SMALL-SCALE TRIAL WITH SABIN ATTENUATED TYPE 1 POLIOVIRUS VACCINE IN A SEMI-CLOSED COMMUNITY*

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The desirable properties of a live poliovirus vaccine are that it should be of attenuated neurovirulence, be genetically stable during growth in the human intestine, and so be safe for the vaccinated individual and the community. It should moreover be able to infect the alimentary tract readily, and elicit an antibody response after such infection (W.H.O., 1960). Although the vaccine viruses developed by Sabin appear to possess most of these qualities, it is known that intestinal passage may give rise to some variation. Nevertheless, no serious consequences have been reported amongst the 100 millions or so that have now received Sabin attenuated vaccine in many parts of the world. A small-scale study with the Sabin type 3 virus has already been reported from Sheffield (Clarke *et al.*, 1958), and the present trial was performed with type 1 vaccine to obtain information about: (1) the spread, within a poliovirus-free semi-closed community, after infection of a number of children with a single dose of living type 1 poliovirus vaccine; (2) the degree and duration of virus excretion; (3) the serological response to vaccination; and (4) the genetic stability of the vaccine virus strain on passage through the human gut. The virus used was the living, attenuated, L Sc, 2ab type 1 vaccine developed by Sabin, and received from him in 1959 in the form of infected monkey-kidney-tissue culture fluid. Since 1959 it had been stored at -70°C .

Trial Procedure

The patients were all under 4 years of age and in a single ward of a long-stay orthopaedic hospital. They were under treatment for various orthopaedic conditions, such as Perthes's disease and congenital dislocation of the hip. None had previously had clinical poliomyelitis. Before the trial began its nature was fully explained to their parents, and their permission obtained. For six months before vaccination stools collected twice-weekly from every child were examined for poliovirus and other viruses. During this control period no poliovirus of any serotype was recovered from any individual in the ward, but E.C.H.O. virus type 1 (two children) and one adenovirus were isolated. The two children excreting E.C.H.O. virus had been discharged from the

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ward before the trial began. At the end of the observation period 12 children remained in the ward, and at this stage samples of blood were withdrawn where possible.

Three ambulant children were selected for initial administration of virus, while the remaining nine were, for orthopaedic reasons, restricted to their cots; in this way, a mobile potential source of infection was provided. On March 15, 1960, each of these infants received, by mouth, 1×10^6 TCD₅₀ (50% tissue-culture infective doses) of virus vaccine fluid, contained in a teaspoonful of orange juice. Daily faecal specimens were then collected from all children in the ward, from those to whom virus had been administered, and from their contacts; wherever possible, this consisted of a stool sample, rectal swabs being taken if no stool had been passed by 5 p.m. Since no attempt at barrier nursing was made there was a reasonable probability of virus spread from child to child.

Laboratory investigation proceeded almost concurrently with the collection of specimens, so that a virtual day-to-day record of the pattern of virus excretion was obtained. However, during the 23 days after feeding the first group of children no poliovirus was recovered from the faeces of any of their contacts (Fig. 1). On April 7, 1960 (23 days later), the nine contact children were therefore given 1×10^6 TCD₅₀ of vaccine virus by mouth in exactly the same way as the first group. Day-by-day observation and collection of specimens continued from all 12 children. The last faecal specimen taken was on May 23, 1960, 46 days after the second group of children had been given virus, and 13 days after the last virus-positive stool had been obtained. A second sample of blood was then taken from each child. During the whole period of the trial no wild type 1 poliovirus was isolated in Sheffield (Public Health Laboratory, Sheffield; personal communication).

The three ambulant children first given vaccine all became infected (Fig. 1), and excreted poliovirus in their stools for at least a month. Virus output per gramme of stool was often considerable (Fig. 2), the highest recorded virus content from any child being 1.1×10^6 P.F.U. (plaque-forming units)/g. Of the nine children receiving vaccine in the second phase of the trial, five subsequently excreted virus, making a total of 8 out of 12 (67%) altogether. Faecal virus excretion continued for up to 33 days in this second group of children, the maximum recorded yield being 5×10^5 P.F.U./g. of stool. At no time during the first six days after feeding was virus recovered from the throats of any of the eight infected children.

The total absence during the trial of any sign of clinical reaction or apparent illness in the children confirms the view that infection with the vaccine, and its subsequent excretion, takes place in the absence of any of the clinical features of poliovirus infection.

Laboratory Methods

Collection of Specimens.—(a) Stools were obtained from each patient in sterile disposable aluminium-foil trays. (b) High rectal swabs were taken by a sleeve-tube technique (Rogers, 1954), and the swab stick was broken off into 4 ml. of transport medium (synthetic "mixture 199" (Morgan *et al.*, 1950) containing 500 units of penicillin, streptomycin, and polymyxin B per ml.), contained in a screw-capped phial. (c) Throat swabs were taken from both tonsils and the posterior pharyngeal wall using a single swab, which was treated like the

rectal swabs. All clinical specimens were stored at approximately -20°C .

