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ANTIGENIC ANALYSIS OF TYPE 1 POLIOVIRUS II. CLASSIFICATION OF THE RECENT EPIDEMIC STRAINS IN JAPAN¹

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SUMMARY Type 1 poliovirus epidemic strains in Japan between 1956 and 1961 were analyzed antigenically by a kinetic neutralization technique with 6 or 7 anti-sera, and the pattern of their NK values were applied to analyze the epidemic of type 1 poliovirus.

Twenty of the 25 type 1 poliovirus strains tested were classified as " '56-type ", " '57-type ", " '58-type " and " '60-'61 type " by their patterns of NK values.

It was suggested that each epidemic was generally caused by antigenically homologous virus, although two different type 1 viruses were observed in a single epidemic in Aomori in 1959.

Five strains could not be classified into any of the 4 types.

INTRODUCTION

Antigenic variability among strains within a given poliovirus type has been noted by several workers. For classification of " wild " or " vaccine-derived " strains required after administration of oral vaccine, McBRIDE (1959) applied the kinetics of the serum neutralization method, WECKER (1959) applied the plaque neutralization technique and MELNICK and BENYESH-MELNICK (1960) introduced another serological technique. An attempt to classify wild strains of type 2 poliovirus antigenically was made by WENNER *et al.* (1956) by the end-

point neutralization technique. According to their results, 18 strains obtained from widely separated places and at different times could be divided into 3 groups. But the relation between the epidemics and the causal virus could not be clarified.

Epidemics of type 1 poliovirus between 1956 and 1961 were observed in 1956 (Ehime and Hokkaido), 1957 (Yamaguchi), 1958 (Kyushu), 1959 (Aomori), 1960 (Hokkaido, Aomori, Ehime, Yamaguchi and Kyushu) and 1961 (Yamaguchi and Kyushu) (KONO, 1960).

In our laboratory the intratypic serodifferentiation test by the kinetic neutralization technique (McBRIDE, 1959) has been applied to antigenic analysis of poliovirus and it was reported that the large epidemic in 1960-61 might be caused by single antigenic viruses (TOYOSHIMA *et al.*, 1964).

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Although the Kawasaki (Hokkaido, 1959) and Ogawa (Osaka, 1961) strains were considered to be antigenically homologous in the previous study, these strains showed different normalized K (NK) values with anti-sera to other strains.

Twenty-five type 1 poliovirus strains were selected for the test on the basis of their distribution in time and place of original isolation or of the results of a screening test of antigenic-

ity with anti-Kawasaki serum. The epidemics were analyzed by comparison of the patterns of the NK values of the strains with 6 or 7 antisera.

MATERIALS AND METHODS

1. *Poliovirus*

The sources of the 25 type 1 poliovirus strains used in this study are given in Table 1. Virus stocks

TABLE 1 Sources of Type 1 Polioviruses

Name	Strain		Supplied by
	Isolated in	Year	
	Place		
Yutani	Hokkaido	1956	M. Kanamitsu
Kita	"	"	R. Kono*, Y. Ashihara*, C. Hamada
			Kyoto University
Ishikura	Ehime	"	K. Kawakami
Tataki	"	"	R. Kono, Y. Ashihara, C. Hamada
			Osaka Medical College
Korosue	Osaka	1957	N. Nakano
Watanabe	Ehime	"	"
Yata	Yamaguchi	"	"
Tamaki	"	"	"
			National Institute of Health, Japan
Sakamoto	Kyushu	1958	"
Yukizaki	"	"	"
Tagami	"	"	R. Kono, Y. Ashihara, C. Hamada
Kawasaki	Hokkaido	1959	M. Kanamitsu
Shiraishi	Aomori	"	N. Nakano
Hakoishi	"	"	"
1150-1	Osaka	"	N. Kawakami
Matsushita	Kyushu	"	N. Nakano
Ishigaki	Hokkaido	1960	M. Kanamitsu
Yudono	"	"	"
A-12-60	Kyushu	"	N. Shingu
M-34-60	"	"	"
			Kurume University
Tanino	Osaka	1961	T. Hotta
Fukuoka	"	"	"
Ogawa	"	"	Osaka Public Health Institute
Y-12-61	Yamaguchi	"	M. Shingu
S-5-61	Kyushu	"	"

