VACCINATION AGAINST POLIOMYELITIS WITH LIVE VIRUS VACCINES*

6. CHANGES IN SABIN TYPE II ORAL VACCINE VIRUS AFTER HUMAN PASSAGE

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Many countries using inactivated (Salk) poliovirus vaccine on a large scale have been maintaining a continuing field surveillance of vaccine safety. If and when these countries embark on programmes involving the use of oral poliovirus vaccines they will almost certainly wish to conduct a similar type of surveillance. The assessment of field safety of live virus vaccines is complicated by the fact that oral vaccines are infectious and therefore the safety of the vaccine to contacts of vaccinees and to the community as a whole requires consideration. This would be particularly true in countries which chose a gradual introduction of live vaccine in preference to mass "area feeding" where the opportunities for vaccine virus spread would be more limited. Knowledge of the changes which occur in the character of the vaccine viruses after human passage is essential for laboratories assisting in surveillance work, because they may be required to distinguish between naturally occurring viruses and viruses of vaccine origin.

In this trial of Sabin type II oral poliovirus vaccine we have attempted to define some of the more common changes which occur in the virus after human passage.

Materials and Methods

Vaccine Virus.—Sabin type II attenuated vaccine virus was obtained through the Medical Research Council's Poliomyelitis Vaccines Committee. The virus was stored at -30°C . and diluted in Earle's salt solution just prior to use. Both the original vaccine virus and the human-passage vaccine virus which was fed

to some children were kept and processed, but not assayed, in a special laboratory in a building adjacent to the Department of Microbiology.

Tissue Culture.—Second-passage rhesus-monkey-kidney cell monolayers in 6 by $\frac{5}{8}$ in. (15 by 1.6 cm.) roller tubes were used for virus isolation, and similar cultures in stationary 3 by $\frac{1}{2}$ in. (7.5 by 1.3 cm.) tubes were used for virus titrations, neutralization tests, and "t" marker tests. Monolayer cultures of HeLa cells in roller tubes were used in addition to monkey-kidney cells for some attempted virus isolations. Lactalbumin medium (Melnick, 1955) was used throughout.

Specimens.—Sera were inactivated at 56°C . for 30 minutes and stored at -20°C . For the collection of faecal samples, disposable nappies were provided for all children taking part in the study: soiled nappies were placed in separate labelled plastic bags and collected daily. Ten per cent. faecal suspensions were prepared on the day of collection and stored at -20°C .

Virus Titrations.—Faecal specimens were titrated using four tubes per $\log_{10}$ dilution. Virus suspensions used for feeding or in tests were titrated using four or eight tubes per $\log_{10}$ dilution. A titration using eight tubes per $\log_{10}$ dilution of a standard suspension of type 1 poliovirus (Brunhilde) was included with each batch of virus titrations, for the purpose of standardization.

Virus Neutralization Tests.—Type I (Brunhilde), type II (Y-SK), and type III (Leon) strains of poliovirus were used. Serum dilutions were incubated with 50 TCD₅₀ of virus for six hours at 37°C . and then left overnight at $+4^{\circ}\text{C}$. before inoculation into tissue-culture tubes. Two tubes were used for each doubling dilution of serum. Cultures were examined on the third and sixth days for cytopathic changes.

Monkey Neurovirulence Tests.—Rhesus monkeys of approximately 5 to 7 lb. (2.3 to 3.2 kg.) weight were inoculated intrathalamically, using the technique described by Bodian *et al.* (1950).

*The five previous papers on "Vaccination Against Poliomyelitis with Live Virus Vaccines" were as follows. (1) "Trial of TN Type II Vaccine" (Dane *et al.*, *Brit. med. J.*, 1957, 1, 59). (2) "Trial of SM Type I Attenuated Poliomyelitis Virus Vaccine" (Dick *et al.*, *Brit. med. J.*, 1957, 1, 65). (3) "Evaluation of TN and SM Virus Vaccines" (Dick and Dane, *Brit. med. J.*, 1957, 1, 70). (4) "Review of Present Position" (Dick and Dane, *Brit. med. J.*, 1958, 2, 1184). (5) "Neutralizing Antibody Levels One Year After Vaccination" (Dane *et al.*, *Brit. med. J.*, 1958, 2, 1187).

T Marker Tests.—The techniques and methods of expressing results described by Benyesh-Melnick and Melnick (1959) were used with the following exception: the results of the tests at 40° C. were standardized by reference to a "wild" *t*— type I poliovirus instead of to a *t*— vaccine virus. This method of standardization was used because the "wild" *t*— type I poliovirus was more sensitive to temperature fluctuation than the vaccine virus. Culture tubes were kept in water baths. Temperature fluctuation in early tests was + or -0.5° C. and in later tests was + or -0.1° C.