Preparation of Specimens.—(1) *Stools*:—Approximately 1 g. of faeces was emulsified in "mixture 199" containing 500 units of each antibiotic per ml. to give a 10% w/v mixture, and immediately centrifuged at 4°C . for 20 minutes at $6,600\times g$. The supernatant fluid (10% faecal extract) was stored at approximately -20°C . (2) *Rectal and throat swabs*:—Swabs were macerated in their transport medium, and centrifuged. The supernatant fluid, regarded as undiluted extract, was stored at approximately -20°C .

Preparation of Monkey-kidney (M.K.) Tissue Cultures.—Primary monolayer tube cultures of rhesus M.K. cells were prepared according to conventional techniques. The growth medium was replaced by an equal volume of maintenance medium as soon as the cultures were confluent. These were usually able to survive for a 14-day period without further attention. The growth medium consisted of Hanks's saline (Hanks, 1948), 95%; lactalbumin hydrolysate, 0.5%; calf serum, 5%; NaHCO_3 , 0.035%; and penicillin, streptomycin, polymyxin B, and nystatin, 100 units/ml. The maintenance medium consisted of Earle's saline (Earle *et al.*, 1943), 98%; lactalbumin hydrolysate, 0.25%; calf serum, 2%; NaHCO_3 , 0.22%; and antibiotics in the same concentration as the growth medium.

Testing of Specimens for Presence of Virus.—0.5 ml. volumes of extract were inoculated into each of four M.K. tubes and rolled at 37°C . for three hours. The fluid was then decanted from each tube, and replaced with 1.5 ml. of fresh maintenance medium. The tubes were then incubated while stationary at 37°C ., and examined daily for up to 10 days for cytopathic effects. Tubes showing 75–100% degeneration were harvested, and the fluids after centrifuging for 10 minutes at $1,300\times g$ stored at approximately -20°C . Occasionally non-specific changes due to toxicity or enzymic activity of the extract appeared within 24 hours of inoculation

of the cultures, in which case the undiluted culture fluid was tested for the presence of virus by subculture to fresh M.K. tubes.

Neutralization Tests.—All sera were examined for type 1 poliovirus antibodies, using the cytopathic test described by Perkins and Evans (1959). The challenge virus used was the type 1 Mahoney strain, and end-points were calculated by the method of Reed and Muench (1938). Recovered viruses were identified serologically, by neutralization with serum prepared in monkeys against Sabin type 1 vaccine virus.

Titration of Virus in Specimens.—(1) *Tube titration*:—Serial tenfold dilutions were prepared in phosphate-buffered saline (P.B.S.) (Dulbecco and Vogt, 1954), and 0.2-ml. volumes of each dilution inoculated into each of four M.K. tubes. The tubes were observed daily for cytopathic changes, and 50% end-points calculated (Reed and Muench, 1938). (2) *Plate titration*:—(a) M.K. monolayers were prepared in $2\frac{1}{2}$ -in. (6.3-cm.) Petri dishes, by seeding with cells suspended in growth medium, and incubating in a 5% CO_2 in air atmosphere. When confluent the medium was removed and the washed monolayers were inoculated with 0.2-ml. volumes of virus dilutions prepared in P.B.S.; after a 45-minute adsorption period at room temperature the monolayer was overlaid with an Earle's saline-based nutrient agar and incubated for three days in a humid atmosphere of 5% CO_2 . Plaques were then made visible by staining with neutral red. (b) Virus assays were also made using agar-cell suspension plates (Cooper, 1955). M.S. ("monkey stable") cells (Kanda and Melnick, 1959), kindly supplied by the Tuskegee Institute of Alabama and maintained in our laboratory by standard subculturing techniques, were used. The nutrient agar was as in (a) above.

Neurovirulence Tests.—Cynomolgus monkeys, weighing between 2 and 3 kg., were inoculated intracerebrally, 0.5-ml. volumes being injected into each thalamus (Bodian *et al.*, 1950), and intraspinally by

FIG. 1.—Pattern of faecal virus excretion. ■ = Virus recovered. □ = Virus not recovered.

injecting 0.05- or 0.1-ml. volumes into the lumbar enlargement of the spinal cord (Sabin, 1959). The monkeys were housed in groups of three. Each animal was exercised daily in an open run in which it was necessary for it to negotiate a low hurdle. All animals were observed for up to 28 days after inoculation for evidence of trauma and poliomyelitis. Animals showing paralysis, and others seriously ill, were anaesthetized with phenobarbitone sodium, and samples of the lumbar enlargement of the spinal cord were removed for attempts at virus isolation. The whole animal, while still anaesthetized, was then perfused intravitaly with 10% formalin containing 1% acetic acid, and the brain and spinal cord were removed and processed for histological examination. Portions of lumbar cord from animals which died without paralysis were also examined for the presence of poliovirus.

