EVALUATION OF SABIN LIVE POLIOVIRUS VACCINE IN JAPAN

III. STUDIES ON THE METHOD OF ADMINISTRATION OF SABIN VACCINE FOR BABIES*

VACCINE ADMINISTRATION SUBCOMMITTEE**

The Japan Live Poliovaccine Research Commission

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SUMMARY: Two methods of administration of Sabin vaccine were investigated in 474 infants mostly 0 to 8 months of age, with two levels of dose, $10^{5.0}$ and $10^{6.0}$ TCID₅₀. In Method 1, monovalent type 1, divalent types 2 and 3, and trivalent vaccine, were administered in this order, and in Method 2, trivalent vaccine was given twice; the interval between consecutive feedings was 6 to 8 weeks. Immunization at the age of 3 months or later by Method 1 was shown to be most effective and, in this case, $10^{5.0}$ TCID₅₀ dose was as satisfactory as $10^{6.0}$ TCID₅₀ dose. The most suitable time for the first feeding of vaccine is considered to be about 3 months after birth. In spite of inferior effectiveness to Method 1, Method 2 is considered to be practically satisfactory enough in infants over 3 months of age, particularly so because the method is less complicated.

INTRODUCTION

In the previous study (Vaccine Administration Subcommittee, 1966) of this series, the effectiveness and safety of Sabin vaccine were verified. However, further studies were urgently requested to elucidate the need and effectiveness of the vaccine for extremely young infants, and the Vaccine Administration Subcommittee of the Commission undertook the study in 1962.

The purpose of the present study was to investigate the following seven items from virological and clinical points of view, and to find out the most adequate feeding procedure of the vaccine for newborns and young infants, including premature babies: (1) need of live poliovirus vaccine for newborns, (2) proper age in months for vaccination, (3) suitable combination of the three types of vaccine and order of feeding, (4) proper number of times of feeding and their interval, (5) vaccine dose, (6) influence of maternal passive immunity and feeding habit, and (7) possible side effects.

MATERIALS AND METHODS

General plan: Fifteen research teams were organized and each team recruited volunteers having no experience of live vaccine. The majority of the participants were under 8 months of age. They were divided into 5 age groups: under one month

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including premature babies, one to 2 months, 3 to 5 months, 6 to 8 months, and 9 months or over. The number of infants was equalized as much as possible among the age groups except the oldest group. The infants were vaccinated by the methods described below. Clinical observation and tests for fecal excretion of viruses and for antibody response were carried out by the members of each team. The data of each vaccinee were recorded on a punch card. The completed cards were sent to Dr. Kono's laboratory, where the compilation and analysis of the data were carried out. The results obtained were discussed by the members of the Subcommittee, and were reported at the general assembly of the Commission, March 16, 1963.

The number of infants in each study group is listed in Tables 1 and 2. The total number of vaccinees was 501, of which 474 were examined thoroughly.

Vaccine: The vaccine used, manufactured by Connaught Laboratories of Canada, was from the same lot as used for the nation-wide mass vaccination in 1962. The vaccine was assayed by the National Institute of Health, Japan; each original monovalent vaccine contained $10^{7.0}$ TCID₅₀/ml of virus. These original monovalent vaccines were diluted with 53.5% sucrose solution before use; the final concentration of each type virus was $10^{5.0}$ or $10^{6.0}$ TCID₅₀/ml, whether the final vaccine was mono-, di-, or trivalent vaccine.

Schedule of vaccine administration: The plan of study and method of vaccine feeding were simplified as much as possible in order to make the cooperative study easy. Two methods of vaccine administration were employed as shown in Table 3. One ml of the diluted vaccine was orally given to each infant using a sterile spoon. Each method involved two groups of infants; one received the $10^{5.0}$ TCID₅₀ vaccine and the other the $10^{6.0}$ TCID₅₀ vaccine (see Tables 1 and 2).

Serum and fecal specimens: Fecal specimens were collected from each infant immediately before the first feeding, and 7 to 10 days after each feeding. Serum specimens were obtained immediately before each feeding, and 6 to 8 weeks after the last feeding; in the Method 2 group, additional specimens were taken 12 to 16 weeks after the last feeding.

Virus isolation and serum neutralization test: Virus isolation from fecal specimens was performed by cell culture method, in which primary monkey kidney cells, FL cells or HEp-2 cells were employed according to convenience of each worker. The techniques were described elsewhere (Vaccine Administration Subcommittee, 1966).

RESULTS

Virus Isolation

Tables 4 and 5 show the results of virus isolation according to the method of feeding and age of vaccinees. The rates of positives for virus isolation are shown in Table 3.

Before the first feeding non-polio enteroviruses were isolated from 16 (3.7%) of 430 subjects examined. There were some cases in which concurrent infection with echovirus 14 occurred. In these cases, echovirus 14 interfered with the multiplication of vaccine virus in the alimentary tract (Hinuma and Murai, 1965).

