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Marker Test of Poliovirus in Relation to Mass Vaccination with Live Oral Vaccines*

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SUMMARY

The characteristics of T marker were first tested on laboratory strains and it was found that plaquing manipulations were often necessary before employing them as standard strains in the T marker test. Then T marker test was employed in a survey of wild strains. Five of the 16 strains of type 1 virus isolated from paralytic patients showed T^- character and these were all found in one group isolated in the same place in the same year. T characters of type 3 wild strains varied between T^- and T^+, but few showed T^+ characters. Tests on the virus excreted in the feces of vaccinees indicated that type 1 and 2 Sabin vaccines were relatively stable, though type 3 virus changed easily from T^- to T^+ during a single passage in the human intestinal tract, and its progeny tended to possess a higher growth capacity at 40°C than wild strains. The role of T marker test in surveys of the progeny of vaccines is discussed.

This test and the intratypic sero-differentiation test were applied on poliovirus obtained from two patients after vaccination. One case excreted type 1 virus and this virus was $T^{\pm}$ and heterologous to the vaccine strain. Another case excreted type 2 viruses continuously and these viruses possessed various thermal characters between T^- and T^+. These were tested by dynamic neutralization and were found to be homologous with the Sabin type 2 strain. The homologous character were maintained even after plaquing of the T^+ substrains, which might increase the possibilities of picking up wild strains. No enteric virus other than vaccine progeny have been isolated from monkey kidney tissue culture up to the present.

INTRODUCTION

In 1961, the benefit and safety of monovalent live poliovirus vaccines (Pfeizer) were tested in a small scale field trial in Japan. Immediately after this trial, mass vaccination was performed with live trivalent vaccines produced in USSR and Connaught. In both cases we tested progeny virus for markers of poliovirus to survey the mutation of the vaccine strains. For this, certain *in vitro* characteristics, so called T, d and MS markers (Lwoff and Lwoff, 1959; Vogt *et al.*, 1957; Kanda and Melnick, 1959), were tested in a field trial by members of the Japan Live Poliovaccine Research Commission comparing results with the monkey

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neurovirulence test and these results were summarized in official reports (1962).

Mass vaccination was performed during the summer months and care was needed to distinguish poliomyelitis caused by wild virus and by live vaccine. In this case, the T marker test was employed for survey of genetic changes, since this marker seemed to be closely related to the virulence of the virus (Benyesh-Melnick and Melnick, 1959; Paul *et al.*, 1959; Cabasso *et al.*, 1960; Baron *et al.*, 1960), and the intratypic sero-differentiation test was performed by the method of McBride (1959), in order to distinguish wild virus from progeny of vaccine strains.

This paper reports results of fundamental studies on the thermal characteristics of the standard strains and their application to the field trial and mass vaccination together with the serological differentiation of some agents isolated. Detailed data on the marker test of the field trial were reported previously.

MATERIALS AND METHODS

1. *Virus*

Standard virulent, attenuated and many wild strains were obtained from many laboratories through the courtesy of many workers. The progeny of Pfeizer monovalent vaccines were isolated in our laboratory from the feces of vaccinees from Osaka University Medical School, Osaka Medical College and Kanazawa University Medical School through the kindness of Drs. Nishizawa, Tatsumi

Table 1 a. Type 1 Strains

Strain Name or Number	Isolation			Obtained from	Remarks
	Year	From	T.C.cell		
Mahoney				NIH, Japan	MK Passage
Brunenders				Osaka Microbiological Research Foundation	〃
Lsc. 2ab				NIH, Japan	〃
Ogawa	1961	Patient	FL	Osaka Public Health Institute	before mass-Vaccintion
Fukuoka	〃	〃	〃	〃	Paralyzed after Vaccination
2	〃	〃	〃	〃	Momoyama Hospital
2	〃	Carrier	MK		before mass-Vaccination
3	1959	Patient	HeLa	The Institute for Infectious Diseases, The Univ. of Tokyo	isolated in Ebara Hospital, Tokyo
7	1960	〃	〃	〃	
2	1961	〃	〃	〃	
1	1960	Carrier	〃	〃	
31	1961	Vaccinee	MK	Osaka Univ. Osaka Med. College Kanazawa Univ.	Pfeizer monevalent Vaccine
11	1961	〃	〃	Osaka Public Health Institute	USSR trivaient Vaccine

and Sagawa. The progeny of trivalent vaccines were isolated in our laboratory from the feces of vaccinees in Osaka Prefecture. All viruses are listed in Tables 1 a-c.