In vitro Genetic Markers.—(1) *Ability to grow at 40° C.*—The ability of virus strains to grow at 40° C. (Lwoff and Lwoff, 1959) was determined by placing M.S. cell-agar plates in a jar containing a humid atmosphere of 5% CO₂ in air, and incubating in a standard electrically heated air incubator fitted with a "sunvic" relay switch. Daily temperature readings showed a maximum variation of $\pm 0.3^\circ$ C. The incubation procedure was as follows: inoculated plates were placed in the jar, which had been warmed to 37° C., and flushed with the gas mixture—also warmed to 37° C.—for exactly 30 minutes in a 37° C. hot-room. The jar was then sealed and transferred to a fixed place in a 40° C. incubator. In almost all experiments the incubator was not opened until the end of three days. (2) *Plaque-size character*—Plaque diameters were measured on M.S. cell-agar plates after 63–66 hours' incubation at 37° C.

Control Virus Strains.—A single ampoule of vaccine virus was diluted in P.B.S. and dispensed in 1-ml. volumes in hard glass ampoules; after sealing, each ampoule was frozen and stored at -78.5° C. Mahoney virus grown in M.K. cultures was diluted, ampouled, and stored in the same way as the vaccine virus. Ampoules of each virus pool were thawed and used once only. Titres of each pool are shown in Table II.

Serological Results

Table I shows antibody titres in the children before and after feeding, their previous history of Salk vaccination, and the duration of vaccine virus excretion. Of the eight children who excreted poliovirus after feeding, five had previously received Salk vaccine, and only one of these possessed demonstrable antibody to type 1 poliovirus. However, virus excretion was associated with at least an eightfold rise in homologous antibody in all sera which could be adequately tested.

Of the four children who did not excrete virus after feeding, three had previously received Salk vaccine. Three possessed an initial high level of homotypic antibody, and in no case did their antibody levels change during the trial.

Properties of Excreted Viruses

The relationship to the original vaccine virus of the viruses present in the faeces was investigated to determine the degree of genetic change occurring during intestinal proliferation. Neurovirulence for the monkey was studied in relation to two *in vitro* characteristics: (1) ability to grow at 40° C.; and (2) plaque size. A

third line of approach—virus growth characteristics in M.S. cells—was tried, but for reasons reported in the next section was abandoned.

Since enrichment of virus suspensions by growth in tissue culture may lead to selection of new virus genotypes, only viruses in faecal specimens, obtained direct from the patient, were examined.

In vitro Characteristics

(a) Growth in M.S. Cells

Kanda and Melnick (1959) reported that attenuated polioviruses grow less well than virulent strains on M.S. cells. However, using the agar-cell suspension overlay plating method, M.S. cultures gave titres of type 1 vaccine virus comparable with those obtained with M.K. monolayer plates and M.K. tubes (Table II). Moreover, such M.S. plates similarly supported growth of virulent Mahoney virus, both at 37° C. and at 40° C. This property of M.S. cells to react indistinguishably from ordinary M.K. assay systems was not confined to adapted laboratory strains of poliovirus, but also held for viruses obtained direct from faeces (Table II). Having established the validity of M.S. cell suspension

TABLE I.—Serological Response of Vaccinated Children

Result of Oral Vaccination	Case No.	Serum Neutralization Titre		Excretion Period (Days)	Previous Salk Vaccination
		Before Vaccine	After Vaccine		
Virus excretion	25/60	<1/4	1/2,048	30	+
	208/59	<1/4	1/256	>31	—
	211/59	<1/4	1/32	25	+
	121/60	<1/32	1/32	>27	—
	76/60	<1/4	1/32	33	—
	215/59	1/4	1/2,048	15	+
	81/60	1/256	1/2,048	6	+
	23/60	Not done	1/256	>18	+
No virus excretion	237/59	1/256	1/256	0	+
	125/60	1/2,048	1/2,048	0	+
	24/60	1/256	1/256	0	—
	207/59	Not done	1/256	0	+

TABLE II.—Comparative Assays of Sabin Type 1 Vaccine, Virulent Mahoney, and Excreted Vaccine Viruses in Different Systems

Mahoney, and Excreted Faecal Viruses in Digestive Systems				
Virus	Temp.	Assay System		
		M.S. Cell-agar Suspension Plates (P.F.U./ml.)	M.K. Monolayer Plates (P.F.U./ml.)	M.K. Tubes (TC ₅₀ /ml.)
Standard Virus Strains				
Virulent Mahoney*	37° C.	$\bar{x} = 1.0 \times 10^7$ S.E. = $\pm 0.16 \times 10^6$ N = 13	$\bar{x} = 6.6 \times 10^6$ N = 2	$\bar{x} = 6.2 \times 10^6$ N = 4
	40° C.	$\bar{x} = 6.6 \times 10^6$ S.E. = $\pm 1.12 \times 10^6$ N = 12	$\bar{x} = 4.7 \times 10^6$ N = 2	1.0×10^6 N = 1
Sabin type 1 vaccine*	37° C.	$\bar{x} = 2.1 \times 10^5$ S.E. = $\pm 0.47 \times 10^5$ N = 10	$\bar{x} = 1.9 \times 10^5$ N = 2	$\bar{x} = 2.4 \times 10^5$ N = 3
	40° C.	$x_{\text{max.}} = < 5.0 \times 10^1$	$< 0.5 \times 10^1$ N = 1	$< 1.6 \times 10^1$ N = 1
Excreted Faecal Viruses				
25 60, RSE2	37° C.	1.5×10^3	1.8×10^3	N.D.
25 60, FE10		2.4×10^4	2.0×10^4	N.D.
25 60, FE14		1.4×10^5	1.4×10^5	N.D.
208 59, FE2		1.1×10^5	N.D.	8.9×10^4
208 59, FE18		7.7×10^2	N.D.	2.5×10^5
215 59, FE9		3.5×10^2	N.D.	1.8×10^2
215 59, FE13		2.0×10^2	N.D.	8.9×10^1
211 59, FE4		1.7×10^3	N.D.	8.9×10^2
211 59, FE19		2.9×10^3	N.D.	5.6×10^2
23 60, FE6		5.0×10^4	N.D.	1.4×10^4
23 60, FE11		4.3×10^3	N.D.	1.8×10^3
76 60, FE1		6.0×10^3	N.D.	1.1×10^2
76 60, FE9		1.9×10^4	N.D.	1.6×10^4
121 60, FE7		4.4×10^1	N.D.	1.1×10^5
121 60, FE20		3.1×10^4	N.D.	8.9×10^3