In Method 1, the rate of type 1 virus isolation after the first feeding with $10^{5.0}$ TCID₅₀ dose was 42% for younger infants, 0 to 2 months of age, and 80% for older infants, 3 to 8 months of age; this marked inhibition of viral growth in younger infants seems to be due to passive maternal immunity or to milk or meconium containing

Table 1. Distribution of vaccines by research team, administration method, vaccine dose, and sex

Research team	Method 1						Method 2						Total of two methods			
	10 ^{5.0} TCID ₅₀			10 ^{6.0} TCID ₅₀			10 ^{5.0} TCID ₅₀			10 ^{6.0} TCID ₅₀			M*	F*	Total tested	
	M*	F*	Total	M*	F*	Total	M*	F*	Total	M*	F*	Total				
Sapporo Medical College	4	8	12	0	0	0	6	7	13	0	0	0	10	15	25	0
Tohoku University	7	4	11	8	4	12	8	5	13	8	2	10	31	15	46	4
Iwate Medical College	10	5	15	12	5	17	11	10	21	8	9	17	41	29	70	0
Tokyo University	5	2	7	4	5	9	5	3	8	4	4	8	18	14	32	0
Keio University	6	5	11	1	6	7	3	5	8	4	5	9	14	21	35	0
Nihon Medical College	7	2	9	6	2	8	3	3	6	2	4	6	18	11	29	0
Tokyo National First Hospital	3	3	6	5	1	6	2	3	5	5	1	6	15	8	23	2
Jikeikai Medical College	4	4	8	4	5	9	5	3	8	5	3	8	18	15	33	0
Shinshu University	5	5	10	2	4	6	5	3	8	6	1	7	18	13	31	0
Kyoto University	4	9	13	7	6	13	6	5	11	3	6	9	20	26	46	21
Osaka Medical College	2	5	7	4	3	7	6	3	9	3	2	5	15	13	28	0
Osaka University	1	7	8	3	3	6	4	2	6	3	5	8	11	17	28	0
Kanazawa University	4	6	10	4	4	8	7	5	12	8	5	13	23	20	43	0
Kumamoto University	4	4	8	4	4	8	4	4	8	3	5	8	15	17	32	0
Total	66	69	135	64	52	116	75	61	136	62	52	114	267	234	501	27

* M : male, F : female.

Table 2. Classification of 474 infants according to administration method, age and sex

Method	Virus dose (TCID ₅₀)	0 month			1-2 months			3-5 months		
		M	F	Total	M	F	Total	M	F	Total
1	10 ^{5.0}	12	12	24	16	16	32	16	12	28
	10 ^{6.0}	8	6	14	14	16	30	21	10	31
2	10 ^{5.0}	14	11	25	17	12	29	23	15	38
	10 ^{6.0}	10	8	18	16	10	26	17	12	29
Total		44	37	81	63	54	117	77	49	126

Method	Virus does (TCID ₅₀)	6-8 months			Over 9 months			Total		
		M	F	Total	M	F	Total	M	F	Total
1	10 ^{5.0}	17	20	37	1	3	4	62	63	125
	10 ^{6.0}	13	14	27	4	1	5	60	47	107
2	10 ^{5.0}	17	16	33	5	2	7	76	56	132
	10 ^{6.0}	14	16	30	3	4	7	60	50	110
Total		61	66	127	13	10	23	258	216	474

Table 3. Schedule of vaccine administration

Method	Feeding order	Vaccine	Interval
1	1	Monovalent, type 1	6-8 weeks
	2	Divalent, types 2+3	
	3	Trivalent	6-8 weeks
2	1	Trivalent	6-8 weeks
	2	Trivalent	

antibodies. However, this inhibition was shown to be overcome by the heavy dose of 10^{6.0} TCID₅₀, as revealed by the isolation rate of 62% in younger infants, and 69% in older infants (Table 6).

After the second feeding of divalent vaccine of types 2 and 3 with the dose of 10^{5.0} TCID₅₀, the rate of poliovirus isolation was 2% for type 1, 38% for type 2, and 58% for type 3 in younger infants and 2, 37, and 65%, respectively, in older infants. With the dose of 10^{6.0} TCID₅₀, the corresponding figures were 11, 49, and 73% in younger infants, and 0, 38, and 79% in older infants. Type 2 virus was less readily recovered than type 3 virus; this may be related to partial heterotypic immunity which had been elicited by the type 1 virus given before. The age difference in the isolation rate, which was marked after the first feeding, was obscured after the second feeding, most probably by the waning passive maternal immunity.