Table 1 b. Type 2 Strains

Strain Name or Number	Isolation			Obtained from	Remarks
	Year	From	T.C.cell		
MEF_1				Osaka Microbiological Research Foundation	MK passage
P_{712} ch2ab				NIH Japan	〃
Okita	1961	Patient	FL	Osaka Publ. Hlth. Inst.	
Hata	〃	〃	MK	〃	Paralyzed after Vaccination
Hirao	〃	〃	〃	〃	〃
2	〃	Carrier	MK	〃	Before mass vaccination
3	1959—61	Patient	HeLa	The Institute for Infectious Diseases, The Univ. ot Tokyo	Isolated in Ebara Hospital, Tokyo
8	1961	Vaccinee	MK	Osaka Univ. Osaka Med. Coll. Kanazawa Univ.	Pfeizer monovalent vaccine
45	〃	〃	〃	Osaka Publ. Hlth. Inst.	USSR trivalent Vaccine

Table 1 c. Type 3 Strains

Strain Name or Number	Isolation			Obtained From	Remarks
	Year	From	T.C.cell		
Saukett				NIH Japan	MK Passage Saukett Y
				Osaka Microbiological Research Foundation	MK Passage Saukett O
				Kurume University	MK Passage Saukett K
Leon				NIH Japan	MK Passage
Leon 12ab				NIH Japan	MK passage
Nakoshi	1961	Patient	MK	Osaka Public Health Institute	
3		Carrier	?	The Institute for Infectious Diseases, The Univ. of Tokyo	Isolated in Kanagawa prefecture.
3	1959—60	Patient	Hela	Institute for Virus Reseach, Kyoto Univ.	Isolated in Ebara Hospital, Tokyo
6	1955	Patient	FL	Osaka Univ. Medical School.	
6	1956	〃	〃		
2	1957	〃	〃		
51	1961	Vaccinee	MK		Pfeizer monovalent Vaccine
30	〃	〃	〃		USSR trivalent Vaccine

Most of the standard and wild viruses received were used after single passages in monkey kidney

tissue cultures at 35°C. Strains from vaccinees and some patients were isolated in monkey kidney tissue cultures, unless otherwise specified.

2. *Tissue culture*

Monkey kidneys (MK) were trypsinized by Bodian's method (1956), suspended in Earle's balanced salts solution supplemented with 0.5 per cent lactalbumin hydrolysate (LE) and 3 per cent bovine serum and seeded in Roux bottles, 50 ml bottles or test tubes. Five or 6 days after seeding, when the monolayers of the MK cells were complete, they were washed once with phosphate buffered saline (PBS) and added with LE containing 0.2 per cent skimmed milk. They were used on the following day.

3. *Virus isolation*

Five to 10 per cent emulsions of feces clarified by centrifugation were inoculated into the MK tissue cultures after discarding the medium, and the cultures were incubated at room temperature for 30 min. Medium 199 or LE without serum was added and then the cultures were incubated at 35°C until maximal cytopathic changes appeared. Isolated agents were stored at −20°C and identifications were performed using 10^{-2} dilutions of infected tissue culture fluid and typing sera containing 20 to 40 units of neutralizing antibodies/0.1 ml. Virus samples containing a single type of poliovirus were used for the marker test.

4. *Virus titration*

Titration was made by the plaque method using 0.2 ml of inoculum and 3 to 5 bottles of 50 ml volume for each dilution. After one hour of adsorption at 35°C, the bottles were overlaid with 4 ml of nutrient agar (0.22 per cent $NaHCO_3$) prepared by Hsiung and Melnick's prescription (1958) containing 0.2 per cent skimmed milk (Snow Brand) rather than serum or bovine albumin.

5. *T marker test*

These tests were carried out by virus titration by the plaque method using different temperatures of incubation and 10 fold serially diluted virus samples. For control titrations, a 35°C air incubator was used and for growth at higher temperatures, a continuously agitated water bath was employed. The variation in the temperature of the water bath was less than ± 0.1°C at the appropriate temperatures. Plaques were counted after 3 and 4 days of incubation at 35°C and after 5 and/or 6 days at higher temperatures.

6. *Preparation of strain specific immune sera*

Anti-LSc 2ab, Ogawa and P 712 Ch 2ab rabbit sera were prepared according to the following schedule. Each antigen was prepared using MK cultures containing Medium 199. Ten ml of antigen containing about 10^9 PFU were inoculated subcutaneously into 4 rabbits for each strain. Two or three times booster does of antigen were inoculated at 3 week intervals and 7 days after the last immunization, animals were bled by heart puncture. After inactivation at 56°C for 30 min. the capacity of the sera to neutralize both homologous and heterologous strains were tested and sera were selected for the intratypic sero-differentiation test.