* Titres = replicate assays of a single virus pool.
N = No. of assays. $\bar{x}$ = Arithmetic mean of N assays. S.E. = Standard error. N.D. = Not done.

plates as a suitable assay system under different temperature conditions for the faecal and vaccine viruses, all experiments designed to study *in vitro* genetic markers were carried out using the same system.

The finding that attenuated type 1 poliovirus can grow as readily in M.S. cells under the conditions described as in M.K. cells in no way contradicts their use in principle as a marker for such viruses when used as described by Kanda and Melnick (1959). However, since the present work was done, one of us (J. M. H.) has found that M.S. cells do, in fact, yield plaques with Sabin type 1 poliovirus when used in a way similar to that described by Kanda and Melnick. Furthermore, the same cell line also has recently been shown to possess human species antigens, and no rhesus monkey species antigens (Franks, Coombs, Beswick, and Winter, 1962, in preparation). It therefore appears that the M.S. cells used in the work reported in this paper are not the M.S. cells used by Kanda and Melnick. As, however, they proved well suited to virus assays, the results will still be reported, but the cells hereafter in this communication will be referred to as "M.S." cells.

(b) Growth at 40° C.

A selection of faecal specimens from all children was examined for ability to yield plaques at 40° C., using the technique described above. To conserve material, all experiments were so done that the minimum detectable amount of virus was 5×10^1 P.F.U./ml. of faecal or rectal swab extract. Replicate "M.S." plates were incubated at 37° C. and 40° C., and in all experiments vaccine virus and virulent Mahoney virus were plated as controls.

Of 60 poliovirus strains obtained direct from faeces only six (10%) were able to grow at 40° C. In the same experiments the control vaccine virus invariably failed to yield plaques. However, the ability of these six strains to grow at 40° C. was not necessarily a

genetically stable feature. Plaques at the limiting infective dilution were picked from plates incubated at 40° C., and were suspended in 2 ml. of P.B.S. for replating at 37° C. and 40° C. under the same conditions as before. Only two of the six strains yielded further plaques at 40° C., while all grew at 37° C. (Table III). Failure of passage with the other four was not due to lack of virus in the primary plaques; all six strains had approximately the same high titre when control assays were done at 37° C. (Table III). Moreover, control Mahoney virus grew as well at 40° C. as at 37° C. (Table II), so that failure to reproduce plaques on replating at 40° C. could not be attributed to change in cell susceptibility.

(c) Plaque-size Characteristics

A selection of faecal specimens from all infected children was examined for plaque-size characteristics. The type 1 vaccine and Mahoney strains were included in each test; the vaccine never gave plaques more than 1 mm. in diameter, while Mahoney plaques were 2-4 mm. in size. Since the large-plaque-producing Mahoney strain always showed some scatter in plaque size, plates yielding fewer than 20 plaques were not used for plaque-size measurement.

Of 36 faecal specimens randomly chosen to include virus strains from each child, 22 (61%) gave plaques quite different in size from those of the vaccine virus, and in 32% of these the plaques were indistinguishable from those of the Mahoney control; 14 (39%) excreted viruses gave plaques which appeared identical to those of the vaccine. When picked and replated near the limiting infective dilution both the large plaques and the vaccine-like plaques from excreted poliovirus strains were genetically stable; that is, they bred true.

Fig. 2 shows the plaque-size characteristics of faecal virus excreted by a single individual (patient 25/60) during the course of 30 days following oral vaccination; the variation in plaque size is discontinuous when plotted against time.

Neurovirulence in Monkeys

At least two virus-containing faecal specimens were selected from each child for direct intrathalamic and intraspinal testing in monkeys, which were not given cortisone. The faecal specimens chosen were those whose viruses showed differences and similarities when compared with the vaccine by *in vitro* methods. From the nature of the material it was not possible to ensure that all monkeys received strictly comparable inocula. Control virus assays were always made on the residual syringe contents immediately after each monkey had been inoculated, and the actual placing of the inoculum itself was subsequently confirmed histologically. Some animals died before the end of the observation period without developing clinical poliomyelitis; these are indicated in the relevant tables.