D Marker Tests.—The techniques and methods described by Benyesh-Melnick and Melnick (1959) were used.

Results

Sabin type II vaccine virus was fed to two children and then peak-titre faecal virus from one of them was fed at various dilutions to children in a residential home, where it spread naturally to other children.

The two children (P.Su. and R.Su.) fed the vaccine were siblings aged 21 months and 3 years. They were given 10^{4.1} TCD₅₀ of vaccine virus by mouth, and faecal specimens were collected from them daily for one week before and for two weeks after feeding. Faecal specimens and throat swabs were also taken daily during this period from the four other members of the family. The two children were quarantined to minimize the chances of viruses spreading into or out of the family. Both became infected with and excreted the vaccine virus, and a mid-peak-titre (fifth day) specimen from child R.Su. was selected as a source of virus for human passage. Specimens were stored and processed in a special laboratory, and the selected faecal specimen (R.Su./5), after purification by centrifugation and filtration, was subjected to tests of purity similar to those used in the preparation of live poliovirus vaccines (Cabasso *et al.*, 1960). The vaccine virus did not spread to other members of the family, and no evidence was found that any other virus was infecting any member of the family during the period of investigation.

The neurovirulence of the selected first-human-passage virus was compared with that of the original vaccine virus by the intracerebral inoculation of rhesus monkeys (Table I).

The next stage in the study of the effects of human passage on the character of the virus was to feed the first human-passage virus at different dilutions to children in a large double ward of a children's home. There were 33 children, aged 5 to 17 months, in the

ward. None of them had previously had any form of poliomyelitis vaccination, but four had serological evidence of previous natural type I poliovirus infections and a few had some residual low-titre maternal antibody (see Fig. 1). Direct contact between them was limited, but there were considerable opportunities for indirect contact. Groups of nine children were fed 1,000, 100, and 10 TCD₅₀ of the first-human-passage virus. The remaining six children were not fed. Faecal specimens were collected daily for one week before, and for 14 days after, feeding, and then again on the 21st and 28th days. The faecal specimens were tested in monkey-kidney cells to detect virus at the level of 10² TCD₅₀ per g., and then the viral content of positive specimens titrated.

All the children fed 1,000 TCD₅₀, four of nine fed 100 TCD₅₀, and two of nine fed 10 TCD₅₀ became infected as a result of feeding. On various days, starting with the eighth day after feeding, five of the six contacts showed evidence of infection. In addition, 11 of the 12 children fed virus who did not excrete it within the first few days after feeding became infected later (see Fig. 1). By comparison with the known contacts, we consider that these children were more likely to have become infected by contact than by the original virus feedings. During the week before any were fed type II poliovirus, slow-growing strains of monkey-kidney-cell cytopathic agents were isolated from six children in the ward, but there was no evidence that these agents interfered in any way with the establishment of type II poliovirus infections.

Titration of the faecal specimens showed that a number of children excreted virus in their faeces for periods of three to four weeks at high levels—that is, above 10⁵ TCD₅₀ per g. (Table II). The daily mean titre of virus excretion for the 15 children infected by feeding is shown in Fig. 2. No differences were observed between the average height or duration of excretion in children infected by feeding or by contact. All children excreting virus developed neutralizing antibody to type II poliovirus (Fig. 1), and the geometric mean titre of antibody in the 15 children infected by feeding was 1 in 325 one month later.

TABLE II.—Proportion of Children Who Excreted 10⁵TCD₅₀ or More of Virus Per Gramme of Faeces on the 7th, 14th, 21st, and 28th Days After Feeding

Day	7th	14th	21st	28th
Proportion of children	11/13	11/14	5/15	4/14

TABLE I.—Intracerebral Neurovirulence in Rhesus Monkeys of Sabin Type II Vaccine Virus and First and Subsequent Human-passage Viruses

Virus	Range of Titre Log ₁₀ TCD ₅₀ /ml.	No. of Monkeys with Paralysis*					No. of Monkeys with Lesions*				
		—	+	++	+++	Total	—	+	++	+++	Total
Vaccine virus	6.6	5	0	0	0	0/5	5	0	0	0	0/5
" " " " " " " "	5.6	5	0	0	0	0/5	5	0	0	0	0/5
1st-human-passage (R.Su./5)	4.0	6	0	0	0	0/6	6	0	0	0	0/6
10% faecal suspension											
1st-human-passage (R.Su./5)	6.8	3	1	0	0	1/4	2	2	0	0	2/4
1st-monkey-kidney passage	5.8	5	0	0	0	0/5	5	0	0	0	0/5
" " " " " " " "	4.8	4	1	0	0	1/5	2	3	0	0	3/5
2nd-human-passage, 17 strains, 1st-monkey-kidney passage	6.0-7.5	21	10	1	1	12/33	9	19	5	0	24/33
3rd or more human-passage, 9 strains, 1st-monkey-kidney passage	5.5-7.5	13	1	4	1	6/19	3	11	5	0	16/19

* — = Nil. + = Slight. ++ = Moderate. +++ = Severe.