7. *Intratypic sero-differentiation test (IST)*

The test was carried out according to the method of McBride (1959). Briefly, serum was diluted to a concentration which reduced a homologous strain of 10^6 PFU/ml to 10^4 PFU or lesser in 20 min., on incubation at 37°C. Virus containing about $10^{6.5}$ PFU/ml and diluted serum were placed in tubes in a 37°C water bath. Aliquots of 0.5 ml of virus and of serum were mixed at zero time and the mixtures incubated in a water bath. At appropriate intervals 0.1 ml of mixtures were introduced into 100 ml of cooled PBS, and then the survival was determined by plaque titration.

RESULTS

1. *Effect of the temperature of incubation on the efficiency of growth*

To select experimental conditions suitable for T marker test, virulent and attenuated strains were tested for their plaque producing capacities at 35°, 39.0°, 39.5°, 40.0° and 40.3°C respectively. Results for each type of virus are summarized in Figs. 1, 2 and 3.

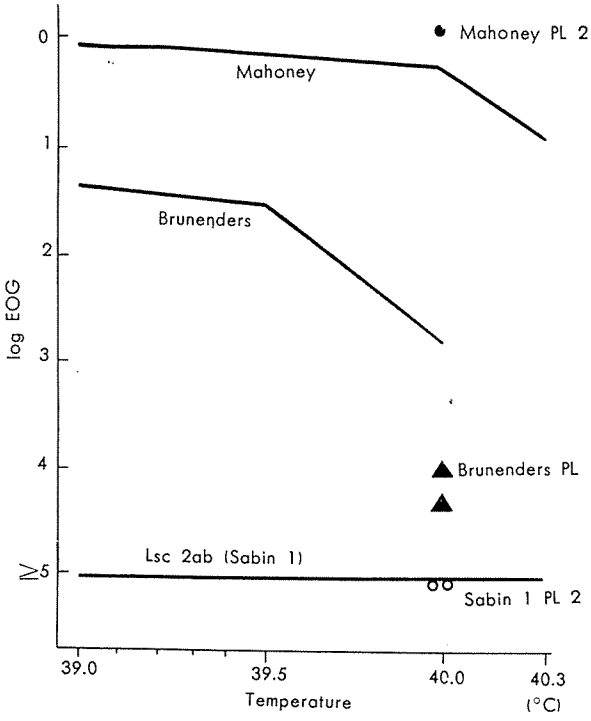

Fig. 1. Temperature Characters of Type I Laboratory Strains
PL: Plaque purified substrain (at 35°C)

As shown in Fig. 1, the Mahoney strain had a stable T+ character which was not affected by plaque purification. The LSc 2ab strains obtained from Pfeizer, USSR and Connaught vaccines all showed T-character to the same extent before and after plaque purification at 35°C. The Brunenders strain was of an intermediate type with regard to its thermal character and showed rct/40-character after plaquing at 35°C.

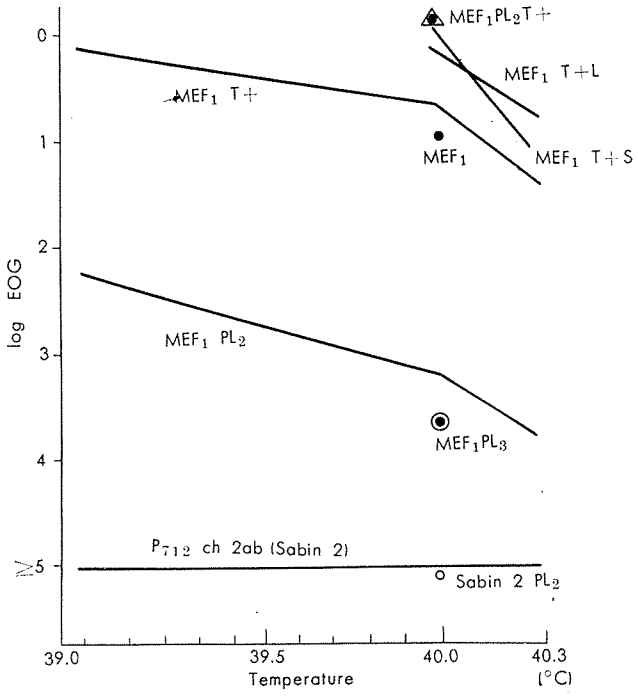

Fig. 2. Temperature Character of Type 2 Laboratory Strains
T+: Manipulated from T+ plaque

With regard to type 2 strains (Fig. 2), MEF 1 strain showed T+ character, but at higher temperatures it produced rather small plaques and the plaque titer was also reduced. MEF 1 purified twice by plaquing at 35°C (MEF 1 PL2) showed an intermediate character and further plaquing at 35°C caused little change in its character (MEF 1 PL3), though rct/40+ substrain was easily obtained from the same clone by plaquing at 39.5°C (MEF 1 PL2 T+). On the other hand, this strain manipulated from a plaque at 40°C showed an evident T+ character (MEF 1 T+) and further manipulation at 35°C yielded stable T+ clones (MEF 1 T+L and T+S). These clones were employed as the T+ control for the type 2 tests. P 712 from three vaccines had T— character at all the high temperatures tested.