TABLE III.—Growth of Excreted Viruses at 40° C. and Subsequent Passage

Faecal Virus Strain	Result with Original Faecal Extract		Result of Replating Single Plaque from Original 40° C. Culture	
	Temp.	Titre (P.F.U./ml. of Extract)	Temp.	Titre (P.F.U./ml. of Plaque Suspension)
25.60, FE10	37° C.	2.4×10^4	37° C.	4.5×10^5
	40° C.	2.5×10^2	40° C.	3.0×10^1
25.60, FE14	37° C.	1.4×10^3	37° C.	1.0×10^5
	40° C.	5.0×10^1	40° C.	$< 0.5 \times 10^1$
121.60, FE14	37° C.	1.4×10^4	37° C.	1.4×10^4
	40° C.	4.5×10^2	40° C.	$< 2.5 \times 10^1$
121.60, FE20	37° C.	3.1×10^4	37° C.	9.1×10^4
	40° C.	5.0×10^2	40° C.	1.1×10^3
208.60, FE12	37° C.	5.4×10^3	37° C.	8.0×10^4
	40° C.	1.0×10^2	40° C.	$< 0.5 \times 10^1$
208.60, FE15	37° C.	2.8×10^4	37° C.	2.2×10^5
	40° C.	4.2×10^2	40° C.	$< 0.5 \times 10^1$

FIG. 2.—Discontinuous variation of excreted virus: plaque size (patient 25/60).

For comparison, assays of the vaccine were made by intrathalamic and intraspinal inoculation. No neurotropic activity was detected in any of a group of 10 monkeys even when 2.5×10^6 TCD50 were inoculated into each thalamus (Table IV). In another group of 14 animals no evidence of neurotropism was found after 2.5×10^5 TCD50 were similarly inoculated. However, when the same virus was tested intraspinally (Table IV), 5×10^3 TCD50 inoculated into the lumbar enlargement produced histological lesions characteristic of poliomyelitis in five out of seven monkeys. Lesions were also found in the cervical cord in three out of five monkeys with lumbar lesions and in the brain-stem of one of these animals, indicating spread of virus beyond the original site of inoculation. Animals inoculated with 1/10 and 1/100 the amount of virus by the same route gave equivocal evidence of activity in one monkey at each dilution. None of the monkeys showed clinical signs of paralysis.

The comparative virulence of the faecal viruses tested intrathalamically is shown in Table V. Clinical paralysis was observed in only 2 out of 50 monkeys inoculated with varying amounts of virus. Histological evidence of neurotropic activity was, however, obtained in 11 out of 21 animals inoculated with faecal specimens from four of the eight infected children. Furthermore, all animals which had lesions in the brain also showed lesions in the cervical and lumbar regions of the cord. In addition, some animals developed cord lesions without

concomitant damage to the brain, indicating spread of virus beyond the original site of inoculation (Fig. 3). The amounts of virus in the various inocula which showed this neurotropic activity varied from 1.4×10^2 TCD50 to 1.1×10^5 TCD50.

Table V also shows the *in vitro* characteristics of these same strains for comparison. Only 3 of the 17 faecal specimens tested showed evidence of growth at 40° C., and two of them caused the only two cases of clinical paralysis observed after intrathalamic inoculation in monkeys. Moreover, the three faecal viruses which grew at 40° C. yielded plaques quite unlike those given by the vaccine.

In Table VI two faecal virus strains are compared for intrathalamic and intraspinal virulence. While the results with strain 23/60, FE6 are not strictly comparable for each route, since the largest intraspinal inoculum was 25 times less than the intracerebral dose, strain 121/60, FE20 appears to possess a similar degree of virulence for each route.

Although attempts were made to recover poliovirus from the lumbar and cervical cords of several animals, particularly from the four which became paralysed,

TABLE IV.—Comparison of Intrathalamic and Intraspinal Virulence of Vaccine Virus

Route of Inoculation	Inoculum Monkey (TCD50)	Observation Period (Days)	Evidence of Poliomyelitis			
			Paralysis	Histological Lesions		
				Lumbar Cord	Cervical Cord	Brain
Intra-thalamic	5.0×10^6	28	0/10*	0/10	0/10	0/10
	5.0×10^5	28	0/14†	0/14	0/14	0/14
Intra-spinal	5.0×10^3	21	0/7	5/7	3/7	1/7
	5.0×10^2	21	0/7	2/7	0/7	2/7
	5.0×10^1	21	0/5	2/5	0/5	0/5

* Numerator=No. of monkeys showing effect. Denominator=No. of monkeys inoculated.

† Four of this group developed dysentery and were killed after 6, 8, 14, and 27 days.

FIG. 3.—Lumbar cord of cynomolgus monkey 28 days after receiving 5×10^6 TCD50 of Sabin type 1 vaccine intrathalamically. Normal appearance. ($\times 400$.)