The primary object of this trial was to determine any changes in the laboratory marker characteristics of the virus that might occur after human passage and after prolonged peak-titre multiplication in the human intestinal tract. It was thought that any changes in the character of the virus after the period of peak-titre multiplication would be of little epidemiological importance, therefore tests of marker characteristics were carried out on peak-titre specimens—that is, faeces containing more than 10^5 TCD₅₀ of type II poliovirus per g.—and all viruses were tested after one passage through monkey-kidney cells.

Monkey Intracerebral Neurovirulence Tests

Twenty-six faecal viruses taken at different intervals from 19 of the children were tested for neurovirulence by the intracerebral inoculation of rhesus monkeys. Each virus was tested initially in two monkeys, using a high-titre inoculum. A definite increase in the neurovirulence of human-passage virus over the original vaccine virus was demonstrated (Table I), together with a progressive change to greater neurovirulence after multiplication in human intestinal tract for a number of weeks, irrespective of the actual number of human passages (Table III, Fig. 3). However, two important facts were observed. Firstly, even when a monkey had severe paralysis in one limb there was little tendency for the paralysis to spread to other limbs as frequently occurs with strains isolated from patients with paralytic poliomyelitis; and, secondly, histological lesions were on the whole less extensive than those caused by strains from paralysed patients. An interesting

finding was that weakness of the lower back muscles was more frequent than we have observed with other strains. Two of the human-passage viruses (D.Re./21st day and J.O'N./28th day) which had caused severe paralysis in the initial monkey neurovirulence tests were titrated by intracerebral inoculation (Table IV). Though the results of these titrations indicated a marked change in neurovirulence as compared with the original vaccine, they differed significantly from the results we have obtained in the past with viruses from paralysed patients.

T Marker Tests

Melnick and Benyesh-Melnick (1960) tested the Sabin vaccine strains before and after multiplication in the human gut and reported changes in the *t* (temperature)

Fig. 2.—Daily geometric mean titre of virus excretion in the 15 children infected by feeding.

Child	Age, months	TCD ₅₀ of 1st.-human-passage virus fed	Antibody %				Virus excretion																			
			Before feeding			1 month after feeding Type II	Days																			
			Type I	Type II	Type III		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	21	28			
B.Lo.	8	1,000	-†	-	-	600																				
M.De.	10		-	-	-	600																				
P.McC.	9		-	-	-	100																				
R.Fa.	7		-	-	-	300																				
P.Di.	10		-	-	-	300																				
P.La.	11		-	-	-	200																				
C.Ma.	15		64	-	-	300																				
D.Re.	15		-	-	-	600																				
M.Ke.	7		16	8	-	80																				
A.McK.	8	100	-	-	-	800																				
E.Ba.	9		-	-	-	80																				
G.Mo.	7		-	-	-	100																				
S.No.	12		>1,024	-	-	4,500																				
W.Fe.	9		-	-	-	100																				
J.O'N.	10		-	-	-	400																				
S.McA.	14		-	-	-	400																				
G.McA.	17		512	-	-	800																				
S.McC.	7		-	-	300																					
G.Hu.	12	10	-	-	-	600																				
M.Re.	15		-	-	-	300																				
S.Os.	10		16	-	-	600																N.S.†				
M.Co.	7		-	-	-	200																				
F.Ke.	15		178	-	-	300																N.S.				
F.Mo.	7		12	-	-	150																				
K.Ri.	9		-	-	-	2,200																				
J.Mo.	8		-	-	-	600																				
B.Co.	7		-	-	-																					
C.Qu.	7	Nil [contacts]	Not tested	Not tested	Not tested	Not tested																				
P.McH.	8																									
G.Ir.	8																									
J.Ma.	5																									
D.McK.	9																									
P.Co.	6																									

* Reciprocal of final dilution neutralizing 50 TCD₅₀ of virus.

† <1:8

‡ No specimen obtained.

