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LARGE-SCALE USE OF SABIN TYPE 2 ATTENUATED POLIOVIRUS VACCINE IN SINGAPORE DURING A TYPE 1 POLIOMYELITIS EPIDEMIC

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In the latter half of 1958 Singapore experienced an epidemic outbreak of poliomyelitis due to the type 1 virus. Eleven weeks after the first case was reported the Minister of Health in the Singapore Government decided, after consultation, to make available the attenuated type 2 vaccine elaborated by Sabin (1957a, 1957b) for children between the ages of 3 months and 10 years. Dr. Sabin agreed to the release of this vaccine on condition that adequate laboratory control could be assured. The following communication gives the reasons for the selection of the type 2 vaccine, the experimental details, and the results of the campaign.

TABLE I.—Incidence of Poliomyelitis in Singapore Since 1946 up to Period of Epidemic

Year	Jan.—March	April—June	July—Sept.	Oct.—Dec.
1946	187	1	—	—
1947	—	—	—	—
1948	—	119	33	20
1949	21	14	23	20
1950	4	3	8	75
1951	30	20	16	28
1952	21	20	11	4
1953	11	11	17	8
1954	36	29	13	7
1955	15	7	2	1
1956	6 (1)	12 (2)	3 (1)	51 (1)
1957	27 (1)	13 (1 and 3)	14 (3)	10 (3)
1958	2 (1 and 3)	3 (1)	—	—

The figures in parentheses indicate the serological type of poliomyelitis isolated during the period.

Previous Experience of Poliomyelitis in Singapore

The incidence of poliomyelitis since 1946 among the locally domiciled population of Singapore is shown in Table I.

There was no seasonal incidence; cases occurred throughout the year, and small epidemic outbreaks were seen in 1946, 1948, and the end of 1950 and beginning

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of 1951. The majority of cases were in children under the age of 2, and the general picture was that of an area in which poliomyelitis was endemic, but with periodic increases in the number of cases.

Paul (1958) drew attention to the fact that this endemic state of poliomyelitis was associated with a high infantile mortality rate, and if the infantile mortality rate fell below 60–80 per 1,000 live births a rise in the number of cases of poliomyelitis could be expected. The infantile mortality rates for Singapore since 1946 are shown in Table II.

This fall in the infantile mortality rate could presage a shift to the direction of increased activity of poliomyelitis and the possible appearance of cases in older children. A serological survey of the population possessing poliomyelitis antibodies (Table III) was conducted in 1956. The proportion of children with poliomyelitis antibodies was relatively low, confirming the possibility of epidemic conditions arising.

Recent Poliomyelitis Epidemic in Singapore

In August, 1958, cases of poliomyelitis due to the type 1 virus began to be reported, numbers increasing rapidly so that epidemic conditions existed. A very significant

TABLE III.—Serological Survey (Singapore)—April, 1956. Poliomyelitis Antibodies

Age Group	Sera Tested	No. Positive			Percentage Positive		
		Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
– 6 months	6	1	0	3	16.7	—	50.0
– 23 "	10	1	1	4	10.0	10.0	40.0
– 3 years	8	5	4	4	62.5	50.0	50.0
– 4 "	12	8	7	11	75.0	58.3	91.7
– 6 "	26	16	16	17	61.5	61.5	68.9
– 8 "	38	28	25	32	73.7	66.6	85.2
– 10 "	16	8	12	9	50.0	75.0	56.3
– 20 "	23	17	16	13	73.9	69.6	56.5
20+ "	138	74	81	59	53.6	58.7	42.7

TABLE II.—Infant Mortality Rates per 1,000 Live Births in Singapore

	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957
Chinese ..	199.52	82.03	79.43	71.02	64.51	75.13	66.69	62.27	58.22	46.80	40.36	33.97	32.81
Malays ..	289.78	140.23	143.25	155.27	119.99	141.72	136.75	120.01	124.38	106.71	104.61	95.46	86.40
Indians ..	290.66	98.31	76.45	76.79	80.89	67.56	70.66	66.19	62.94	60.76	44.15	34.27	39.64
Europeans } ..	224.81	53.98	57.69	43.10	19.61	29.82	23.19	31.70	25.79	21.37	17.42	16.41	17.54
Eurasians } ..			77.99	46.24	35.33	52.96	36.65	47.35	73.85	35.93	33.24	24.86	27.78
Others ..	182.48	143.68	113.51	61.90	86.02	83.33	92.04	78.28	60.12	63.06	57.72	29.95	37.61
Total ..	215.74	89.69	87.33	80.79	72.04	82.23	75.15	69.97	67.04	56.10	49.67	42.66	41.11

finding was the occurrence of cases in children over 8 years old, a further indication of the changing pattern of the poliomyelitis picture. The weekly case incidence of paralytic poliomyelitis cases in age groups and by date of onset of symptoms is shown in Table IV.

TABLE IV.—Paralytic Poliomyelitis Cases by Date of Onset of Symptoms

Date of Onset	Total	Age in Years										
		< 6/12	7/12-	2-	3-	4-	6-	8-	10-	15-	20+	
Aug. 3-9	3		1		2							
" 10-16	—			1								
" 17-23	2		1	1								
" 24-30	5		3	2								
" 31-												
Sept. 6	9		5	3		1						
Sept. 7-13	5		1	4								2
" 14-20	13		7	1	1				1		1	
" 21-27	10	1	3	2	1	1	1					1
" 28-												
Oct. 4	23	2	8	5	4	2	1	1				
Oct. 5-11	28	2	8	6	3	5	1		1			2
" 12-18	49	2	18	13	6	6	2				1	1
" 19-25	54	3	20	10	8	1	1	4	3			4
" 26-												
Nov. 1	42	2	10	13	2	3	2	2	2			6
Nov. 2-8	27		17	6	1							3
" 9-15	20	2	7	6	1	2					1	1
" 16-22	25	2	15	2	3	1	1					1
" 23-29	26	3	16	4	1				1			1
" 30-												
Dec. 6	16		12	1		1	1	1				
Dec. 7-13	16		8	5	2	1						
" 14-20	8		4	2	1	1						
" 21-27	7		3	4								
" 28-												
Jan. 3	7		2	1	2		1					1
Jan. 4-10	1		1									
" 11-17	4		2	1	1							
" 18-24	2				1	1						1
" 25-31	7	1	4	1	1							
Feb. 1-7	1					1						
" 8-14	—											
" 15-21	2			1		1						
" 22-28	2				2							
Mar. 1-7	—											
" 8-14	1			1								
Total	415	20	176	95	43	27	11	8	8	3		24

TABLE V.—Racial Incidence of Poliomyelitis

Race	Population	No. of Cases	Cases per 10 ⁵ Population
Chinese	1,090,595	314	28.7
Malays	197,060	46	23.3
Indian, Pakistanis, Ceylonese	129,510	43	33.3
Europeans	10,826	8	73.9
Eurasians	11,382	4	35.1

The total and racial incidence of cases per hundred thousand of the population is shown in Table V.