In Method 1, the isolation rate was significantly lower after the third feeding with trivalent vaccine than after the earlier feedings; this was expected as the earlier feedings

Table 4. Virus isolation from feces, Method 1 Group

Age	Virus isolated	Virus dose									
		10 ^{5.0} TCID ₅₀					10 ^{6.0} TCID ₅₀				
		Specimen No.*					Specimen No.*				
		1	2	3	4	Total	1	2	3	4	Total
5 month	Polio 1		6	1	4	11		6			6
	2		1	2		3			1		1
	3			5	3	8			5	2	7
	1+2			1		1				1	1
	2+3			4	2	6			3		3
	1+3				1	1					0
	1+2+3					0					0
	Non-polio					0					0
1-2 months	None	22	15	7	10	54	13	7	3	3	26
	Not tested	2	2	4	4	12	1	1	2	8	12
	Polio 1		15		2	17		18		3	21
	2			3	5	8			3	1	4
	3		1	11	3	15			7	4	11
	1+2				2	2				1	1
	2+3			8		8			8	2	10
	1+3					0			1		1
3-5 months	1+2+3					0			3	1	4
	Non-polio	1	1		1	3		2			2
	None	29	12	6	13	60	28	8	3	15	54
	Not tested	0	1	2	4	7		0	3	1	4
	Polio 1		22		2	24		18		3	21
	2		1	6	2	9			4	2	6
	3			7	2	9		1	16	1	18
	1+2				0	0					0
6-8 months	2+3			3	3	6			4		4
	1+3				0	0					0
	1+2+3					0					0
	Non-polio		1			1		3	1	2	6
	None	27	3	6	17	53	28	7	1	19	55
	Not tested			5	1	6	1	0	3	2	6
	Polio 1		27		1	28		18			18
	2			1	3	4		1	2		3
Over 9 months	3			15	8	23			9	3	12
	1+2					0					0
	2+3			8		8			8	2	10
	1+3					0					0
	1+2+3			1	1	2					0
	Non-polio	2	1		2	5	1	0		1	2
	None	32	6	5	17	60	23	4	2	16	45
	Not tested	1	1	5	3	10	1	2	4	3	10
Over 9 months	Polio 1		2			2		3			3
	2		1			1					0
	3			2	1	3			2		2
	1+2		1			1		2			2
	2+3					0					0
	1+3					0					0
	1+2+3					0					0
	Non-polio			2		2			2		2
Over 9 months	None	4			3	7	5		1	4	10
	Not tested					0				1	0

* No. 1: just before the first feeding,
No. 3: after the second feeding,

No. 2: after the first feeding,
No. 4: after the third feeding.

Table 5. Virus isolation from feces, Method 2 Group

Age	Virus isolated	Virus dose									
		10 ^{5.0} TCID ₅₀					10 ^{6.0} TCID ₅₀				
		Specimen No.*					Specimen No.*				
		1	2	3	4	Total	1	2	3	4	Total
0 month	Polio 1		2	5	2	9					0
	2		6	1	1	8		4	2		6
	3		2	4		6		2	1	1	4
	1+2			1		1		1	3		4
	2+3		1	4		5		1	1		2
	1+3		1			1		2	1		3
	1+2+3		1			1		1			1
	Non-polio								1		1
1-2 months	None	22	12	5	7	46	14	7	4	6	31
	Not tested	3		5	15	23	4		5	11	20
	Polio 1			3		3			4		4
	2		7	2		9		4		1	5
	3		3		1	4		1	4	1	6
	1+2					0		2	1		3
	2+3		5	2	1	8		4	1		5
	1+3					0		1			1
3-5 months	1+2+3		1	2		3		3	1		4
	Non-polio					0	1	2	1		4
	None	26	10	13	11	60	23	7	7	7	44
	Not tested	1	1	5	14	21			5	15	20
	Polio 1		1	7	1	9			5		5
	2		9	3	1	13		5	1		6
	3		1	4		5		4	4		8
	1+2		3	2		5		4			4
6-8 months	2+3		6	1		7		4	1		5
	1+3		1	1		2			2		2
	1+2+3		4	1		5		3	1		4
	Non-polio	2	3		1	6	2	3		1	6
	None	32	7	14	14	67	24	3	5	10	42
	Not tested	2	1	3	19	25	1	1	8	16	26
	Polio 1			9		9		1	2		3
	2		6	2		8		5			5
Over 9 months	3		10	2	2	14		6	8		14
	1+2		1	2		3		5	1		6
	2+3		4	1		5		1			1
	1+3			1		1		2			2
	1+2+3		4	2		6		5	2		7
	Non-polio	4	1		1	6	2				2
	None	27	4	10	13	54	26	4	13	13	56
	Not tested	1	2	3	16	22	1		3	16	20
Over 9 months	Polio 1			2		2			3		3
	2		2			2					0
	3					0		1			1
	1+2					0		1			1
	2+3		2	1		3		2	1		3
	1+3					0					0
	1+2+3		2	1		3		3	1		4
	Non-polio	1	1	1		3			1		1
Over 9 months	None	6		1		7	7				7
	Not tested			1	7	8			1	7	8

* See the footnote of Table 5.