Fig. 1.—Virus excretion and antibody response of children fed first-human-passage virus and of their contacts.

TABLE III.—Effect of Duration of Multiplication of Virus in the Intestinal Tract on the Intracerebral Neurovirulence in Rhesus Monkeys

Week of Virus Multiplication in Intestinal Tract	No. of Virus Strains Tested	Range of Titre Log ₁₀ TCD ₅₀ ml.	No. of Monkeys with Paralysis*					No. of Monkeys with Lesions*				
			—	+	++	+++	Total	—	+	++	+++	Total
0 (original vaccine)	1	5.6-6.6	10	0	0	0	0/10	10	0	0	0	0/10
1st	1	5.8-6.8	8	1	0	0	1/9	7	2	0	0	2/9
2nd	1	6.1	2	0	0	0	0/2	2	0	0	0	0/2
3rd	9	6.0-7.3	12	5	0	0	5/17	5	9	3	0	12/17
4th	6	6.8-7.3	8	3	0	1	4/12	3	9	0	0	9/12
5th	10	5.8-7.1	12	3	5	1	9/21	2	12	7	0	19/21

* — Nil. + Slight. ++ Moderate. +++ Severe.

TABLE IV.—Intracerebral Titrations of Second and Third Human-passage Viruses in Rhesus Monkeys

Virus	Human Passage	Titre Log ₁₀ -TCD ₅₀ ml.	No. of Monkeys with Paralysis*					No. of Monkeys with Lesions*				
			—	+	++	+++	Total	—	+	++	+++	Total
D. Re. 21	2nd	7.1	0	1	0	1	2/2	0	2	0	0	2/2
		6.1	2	0	0	0	0/2	2	0	0	0	0/2
		5.1	1	1	0	0	1/2	0	2	0	0	2/2
		4.1	1	1	0	0	1/2	0	1	0	1	2/2
J. O'N. 28	3rd	6.8	1	0	0	1	1/2	0	1	1	0	2/2
		5.8	0	1	1	0	2/2	0	1	1	0	2/2
		4.8	1	0	0	1	1/2	1	0	0	1	1/2
		3.8	1	1	0	0	1/2	1	0	1	0	1/2

* — Nil. + Slight. ++ Moderate. +++ Severe.

marker character of the viruses after human passage. They found that an increased ability of a strain to multiply at 40° C. was usually associated with an increase in neurovirulence for monkeys. They classified strains of virus as *t+*, *t±*, and *t-*: the *t+* strains being those that grew well at 40° C., the *t-* strains being those that grew poorly, and the *t±* strains being those that were intermediate.

The value of the *t* marker appeared to lie firstly in its use as a measure of change in the vaccine virus after human passage, and secondly as a means of distinguishing "wild" strains of poliovirus from strains of vaccine origin. Little information had been published on the *t* marker character of "wild" strains, therefore we tested a number of the naturally occurring polioviruses isolated in Ireland during the past few years. The results of testing 89 viruses (63 from paralytic cases, 15 from non-paralytic cases, and 11 from other children) indicated that *t+*, *t±*, or *t-* strains may be isolated both from cases of acute poliomyelitis and from other children (Tables V and VI). There was a tendency for viruses isolated at approximately the same time and place to have the same *t* character, but in 1957, which was an epidemic year in Northern Ireland, both *t+* and *t-* type I viruses were found, suggesting that this marker is not always stable in naturally occurring strains. The severity of paralysis in patients was not related to whether a *t+* or a *t-* virus had been found in the faeces.

Seventy-five human-passage type II vaccine virus strains from the trial were tested, with the results shown in Table VII. Eight of these strains were *t±*, but none were *t+*. More *t±* viruses were found among the later isolates than among the earlier ones.

The original vaccine virus grows poorly at 39° C., and a number of other viruses were retested for their ability to grow at this temperature. "Wild" *t-* (40° C.) strains from paralysed patients were found to be *t+* (39° C.), but after human passage the type II vaccine virus also grew better at 39° C. than at 40° C. The results of *t* tests at 39° C. and 40° C. on vaccine viruses isolated at intervals from two children are shown in Table VIII. There was a gradual shift from *t±* to *t+* (39° C.).

FIG. 3.—Relationship between duration of virus multiplication in the intestinal tract and percentage of inoculated monkeys showing paralysis and lesions.