With the exception of the Europeans, the incidence was much the same in all the racial groups. The higher rate in the former group was expected, as previous experience had shown that the relative number of poliomyelitis cases among the expatriate European population was always higher in any year in which poliomyelitis showed an increase in activity than among the locally domiciled population.

Decision to Use Attenuated Poliomyelitis Vaccine

The second report of the World Health Organization Expert Committee on Poliomyelitis (1958) suggested that in the face of an impending epidemic or where poliomyelitis of the infantile type is endemic, especially where signs are indicative of an imminent shift to the epidemic form of the disease, a large-scale trial of attenuated vaccine might be attempted. Both these conditions existed in Singapore, and the Minister of Health decided to offer, on a purely voluntary basis, immunization with the attenuated poliomyelitis virus vaccine.

Although the epidemic was due to the type 1 virus and all reported cases were shown to be infected with this

serological type it was decided to use a vaccine of the type 2 attenuated virus for the following reasons.

(a) Any large-scale use of attenuated virus vaccine at this stage should be such that it would be possible to arrive at a conclusion at the end of the trial regarding the safety of the vaccine not only for the vaccinees but also their contacts who became secondarily infected. All cases of poliomyelitis that had occurred in Singapore since 1956 had been examined to establish the serological type of the virus responsible, and Table I shows that cases due to the type 2 virus had not been found since June, 1956, and, as every case was examined by laboratory procedures, any due to the type 2 virus would have been spotted immediately in this type 1 epidemic. Had type 2 cases occurred they would have been assumed to result from the introduction of the vaccine strain into the population, especially had there been any significant number of such cases.

(b) Feeding of attenuated strains of all three types simultaneously in chimpanzees (Sabin, 1956) resulted in a complete suppression of multiplication of the type 3 virus. Interference of one serological type with the establishment of a second serological type in the alimentary tract was the possibility that resulted in the suggested administration of one serological type of attenuated vaccine at one time. It was hoped that children in whom the attenuated type 2 strain was established would show this interference phenomenon if exposed to the type 1 strain.

(c) Persons who have experienced poliomyelitis infection often show some heterologous protection against the other types. Second paralytic attacks of poliomyelitis, although theoretically possible, are extremely rare. Experimentally, Sabin (1956) and Koprowski (1955) showed that the dose of an attenuated virus necessary to establish an alimentary infection was greater in the person with heterotypic antibody than those devoid of all antibody. Vaccination would ensure that all vaccinees had type 2 antibodies and this heterotypic protection might be invoked.

(d) The large-scale use of the vaccine would result in dissemination of large quantities of attenuated virus throughout the community, and this virus could interfere with the natural transmission of the prevalent epidemic strain.

Technical Methods

Vaccination

The type 2 attenuated vaccine was obtained from Dr. Sabin, who supplied the data which can be found in the Addendum to this paper. The Health Department of the Singapore Government set up a number of vaccination centres each in charge of a medical officer; the details of the organization involved will be reported elsewhere. The temperature of children attending the centres was taken, and if this was normal they were given 0.1 ml. of a 1/10 dilution of the tissue culture fluid in a teaspoonful of syrup. simplex B.P. Exclusion of children with a raised temperature was an attempt to weed out those who might be incubating poliomyelitis.

Isolation of Virus from Faeces

A 10% suspension of faeces was prepared by grinding in a mortar with distilled water containing 2,000 units of penicillin and 2,000 µg. of streptomycin per ml. This suspension was clarified in an angle centrifuge at 2,500 r.p.m. for 10 minutes. The clear fluid was removed and centrifuged for 30 minutes at 10,000 r.p.m. in a "spinco"

ultracentrifuge, the supernatant fluid being used for virus isolation.

Poliovirus.—The supernatant fluid was inoculated into monkey-kidney-tissue culture tubes, 0.1 ml. per tube. The tubes were examined daily, and when cells showed marked cytopathogenic changes the fluid was harvested and clarified by a light spinning. The virus was identified by the ability of specific immune sera to neutralize this cytopathogenic effect.

Enteric Viruses.—As specific antisera for this group were not available to us at this time we carried out a rough-and-ready classification. The supernatant fluid obtained from the faeces as described above was inoculated into monkey-kidney tissue culture tubes and also into a litter of suckling mice (0.02 ml. intracerebrally and a further 0.05 ml. subcutaneously into the tissues of the neck as the needle was withdrawn). A virus that gave rise to cytopathogenic change in the tissue culture and was pathogenic to suckling mice was considered to be Coxsackie A9 or B type. One, pathogenic to suckling mice only, was designated a Coxsackie type A. Any virus that was cytopathogenic in tissue culture, was not neutralized by any of the three types of polio antisera, and was non-pathogenic to suckling mice was classified as an E.C.H.O. virus. We realized that an adenovirus which might be isolated from faeces by this technique would have been classified by us as an E.C.H.O. virus.

Estimate of Poliomyelitis Neutralizing Antibody Present in Sera

This was carried out by the method described in the second Report of the World Health Organization Expert Committee on Poliomyelitis (1958); with one exception serum and virus mixture or virus titrations were held overnight in the refrigerator and then one hour at 37° C. before inoculation of tissue culture tubes. (For serological surveys a 1/3 dilution only of serum was used, but antibody responses were measured by the titre of neutralizing antibodies present in the serum.)

Pathogenicity of Poliomyelitis Virus Excreted by Vaccinees and Contacts

Virus for pathogenicity tests was grown in the media described by Sabin (1959), and intraspinal and intracerebral inoculations in monkeys were made, using the techniques described in that article.

Investigations Prior to the Commencement of Vaccination Campaign

(a) Serological Survey

It was necessary to ascertain what proportion of the children who were to receive the vaccine would be susceptible to the type 2 strain and how many susceptible to type 1 virus were also susceptible to type 2 virus. It was only in this latter group that an interference phenomenon could be expected to play a part in protection. A number of children were bled just before administration of the vaccine and the type of poliomyelitis antibodies present in their sera was ascertained. Table VI shows the percentage of children with antibodies against each type of virus, and Table VII the comparison of the two main races, the Chinese and Malays.

The percentage of children susceptible to type 1 and also type 2 infection is illustrated in Table VIII.