Table 6. Results of virus isolation classified according to administration method and age of vaccinees

Method	Virus dose (TCID ₅₀)	Age in months	Incidence of poliovirus in feces, No. of excreters over No. tested (%)								
			After the first dose			After the second dose			After the third dose		
			Type 1	Type 2	Type 3	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
1	10 ^{5.0}	0-2	21/50 (42.0)			1/48 (2.1)	18/48 (37.5)	28/48 (58.3)	9/46 (19.6)	9/46 (19.6)	9/46 (19.6)
		3-8	49/61 (80.3)			1/52 (1.9)	19/52 (36.5)	34/52 (65.4)	4/58 (6.9)	9/58 (15.6)	14/58 (24.2)
	10 ^{6.0}	0-2	24/39 (61.5)			4/37 (10.8)	18/37 (48.7)	27/37 (73.0)	6/33 (18.2)	6/33 (18.2)	9/33 (27.3)
		3-8	36/52 (69.2)			0/47 (0)	18/47 (38.3)	37/47 (78.7)	3/49 (6.1)	4/49 (8.2)	6/49 (12.2)
2	10 ^{5.0}	0-2	5/51 (9.8)	21/51 (41.2)	14/51 (27.5)	11/42 (26.2)	12/42 (28.6)	12/42 (28.6)			
		3-8	14/65 (21.6)	37/65 (56.9)	30/65 (46.2)	25/62 (40.3)	21/62 (33.9)	13/62 (21.0)			
	10 ^{6.0}	0-2	10/41 (24.4)	20/41 (48.8)	15/41 (36.6)	11/32 (34.4)	9/32 (28.1)	9/32 (28.1)			
		3-8	20/54 (37.1)	32/54 (59.3)	25/54 (46.3)	13/45 (28.9)	6/45 (13.3)	18/45 (40.0)			

had induced immunity. The isolation rate of type 1 virus was lower in the older babies than in the younger ones; this difference seems to be a reflection of the age difference in the immunity induced by the first feeding.

In Method 2, after the first administration of trivalent vaccine, type 2 virus was most readily recovered from feces, type 3 virus came next and type 1 virus was the last. As observed in Method 1, younger babies excreted vaccine viruses less frequently than older ones, although the age difference was less conspicuous among the babies fed with 10^{6.0} TCID₅₀.

After the second feeding in Method 2, the rate of virus isolation was much higher in the groups which showed low rates of virus isolation after the first feeding, whereas it was greatly reduced in the groups which showed high rates of virus isolation after the first feeding. Thus, the rate was much increased for type 1 virus in the babies receiving 10^{5.0} TCID₅₀ of virus and in the younger babies vaccinated with 10^{6.0} TCID₅₀. The opposite was shown for type 1 virus in the older babies fed with 10^{6.0} TCID₅₀, and for types 2 and 3 in all the groups of babies, except for type 3 virus in the younger age group fed with 10^{5.0} TCID₅₀, in which no significant difference was noted in the rate between the first and second feedings.

Antibody Response

The results obtained are summarized in Table 7. They are based on the data obtained 6 to 8 weeks after the last feeding. With the babies initially seronegative at 1:4 serum dilution, the conversion rate is listed, while with the initially seropositive babies the rate of titer rise is shown. The combined rate of conversion and titer rise

Table 7. Antibody response to vaccination,

Administ- ration method	Virus dose (TCID ₅₀)	Antibody response	Type 1					Total
			0 mos.	1-2 mos.	3-5 mos.	6-8 mos.	9- mos.	
1	10 ^{5.0}	Conversion	7/9*	10/11	16/18	34/34	3/3	70/75
		(%)	(77.7)	(90.9)	(88.9)	(100.0)	(100.0)	(93.1)
		Titer rise	10/15**	12/18	8/8	—	0/1	33/42
		(%)	(66.6)	(66.6)	(100.0)	—	—	(71.4)
		Total	17/24	22/29	24/26	34/34	3/4	100/117
		(%)	(70.8)	(78.7)	(92.4)	(100.0)	(75.0)	(85.5)
	10 ^{6.0}	Conversion	2/3	8/8	21/25	22/22	5/5	58/63
		(%)	(66.7)	(100.0)	(89.0)	(100.0)	(100.0)	(92.1)
		Titer rise	3/10	16/20	6/6	—	—	25/36
		(%)	(33.3)	(80.0)	(100.0)	—	—	(69.5)
		Total	5/13	24/28	27/31	22/22	5/5	83/99
		(%)	(38.5)	(85.6)	(87.1)	(100.0)	(100.0)	(83.9)
2	10 ^{5.0}	Conversion	2/5	7/13	24/27	21/31	4/6	58/82
		(%)	(40.0)	(53.8)	(88.8)	(67.7)	(66.6)	(70.7)
		Titer rise	7/16	3/11	7/7	1/1	—	18/35
		(%)	(43.7)	(27.3)	(100.0)	—	—	(51.4)
		Total	9/21	10/24	31/34	22/32	4/6	76/117
		(%)	(42.9)	(41.7)	(91.2)	(68.6)	(66.6)	(65.0)
	10 ^{6.0}	Conversion	2/3	11/12	18/21	25/29	6/6	62/71
		(%)	(66.6)	(91.6)	(85.6)	(86.2)	(100.0)	(85.9)
		Titer rise	7/13	6/11	4/4	—	0/1	17/29
		(%)	(53.8)	(54.6)	(100.0)	—	—	(61.3)
		Total	9/16	17/23	22/25	25/29	6/7	79/100
		(%)	(56.2)	(73.9)	(88.0)	(86.2)	(85.7)	(79.0)

* Number of converted babies over number of babies initially seronegative at 1:4 serum dilution.