Type 3 viruses, however, differed from the two other types (Fig. 3). Both Leon and Saukett strain from the National Institute of Health, Japan (Saukett Y) showed T+character, but tended to show T± character at 40.3°C and could not be used as T+ standards. Two Saukett strains obtained from other laboratories (Saukett B and Saukett k) were tested during selection of a T+ standard strain, but they showed similar temperature characters to those described above.

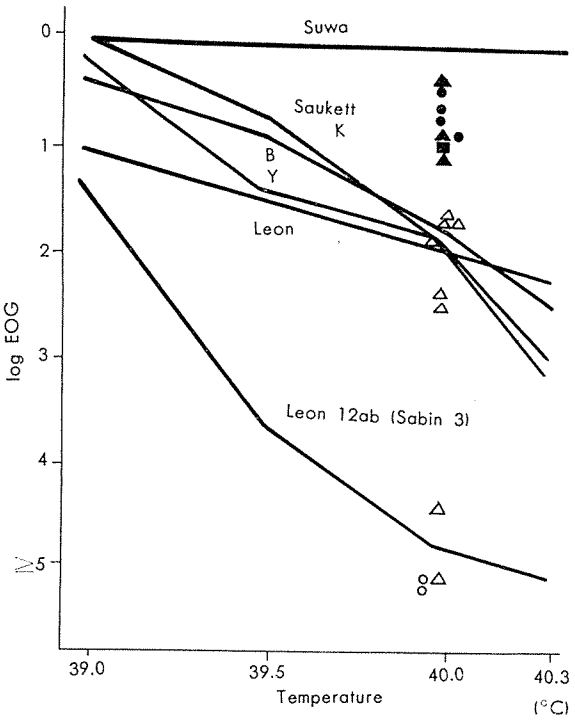

Fig. 3. Temperature Character of Type 3 Laboratory Strains

○ and ● : Substrains of Leon 12ab manipulated from plaques at 35°C and at 39.5°C
△ and ▲ : Substrains of Saukett manipulated from plaques at 35°C and at 39.5°C
■ : Substrain of Leon manipulated from a plaque at 39.5°C

From these results the Suwa strain, which had been determined as a T+ strain at the National Institute of Health, Japan, was tested and used as the T+ standard strain of type 3 in the following marker tests. As can be seen in Fig. 3, Leon 12 ab, which was not plaque-purified in our laboratory, showed rct/40.3-, rct/40- and rct/39.5± characters. Thus a 40.3°C water bath was employed for T marker test of type 3 viruses concomitantly with a 40.0°C water bath. Substrains of type 3 virus were tested after manipulation from plaques at 35°C and 39.5°C, because all these strains, except the Suwa strain, produced only small, hazy plaques at 40°C and their titers also fluctuated considerably at higher temperatures. A common phenomenon among these strains was that rct/40+ substrains were constantly obtained from plaques at 39.5°C and they produced larger plaques at 40°C than the original strains. On plaquing the Saukett strain at 35°C substrains varying from rct/40 + to rct/40− were obtained, but substrains obtained from Leon 12 ab did not produce any plaques at 40°C.

2. *Thermal characteristics of wild strains*

Sixteen type 1 and four type 2 strains isolated from paralytic patients, and three type 1 and two type 2 strains from healthy carriers were tested. The results are summarized in Table 2.