It was apparent that there had been an increase in the percentage of children with poliomyelitis antibodies since the 1956 survey was conducted. The increase in

TABLE VI.—Antibody Survey of Population Just Before Beginning Vaccination

Age Group	No. Tested	Antibodies			Percentage Positive		
		Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
3-6 months	25	11	9	7	44	36	28
-23 "	71	23	9	18	32.4	12.7	25.4
-3 years	60	34	15	33	56.7	25	55
-4 "	56	42	33	33	75	58.9	58.9
-6 "	71	53	53	54	74.6	74.6	76.1
-8 "	70	52	61	62	74.3	87.1	88.6
-10 "	83	68	79	77	81.9	95.2	92.8

TABLE VII.—Comparison of Antibodies, Chinese and Malays, at Beginning of Vaccination Campaign

Age Group in Years	Race	No. Tested	Antibodies			Percentage Positive		
			Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
3-6/12	Chinese	14	6	8	4	42.9	57.1	28.6
	Malays	8	4	1	3	50.0	12.5	37.5
-23/12	Chinese	39	9	6	9	23.1	15.4	23.1
	Malays	28	14	3	9	50.0	10.7	32.1
-3	Chinese	26	13	3	11	50.0	23.1	42.3
	Malays	32	20	11	21	62.5	34.4	65.6
-4	Chinese	22	14	10	6	63.6	45.5	27.3
	Malays	32	23	22	19	71.9	68.7	59.4
-6	Chinese	40	30	30	28	75	75	70
	Malays	26	19	21	17	73.1	76.9	65.4
-8	Chinese	35	24	31	32	68.6	88.6	91.4
	Malays	32	27	27	30	84.4	84.4	93.7
-10	Chinese	43	33	40	39	76.7	93	90.7
	Malays	29	26	28	27	89.7	96.6	93.1

TABLE VIII.—Analysis of Polio Type 1 Susceptibles

Age Group in Years	No. Tested	No. Susceptible Type 1	Type 1 Susceptible, No Type 2 or 3 Antibody	Type 1 Susceptible, No Type 2 Antibody
3-6/12	25	14 (56.0%)	8 (32%)	14 (56%)
-23/12	71	48 (67.6%)	40 (56.3%)	47 (66.2%)
-3	60	26 (43.3%)	10 (16.7%)	23 (38.7%)
-4	56	14 (25.0%)	2 (3.6%)	6 (10.7%)
-6	71	18 (25.4%)	2 (2.8%)	6 (8.5%)
-8	70	18 (25.7%)	2 (2.8%)	2 (2.8%)
-10	83	15 (18.1%)	0	1 (1.2%)

immunity to the type 1 virus was an indication of its circulation in the community, as the survey was begun only after the epidemic had reached its twelfth week. Unfortunately many of the population have serious objections—some religious, some superstitious—to the bleeding of their children. As a result of this our survey could only be completed over a period of a few weeks, and during this period popular demand was such that the vaccination campaign had to proceed. This was indicated in the survey figures as a rise in the number of children with type 2 antibodies due to contact with vaccinees. In 40 cases we found the apparently anomalous result of children with type 2 antibodies present in their sera when the vaccine was given, and yet the type 2 virus was found in their faeces tested 10 days later. A second specimen of serum taken three to four weeks after vaccination in all these cases showed a fourfold to thirty-twofold rise in the titre of neutralizing antibodies present. We concluded that these were children who were already infected at the time of vaccination, presumably from contact with a vaccinee.

(b) Excretion of Enteric Viruses in the Child Population

Sabin (1958) reported several patterns of interference with multiplication of attenuated poliovirus in some children who were carriers of non-poliomyelitis enteric viruses prior to vaccination. In view of this it was decided to ascertain the degree of enteric non-poliomyelitis virus excretion in our child population to

determine, if possible, the effect of this on the vaccination programme. Faeces of children from birth to 4 years old were collected and examined for the presence of enteric viruses, and the results are shown in Table IX. (This survey also indicated the dissemination

of the type 1 strain in the population.) Table X shows that a greater proportion of Malay children excreted these viruses than Chinese children, and the fact that the majority of Chinese lived in urban surroundings whilst the Malays lived in rural areas might have accounted for this difference. A comparison of urban and rural Chinese (Table XI), however, indicated very little difference between these groups. The difference between Malays and Chinese is therefore not due to urban or rural surroundings, but is a reflection of the rather more primitive conditions of Malay life that probably afforded greater opportunities of faecal infection.

TABLE IX.—Excretion of Enteric Viruses by Healthy Children Immediately Prior to Vaccination Campaign

Age Group in Years	No. Tested	Virus Isolations				% of Non-Polio Enteric Virus Excretors	% of Polio Type 1 Excretors
		Coxsackie		E.C.H.O.	Polio Type 1		
		Type A	Type B or A9				
< 3/12	19	2	0	3	0	26.3	0
-6/12	42	2	3	2	1	16.1	2.4
-23/12	71	5*	3	2	3*	14.1	4.2
-3	57	6	3	7	2	28.1	3.5
-4	31	1	1	3	1	16.1	3.2
-6	28	1	0	1	0	7.1	0
Total	248	17*	10	18	7*	18.1	2.8

* One child was excreting polio type 1 virus and a Coxsackie type A.

TABLE X.—Excretion of Enteric Viruses by Healthy Malay and Chinese Children

Age Group in Years	Race	No. Tested	Virus Isolations				% of Non-Polio Enteric Virus Excretors	% of Polio Type 1 Excretors
			Coxsackie		E.C.H.O.	Polio Type 1		
			Type A	Type B or A9				
< 3/12	Chinese	15	1	—	2	—	20.0	—
	Malays	3	1	—	1	—	66.7	—
-6/12	Chinese	27	1	1	2	1	18.5	3.7
	Malays	11	1	2	—	—	27.3	—
-23/12	Chinese	35	2	—	—	1	5.7	2.8
	Malays	31	2*	3	2	2*	22.6	6.4
-3	Chinese	19	2	1	—	1	15.8	5.3
	Malays	35	4	2	7	1	37.1	2.9
-4	Chinese	15	—	1	—	—	6.7	—
	Malays	13	1	—	3	—	30.8	—
-6	Chinese	15	—	—	—	—	—	—
	Malays	10	1	—	1	—	20.0	—
Total	Chinese	126	6	3	4	3	10.3	2.4
	Malays	103	10	7	14	3	30.1	2.9

* One child was excreting polio type I virus and a Coxsackie type A.