(hereafter referred to as antibody response rate) is also included in the table. To facilitate analysis, the data in Table 7 are condensed in Table 8.

In Method 1, the response rate was higher in the older age groups than in the younger age groups, but did not differ significantly between the two levels of vaccine dose. The response rate was not much different depending upon the virus types, although the response to type 1 was somewhat inferior in younger babies.

In Method 2, the response rate of each type was higher in older infants than in younger ones; the rate ranged from 42 to 73% in younger babies and from 80 to 92% in older babies with the dose of 10^{5.0} TCID₅₀, and from 87 to 96% in older babies with 10^{6.0} TCID₅₀ dose. The response rate was lower for type 1 than for the other types. Compared with Method 1, Method 2 gave a somewhat inferior antibody response, particularly in younger babies.

Table 9 shows the mean antibody titers of consecutive bleedings according to the

6 to 8 weeks after the last feeding

Type 2						Type 3					
0 mos.	1-2 mos.	3-5 mos.	6-8 mos.	9- mos.	Total	0 mos.	1-2 mos.	3-5 mos.	6-8 mos.	9- mos.	Total
13/13	17/17	15/15	31/31	3/3	79/79	10/10	20/20	22/22	31/32	4/4	87/88
(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(100.0)	(96.7)	(100.0)	(99.0)
7/11	10/12	10/11	3/3	0/1	30/38	10/14	8/9	3/4	2/2	—	23/29
(63.6)	(83.4)	(90.9)	(100.0)	—	(79.0)	(71.5)	(88.9)	(75.0)	(100.0)		(79.3)
20/24	27/29	25/26	34/34	3/4	109/117	20/24	28/29	25/29	33/34	4/4	100/117
(83.4)	(93.2)	(96.3)	(100.0)	(75.0)	(93.2)	(83.4)	(96.6)	(96.2)	(97.1)	(100.0)	(94.0)
8/10	9/11	21/21	19/19	4/4	61/65	6/7	12/13	25/26	18/19	2/2	63/67
(80.0)	(81.8)	(100.0)	(100.0)	(100.0)	(94.0)	(85.7)	(92.3)	(96.1)	(94.6)	(100.0)	(94.0)
3/3	15/17	9/10	2/3	1/1	30/34	5/6	12/15	5/5	2/3	2/3	26/32
(100.0)	(88.2)	(90.0)	(66.6)	—	(88.3)	(83.4)	(80.0)	(100.0)	(66.6)	(66.6)	(81.2)
11/13	24/28	30/31	21/22	5/5	91/99	11/13	24/28	30/31	20/22	4/5	89/99
(84.6)	(85.6)	(96.7)	(95.5)	(100.0)	(91.9)	(84.6)	(85.6)	(96.7)	(91.9)	(80.0)	(90.0)
5/6	12/13	27/28	28/28	6/6	78/81	6/10	13/15	25/30	27/31	5/5	76/91
(83.3)	(100.0)	(96.4)	(100.0)	(100.0)	(96.3)	(60.0)	(86.7)	(83.4)	(87.2)	(100.0)	(83.5)
8/15	6/11	5/6	1/4	—	20/36	8/11	6/9	3/4	1/1	0/1	18/26
(66.6)	(63.6)	(83.3)	(25.0)	—	(63.9)	(72.6)	(66.6)	(75.0)	—	—	(69.3)
13/21	18/24	32/34	29/32	6/6	98/117	14/21	19/24	28/34	28/32	5/6	94/117
(61.9)	(75.0)	(94.1)	(84.4)	(100.0)	(83.7)	(66.7)	(79.1)	(82.4)	(87.5)	(83.3)	(80.3)
3/3	10/11	15/16	22/23	7/7	57/60	3/6	13/15	20/20	23/25	6/6	65/72
(100.0)	(90.0)	(93.7)	(95.6)	(100.0)	(95.0)	(50.0)	(86.6)	(100.0)	(92.0)	(100.0)	(90.2)
10/13	10/12	7/9	5/6	—	32/40	5/10	7/8	5/5	4/4	1/1	22/28
(77.0)	(83.3)	(77.7)	(83.3)	—	(80.0)	(50.0)	(77.7)	(100.0)	(100.0)	(100.0)	(78.6)
13/16	20/23	22/25	27/29	7/7	89/100	8/16	20/23	25/25	27/29	7/7	87/100
(81.3)	(87.0)	(88.0)	(93.1)	(100.0)	(89.0)	(50.0)	(87.0)	(100.0)	(93.1)	(100.0)	(87.0)

** Number of babies showing 4-fold or greater titer rise over number of babies initially seropositive.

method and age. Waning of maternal passive immunity was discernible as decreasing initial mean titers with increasing age. Similarly, in younger age groups, antibodies passively transferred from mothers decreased for types 2 and 3 after the type 1 feeding in Method 1. Each feeding generally induced increase in mean titer for the homologous antibodies, although some instances of younger age failed to show titer rise. The final mean titer tended to be increasingly higher with the age of vaccinees.