TABLE V.—*T*-character of 78 Strains of Poliovirus from Patients With Acute Poliomyelitis

Year	Type I				Type II				Type III			
	Total Tested	<i>t+</i>	<i>t±</i>	<i>t-</i>	Total Tested	<i>t+</i>	<i>t±</i>	<i>t-</i>	Total Tested	<i>t+</i>	<i>t±</i>	<i>t-</i>
1954	0				2	2	0	0				
1955	5	0	3	2	5	4	1	0				
1956	6	2	2	2	16	12	3	1				
1957	11	3	0	8					10	4	3	3
1958	7	3	2	2								
1959	6	6	0	0					6	6	0	0
1960	4	4	0	0								
Total	39	18	7	14	23	18	4	1	16	10	3	3

TABLE VI.—*T*-character of 11 Strains of Poliovirus Isolated from Children Who Did Not Have Acute Poliomyelitis

Year	Type I				Type II				Type III			
	Total Tested	<i>t+</i>	<i>t±</i>	<i>t-</i>	Total Tested	<i>t+</i>	<i>t±</i>	<i>t-</i>	Total Tested	<i>t+</i>	<i>t±</i>	<i>t-</i>
1957-61	5	2	1	2	4	0	0	4	2	2	0	0

TABLE VII.—*T-character of 75 Strains of Human-passage Virus After 1-5 Weeks' Multiplication in the Intestinal Tract*

No. of Weeks in Intestinal Tract	Minimum No. of Human Passages	No. of Strains Tested	<i>t</i> Marker: 40° C.		
			<i>t</i> -	<i>t</i> ±	<i>t</i> +
1-2	2	5	0	0	5
2-3	2	7	0	0	7
3-4	1	7	0	0	7
	2	16	0	4	12
	3	14	0	2	12
4-5	1	2	0	0	2
	2	12	0	1	11
	3	12	0	1	11
Total		75	0	8	67

TABLE VIII.—*Comparison of the T-character at 39° C. and 40° C. of Two Human-passage Viruses*

Child	Days after Feeding	Total No. of Days in Intestinal Tract	<i>t</i> Marker	
			40° C.	39° C.
D. Re.	5	10	<i>t</i> -	<i>t</i> ±
	12	17	<i>t</i> -	<i>t</i> +
	21	26	<i>t</i> -	<i>t</i> +
C. Ma.	6	11	<i>t</i> -	<i>t</i> ±
	12	17	<i>t</i> -	<i>t</i> ±
	21	26	<i>t</i> ±	<i>t</i> ±
	28	33	<i>t</i> ±	<i>t</i> +

D Marker Tests

A character of certain poliovirus strains is that plaque formation is delayed in the presence of a low bicarbonate concentration in the overlay medium. Those strains showing a low efficiency of plating under these conditions have been designated *d* -, and those which produce plaques equally well, whether a high or low bicarbonate concentration is employed, have been designated *d* +. Intermediate strains are *d* ±.

Benyesh-Melnick and Melnick (1959) reported changes in the *d* character of Sabin vaccine viruses after multiplication in the human gut. They found that the original vaccine viruses were *d* -, but that a proportion of human passage strains were *d* ± or *d* +.

Twelve human-passage strains of Sabin type II virus isolated from four children taking part in the present trial were tested for the *d* marker. Three were found to be *d* - like the original vaccine virus, but the remaining nine were *d* ±, indicating that a change in the character of the virus had occurred (Table IX).

TABLE IX.—*D-character of 12 Strains of Human-passage Virus*

Virus	No. of Human Passages	Days after Feeding	<i>d</i> Marker
Original vaccine	Nil	0	<i>d</i> -
M. De.	2	6	<i>d</i> ±
		13	<i>d</i> ±
		21	<i>d</i> ±
B. Lo.	2	6	<i>d</i> -
		12	<i>d</i> -
		21	<i>d</i> ±
C. Ma.	2	6	<i>d</i> ±
		12	<i>d</i> ±
		21	<i>d</i> ±
		28	<i>d</i> ±
P. McH.	3	11	<i>d</i> -
		21	<i>d</i> ±

Antigenic Marker

Six strains of type II poliovirus were tested for intra-typic antigenic differences by cross-neutralization tests, using antisera prepared in guinea-pigs. The six strains chosen were: Sabin type II vaccine virus, two second-human-passage derivatives of this virus, a naturally occurring avirulent type II virus isolated in 1960, a "wild" virulent type II virus isolated from a paralysed child in 1956, and the laboratory strain Y-SK. Y-SK virus

was readily distinguishable from the other five viruses by this means, but the other five viruses could not be distinguished from one another. It was concluded, therefore, that attempts at strain differentiation with the use of this technique might have little value. More extensive tests with different techniques are in progress.