TABLE XI.—Faecal Virus Excretion by Chinese Children—Urban and Rural

Age Group in Years		No. Tested	Virus Isolations				% of Non-Polio Enteric Virus Excretors	% of Polio Type 1 Excretors
			Coxsackie		E.C.H.O.	Polio Type 1		
			Type A	Type B or A9				
< 3/12	Urban	9	2	—	—	—	22.2	—
	Rural	8	—	—	2	—	25.0	—
-6/12	Urban	18	1	1	2	1	22.2	5.6
	Rural	14	—	—	—	—	—	—
-23/12	Urban	34	2	—	—	—	5.9	—
	Rural	11	1	—	—	1	9.1	9.1
-3	Urban	19	1	1	—	1	10.5	5.3
	Rural	6	1	—	—	—	16.7	—
-4	Urban	11	—	1	—	1	9.1	9.1
	Rural	7	—	—	—	—	—	—
-6	Urban	16	—	—	—	—	—	—
	Rural	10	—	—	1	—	10.0	—
Total	Urban	107	6	3	2	3	10.3	2.8
	Rural	56	2	—	3	1	8.9	1.8

TABLE XII.—Excretion Rate of Type 2 Virus by Vaccinees

Age Group in Years	No. Tested	Excreting Type 2 Virus	
		No.	%
3-6/12	36	21	58.3
-23/12	95	53	55.8
-3	70	38	54.3
-4	63	30	47.6
-6	106	25	23.6
-8	83	12	14.5
-10	82	16	19.5

Excretion of Type 2 Virus by Vaccinees

The excretion of the type 2 virus by vaccinees was followed up in a number of cases. Table XII shows this rate of excretion, and, as was expected, the percentage of excretors in each group corresponded very closely to the percentage in each group shown to be susceptible by serological tests.

Degree of Antibody Response to Vaccination by Type 2 Susceptibles

Children bled prior to vaccination whose sera contained no detectable type 2 antibody were selected for further study. Faeces collected at intervals of time were tested for the presence of the type 2 virus, and at the end of three to four weeks a second serum was examined for the presence and titre of type 2 antibody. The results of this study are shown in Table XIII.

TABLE XIII.—Antibody Response to Vaccination

Age Group in Years	No. of Children without Type 2 Antibody	No. with Type 2 Antibody 3-4 Weeks after Oral Vaccine	Percentage Response	No. of Children	Titre*
3-6/12	10	7	70.0	2	50
				2	112
				2	250
				1	564
				1	2
-23/12	44	38	86.4	1	10
				5	22
				3	50
				4	112
				2	250
-3	32	31	96.9	8	564
				5	1,250
				2	> 1,250
				9	2
				4	10
-4	18	17	94.4	4	16
				5	22
				4	50
				3	112
				3	250
-6	14	13	92.9	4	564
				1	1,250
				1	> 1,250
				1	2
				1	22
-8	5	5	100	2	50
				1	112
				1	250
				1	> 1,250
				1	2
-10	2	2	100	1	22
				1	564

* Titre is expressed as the reciprocal of the highest dilution of the serum neutralizing 100 TCD₅₀ of the type 2 virus (0.1 ml. of serum used in control).

Spread of Vaccine Strain Among the Contacts of Vaccinees

From several child welfare clinics in widely different areas of the island faeces were collected from children who had not received vaccine but were reporting on account of minor illness or for a medical check-up. Of 633 children tested, 14 were found to be excreting the type 2 virus and 23 the type 1 strain, which was a clear indication that the vaccine strain spread through the population as readily as the epidemic type 1 strain. This spread of the vaccine strain among contacts was also confirmed, as stated earlier, by the finding of attenuated type 2 virus excretion in 40 children whose sera prior to vaccination revealed type 2 antibody but sera titrated three to four weeks later showed a fourfold to thirty-two fold increase in antibody titre.

Pathogenicity of Type 2 Strains Excreted

Virus obtained from vaccinees of various ages and at different periods of time after vaccination were tested for pathogenicity in monkeys; the results are shown in Table XIV. Pathogenicity tests were also carried out on type 2 viruses isolated from contacts who had not received vaccine (Table XV).

TABLE XIV.—*Monkey Pathogenicity Tests. Post-vaccine Type 2 Isolates*

Age	Days after Taking Vaccine Virus Isolated	Dose × Log ₁₀ TCD ₅₀	Route Inoculation	Result*	
5 months	8	5.0	IS	0/2	
6 "	8	5.5	IS	0/2	
7 "	11	4.5	IS	1/2	
		3.5	IS	0/2	
8 "	12	5.2	IC	0/2	
		5.0	IS	0/2	
10 "	23	5.33	IS	0/2	
17 "	8	5.33	IS	0/2	
2 years	5	5.33	IS	1/2	
		4.33	IS	1/2	
		6.03	IC	0/2	
2 "	5 months	11	5.33	IS	0/2
2 "	10	5	5.0	IS	1/2
		5	4.0	IS	0/2
		5	5.7	IC	0/2
4 "	5	4.23	IS	0/2	
4 "	8	4.5	IS	1/2	
		3.5	IS	0/2	
		5.2	IC	0/2	
4 "	7 months	8	5.66	IS	1/2
		8	4.66	IS	1/2
		8	6.36	IC	0/2
6 "	11	11	5.0	IS	0/2
7 "	8	8	5.5	IS	0/2
7 "	8 months	8	5.5	IS	0/2
8 "	2	8	5.5	IS	0/2
8 "	2	8	5.0	IS	1/2
		8	4.0	IS	0/2
		8	5.7	IC	0/2
8 "	5	8	5.5	IS	0/2

* Numerator = No. of monkeys paralysed. Denominator = No. of monkeys inoculated.

Cases of Poliomyelitis Among Vaccinated Children

The cases of paralytic poliomyelitis that occurred in children who had received the vaccine are analysed in Table XVI.

Discussion

A virus of attenuated virulence to be used as a vaccine must give rise to no illness or upset in the person inoculated or infected with the material. The attenuated type 2 poliovirus used in this instance was administered to 198,965 children between the ages of

TABLE XV.—*Monkey Pathogenicity Tests. Virus Isolated from Children Who Have Become Infected from Others*

Age	Dose × Log ₁₀ TCD ₅₀	Route Inoculation	Result*	
3 months	5.23	IS	0/2	
	4.23	IS	1/2	
8 "	3.23	IS	0/2	
	4.93	IC	0/2	
8 "	4.5	IS	0/2	
14 "	4.5	IS	0/2	
19 "	5.33	IS	0/2	
20 "	4.5	IS	0/2	
3 years	5.0	IS	0/2	
3 "	1 month	5.33	IS	0/2
		6.33	IS	1/2
8 "		5.33	IS	1/2
		5.33	IC	0/2

* Numerator = No. of monkeys paralysed. Denominator = No. of monkeys inoculated.

