 9}$ to $10^{2 \cdot 9}$ TCD50 (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*

Dilution of Tissue Culture Fluid	Log10 TCD50 Inoculated	No. of Monkeys Inoculated	Paralytic Ratio	Remarks
Undiluted	5·9	3	0/3	
10⁻¹	4·9	4	0/4	1 monkey died day 9; weak monkey
10⁻³	2·9	4	0/4	

All monkeys inoculated intracerebrally 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*

No.	Age	Sex	*Temp. (°F.) Rise, on Day ( )	Symptoms and Signs on Day ( )	Remarks
349	2/12	M	None	Colic (8)	—
242	3/12	M	,,	Off feed (8)	—
338	2	M	101° (3)	Off colour for 8 weeks	Contact of 337 (see text)
337	4	F	103° (6,7)	Fever	Non-polio virus recovered from throat (day 6) (see text)
235	25	M	None	Sore throat (4)	No virus in throat
353	24	F	,,	Backache (2–4)	—
331	31	M	,,	Feeling unwell (0–3)	Anxiety
218	39	M	,,	Occasional loose stool (3–5)	—
326	53	M	,,	Easily tired (0–14)	—

* 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 titres 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.

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3. THE EVALUATION OF TN AND SM VIRUS VACCINES*

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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, Nelsen, 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. = 4.7081). 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 TCD_{50} of SM (type I) vaccine developed antibody, but only one out of three adults who were fed 54 TCD_{50} developed antibody.† On the other hand, Koprowski 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 (Peart, 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 TCD_{50} 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
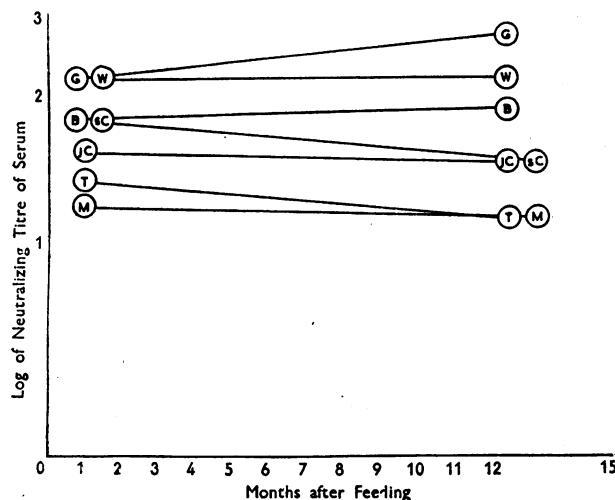

FIG. 1.—Antibody levels months after vaccination with SM type I virus. (Drawn from results presented by Koprowski, 1955).

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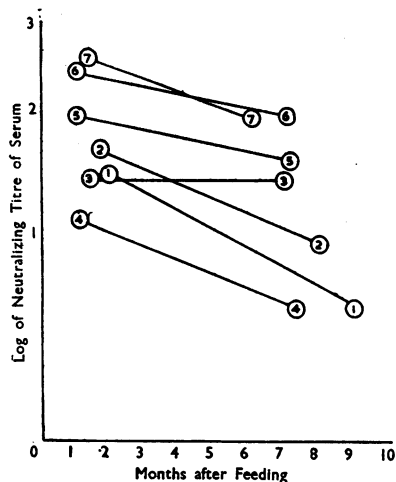
FIG. 2.—Antibody levels months after vaccination with SM type I virus. (Dick et al., 1957).

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.

Excreted Viruses

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 neoblastic) origin. Their tests for faecal virus were accordingly made by inoculating faecal extracts intraspinally 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 10^3 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 PRI 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 10^5 to more than 10^6 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 may 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 II antibody might have prevented type I virus entering the blood stream in the majority of the subjects vaccinated. If viraemia is important in the pathogenesis of paralytic poliomyelitis, it might be that circulating type II antibody reduces the chance of paralysis in 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 II strain. 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 PD_{50} 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 PD_{50} of TN vaccine 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 $10^{6.8}$ as compared with a titre of $10^{6.8}$ TCD_{50} in the absence of the vaccine.

So far as we know, cyotpathogenic 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 fulfil 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 affinity of the viruses 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 monkey virulence, 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 can often 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 Koprowski'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 vaccine 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.

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EFFECT OF HYPERMETABOLISM ON THE GOITRE OF A SPORADIC CRETIN

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It is well known that non-toxic nodular goitres may decrease in size as the result of thyroid administration, though to a much less degree than diffuse thyroid enlargements. That this response may be especially evident with hypothyroid nodules was the subject of two of our own recent communications (Zondek *et al.*, 1955; Zondek and Leszynsky, 1956). It was shown that in two siblings with familial sporadic cretinism and large, partly diffuse, and partly nodular goitres the induction and maintenance of a euthyroid state succeeded in one case in considerably reducing the size of the nodules, and in the other in causing the disappearance of four out of five nodules. The diffuse enlargement subsided entirely in both patients.

The purpose of this communication is to show how hypothyroid nodules respond to short-termed overdosage —that is, to the induction of " spurious alimentary hyperthyroidism."

Case Report

The subject of the study was one of the above-mentioned siblings, a girl aged 14. She was first seen at the age of 12. At that time she had, together with the typical features of childhood myxoedema, a large diffuse goitre in which three nodules varying in size between large plums and peaches were embedded. The heart shadow was greatly enlarged (transverse diameter 14 cm.), the B.M.R. was −13% and the blood cholesterol was 310 mg. per 100 ml. Electrocardiography was within normal range. After 1,020 μg. of tri-iodothyronine given within eleven days the clinical picture had in every respect reverted to normal. Both the diffuse and nodular parts of the goitre had at that point greatly decreased in size, and continued doing so during the subsequent four months of maintenance therapy (though irregularly carried out) with tri-iodothyronine and desiccated thyroid. The nodules failed, however, to disappear completely. The patient stopped substitution therapy entirely during the following twelve months.