Table 2. Temperature Markers of Type 1 and 2 Wild Strains

Type	Isolated from	log EOG ≥5	5—4	4—3	3—2	2—1	1—0
I	Patient	1*	4*		3	3	5
	Carrier	1	1				1*
II	Patient		1			1	2
	Carrir	1		1			
Character		rct/40—		rct/40±		rct/40+	

* : isolated from patients in Tokyo in 1960

* : isolated from a family contact

Five type 1 strains isolated from paralytic patients showed rct/40— character. All these 5 T—strains from patients were concentrated in strains isolated in Tokyo in 1960. On the other hand, three rct/40+ strains were isolated in the same year in Tokyo. Two of them were obtained from patients, one in summer and one

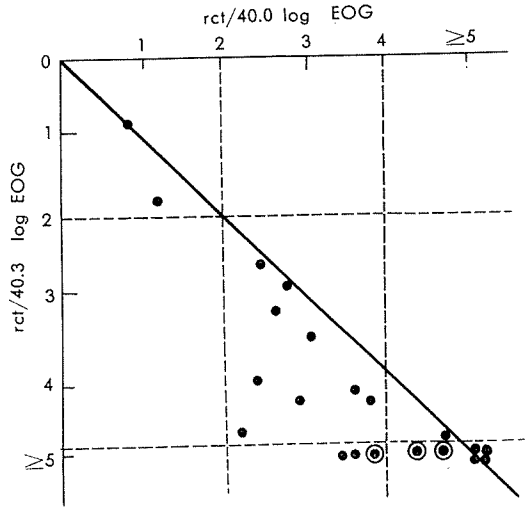

Fig. 4. Comparison between rct/40 and rct/40.3 Characters of Type 3 Wild Strains

● Strains isolated from patients

◉ Strains isolated from healthy carriers

toward the end of the year. The remaining strain was isolated from a healthy child who had come into contact with a brother excreting the rct/40+ strain. Other type 1 strains isolated from patients exhibited rct/40+ character and those obtained from healthy carriers had rct/40- characters. Type 2 strains showed a similar tendency to type 1 wild strains though too few were examined to analyze the results.

Both the rct/40 and rct/40.3 characters of type 3 strains were studied. As shown in Fig. 4, only 2 strains showed T+ character although 18 strains were distributed in various ways between rct/40− and rct/40.3+. Three strains isolated from healthy children showed T− character. In addition, rct/40.3− character did not seem to be directly associated with rct/40− character.

3. *Tests on virus excreted in the feces of vaccinees*

The progeny of the Sabin type vaccine strains were tested for their characters after single passage in MK cultures at 35°C. The results are summarized in Tables 3 and 4. Most progeny of type 1 and 2 strains showed nearly equal thermal characteristics to the original virus though some showed rct/40± character (Table 3). None of them showed the rct/40+ character and they seemed to be relatively stable with regard to thermal characteristics. No remarkable difference was observed between USSR and Pfizer vaccines.

On the contrary, the thermal characteristics of type 3 vaccine strains changed easily from rct/40.3− to rct/40.3+ by single passage in the human intestinal tract and moreover these strains tended to grow better at 40.0° and 40.3°C than virulent wild strains. Since the progeny of Pfeizer type 3 vaccine had been tested at 40.0°C,

Table 3. Temperature Makers of Type 1 and 2 Vaccine Progeny

Vaccinated with		Isolated	log EOG					
			≥5	5~4	4~3	3~2	2~1	1~0
Type 1	Pfeizer	1~3 W	12	3		1		
		≥4 W	8	5	1	1		
	USSR	1 W	7					
		≥1 M	4					
Type 2	Pfeizer	1~3 W	4		2			
		≥4 W	2					
	USSR	1 W	12	1	3	2		
		1 M	23	3		1		
Character			rct/40		rct/40			

W=week M=month

Table 4. Temperature Markers of Type 3 Vaccine Progeny

Vaccinated with	tested at (°C)	isolated after	log EOG ≥5	5~4	4~3	3~2	2~1	1~0
Pfeizer	40.0	1~3 W	1		1	4	7	5
		≥4 W				1	6	8
USSR	40.0*	1 M				4	7	7
Character			rct/40.0−		rct/40.0±		rct/40.0+	
USSR	40.3	1 W		2	3	5		
		1 M	3	2	4	6	4	1
Character			rct/40.3−		rct/40.3±		rct/40.3+	

* These samples were included in samples at 40.3°C
W=week M=month

various USSR vaccine progeny were also tested at 40.0°C and their stabilities were compared with those of Pfeizer vaccines. As shown in Table 4, again no remarkable differences were observed.