Effects of Feeding Habit and Birth Weight on Vaccination

The feeding habit at the time of the first vaccination was classified into breast, bottle, and mixed feedings. The vaccinees were divided into premature babies whose birth weight was less than 2.5 kg and mature babies whose birth weight was more than 2.5 kg. According to these classifications the virus isolation rate and antibody response rate are listed in Tables 10 and 11. In these tables, results of the first feeding are shown

Table 8. Summary of antibody response, 6 to 8 weeks after the last feeding

Method	Virus dose (TCID ₅₀)	Age in months	Antibody response rate* for		
			Type 1	Type 2	Type 3
1	10 ^{5.0}	0-2	37/53 (73.6%)	47/54 (87.0%)	48/53 (90.6%)
		3-8	58/60 (96.7%)	59/60 (98.2%)	58/63 (92.1%)
	10 ^{6.0}	0-2	29/41 (70.7%)	35/41 (85.4%)	35/41 (85.4%)
		3-8	49/53 (92.5%)	51/53 (96.2%)	50/53 (94.3%)
	10 ^{5.0}	0-2	19/45 (42.2%)	31/45 (66.7%)	33/45 (73.3%)
		3-8	53/66 (80.3%)	61/66 (92.4%)	56/66 (84.8%)
2	10 ^{6.0}	0-2	26/39 (66.7%)	33/39 (84.6%)	28/39 (71.8%)
		3-8	47/54 (87.0%)	49/54 (90.7%)	52/54 (96.3%)

* Number of babies showing conversion or titer rise over number of babies tested.

for babies 0 to 2 months of age, in which the difference due to feeding habit and birth weight may appear most pronouncedly; results of the second feeding was discarded, since the feeding habit may change with increasing age, and influence of the first feeding of vaccine may confuse the result. Cases harboring non-polio enteroviruses are not included in the tables.

The different methods of feeding showed no significant difference in the rate of virus isolation and antibody response, although the virus isolation rate among breast fed babies seemed lower in comparison with that among bottle fed babies.

As for birth weight, the virus isolation rate was higher in premature babies and the antibody response rate was higher in mature babies, but the differences could not be regarded statistically significant.

Effect of Maternal Passive Immunity on Vaccination

The rate of virus isolation and antibody response after vaccine feeding were investigated in connection with the antibody titer before vaccination, since most of the younger babies possessed maternal antibody beforehand. The results are shown in Tables 12 and 13, which include only data on the first feeding of young babies, 0 to 2 months of age.

After monovalent type 1 vaccine the rate of virus isolation was 53 and 69% for 10^{5.0} and 10^{6.0} TCID₅₀ doses of vaccine, respectively, among babies with initial antibody titers of 1:4 or lower. In contrast, the rate was lower, 36 and 58%, respectively, for vaccinees whose initial antibody titers were 1:8 or higher. The antibody response rate also showed a similar tendency.

After giving the trivalent vaccine, comparison was made only for type 2 vaccine virus and its antibody, since response to type 2 vaccine virus was dominant as described before. The rate of virus isolation was 48 and 59% for doses of 10^{5.0} and 10^{6.0} TCID₅₀,

Table 9. Mean antibody titers

Method	Virus dose (TCID ₅₀)	Antibody type	0 month				1-2 months				3-5 months				6-8 months			
			Specimen No.*				Specimen No.				Specimen No.				Specimen No.			
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	10 ⁵	No. tested	24	22	22	22	30	27	26	24	26	22	23	26	35	30	29	27
		1*	2.9**	3.6	3.9	5.9	2.8	4.6	5.2	6.3	1.2	5.8	6.7	7.2	0	6.0	6.8	7.7
		2	2.4	1.7	4.8	5.8	1.9	1.5	5.4	6.9	1.5	1.5	6.6	7.8	0.3	0.7	5.9	7.4
	10 ⁶	3	2.2	1.6	4.3	6.1	1.2	0.6	6.3	7.0	0.6	1.2	5.2	6.7	0.2	0.9	5.9	7.4
		No. tested	14	13	12	10	28	24	22	24	31	26	27	26	23	21	19	19
		1	4.5	4.6	5.2	6.3	3.3	4.4	4.9	7.4	5.5	5.7	6.4	6.9	0.2	4.8	6.2	7.6
		2	1.4	1.3	3.7	5.1	2.6	1.5	5.6	7.2	1.1	1.4	5.5	6.5	0.6	1.3	6.1	6.5
		3	2.3	1.8	4.3	5.9	1.9	1.0	6.0	6.7	0.5	0.7	6.6	6.7	0.4	1.2	5.7	7.1
	10 ⁵	No. tested	25	21	17	4	26	20	21	9	35	27	30	13	32	25	30	13
		1	3.5	3.0	3.8		2.0	1.9	3.4	3.6	0.4	1.8	5.1	5.2	0.2	1.3	4.9	5.4
		2	3.4	2.6	4.4		2.1	4.1	6.4	7.2	0.5	4.3	6.3	6.5	0.7	5.1	6.7	7.6
2	10 ⁵	3	2.3	2.0	4.4		1.5	4.3	6.4	6.1	0.3	2.8	6.1	6.5	0.2	4.8	6.3	6.5
		No. tested	18	14	11	5	24	18	18	9	26	19	22	9	29	24	26	12
	10 ⁶	1	3.9	3.4	5.0		1.8	3.7	5.3	4.7	0.4	1.8	5.3	5.0	0	1.8	5.5	5.3
		2	3.6	3.6	5.6		2.3	4.3	6.1	7.6	1.3	5.4	6.8	6.6	0.7	4.9	6.9	7.9
		3	2.6	2.9	4.2		1.3	3.7	5.2	6.8	0.6	3.6	7.0	6.6	0.4	3.8	6.6	6.4