TABLE V.—*In vitro* and *in vivo* Characteristics of Excreted Viruses

Virus	Duration of Excretion (Days)	Specimen and Days after Feeding	Growth at 40° C.	Plaque Size	Inoculum/ Monkey (M.K. TCD50)	Intrathalamic Virulence for Cynomolgus Monkeys				
						Observation Period	Evidence of Poliomyelitis			
							Paralysis	Lumbar Cord	Cervical Cord	Brain
25/60	30	RSE2	—	LV	1.2×10^3	21	0/1	0/1	ND	ND
		FE10	+	UV	1.75×10^3	21	0/1	0/1	ND	ND
		FE14	+	UV	1.1×10^4	21	0/2	1/2	ND	ND
					7.8×10^3	21	0/2	0/2	ND	ND
					1.4×10^3	21	1/2	1/2	ND	ND
208/59	> 31	FE2	—	LV	8.9×10^4	21	0/3	0/3	0/3	0/3
		FE18	—	LV	5.6×10^3	21	0/3	1/3	1/3	1/3
211/59	25	FE4	—	UV	8.9×10^3	28	0/3*	0/3	0/3	0/3
		FE19	—	UV	5.6×10^3	28	0/3†	0/3	0/3	0/3
215/59	15	FE9	—	ND	1.8×10^3	21	0/3	0/3	0/3	0/3
		FE13	—	ND	1.1×10^3	21	0/3	0/3	0/3	0/3
23/60	> 18	FE6	—	UV	1.4×10^4	28	0/3	2/3	2/3	1/3
		FE11	—	UV	1.9×10^3	28	0/3	2/3	1/3	0/3
76/60	33	FE1	—	LV	1.1×10^3	28	0/3	0/3	0/3	0/3
		FE9	—	UV	2.8×10^3	28	0/3	0/3	0/3	0/3
81/60	6	FE3	—	ND	2.8×10^3	21	0/3	0/3	0/3	0/3
		FE7	—	ND	7.1×10^3	21	0/3	0/3	0/3	0/3
121/60	> 27	FE7	—	UV	1.1×10^3	28	0/3	3/3	2/3	2/3
		FE20	+	UV	8.9×10^3	28	1/3†	1/3	1/3	1/3
Vaccine			—		5.0×10^6	28	0/10	0/10	0/10	0/10
				LV	5.0×10^5	28	0/14	0/14	0/14	0/14

* Two monkeys in this group died with pneumonia after 3 and 10 days. † Two monkeys in this group died with pneumonia after 3 and 14 days.

‡ One monkey in this group died with meningitis after 3 days.

FE=Faecal extract. RSE=Rectal swab extract. LV=Plaques like vaccine. UV=Plaques unlike vaccine. ND=Not done.

success was obtained in only one instance (see Table VI). Paradoxically, this animal showed no histological lesions, while the virus yield showed unequivocally that multiplication had occurred (virus recovered: 1.4×10^4 P.F.U./g. of cord; virus inoculum: 5.6×10^3 P.F.U.).

Discussion

Although the group of children initially given virus provided a mobile source of infection, no spread occurred to contact children who were subsequently shown to be susceptible to the vaccine virus. Horsmann *et al.* (1957) and others have shown that under appropriate conditions considerable spread of Sabin type 1 vaccine virus can occur in children confined to mental institutions and to the parents and siblings of small children at home. However, the factors governing the transmission of polioviruses under experimental or natural conditions are still relatively obscure, and nothing is known of the comparative infectivity for man of any of the poliovirus strains described.

The serological results show that infection, as measured by subsequent excretion of live virus, may result in a marked increase in homotypic antibody. Although previous inoculation with a killed virus vaccine *per se* does not prevent uptake of a living attenuated virus (Lipson *et al.*, 1956) the presence of a high level

of homologous antibody appeared in all but one instance to prevent infection with the vaccine virus in the present study. The one exception, patient 81/60, suggests that where such infection does take place the duration of excretion may be drastically reduced.

The studies of the properties of the excreted viruses appear to confirm those of other workers, and variation in three characteristics (ability to grow at 40° C., plaque size and monkey neurovirulence) was found. Fewer strains were found which would grow at 40° C. (10%) than showed plaque-size variation (61%). The ability to grow at 40° C. appeared to be correlated with a capacity to yield plaques unlike those of the vaccine, although viruses which gave non-vaccine-like plaques were not necessarily able to replicate at 40° C.; in fact, the correlation was one-way.

The inability of some plaques picked from plates incubated at 40° C. to yield plaques when tested again at the same temperature was not due to change in cell susceptibility or to failure of virus yield in the primary plaques. Virus growth of an abortive nature cannot explain the phenomenon; it should be remembered that the primary plaques themselves are the result of several cycles of virus growth, so that the observed facts are not readily interpreted and must await further study. Ability to grow at 40° C. cannot always be explained by the appearance of a genetically stable mutant.