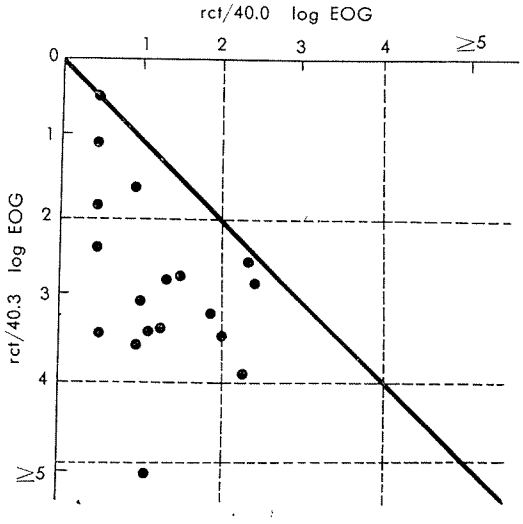

Fig. 5. Comparison between rct/40 and rct/40.3 Characters of Type 3 Vaccine Progeny

A comparison between rct/40.3 and rct/40.0 was made with some type 3 vaccine progeny (Fig. 5). Most strains tested were rct/40+ and the characters seen at 40.3° were not always proportional to the rct/40.0 characters.

4. *Application of the marker test to post vaccinal paralytic patients*

Feces were collected from three paralytic patients hospitalized immediately after mass vaccination (Table 5). One of these, the third case, was diagnosed clinically as a case of intracranial hemorrhage, and strains isolated were included in the test as control progeny virus.

Table 5. Paralytic Patients after Administration of Trivalent Vaccine

Name	Age	Date of Vaccination	Onset of Illness	Hospitalization	Prognosis
Fukuoka	4	July, 23	July, 25	July. 28	Died on July. 30
Hata	5	July, 24	July, 26?	July, 28	
Hirao	4	July, 21	August, 9	August, 9	Clinical Diagnosis: Intracranial Hemorrhage

Table 6 Temperature Markers of Viruses Isolated from Patients after Vaccination

Strain		Type	Thermal Character		
Fskuoka	729	1	$\frac{35}{40}=\frac{7.01}{4.97}$		rct/40±
Hata	803	1	$\frac{35}{40}=\frac{7.20}{<1.70}$		rct/40−
	811	2	$\frac{35}{40}=\frac{6.10}{2.70}$		rct/40±
	812	2	$\frac{35}{40}=\frac{7.22}{<1.70}$		rct/40−
	815	2	$\frac{35}{40}=\frac{7.22}{<1.70}$	$\frac{7.95}{2.28}$	rct/40−
	817	2	$\frac{35}{40}=\frac{6.67}{4.09}$	$\frac{7.23}{5.30}$	rct/40±～+
	819	2	$\frac{35}{40}=\frac{7.30}{4.64}$	$\frac{7.70}{5.65}$	rct/40±
	822	2	$\frac{35}{40}=\frac{7.00}{2.00}$		rct/40−
	824	2	$\frac{35}{40}=\frac{7.65}{<1.70}$		rct/40−
	825	2	$\frac{35}{40}=\frac{7.81}{3.79}$	$\frac{8.00}{3.26}$	rct/40−
Hirao	814	2	$\frac{35}{40}=\frac{6.65}{5.77}$	$\frac{7.81}{6.64}$	rct/40+
	820	2	$\frac{35}{40}=\frac{75.9}{<1.70}$	$\frac{7.70}{<1.70}$	rct/40−

From the feces of the first case, type 1, from the second case, types 1 and 2 and from the third case, type 2 viruses were isolated. The results of the T marker test on these viruses are listed in Table 6. Type 1, Fukuoka strain showed rct

/40± characters. In the second case, the thermal characteristics of type 2, Hata strains isolated from the feces on different days varied from day to day, but the characteristics inclined to rct/40+ character when compared with other Sabin type 2 progeny strains (see Table 3).

Preliminary tests on their antigenic characters were made by the dynamic neutralization test according to McBride's method. The antigenic character of the type 1 Fukuoka strain was identified as heterologous to LSc 2 ab (Fig. 6) and homologous to Ogawa strain which had been isolated in our laboratory from a patient in Osaka about 1 month before the mass vaccination.