3 months and 10 years without causing any cases of paralytic poliomyelitis within the group. This finding demonstrates the safety of this vaccine for the vaccinee. In other trials (Sabin, 1956, 1957a, b, 1958; Verlinde *et al.*, 1958) no untoward effects resulted in vaccinees from the use of attenuated virus. We were able to follow carefully only a limited number of children after vaccination, but in all instances of children excreting the virus we observed no ill effects; they remained perfectly fit and afebrile, and continued a normal life. Young children in Singapore suffer from constipation and diarrhoea, and run temperatures from colds, etc., as they do elsewhere, but it must be realized that these incidents present one of the minor difficulties of a mass campaign, as mothers usually ascribe these symptoms to the vaccine if they follow its administration.

One of the great problems in the use of attenuated poliovirus vaccine is that of virus excretion by the susceptible person which often results in infection of contacts, and the danger is that a mutant virulent strain will arise. Our results showed quite clearly that the vaccine strain spread rapidly through the susceptible members of the community. Many small-scale experiments have been made in which attenuated virus was fed to volunteers and the excreted virus tested for pathogenicity; and several workers (Sabin, 1957a; Dick *et al.*, 1957; Dick and Dane, 1957; Clarke *et al.*, 1958) have reported the isolation of strains of increased pathogenicity. Our results also showed some strains with slight increase in pathogenicity, but there was no evidence whatsoever that the alimentary tract was selective for this type of variant. Tests on strains isolated from contacts where the virus must have had at least one human passage gave the same picture of occasional increase in pathogenicity, but these passaged strains showed no greater increase in pathogenicity than those obtained direct from vaccinees.

The age of the child, the question of previous poliomyelitis experience, and the time lapse after vaccination that the virus was isolated did not appear to be the factors determining excretion of more pathogenic variants. It cannot be overemphasized that this increase in virulence is a laboratory measurement, using the extremely sensitive monkey-lumbar-spinal-cord neurones. The real question at issue is not that of pathogenicity to monkey-lumbar-cord neurones but the capacity to cause disease in man. Strains of greater virulence than that exhibited by excreted strains of this vaccine have been isolated from perfectly healthy children during interepidemic periods. Smorodintsev *et al.* (1958) reported studies on triple-negative children fed the type 1 vaccine strain supplied by Dr. Sabin. While they observed a slight increase in neurotropism

of the excreted virus it was no greater after an estimate of four to five natural human passages than it was in the stools of the children receiving the original culture. With the type 2 strain used in the campaign we have described, these workers found that in the course of five experimental consecutive passages in triple-negative children there was a partial increase in both cerebral and spinal neurotropism in the stools of the fourth passage, but this was no longer found at the fifth passage.

In this trial 198,965 children were vaccinated and a large percentage excreted virus which spread rapidly through the community, an ideal situation by which to estimate the theoretical danger discussed above. One

case of paralytic poliomyelitis due to the type 2 virus in a 4-year-old child occurred in the twentieth week of the epidemic, nine weeks after starting vaccinations. It was an isolated case, and if it had resulted from infection by an excreted vaccine strain many more cases would have been expected. Although type 2 virus had not been isolated from paralytic cases in Singapore since June, 1956, such cases were occurring in Malaya even at the time the type 1 epidemic started in Singapore. At this time several strains of type 2 poliovirus were isolated from healthy children in Johore Bahru (the neighbouring town in Malaya separated from Singapore by the causeway). Pathogenicity tests of these strains are shown in Table XVII, and there seems little doubt that

TABLE XVI.—Analysis of Paralytic Poliomyelitis Cases with History of Having Taken Sabin Vaccine

Name (Lab. No.)	Sex	Age (Years)	Date of Sabin Vaccination	Onset of Symptoms	Signs and Symptoms	C.S.F.	Faeces Isolation	Neutralizing Antibodies in 1st and 2nd Specimens of Sera				Days	Remarks
								Type 1		Type 2			
								1	2	1	2		
L. S. M. (632)	F	2½	5/11/58 Kallang YSC	4/11/58	Adm. 10/11/58. Flaccid paralysis both lower limbs	Pos.	Polio 1						Vaccine taken during incubation period of Type 1
K. K. (694)	F	22/12	7/11/58 I.O.H.	15/11/58	Adm. 17/11/58. Paraly- sis rt. leg; weak lt. quadric.	"	" 1						" " "
W. M. L. (840)	F	5/12	26/11/58 New World	27/11/58	Adm. 5/12/58. Paralysis lt. upper limb; weak rt. deltoid and biceps. Neck rigidity ++. Ext. paralysis lower limbs. Bladder dis- tended	"	Neg.	5	56	<5	<5	30	" " "
S. Y. T. (858)	M	1	4/12/58 New World	6/12/58	Adm. 8/12/58. Ext. flaccid paralysis lt. lower limbs	"	Polio 1 and 2	25	625	<5	<5	49	Vaccine taken during incubation period of Type 1. Pathogenicity test of Type 2 showed it still attenuated; it seemed to be supplant- ing Type 1
F. B. S. (871)	F	22/12	2/12/58 Changi	5/12/58	Adm. 11/12/58. Ext. flaccid paralysis both lower limbs with ab- sent jerks. Neck rigidity ++	"	Polio 1						Vaccine taken during incubation period of Type 1
H. B. H. (841)	F	1	25/11/58 Sembawang	28/11/58	Adm. 5/12/58. Paralysis rt. lower limb. Weak lt. hip	"	" 1						" " "
L. Y. H. (850)	M	2	28/11/58 I.O.H.	30/11/58	Adm. 6/12/58. Flaccid paralysis rt. lower limb. Weak lt. quad- riceps	Normal	" 1						" " "
N. S. (868)	F	11/12	3/12/58 Queenstown	27/11/58	Adm. 8/12/58. Cannot move rt. leg. Weak foot, evertous both sides	Pos.	Polio 1 and 2						Patient actually para- lysed when given vac- cine. Shows Type 2, which proved to be still attenuated
N. S. H. (3)	F	15/12	2 mths ago Sims Avenue	21/11/58	Adm. 31/12/58. Weak lt. quad.; absent K.J.	Normal	Polio 1	56	625	56	56	21	Represents a true Type 1 infection in a vac- cinated person
L. C. B. (918)	M	2	5 wks. ago. Lim Ah Pin	13/12/58	Adm. 17/12/58. Paraly- sis lower limbs. Neck rigidity ++. Cyano- sis. Diaphr. and inter- costal paralysis	Pos.	Neg.	<5	282	56	56	22	" " "
C. C. (912)	F	10/12	10/12/58 Island Team	12/12/58	Adm. 16/12/58. Lt. facial paralysis. Wk. lt. hip adductor	"	Polio 1						Vaccine taken during incubation period of Type 1
A. T. T.	M	1	4/12/58 Lim Ah Pin	23/12/58	Adm. 25/12/58. Rt. intercostal paralysis. Paralysis both lower limbs. Phlegm in throat	"	No specimen received. Patient died						Would appear to be a true infection in a vaccinated person
W. C. (917)	F	2	6/12/58 Boon Lay	12/12/58	Adm. 17/12/58. Lower limbs flaccid; weak- ness, with absent jerks. Neck rigid	Normal	Polio 1						Vaccine taken during incubation period of Type 1
R. B. R. (63)	F	1	27/11/58 Sembawang	12/1/59	Adm. 16/1/59. Flaccid paralysis lt. lower limb. Neck and spine rigid. Cannot sit or stand	Pos.	" 1						A true infection in a vaccinated person
A. H. G. (153)	F	1	14/11/58 Kallang YSC	10/1/59 (approx.)	Adm. 24/2/59. 1½ mths., inability to move lt. leg. Flaccid paralysis lt. lower limb, with absent jerks	Normal	Cox. B/A9	250	250	22	22	14	Late case of Type 1 poliomyelitis in which poliovirus excretion stopped
N. L. K. (62)	M	3	28/11/58 Great World	19/1/59	Adm. 22/1/59. First sent to G. H. (17/1/59) with history of 10 days' illness. Weak- ness of legs. Phlegm in throat. Absent K.J. and A.J. both sides. Upper limbs weak. Rt. reflexes absent	"	Neg.	<10	250	564	564	11	A case of Type 1 polio- myelitis. A break through the vaccina- tion