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were obtained by growth in cynomolgous monkey (*Macaca irus*) kidney tissue cultures maintained under medium 199, and were stored at -20°C . Virus was diluted with an equal volume of phosphate buffered saline (PBS), and stored at 4°C until required.

2. Anti-sera

Anti-Yutani (Hokkaido, 1956), anti-Ishikura (Ehime, 1956), anti-Shiraishi (Aomori, 1959), anti-1150-1 (Osaka, 1959), anti-Kawasaki (Hokkaido, 1959), anti-Ogawa (Osaka, 1961) and anti-Y-12-61 (Yamaguchi, 1961) sera were prepared by immunization of 2 to 4 rabbits according to the schedule described previously (TOYOSHIMA *et al.*, 1964).

Immune sera heated at 56°C for 30 minutes were tested by kinetic neutralization with isologous and heterologous strains and the sera possessing high specificity were selected for the test.

3. Tissue cultures

Primary cultures of cynomolgous monkey kidney were prepared by Bodian's method and grown in plaque bottles in a medium composed of 0.5 per cent lactalbumin hydrolysate in Earle's solution (LE) and 3 per cent bovine serum. Five to 6 days after seeding, the cells were washed with PBS and incubated in 7 ml of LE containing 0.2 per cent skimmed milk. These cultures were used on the following day.

4. Virus assay

Viruses were assayed by the plaque method with half log dilution. The plaque count was made 4 and 5 days after inoculation.

5. Neutralization test

The kinetic neutralization test was performed according to McBride's method as described previously (TOYOSHIMA *et al.*, 1964).

RESULTS

Antigenic Analysis by Kinetic Neutralization

1. Four strains isolated in 1956

The Yutani and Kita strains were isolated from small epidemic cases in Hokkaido in 1956, and the Ishikura and Tatakai strains were isolated from epidemic cases in Ehime in 1956.

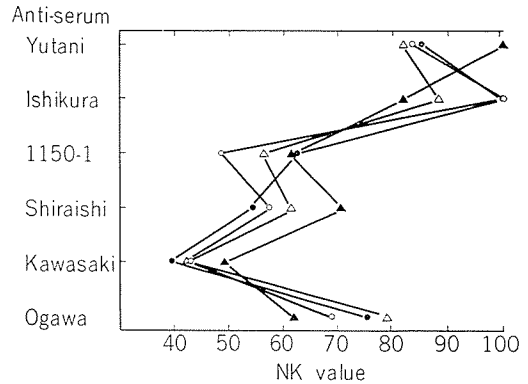


FIGURE 1 Patterns of NK Values with 6 Anti-sera of Yutani (\blacktriangle — \blacktriangle), Kita (\triangle — \triangle), Ishikura (\bullet — \bullet) and Tatakai (\circ — \circ) Strains Isolated in 1956.

These 4 strains were tested with 6 anti-sera, and showed similar antigenicities (Fig. 1). Although the NK value of the Yutani strain was slightly higher than those of the other 3 strains with anti-Yutani and anti-Ogawa sera (Table 2), the difference between the NK values was within the range of error.

2. Four strains isolated in 1957

The Korosue and Watanabe strains were isolated from sporadic cases in Osaka and Ehime, and the Tamaki and Yata strains were isolated from epidemic cases in Yamaguchi. These 4 strains isolated in 1957 were tested with 7 anti-sera and their NK values are given in Table 2.

The Yata and Tamaki strains showed quite similar NK values with 7 anti-sera.