The significance of the discontinuity in time of the plaque-size variation is also not easily interpreted. A discontinuous change similar to that shown in Fig. 2 has been reported by Sabin (1956), where a faecal poliovirus specimen of reduced neurovirulence was preceded and succeeded by specimens of greater virulence for monkeys tested directly. Whatever the wider significance of such discontinuous changes, it is significant that variation of this kind may lead to a to-and-fro displacement of vaccine-like by non-vaccine-like particles, and that the change does not necessarily imply a once-and-for-all replacement of the vaccine virus by that of another, possibly more dangerous, kind.

The results following direct inoculation of massive amounts (5×10^6 TCD₅₀) of the vaccine virus into the thalamus of cynomolgus monkeys confirm that Sabin type 1 vaccine possesses no demonstrable neurotropic activity when tested by this route. However, 1/500 of the vaccine inoculum was able to produce histological lesions in the cervical cord and brain of monkeys inoculated into the lumbar enlargement, indicating at least some spread of virus beyond the original site of inoculation. These results are in accord with those of Murray *et al.* (1959), who reported some neurotropism after intraspinal inoculation into monkeys receiving 1/10 of this amount of Sabin type 1 vaccine virus.

Evidence of the variation that was found when excreted polioviruses were tested by *in vitro* methods was also found when the same viruses were tested directly in monkeys. Although only two monkeys exhibited clinical paralysis, histological evidence of neurotropic activity was obtained when faecal specimens from four of the eight infected children were inoculated intrathalamically. These results must be considered valid, since there was a complete absence of lesions in animals receiving much larger vaccine inocula by the same route. The number of animals showing evidence of neurotropism when an intrathalamically virulent faecal strain (121/60, FE20) was tested intraspinally is small, but the results (Table VI) suggest that during

FIG. 4.—Lumbar cord of cynomolgus monkey which became paralysed after intrathalamic inoculation of 8.9×10^3 TCD₅₀ of poliovirus recovered from stool of patient 121/60, who had been fed 20 days earlier with Sabin type 1 vaccine. Loss of neuronal Nissl substance. ($\times 400$.)

TABLE VI.—Comparison of Intrathalamic and Intraspinal Virulence of Excreted Viruses

Virus	Specimen and Days after Feeding	Route of Inoculation	Inoculum/ Monkey (TCD ₅₀)	Observation Period (Days)	Evidence of Poliomyelitis			
					Paralysis	Histological Lesions		
						Lumbar Cord	Cervical Cord	Brain
23/60	FE6	Intra-thalamic	1.4×10^4	28	0/3	2/3	2/3	1/3
		Intra-spinal	5.6×10^3	21	0/5*	0/5	0/5	0/5
			5.6×10^3	21	0/5†	0/5	0/5	0/5
			2.8×10^0	21	0/5	0/5	0/5	0/5
121/60	FE20	Intra-thalamic	8.9×10^3	28	1/3‡	1/3	1/3	1/3
		Intra-spinal	5.6×10^3	21	2/5§	1/5	1/5	1/5
			5.6×10^3	21	0/5	21/5	0/5	0/5
			5.6×10^1	21	0/5	21/5	0/5	0/5

* Two monkeys in this group died with meningitis after 15 and 18 days.

† One monkey in this group died without symptoms after 6 days.

‡ One monkey in this group died with meningitis after 3 days.

§ One monkey in this group died with meningitis and pneumonia after 13 days.

|| One of these animals showed no histological lesions in the central nervous system, although poliovirus was recovered from its spinal cord.

passage of vaccine through the human gut there may be no dramatic increase in intraspinal over intracerebral virulence. However, the dose-response curve of Sabin type 1 vaccine assayed intraspinally in monkeys is shallow (Murray *et al.*, 1959), and this may blur the picture of faecal virus virulence.

In general, the monkey-virulent faecal virus strains possess *in vitro* properties deviating from those of the vaccine virus, but this is not always true. Thus, strains 76/60,FE9, 211/59,FE4, and 211/59,FE19, which show non-vaccine-like plaques, were apparently avirulent when tested intrathalamically. By contrast, one of three monkeys inoculated with virus 208/59, FE18, which is apparently similar to the vaccine, developed lesions throughout the central nervous system when tested by the same route. Nevertheless, the only three virus strains shown (Table V) which were able to grow at 40° C. were also able to induce damage to the central nervous system. Two of these were the only viruses to produce paralysis in monkeys after intracerebral inoculation.

The degree of genetic stability of attenuated polioviruses as they undergo replication in the alimentary tract of vaccinated individuals is of the utmost importance, both to the individual concerned and to the community. It is unfortunate that conclusions concerning the stability of such viruses have been most often based on examination of the progeny of faecal viruses, derived in tissue culture, and not on a study of the faecal viruses themselves. We regard this as no mere academic quibble, since technical manoeuvres of this kind are known to select variant virus particles. Nothing is known of the extremely rapid changes which may take place in the virus population during replication within the gut itself. Clarke *et al.* (1958) reported the excretion of virus with increased neurovirulence within 48 hours of feeding Sabin type 3 vaccine. Nor is anything known of the nature of the event, or sequence of events, manifested in the discontinuous variation in time in plaque size of excreted viruses, reported in this paper. Is it possible that the host cells of the alimentary tract may in some way direct the genetic make-up of the virus particles they produce, as apparently occurs when a Patas monkey-kidney-adapted strain of Coxsackie A-9 virus grows in rhesus kidney cells (Hsiung, 1960; see also Hoskins, 1960)? We do not know the answers to these questions, but in a study of the variation which takes place with polioviruses during growth in the human gut the virus to study is found in the gut; not one sophisticated in tissue culture.