Comparative Infectivity of the Original Vaccine Virus and Early Human-passage Virus

The infectivity of the original vaccine was titrated in groups of children attending immunization clinics. Faecal specimens, which were collected for three days before and for seven days after feeding virus, were tested for the presence of virus in monkey-kidney cells. Fourteen children excreting viruses at the time of feeding were excluded from the study. None had actively acquired immunity to type II poliovirus. In order to diminish the chances of cross-infection between children (some of whom were close neighbours) influencing the results, the presence of type II virus in the faeces during the first week after feeding and the development of type II antibody after one month were taken as evidence of infection at the time of feeding.

A comparison between the infectivity of the original vaccine virus and the first-human-passage virus (R.Su./5) is shown in Table X. Both viruses were highly infective, but no significant difference in infectivity was observed between them. This comparison would have been of

TABLE X.—*Comparison of Infectivity of Sabin Type II Vaccine Virus and First-human-passage Virus*

Virus	Dose	Log ₁₀ TCD ₅₀	Children Infected
Original vaccine	4-6	4-6	7 7*
	3-6	3-6	9 9
	2-6	2-6	6 9
1st human passage (R.Su. 5)	3-0	3-0	9 9
	2-0	2-0	4 9
	1-0	1-0	2 9

* Number of children infected number of children f.d.

more interest if the infectivity of a later human-passage virus showing more pronounced changes in marker characteristics had also been titrated.

Community Surveillance

An attempt was made to gauge the long-term spread of virus in the children's home. Fourteen weeks after the children were fed the human-passage virus, faecal specimens from all the 82 children in the home were tested for virus in monkey-kidney cells. Type II poliovirus was isolated from the faeces of four children. For one month after the virus was fed there had been no movement of children into or out of the ward, but soon afterwards some of the older children were moved to other parts of the home. Three of the children (M. Ne., T. Ow., M. El.) from whom type II was isolated had not taken part in the original trial, but had been in contact with children moved from the ward. None of these three children was excreting much virus, the titres ranging from 10² to 10³ TCD₅₀ per g. of faeces. Seven months after the original virus feeding a number of children not known to have been infected with type II poliovirus during the previous studies were bled and found to have naturally acquired immunity to this virus, suggesting that cross-infection between children in the home may have been rather more extensive than the screening for faecal carriers after 14 weeks indicated.

During the period when the trials were conducted, samples of faeces were collected from 888 children on

the day of their admission to the Royal Belfast Hospital for Sick Children. Children with infectious diseases are not intentionally admitted to this hospital, and it was hoped that if there had been relatively avirulent polioviruses circulating in the community it might have been possible to isolate them from this source which was convenient. Between the end of March, 1960, and the end of March, 1961, three strains of type II poliovirus and one type I virus were isolated. The clinical diagnoses in the three children (R. Cr., E. McW., and P. Li.) excreting type II virus were meningitis, inguinal hernia, and burns, respectively.

In addition to the screening of faecal specimens from children at the Royal Belfast Hospital for Sick Children, the usual routine faecal specimens were obtained from all cases of possible acute poliomyelitis and aseptic meningitis admitted to the fever hospitals. Type II poliovirus was recovered from the faeces of only one fever hospital patient. This child (W. Bl.) had aseptic meningitis, but Coxsackie B₅ as well as type II poliovirus was recovered from the faecal specimens. Coxsackie B₅ was responsible for many cases of aseptic meningitis during 1960 in Northern Ireland, and it was therefore likely to have been the cause of the aseptic meningitis in this instance.

The monkey neuropathogenicity and *t* (40° C.) tests for the type II polioviruses isolated in the surveillance programme are shown in Table XI. The possibility that these four viruses recovered from the community might have been of vaccine virus origin was investigated. In two instances (W. Bl. and R. Cr.) time and place relationships suggested that the viruses might have been of vaccine origin, but it seemed much more probable that the relatively avirulent type II polioviruses which these four children were excreting were in fact wild strains. It is of interest that no type II polioviruses have been isolated from cases of acute poliomyelitis in Northern Ireland since 1956, though there was one probable mild paralytic case in a child in November, 1959. Only a late serum specimen was available, and therefore the diagnosis could not be certain.

Illnesses in Vaccinated Children and Their Contacts

In this series of trials 102 children were known to have become infected with the vaccine virus or its progeny. One child aged 9 months suffered from convulsions five days after receiving 10^{3.6} TCD₅₀ of the original vaccine virus. He excreted the vaccine virus from the second day after feeding until at least the 14th day. He had previously suffered from convulsions, and it was thought unlikely that the vaccine virus was responsible for precipitating this further attack.

No other illnesses of significance were noted.