C.S.F.:—Pos. indicates increased cell count and raised protein in later specimens.

TABLE XVII.—*Monkey Pathogenicity Tests of Type 2 Virus Found in Healthy Children in Johore Bahru at Beginning of Vaccination Campaign in Singapore*

Age	Dose × Log ₁₀ TCD ₅₀	Route of Inoculation	Result*
3 months ..	5.33	IS	2/2
	4.33	IS	2/2
	6.03	IC	1/2
1 year ..	5.33	IS	0/2
2 years 2 months	6.0	IS	1/2
	5.0	IS	1/2
	6.7	IC	0/2
3 ,, 1 month	5.0	IS	2/2
	4.0	IS	2/2
	5.7	IC	2/2

* Numerator = No. of monkeys paralysed. Denominator = No. of monkeys inoculated.

"wild" virulent strains were circulating in this area. It is therefore highly probable that the one case reported in Singapore resulted from infection with one of these "wild" strains.

After the safety of the vaccine the most important characteristic is the degree of immunity it will confer on an individual. In this trial, for reasons already stated, it was decided to use a type 2 vaccine that could give only a heterologous protection and possibly an interference phenomenon against the current type 1 epidemic strain. The degree of homologous protection could be assessed only by the conventional laboratory method of estimating the antibody response in children susceptible to type 2, who were fed the vaccine strain. The antibody titres attained in these children showed the expected scatter, and titres had no observable relationship to the age of the child or their previous experience of poliomyelitis infection.

In the younger age groups the percentage responding was lower than in the older age groups, and the possible reasons for this are somewhat difficult to elucidate. It might be that such children were actually infected, but virus excretion was transitory, and antibody response unmeasurable by our technique. Susceptible cells in these children may, however, have undergone such change that they were no longer capable of reinfection with that particular serological type of virus. Alternatively, it may represent a true failure of the vaccine strain to cause infection. Sabin (1958) showed that E.C.H.O. 9 and a virus isolated in E.R.K. cells interfered with multiplication of the type 3 vaccine strain. The proportion of non-poliomyelitis enteric viruses excreted was found to be higher in the younger than in the older age groups. However, in cases which we were able to follow closely, children excreting Coxsackie type A, Coxsackie type A9 or B, or E.C.H.O. viruses, which unfortunately we were not in a position to classify further, became infected by the type 2 strain when given vaccine, and their antibody responses were of the same order as those found in other children.

In no case did we find any interference with the establishment of the type 2 virus; in fact, although it was easy to isolate the non-poliovirus prior to vaccination, four days later, when the type 2 strain was established, we failed to isolate the strain found before vaccination however carefully we tried. Nevertheless it could well be that, as we used only monkey-kidney cells, we failed to detect viruses that interfered with the establishment of the type 2 vaccine strain. Refeeding at a later date of those children who failed to respond should decide whether, despite a failure to detect antibody, they are immune or that there was a true failure of the vaccine strain to infect.

The protection afforded by this type 2 vaccine strain against the type 1 epidemic strain is difficult to interpret, as vaccinations proceeded over a period of weeks and a proportion of the theoretically non-vaccinated population actually become vaccinated as the result of infection contracted from vaccinees. During the course of the epidemic six cases of paralytic poliomyelitis due to the type 1 strain developed among 198,965 vaccinated children (this figure excludes the cases incubating type 1 infection when given the vaccine). During the same period from the first week after the beginning of the vaccination campaign there were 179 paralytic type 1 cases in approximately 300,000 non-vaccinated children of the same age group as the vaccinees. The age distribution of children was approximately the same in vaccinated and non-vaccinated groups. These figures are probably biased against showing protective effects of the vaccine because a proportion of the 300,000 non-vaccinated children were in fact secondarily vaccinated. As there were no grounds to suppose that these secondarily vaccinated children would not have the same protection shown by the primarily vaccinated, the difference between vaccinated and non-vaccinated groups was probably much greater.

On the other hand, it can quite rightly be stated that any child whose parents intended that it should be vaccinated but who developed poliomyelitis before this would be automatically included in the non-vaccinated group. This would result in a slight rise in the paralytic case ratio in the non-vaccinated group. Although most of the vaccinations were completed in seven weeks, centres remained open as long as the public demanded it, so that it was not until January 11, 1959, that the last vaccination was completed. By this time the epidemic was very much on the wane, which was no doubt the reason that there were no further requests for vaccination. Two weeks after the last vaccination was performed there were 13 paralytic cases, all in non-vaccinated children. These cases must have become infected after vaccinations had ceased, and it does represent, therefore, a relatively uncomplicated comparison between vaccinated and non-vaccinated children.