The most urgent epidemiological problem is to find some way of recognizing the vaccine virus once it has been released into a population. Many genetic "markërs" have been described which, taken together, show fair agreement with monkey neurotropic activity. It is pertinent to recall the inherent capacity for variation among viruses. Hard-and-fast lines can rarely be drawn. Even the property of neurovirulence itself is not an all-or-none feature. A genetic marker absolutely correlated to neurovirulence is a will-o'-the-wisp. The best we are likely to get is a series of markers which, taken together, can enable us to establish with a greater degree of certainty the relationships between the viruses.

Summary

Living, attenuated, L Sc, 2ab type 1 poliovirus vaccine (Sabin) was fed to 3 out of 12 children in a hospital

ward. Virus excretion was considerable and prolonged, but no spread took place to contacts, some known to be susceptible. When, subsequently, the contacts were also given the vaccine 8 out of the 12 children were shown to be capable of infection. No child became ill.

Antibody to the fed vaccine developed in all virus excretors who could be adequately tested. Previous Salk vaccination *per se* appeared to make no difference to excretion. With one exception, all children with an initially high antibody titre failed to become infected. The exception excreted virus for a short time only. No change in antibody level was recorded in children who did not excrete virus.

The excreted polioviruses were examined for their ability to grow at 40° C. and for their plaque-forming properties using "M.S." cells. Many were also examined for neurovirulence by intrathalamic and intraspinal inoculation of monkeys.

Variation from the vaccine virus was found in all three properties, and change in one character was often, though not always, associated with variation in another. Although very few viruses were found which grew at 40° C., they all gave plaques unlike those yielded by the vaccine. However, not all those faecal viruses which grew at 40° C. on primary plating were able to yield plaques on recultivation at the same temperature. The reason for these findings is obscure. The increase in plaque size was itself discontinuous in time.

There was no evidence of neurotropic activity when massive doses of vaccine virus were inoculated intrathalamically into cynomolgus monkeys, although much smaller doses of the vaccine produced histological lesions when tested intraspinally. However, the data reported indicate an increase in intrathalamic virulence for the monkey after replication of the vaccine virus in the human alimentary tract.

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REFERENCES

- Bodian, D., Morgan, I. M., and Schwerdt, C. E. (1950). *Amer. J. Hyg.*, **51**, 126.
 Clarke, S. K. R., Goffe, A. P., Stuart-Harris, C. H., and Herzog, E. G. (1958). *Brit. med. J.*, **2**, 1188.
 Cooper, P. D. (1955). *Virology*, **1**, 397.
 Dulbecco, R., and Vogt, M. (1954). *J. exp. Med.*, **99**, 167.
 Earle, W. R., Schilling, E. L., Stark, T. H., Straus, N. P., Brown, M. F., and Shelton, E. (1943). *J. nat. Cancer Inst.*, **4**, 165.
 Hanks, J. H. (1948). *J. cell. comp. Physiol.*, **31**, 235.
 Horstmann, D. M., Niederman, J. C., Melnick, J. L., and Paul, J. R. (1957). *Trans. Ass. Amer. Physns.*, **70**, 91.
 Hoskins, J. M. (1960). *Bull. schweiz. Akad. med. Wiss.*, **16**, 141.
 Hsiung, G. D. (1960). *J. Immunol.*, **84**, 285.
 Kanda, Y., and Melnick, J. L. (1959). *J. exp. Med.*, **109**, 9.
 Lipson, M. J., Robbins, F. C., and Woods, W. A. (1956). *J. clin. Invest.*, **35**, 722.
 Lwoff, A., and Lwoff, M. (1959). *C.R. Acad. Sci. (Paris)*, **248**, 1725.
 Morgan, J. F., Morton, H. J., and Parker, R. C. (1950). *Proc. Soc. exp. Biol. (N.Y.)*, **73**, 1.
 Murray, R., Kirschstein, R., Van Hoosier, G., and Baron, S. (1959). In *Live Poliovirus Vaccines: First International Conference, Washington, 1959*, p. 39. Scient. Publ. No. 44 of the Pan American Sanitary Bureau, Washington, D.C.
 Perkins, F. T., and Evans, D. G. (1959). *Brit. med. J.*, **1**, 1549.
 Reed, L. J., and Muench, H. (1938). *Amer. J. Hyg.*, **27**, 493.
 Rogers, K. B. (1954). *Brit. med. J.*, **1**, 147.
 Sabin, A. B. (1956). *J. Amer. med. Ass.*, **162**, 1589.
 — (1959). *Brit. med. J.*, **1**, 663.
 World Health Organization (1960). *Tech. Rep. Ser.*, No. 203, p. 32.