The Watanabe strain showed similar NK values to the two strains of Yamaguchi with anti-Kawasaki, anti-Ogawa and anti-Y-12-61 sera (Fig. 2) but this strain was more readily neutralized with anti-Yutani and anti-Ishikura sera than the strains from Yamaguchi.

The Korosue strain showed a different pattern to the other strains, but the pattern of this strain was somewhat similar to those isolated in 1956.

TABLE 2 *Normalized K Values of 25 Type 1 Poliovirus Strains with 6 or 7 Anti-sera by Kinetic Neutralization Test*

Strain	Normalized K Value with Anti-						
	Yutani	Ishikura	1150-1	Shiraishi	Kawasaki	Ogawa	Y-12-61
Yutani	100	82	61	71	49	96	102
Kita	82	89	56	61	42	79	N.D.
Ishikura	85	100	48	57	42	69	104
Tataki	83	100	62	54	39	76	N.D.
Korosue	100	100	76	61	50	88	N.D.
Watanabe	100	100	91	100	57	87	N.D.
Yata	84	78	87	98	61	85	84
Tamaki	84	80	86	87	56	80	90
Sakamoto	68	105	91	79	34	72	89
Yukizaki	62	98	83	93	49	76	85
Tagami	67	102	86	86	52	79	95
Kawasaki	67	71	38	48	100	97	113
Shiraishi	82	71	77	100	52	79	87
Hakoishi	77	100	71	62	62	83	N.D.
1150-1	100	91	100	59	33	54	87
Matsushita	62	66	79	72	97	104	84
Ishigaki	68	80	81	77	95	89	93
Yudono	68	80	85	86	100	95	88
A-12-60	67	97	84	74	92	101	122
M-34-60	69	100	88	72	108	107	98
Tanino	72	81	83	83	90	98	94
Fukuoka	73	88	83	82	99	111	96
Ogawa	75	100	99	73	96	100	100
Y-12-61	73	79	82	86	102	98	100
S-5-61	74	79	82	89	85	105	90

N.D.: Not determined

3. *Three strains isolated in 1958*

The Sakamoto, Yukizaki and Tagami strains were isolated from epidemic cases in Kyushu in 1958. These strains showed quite similar patterns (Fig. 3).

4. *Five strains isolated in 1959*

The Kawasaki, 1150-1 and Matsushita strains were isolated from sporadic cases in

Hokkaido, Osaka and Kyushu, and the Shiraishi and Hakoishi strains were isolated from epidemic cases in Aomori in 1959. Some similarity was noted in two (Kawasaki and Matsushita) of the 5 strains and these patterns were rather like those of the viruses isolated in 1960 and 1961. With the three others no similarity could be observed in the antigenic patterns (Fig. 4).

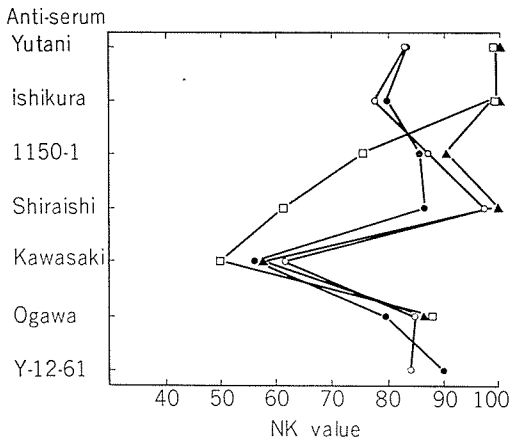


FIGURE 2 Patterns of NK Values with 7 Anti-sera of Korosue (□—□) Watanabe (▲—▲), Yata (○—○) and Tamaki (●—●) Strains Isolated in 1957.