* See the footnote of Table 5.

** The geometric mean titer expressed as logarithm to base 2.

Table 12. Virus isolation rates and antibody titers before vaccination.

(The compilation of data was confirmed to the results obtained by the first feeding of young infants whose ages were from 0 to 2 months old)

Method	Virus type	Virus dose (TCID ₅₀)	Homotypic antibody titer before vaccination								
			<4	4	8	16	32	64	128	256	>256
1	1	10 ⁵	9/17	1/2	2/9	1/3	3/10	3/5	2/2	—	0/2
			10/19	(52.6%)			11/31	(35.5%)			
		10 ⁶	7/11	2/2	4/6	2/4	3/5	3/4	1/4	1/2	1/1
			9/13	(69.2%)			15/26	(57.7%)			
2	1	10 ⁵	1/21	—	1/8	2/6	1/9	0/4	0/2	0/1	—
			1/21	(4.8%)			4/30	(13.3%)			
		10 ⁶	2/16	2/5	2/5	1/3	2/7	1/2	0/2	—	0/1
			4/21	(19.0%)			6/20	(30.0%)			
	2	10 ⁵	12/24	1/3	1/5	2/4	1/7	1/2	1/3	—	2/3
			13/27	(48.2%)			8/24	(33.3%)			
		10 ⁶	8/14	2/3	1/6	5/8	1/3	2/4	0/2	—	1/1
			10/17	(58.8%)			10/24	(41.6%)			
	3	10 ⁵	7/30	0/3	3/4	1/6	0/3	0/1	2/2	—	1/2
			7/33	(21.2%)			7/18	(38.9%)			
		10 ⁶	8/21	1/5	2/6	2/3	2/3	0/2	—	0/1	—
			9/26	(34.6%)			6/15	(40.0%)			

respectively, among babies with initial titers of 1 : 4 or lower. Reduced rates, 33 and 42 % were obtained for vaccinees with initial titers of 1 : 8 or higher. However, these differences were not statistically significant ; anyway, the inference is very limited since the number of babies available for these analyses was very small.

Although further investigations are required to solve these problems, the factors of feeding habit, birth weight and maternal passive immunity can be neglected from the practical point of view, if the vaccine is fed to babies at the age of 3 months or later.

DISCUSSION

After the completion of mass vaccination with live oral poliovirus vaccine which was accomplished in U. S. S. R., Czechoslovakia and Japan, the next stage consists in the vaccination of infants born annually. The present study was undertaken to seek the most effective practical method of vaccination for these infants. After due consideration, two methods of vaccination were studied with two levels of vaccine dose, 10^{5.0} and 10^{6.0} TCID₅₀.

Method 1 (monovalent type 1 vaccine—divalent types 2 and 3 vaccine—trivalent vaccine) proved more effective than Method 2 (trivalent—trivalent vaccine). No difference in antibody response was found between the virus doses in Method 1, but there was an advantage of the dose of 10^{6.0} TCID₅₀ in Method 2. Particularly the higher

Table 13. Rates of antibody rise and antibody titers before vaccination

Method	Viruses dose	Virus dose (TCID ₅₀)	Homotypic antibody titer before vaccination								
			<4	4	8	16	32	64	128	256	>256
1	1	10 ⁵	10/18	1/2	3/7	1/2	3/9	0/5	0/2	—	—
			11/20 (55.0%)				7/25 (28.0%)				
		10 ⁶	6/10	1/3	3/4	2/4	0/4	0/4	0/4	1/2	—
			7/13 (53.8%)				6/22 (27.3%)				
2	1	10 ⁵	3/18	—	1/4	1/5	0/7	2/4	—	—	—
			3/18 (16.7%)				4/20 (20.0%)				
		10 ⁶	8/14	1/3	1/3	1/2	0/5	0/1	0/2	—	—
			9/17 (52.9%)				2/13 (15.4%)				
	2	10 ⁵	8/16	0/1	1/5	1/4	1/4	0/2	0/3	—	—
			8/17 (47.1%)				3/18 (16.7%)				
		10 ⁶	7/13	2/3	2/4	3/5	0/1	0/3	1/2	—	—
			9/16 (56.2%)				6/15 (40.0%)				
		10 ⁵	10/22	0/1	2/3	0/5	0/2	0/1	0/2	—	—
			10/23 (43.5%)				2/13 (15.4%)				
		10 ⁶	8/17	2/4	1/4	2/2	0/1	0/2	—	0/1	—
			10/21 (47.6%)				3/10 (30.0%)				