Discussion

Sabin type II vaccine virus was shown by Murray *et al.* (1959) and by Melnick and Brennan (1959) to

produce no paralysis and no histological lesions when large amounts were inoculated intrathalamically into monkeys. Our own tests on a smaller number of monkeys confirmed these observations. Tests on the vaccine after virus multiplication in the human intestinal tract show that it acquires a measurable degree of neurotropism. To some extent this tendency to greater neurotropism appeared to be progressive. It is quite possible that the five weeks of peak-titre virus multiplication, representing two or more human passages, that we studied, represented the period of maximum virus change and that further changes to greater neurotropism would not have occurred. The three type II polioviruses isolated after 15 weeks' multiplication in the human intestinal tract, and at least three human passages, had no higher neurotropism than those found after four and five weeks. However, these three strains were found in low titre and probably represented post-peak-titre virus and therefore were not truly comparative.

The direct significance of the intracerebral monkey inoculation test is not known, but it has been used to compare "wild" strains from paralytic cases and attenuated vaccine strains, and also to compare different vaccine strains. After several weeks' passage in the human intestinal tract Sabin type II vaccine virus is much less neurovirulent than any strain we have tested from paralysed patients, but it is similar to some strains isolated from healthy children, and has approximately the same degree of neurotropism as some vaccine strains discarded after field trials.

The *t* marker appears likely to be of only limited value in resolving surveillance problems. Comparing virus titrations performed at 36° C. and 40° C., we were unable to differentiate the vaccine virus from a number of strains isolated from paralytic cases. Using 36° C. and 39° C. the original vaccine virus strain could be clearly differentiated from wild strains which had caused paralytic poliomyelitis, but after passage through the human intestine this was no longer so. In other words, the *t* - marker was lost quite rapidly using the 39° C. test and was of little significance using the 40° C. test, and therefore neither test appeared to be suitable for identifying strains of vaccine origin in the field.

The *d* marker of the original vaccine virus frequently changed to *d*_± after human passage. The significance of this observation, apart from the fact that it indicated some change in the character of the virus, is not known.

The poliomyelitis community surveillance formed a necessary part of the trial. No type II paralytic cases have occurred in Northern Ireland either during or since the trial (12 months). Type II polioviruses which by marker characteristics resembled Sabin type II human-passage virus were isolated from two cases of meningitis. In one of these cases Coxsackie B₅ was also present in the patient's faeces and was thought to be the more probable cause of the meningitis. In both

TABLE XI.—*Neurovirulence for Rhesus Monkeys and T-character of the 7 Type II Poliovirus Strains Isolated in the Surveillance Programme*

Child	Virus Titre Log ₁₀ - TCD ₅₀ ml.	<i>t</i> Marker: 40° C.	No. of Monkeys with Paralysis*					No. of Monkeys with Lesions*				
			—	+	++	+++	Total	—	+	++	+++	Total
M. Ne.	5.6	<i>t</i> —	2	0	0	0	0/2	0	2	0	0	2/2
T. O.w.	6.8	<i>t</i> —	1	0	0	0	1/2	1	0	1	0	1/2
M. Fl.	6.1	<i>t</i> —	2	0	0	0	0/2	2	0	0	0	0/2
R. Cr.	6.0	<i>t</i> —	2	0	1	0	1/3	1	0	2	0	2/3
E. McW.	6.4	<i>t</i> —	2	0	0	0	0/2	0	1	1	0	2/2
P. Li.	6.0	<i>t</i> —	2	0	0	0	0/2	1	1	0	0	1/2
W. Bl.	6.0	<i>t</i> —	2	0	0	0	0/2	2	0	0	0	0/2

* — = Nil. + = Slight. ++ = Moderate. +++ = Severe.

instances there was some time and place association with the use of Sabin type II vaccine. Two other rather similar type II viruses were isolated from children admitted to hospital with hernia and burns. We consider that, on the evidence available to us, all these four type II strains isolated from the community represented naturally occurring viruses of low virulence. However, our experience does serve to emphasize the difficulties which may arise if attempts are made to assess the community safety of infectious vaccine viruses.

Tests are in progress to determine whether the homo-typic antigenic marker described by McBride (1959) could be of value in distinguishing wild viruses from those of vaccine origin. If they prove successful this marker characteristic may be of great value in sorting out the epidemiological coincidences that are likely to arise when oral vaccines are in use. Simple cross-neutralization tests were found to be unsatisfactory in distinguishing between strains of type II poliovirus of different origin.