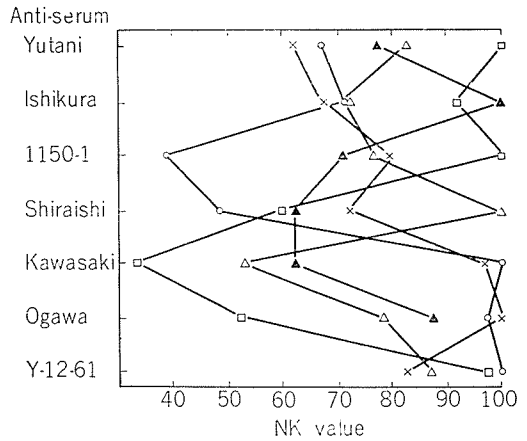


FIGURE 4 Patterns of NK Values with 7 Anti-sera of Kawasaki (○—○), Shiraishi (△—△), Hakoishi (▲—▲), 1150-1 (□—□) and Matsushita (×—×) Strains Isolated in 1959.

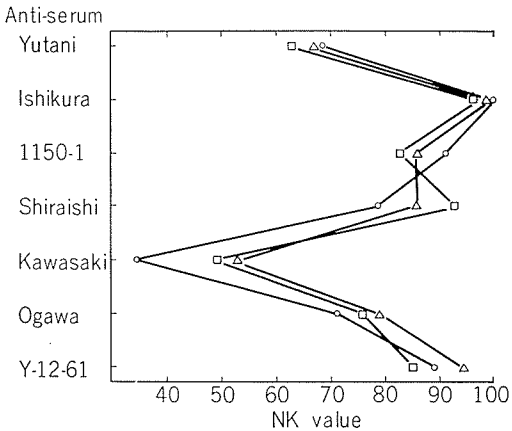


FIGURE 3 Patterns of NK Values with 7 Anti-sera of Sakamoto (○—○), Yukizaki (□—□) and Tagami (△—△) Strains Isolated in 1958.

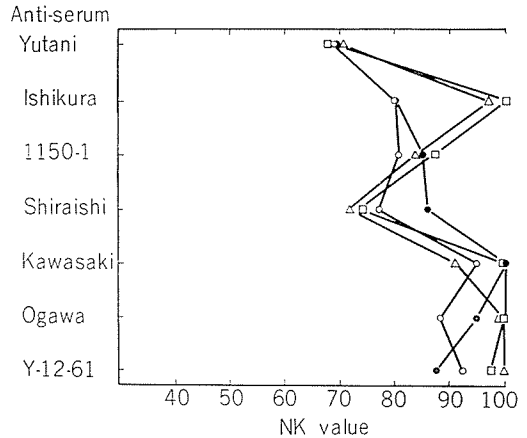


FIGURE 5 Patterns of NK Value with 7 Anti-sera of A-12-60 (△—△), M-34-60 (□—□), Ishigaki (○—○) and Yudono (●—●) Strains Isolated in 1960.

5. Four strains isolated in 1960

The Yudono and Ishigaki strains were isolated in Hokkaido and the A-12-60 and M-34-60 strains were isolated from epidemic cases in Kyushu in 1960.

Although these 4 strains showed somewhat different NK values with some anti-sera (Table 2), they showed similar patterns to each other (Fig. 5).

6. Five strains isolated in 1961

The Ogawa, Tanino and Fukuoka strains were isolated from sporadic cases in Osaka were selected as those showing high NK values with anti-Ogawa serum. The Y-12-61 and S-5-61 strains were isolated from epidemic cases in Yamaguchi and Kyushu in 1961. These strains were tested with 7 anti-sera, and were found to possess antigenically similar patterns (Fig. 6).