One significant finding was that none of the six cases of paralytic poliomyelitis among vaccinees occurred within the period of 10 to 20 days after vaccination. This is the period when any interference effect of the type 2 virus would be expected to be maximal. Type 1 virus appeared to exert very little interference on the type 2 virus. One child (N.S. 868, Table XVI) was paralysed when presented for vaccination, although this was not spotted until just after the vaccine was given. At the time of vaccination type 1 virus was being excreted, but within four days the vaccine strain had established itself and both types 1 and 2 virus were found in the faeces. This superimposed infection of type 2 was also seen in one other case (S.Y.T. 858), and the interesting feature of this case is that serum taken 49 days after vaccination showed a 125-fold rise of type 1 neutralizing antibodies but no detectable type 2 antibody in a 1 in 5 dilution of the serum, although the type 2 strain had infected the alimentary tract.

Summary

An epidemic outbreak of poliomyelitis due to a type 1 strain occurred in Singapore during the latter months

of 1958 and first two months of 1959. A very definite trend in cases in older children was noticed; previous outbreaks had always been in the locally domiciled children under about 2-3 years old. This is discussed, and the suggestion is made that Singapore is changing from an area where poliomyelitis is endemic and cases of the infantile type occur, to one in which epidemics might be expected and older children would be involved.

A decision was made at Government level to offer an attenuated type 2 vaccine supplied by Dr. Sabin for voluntary vaccination. The experimental organization and results of this campaign are discussed. Of 198,965 children vaccinated only six developed paralysis due to the type 1 virus. During the same period of time—that is, one week after the start of the vaccination campaign to the close of the epidemic—there were 179 paralytic type 1 cases in approximately 300,000 non-vaccinated children of the same age group.

No untoward effects followed the vaccination and no case of type 2 paralysis occurred in the vaccinees. Antibody response to the vaccine was highly satisfactory, although a small proportion of children in the younger age groups failed to give detectable antibodies after vaccination. Possible reasons for this are discussed.

Some of the virus strains excreted by vaccinees showed slight increases in virulence to monkeys tested by intraspinal inoculation. This was not a progressive phenomenon, and strains isolated from contacts, where it could be assumed that at least one human passage had taken place, were no more virulent than those obtained direct from vaccinees.

Only one case of type 2 paralytic poliomyelitis occurred in a non-vaccinated child, and, as it was shown that wild virulent type 2 strains were circulating in a neighbouring community, it was thought that this case could well have resulted from infection with such a wild strain.

We thank Dr. A. B. Sabin, of the Children's Hospital Research Foundation, Cincinnati, for making the vaccine available, but more for his advice and encouragement so freely given; the Rockefeller Foundation of New York, whose grant to the Department of Bacteriology of the University of Malaya was very largely used to defray expenses of this work; Dr. Phoon Wai On, of the Medical Department, Singapore, and the voluntary workers who made the field work possible; and the technicians in the Department of Bacteriology, who gave untiring service without complaint during a very busy period.

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ADDENDUM

DATA ON LARGE LOTS OF LIVE ATTENUATED TYPE 2 POLIOVIRUS VACCINE DISTRIBUTED BY DR. A. B. SABIN, THE CHILDREN'S HOSPITAL RESEARCH FOUNDATION, UNIVERSITY OF CINCINNATI COLLEGE OF MEDICINE, CINCINNATI, OHIO

Preparation.—Approximately 22 to 25 litres of type 2 were prepared at the Merck Sharp and Dohme Research Laboratories, West Point, Pennsylvania, December, 1956, under the direction and supervision of Dr. A. B. Sabin.

Strains Used.—Progeny of specially selected single plaques, purified by three consecutive plaque passages (see A. B. Sabin, *J. Amer. med. Ass.*, 1957, **164**, 1216, for selection and properties of strains used): Type 2 (P 712, Ch, 2 ab).

Procedure.—Trypsinized rhesus-kidney cells grown in 0.5% lactalbumin hydrolysate medium with 2% heated calf serum were used. After inoculation of virus, the maintenance medium consisted of 0.5% lactalbumin hydrolysate in Earle's solution gassed with CO₂ to pH 7.5. Ten per cent. of the bottles prepared from each lot of trypsinized monkey kidneys were *not* inoculated with poliovirus, and were observed for the appearance of spontaneous simian viruses. No evidence of spontaneous virus activity was found in any of the original bottles, or on passage of the harvested fluids to human amnion and monkey-kidney cells. The passages in human amnion cells were carried out to check on the possible presence of measles virus. The harvested culture fluids were centrifuged and passed through K 6 and S 1 Seitz filter pads in series. The Seitz-filtered fluids constitute the vaccine and have been stored at approximately -20° C.

Tests for Bacterial Sterility.—The tests prescribed by the National Institutes of Health for aerobic and anaerobic bacteria, as well as for tubercle bacilli in guinea-pigs, were carried out with negative results.

Tests in Mice.—Twenty adult mice were inoculated intracerebrally (0.03 ml.) and intra-abdominally (0.5 ml.) as a check chiefly for the absence of the virus of lymphocytic choriomeningitis as well as of certain pathogenic bacteria. These tests were negative. Twenty adult mice were inoculated intraspinally with 0.02 ml. of undiluted culture fluid, as a check on the properties of the polioviruses used which are not pathogenic for mice. These tests were negative.

Tests in Rabbits.—A total of 60 ml. was inoculated in six rabbits (1 ml. intracutaneously and 9 ml. subcutaneously in each rabbit). These tests, to exclude the presence of B virus, were all negative.

Potency.—The potency of the vaccine is determined by titration in trypsinized monkey-kidney-tissue-culture tubes and by plaque count by the Hsiung-Melnick technique in stoppered bottles. Titration by the pH test is not suitable, because these strains are affected by the acid pH and erroneous low titres are obtained. Since the titre is influenced by the pH of the medium it is also essential that the maintenance medium in the cultures used for titration contains at least 0.22% of NaHCO₃ (as in Earle's solution). This alkaline medium is gassed with CO₂ to a pH of about 7.5. Similarly, for the plaque count it is essential that the acid medium be washed away from the bottles and that the virus dilutions be prepared in the maintenance medium containing 0.22% NaHCO₃. The maintenance medium used in the tube titrations should not contain serum. The titrations are carried out at 0.5 log dilution intervals. The following results were obtained:

Pooled Lots Tested December, 1956: 3.6 × 10⁷ plaque-forming units/ml. 5 × 10⁷ TCD₅₀/ml.

Tests on Ampoules November, 1957: 2 × 10⁷ TCD₅₀/ml.