These who have antibody titer over 1:256 before vaccination were discarded from compilation, because they were hard to determine whether they exerted antibody rise or not.

dose seems advantageous to overcome resistance in infants under 2 months of age who are more insensitive to the antigenic stimulus. However, the ordinary dose of 10^{5.0} TCID₅₀ was shown to be quite satisfactory to provoke immunity in infants over 3 months of age. Method 2 was obviously less satisfactory than Method 1, but it is considered to be practically satisfactory enough in infants over 3 months of age, particularly so because the method is less complicated.

The exact nature of the refractoriness of young infants to the immunizing effect of live vaccine is still obscure, but several factors are considered to be involved: (1) high acidity of gastric juice, (2) maturity grade at birth, (3) feeding habit of babies, and influence of maternal passive antibodies which are found to occur in amniotic fluid and colostrum, and/or considered to pass to the bowels through alimentary mucosa from the circulation. In the present study differences were observed between the groups of infants classified by the birth weight, feeding habit and antibody titer, indicating that such factors might be involved, but they were not statistically significant. Other works showed definite influence of maternal immunity through various channels in neonatal babies (Holguin, Reeves and Gelfand, 1962; Lepow et al., 1961, 1962; Plotkin, Pagano and Koprowski, 1960; Sabin et al., 1963a, b). It may be natural that we could not obtain definite difference, since the age of our vaccinees was much older when compared to that in the above cited studies. At any rate, the immunological refractoriness of young infants does not seem so strong as that observed after the natural infection in older

children and adults.

In the present procedure of vaccination with Sabin vaccine in this country, the first feeding is administered at the age of 3 months: this is considered to be sound from the present study. In the present epidemiological situation of poliovirus infection in this country, characterized by a wide spread vaccination with Sabin vaccine and few cases of wild poliovirus, it is not necessary to begin immunization earlier than this.

REFERENCES

- HINUMA, Y. AND MURAI, Y. (1965): Two outbreaks of echovirus infection: A possible interference with oral poliovirus vaccine and a probable association with aseptic meningitis. *J. Hyg., Camb.*, **63**, 277-284.
- HOLGUIN, A. H., REEVES, J. S. AND GELFAND, H. M. (1962): Immunization of infants with Sabin oral poliovirus vaccine. *Am. J. Publ. Hlth.* **52**, 600-610.
- LEPOW, M. L., WARREN, R. J., GRAY, N., INGRAM, V. G. AND ROBBINS, F. C. (1961): Effect of Sabin type 1 poliomyelitis vaccine administered by mouth to newborn infants. *New Eng. J. Med.* **264**, 1071-1078.
- LEPOW, M. L., WARREN, R. J., INGRAM, V. G., DAUGHERTY, S. C. AND ROBBINS, F. C. Sabin type 1 (LSc2ab) oral poliomyelitis vaccine. Effect of dose upon response of newborn infants. *Am. J. Dis. Child.* **104**, 67-71.
- PLOTKIN, S. A., PAGANO, J. S. AND KOPROWSKI, H. (1960): Vaccination of full-term infants with attenuated polioviruses. in *Live Poliovirus Vaccines; Papers Presented and Discussion Held at the 2nd International Conference on Live Poliovirus Vaccines*, Pan American Hlth. Org. 294-301.
- SABIN, A. B., MICHAELS, R. H., KRUGMAN, S., EIGER M. E., BERMAN, P. H. AND WARREN, J. (1963a): Effect of oral poliovirus vaccine in newborn children. I. Excretion of virus after ingestion of large doses of type 1 or of mixture of all three, types, in relation to level of placentally transmitted antibody. *Pediatrics* **31**, 623-640.
- SABIN, A. B., MICHAELS, R. H., ZIRING, P., KRUGMAN, S. AND WARREN, J. (1963b): Effect of oral poliovirus vaccine in newborn children. II. Intestinal resistance and antibody response at 6 months in children fed type I vaccine at birth. *Pediatrics* **31**, 641-650.
- VACCINE, ADMINISTRATION, SUBCOMMITTEE, (1966): Evaluation of Sabin live poliovirus vaccine in Japan. II. Clinical, virologic and immunologic effects of vaccine in children. *Jap. J. Med. Sci. Biol.* **19**, 277-291.