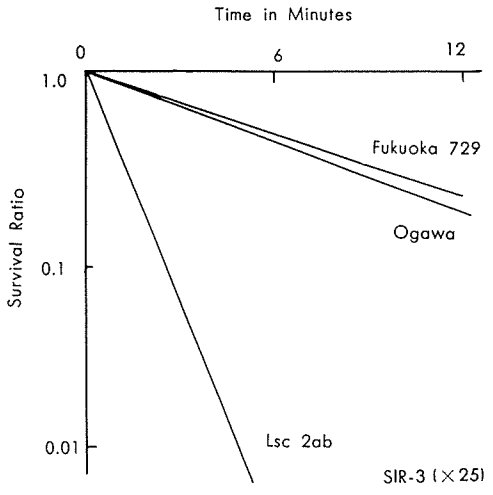

Fig. 6. Dynamic Neutralization with Anti-Lsc 2ab Rabbit Serum

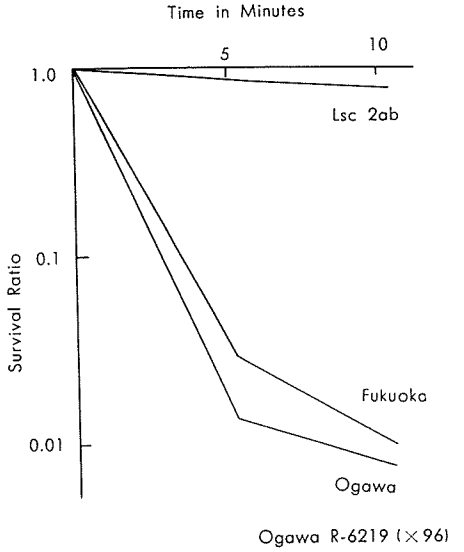

Fig. 7. Dynamic Neutralizationwith Anti-Ogawa Rabbit Serum

On the other hand, all Hata strains of type 2 possessed homologous characters to P 712 showing K values of 84-106 per cent of the K value of isologous virus with anti-P 712 serum. This character was identical to other vaccine progeny strains, and wild viruses isolated in the same year (Okita strain) showed quite different serological properties. Further identification was made on 6 clones picked out from plaques grown at 40°C. All thees clones again showed homologous properties to P 712.

One Hata strain of type 1 isolated from the same patient was rct/40- and homologous to L Sc 2ab.

Therefore, efforts were made to obtain other enteric viruses from the feces of the patient after neutralization of the polioviruses, but no virus has been isolated in MK cultures up to the present. Isolation using suckling mice has not yet been tested.

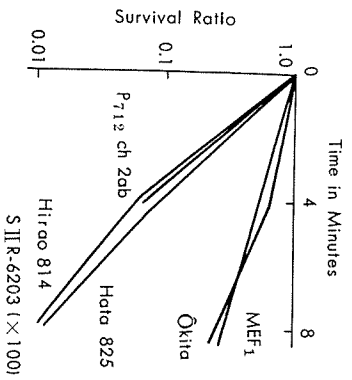

Fig. 8. Example of Dynamic Neutralization with Anti-P 712 ch 2ab Rabbit Surem

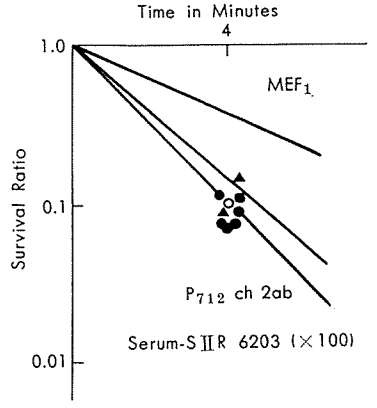

Fig. 9. Dynamic Neutralization of T+ Substrains manipulated from Starins of Patients with anti-P 712 ch 2ab Rabbit Serum

○ : P 712 ch 2ab
▲ : T+substrains obtained from Hirao 814
● : T+substrains obtained from Hata 811, 817 and 819

DISCUSSION

To detect genetical changes, the T marker test was employed since it gave the best correlation with neurovirulence in monkeys, as reported by many workers (Benyesh-Melnick and Melnick, 1959; Paul *et al.*, 1959; Melnick and Benyesh-Melnick, 1960; Cabasso *et al.*, 1960; Baron *et al.*, 1960). However, there have been relatively few reports on the application of the T marker test, and different conditions of temperature were employed by a various workers (Sabin, 1959; Benyesh-Melnick and Melnick, 1959; Paul *et al.*, 1959; Yoshioka *et al.*, 1959; Melnick and Benyesh-Melnick, 1960; Cabasso *et al.*, 1960; Baron *et al.*, 1960; Nakagawa *et al.*, 1961; Report of Japan Live Poliovaccine Research Commission; 1962). In the present work the thermal characters of laboratory strains were first examined using different temperatures for their growth. The Mahoney strain, which was considered to be fully virulent, showed the T+ character even at 40.3°C and the L Sc 2ab strain was T− at 39°C. Brunenders strain, which was a partly attenuated substrain of the Brunhilde strain, showed an intermediate character. With regard to type 2 strains, MEF 1 was rct/40+, but it produced smaller plaques and gave a lower titer at 40°C than at 35°C. Substrains obtained from plaques at 40°C produced larger plaques at 40°C and had a definite T+ character. This substrain was replaqued at 35°C and stable T+ clones were obtained. On the other hand, plaquing of original strain at 35°C resulted in the isolation of a rct/40± substrain. These results may be explanined by supposing that the original MEF 1 strain contained a mixed population, consisting largely of T± substrains with some T+ substrains. Plaquing at 35°C Picked up a substrain from the majority

of the population having T± character though plaquing at 40°C selected a T+ mutant from the minority of the population which had T+ character.