Identification of Virus.—Potent antipoliiovirus rabbit antisera, rather than monkey antisera, are used for identification for two reasons: (a) to permit a test on the undiluted culture fluids which would allow the detection of small amounts of cytopathogenic agents other than the

polioviruses; and (b) to avoid the potential neutralizing effect of monkey antisera against simian viruses. Undiluted culture fluid was mixed with an equal quantity of the type-specific rabbit antiserum, and after an incubation period of one hour at room temperature 0.2 ml. was distributed in each of 10 monkey-kidney-culture tubes. At the end of seven days the medium is changed to permit observation of the tubes for another seven days for the detection of minimal amounts of non-poliovirus cytopathogenic agents. It is essential that the fresh medium contain 0.1 ml. of the specific rabbit antiserum per tube to prevent the emergence of poliovirus. Complete neutralization was obtained, and no other cytopathogenic agent was found. However, if a cytopathogenic effect had appeared, it would be necessary to check whether it was poliovirus that escaped neutralization or some other agent.

Tests for Residual Neurotropism in Monkeys.—The fluid was tested in 35 cynomolgus monkeys by intracerebral (1 ml.) and intraspinal (0.1 ml.) inoculation of the undiluted culture fluid and of the dilutions indicated below. The diluted, as well as undiluted, culture fluids are tested as a check against a zone phenomenon, which has been observed with certain strains or with cultures containing mixtures of virus of differing degree of neurotropism. The intracerebrally inoculated monkeys must be observed for at least four weeks, and the intraspinally inoculated monkeys for at least three weeks. The results of these tests, published in *J. Amer. med. Ass.*, 1957, 164, 1216, were as follows:

Paralytic Effect in Monkeys Inoculated Intracerebrally with 1 ml. of Indicated Dilution		Paralytic Effect in Monkeys Inoculated Intraspinally with 0.1 ml. of Indicated Dilution		
Undiluted	10 ⁻¹	Undiluted	10 ⁻¹	10 ⁻²
0/10	0/5	1/10	0/5	0/5

Test for Absence of Neurotropism in Spinally Inoculated Chimpanzees.—Although this need not be a standard test, fluid was tested in three chimpanzees devoid of spontaneously acquired antibody for any of the three types of poliovirus. Chimpanzees with spontaneously acquired antibody for any of the three types of poliovirus might also have some lesions in the spinal cord as a result of the natural infection, and therefore are not suitable for this type of test. Each chimpanzee was inoculated intraspinally in the region of the lumbar enlargement with 0.2 ml. of undiluted culture fluid (approximately 8 × 10⁸ P.F.U.). They were observed for at least three weeks prior to sacrifice for histological examination. Neither paralysis nor lesions were observed in any of the nine chimpanzees used in these tests, and sections through the lumbar spinal cord indicated that the inoculum had been properly placed in the grey matter.

Tests in Human Beings.—Aliquots were fed to human beings between January and May, 1957, as described in *J. Amer. med. Ass.*, 1957, 164, 1216. The dose consisted of 0.01 ml. (0.1 ml. of a 1:10 dilution) of culture fluid added to a teaspoonful of cherry syrup just before administration. No illness resulted and no cytopathogenic agents, other than

Excretion of Virus and Development of Antibody by 5 Children Without Low-avidity or High-avidity Neutralizing Antibody for Any of the 3 Types of Poliovirus Following Feeding of 0.01 ml. of Culture Fluid (100,000 TCID) of the Type 2 Attenuated Strain at 3-Week Intervals

Child	Age in Years	Excretion of Indicated Type of Virus		Antibody Development† for Indicated Type	
		2		Low-avidity+ High-avidity pH Test	High-avidity Cytopathogenic Test
		Peak Titre	Duration Days		
A. S.	5	5.2	53*	256	200
D. S.	7	4.7	32	512	320
S. F.	5	5.7	32	512	320
P. F.	9	5.7	52†	512	320
K. F.	11	4.2	21	512	320

* Titre = Log₁₀ TCD₅₀ per gramme of faeces.

† Serum obtained 77 days after feeding of first type and 36 days after last.

the administered polioviruses, were recovered from the stools. The type 2 virus multiplied well in the alimentary tract and antibody formation was demonstrated. A summary of the results obtained in five children, without prior antibody for any of the three types of poliovirus, is shown in the Table. The results of extensive tests on the neurotropism of the excreted virus were published in *J. Amer. med. Ass.*, 1957, 164, 1216. Further human tests with aliquots of these lots are now in progress in the U.S.A. by three different groups of investigators.

Stability of the Vaccine.—The activity of the viruses is maintained for long periods of time by storage at approximately -20° C. Tests carried out by Dr. P. A. D. Winter, of the Poliomyelitis Research Institute, Johannesburg, South Africa, while a visiting investigator in this laboratory, indicated that the 1:10 dilution of the vaccine used for feeding can be maintained in the fluid state in ordinary wet ice without loss of titre for at least one week.

NOTE.—This experimental vaccine fulfils the requirements of the Expert Committee on Poliomyelitis of the World Health Organization (Report of July, 1957) for an attenuated live poliovirus vaccine for use in large-scale tests on human beings (see W.H.O. Technical Report Series No. 145).

BRITISH STANDARD POLIOMYELITIS ANTISERA TYPES 1, 2, AND 3

BY

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There is clearly a need for standard antisera to each of the three poliomyelitis virus types. Such sera would enable the neutralizing potency of other sera to be expressed in terms of the standard and allow a valid comparison to be made between the results obtained by different workers. In 1955 reference poliomyelitis antisera were prepared to serve as controls in titrating poliomyelitis antibodies in both human and animal sera (Medical Research Council, 1957; Biological Standards Control Laboratory, 1957; Perkins, 1957; Perkins, Sousa, and Tobin, 1958). These antisera were used essentially in ensuring the consistency of the titration system and not in assaying sera in terms of units. For a number of reasons they were unsuitable for use as standards to which a unitage could be assigned. New freeze-dried preparations of antisera to each of the three poliomyelitis virus types were therefore made, but before establishing them as standards a collaborative study* was arranged. The study was designed to determine whether different workers were able to obtain similar potency values when sera were assayed in terms of the freeze-dried preparations. As a result of the study, which is described in this report, it

*Participants in the study.—Cytopathic test: Miss J. O. R. Day, Glaxo Laboratories, Sefton Park, Stoke Poges, Bucks; Dr. R. Heath, Messrs. Pfizer Ltd., Richborough, Sandwich, Kent; Mrs. J. McCapra, Virus Reference Laboratory, Central Public Health Laboratory, Colindale, London; and Dr. F. T. Perkins and Miss R. Yetts, with the assistance of Miss K. South and Miss P. Tumber, Biological Standards Control Laboratory, Hampstead, London. Colour test: Dr. J. O'H. Tobin and Miss O. Stanbridge, Biological Standards Control Laboratory, Hampstead, London.