Our interpretation of the results reported in this trial is that after a few weeks' multiplication in the human intestinal tract Sabin type II vaccine virus appears to revert to a form indistinguishable from naturally occurring avirulent type II polioviruses. This is no reason *per se* for considering that the virus is likely to become more neurotropic after further human passage or that it is unsafe for community contacts.

Summary

Sabin type II poliomyelitis vaccine virus was fed to a child, and peak-titre faecal virus recovered from this child was then fed in varying amounts to 27 of 33 children living in a ward of a children's home. All but two of the 33 children became infected with the virus during the next four weeks either as a result of the feeding or by contact. In an attempt to measure the stability of the vaccine virus after human passage, laboratory marker characteristics of faecal virus strains isolated from these children were compared with those of the original vaccine virus. Viruses representing the second, the third, and possibly the fourth human passage were available for study. They had been isolated after periods of up to five weeks' multiplication in the human intestinal tract. Tests for marker characteristics were confined to peak-titre isolates because it was thought that changes in the virus after the period of infectivity had ceased were less likely to be of importance.

The original vaccine virus produced no paralysis or histological lesions when inoculated intracerebrally into rhesus monkeys. Comparative inoculations were carried out with strains of human-passage virus, and these were found to produce paralysis and histological lesions in a proportion of the monkeys inoculated. The change to greater neurotropism appeared to be progressive over the study period of five weeks.

The *t* marker character of the original vaccine virus, human-passage strains of this virus, and a number of naturally occurring polioviruses were compared. It was found that the *t* marker is only of limited value in differentiating the human-passage vaccine viruses from virulent polioviruses.

Tests for the *d* marker on human-passage strains of the vaccine virus showed that some were *d*±, whereas the original vaccine virus is *d*—.

The infectivity for children of the original vaccine virus and the first human-passage virus were found to be approximately the same.

The community surveillance which formed part of the trial is described. The most important finding was that no case of paralytic poliomyelitis caused by type II poliovirus occurred in Northern Ireland in the year following the trial.

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

REFERENCES

- Benyesh-Melnick, Matilda, and Melnick, J. L. (1959). In *First International Conference on Live Poliovirus Vaccines*, p. 179. Pan American Sanitary Bureau, Washington, D.C.
- Bodian, D., Morgan, Isabel M., and Schwerdt, C. E. (1950). *Amer. J. Hyg.*, **51**, 126.
- Cabasso, V. J., Jervis, G. A., Moyer, A. W., Roca-Garcia, M., Orsi, E. V., and Cox, H. R. (1960). *Brit. med. J.*, **1**, 373.
- McBride, W. D. (1959). *Virology*, **7**, 45.
- Melnick, J. L. (1955). *Ann. N.Y. Acad. Sci.*, **61**, 754.
- and Benyesh-Melnick, Matilda (1960). In *Second International Conference on Live Poliovirus Vaccines*, p. 12. Pan American Health Organization, Washington, D.C.
- and Brennan, J. C. (1959). In *First International Conference on Live Poliovirus Vaccines*, p. 65. Pan American Sanitary Bureau, Washington, D.C.
- Murray, R., Kirschstein, R., Van Hoosier, G., and Baron, S. (1959). *Ibid.*, p. 39.

In the course of a discussion on scientific creativity during the third congress of psychiatry held recently in Montreal, Dr. A. SZENT-GYÖRGYI, a Hungarian who now works in the United States, observed that nature itself was not democratic and had not made everyone intellectually equal. "Everything is being done [in the United States] to correct this undemocratic attitude of nature and make us all equals also in the domain of the intellect, lifting the retarded and knocking down the outstanding to the common level." Before the political changes after World War II, Dr. Szent-Györgyi said, he had planned to create a flourishing climate for the development of genius in Hungary in his capacity as chairman of the National Council of Education. But he was frustrated with the coming of Communist domination. And he had found, he said, that his ideas flourished equally poorly in the United States, where he works at the Institute for Muscle Research, Marine Biological Laboratory, Woods Hole, Massachusetts. "If we had spent half as much money and thought on the outstanding as we have on the retarded children, leadership would not be such a scarce commodity," he asserted. Dr. Szent-Györgyi gave his own formulae for the creative scientist: he must do research because he cannot do otherwise ("a problem solved is no problem at all"); he must have standards shaped at an early age ("I often wonder into what sort of people the youngster will develop who grows up on T.V. violence and murder and deceptive advertising, often without a family tradition which could compensate"); and he must be an egotist ("If a student comes to me and says he wants to be useful to mankind and go into research to alleviate human suffering, I invariably advise him to go, rather, into charity."). (*New York Times*, June 9.)