Similar findings were observed with the type 3 Saukett strain, but in this case, rct/40+ substrains were isolated from T+ plaques at 39.5°C, because this strain produced only small and ill-defined plaques at 40°C. Substrains obtained from plaques at 35°C showed various T characters varying from T− to T+. Although Leon 12 ab strain was largely rct/40− virus, it produced clear plaques at 39.5°C, and rct 40+ mutants were easily obtained from plaques grown at this temperature. These T+ mutants, however, produced very small plaques at 40.3°C and the number of plaques formed were difficult to determine. Plaque-purified substrains which did not produce any plaques at 40.0°C, included a T+ mutant after a second passage at 38°C. This suggests the genetical instability of this virus with regard to thermal characteristics.

The T characters of the wild strains were tested. Since these strains had various passage histories some strains isolated in our laboratory were tested at various passage levels including a first passage in MK cultures, but no remarkable change in the thermal characteristics was observed.

Type 1 wild strains were tested at 40°C and 5 out of the 16 strains isolated from patients showed rct/40- character. This is not peculiar as Sabin (1960) had already pointed out that T− strains could be isolated from patients and such strains might be very paralytogenic for monkeys. It is interesting that we found paralytic patients excreting T− viruses only in one year and in a single district. Another interesting finding is that only 3 patients excreated T+ viruses during this year in this same place and a healthy child who came into contact with one of these patients also excreted T+ virus. Further analysis is now in progress on these points.

Type 1 and type 2 vaccines were relatively stable with regard to their thermal characters. From these results and good correlation of T marker with neurovirulence, this marker seems to be a convenient tool for surveys of the progeny of vaccines of types 1 and 2. Type 3 virus was easily changed to T+ after a single passage in the human intestinal t act. This tendency has already been noted by Sabin (1959). Moreover, these T+ character seemed to be stronger than those of wild strains isolated from patients, and many T±～T−strains of type 3 viruses were isolated from patients and from healthy carriers. From these results and the low grade of correlation between this marker and neurovirulence in type 3 virus (Baron *et al.*, 1960), the role of the T marker test in the survey of the progeny of this vaccine is dubious. Some other type 3 strains, however, possessed a stable thermal character, *e. g.* the Suwa strain is T+and this character does not change during plaque purification while the Nakoshi strain is T−before and after plaquing and this character is stable on passage at 38°C. These strains seemed to be separable on the basis of their stable thermal characteristics from the Leon 12 ab strain in which the thermal characteristics are changeable. The Saukett strain, which

may belong to the latter group. Although these differences may be caused by differences in the passage level, the Mahoney strain of type 1 is stable in spite of its long passage history and the T—variant of this strain, L Sc 2ab, is also relatively stable.

Polioviruses were isolated from two patients hospitalized in Momoyama Hospital, Osaka, immediately after vaccination. Type 1 virus was isolated from a fatal case and this was serologically identified as wild type virus which was present in Osaka in the same year. The other case excreted mainly type 2 strains with various thermal characters. The day to day variation in these strains showed no regular tendency. This may be due to the random selection from a mixed population during isolation. The antigenic characters of these strains were tested by dynamic neutralization and they were found to be homologous with the P 712 strain. In this serological test these strains might have contained a small amount of wild virus. T^+ viruses were selected by plaquing to increase the possibilities of detection of wild virulent virus. Six substrains with T^+ character were tested by dynamaic neutralization, but again they showed similar characters to the Sabin type 2 vaccine strain's.

Therefore attempts were made to isolate non-polio-enteric viruses using MK cultures of the feces of this patient after neutralizing the polioviruses, but no such agent has been isolated yet. Isolation of Coxsackie A viruses using suckling mice has not been carried out yet. Although there may be other explanations, it seems likely that in this patients, type 2 virus of vaccine progeny may also have caused the illness, since type 2 vaccine can caused viremia (Report of Japan Live Poliovaccine Research Commission, 1962) and febril reaction (Mitsuda, to be published) in vaccinees. It is regretable that no sera could be obtained from the patient, though serological examinations were also necessary for studying the cause of the disease. The very early onset of the illness after administration of vaccine in this case, does not necessarily exclude the possibility that the paralysis may have been caused by vaccine progeny, since vaccination had been started several days before the administration of the vaccine to this patient.

The use of this vaccine is very hopeful, but its administration should be planned carefully, especially in virgin soil for oral vaccine.

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