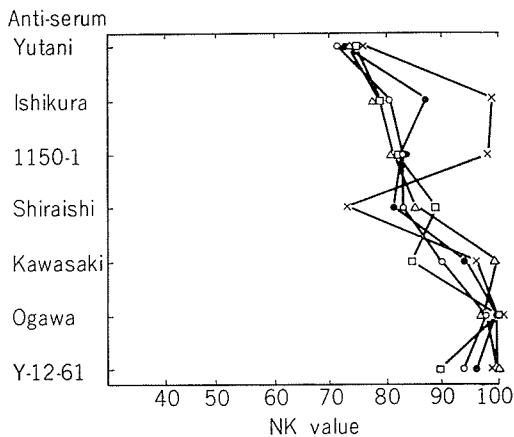


FIGURE 6 Patterns of NK Values with 7 Anti-sera of S-5-61 (□—□), Tanino (○—○), Fukuoka (●—●), Ogawa (x—x) and Y-12-61 (△—△) Strains Isolated in 1961.

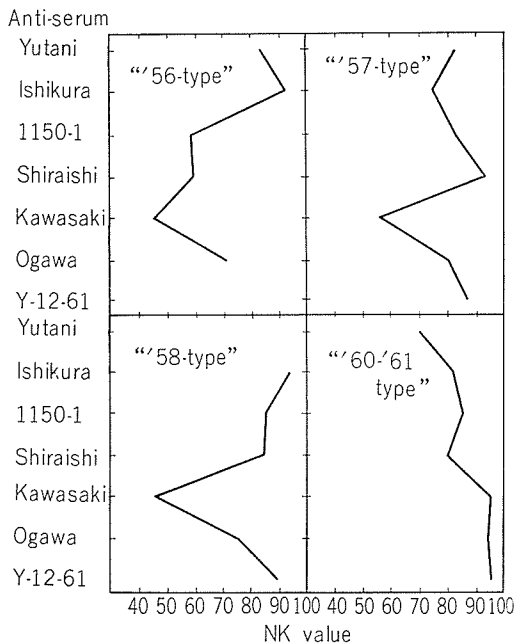


FIGURE 7 Representative Patterns of the Strains Isolated from 1956 through 1961.

Classification of the Epidemic Strains between 1956 and 1961

Most of the strains tested could be classified into 4 groups by their antigenic patterns (Fig.7).

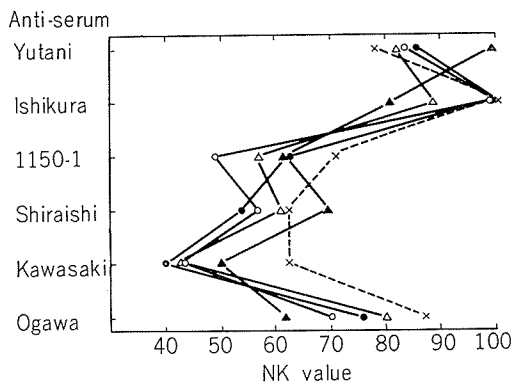


FIGURE 8 Patterns of NK Values with 6 Anti-sera of Yutani (▲—▲), Kita (△—△), Ishikura (●—●), Tataka (○—○) and Hakoishi (x—x) Strains Classified as "56-type".

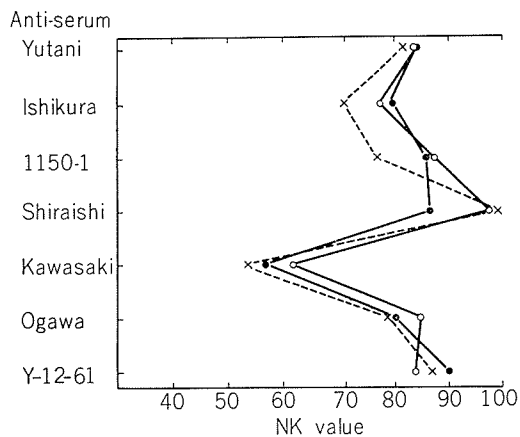


FIGURE 9 Patterns of NK Values with 7 Anti-sera of Yata (○—○), Tamaki (●—●) and Shiraishi (x—x) Strains Classified as "57-type".

1. "56-type"

All the strains in 1956 (Yutani, Kita, Ishikura and Tataka) showed similar antigenicity so that they were classified as "56-type".

The Hakoishi strain was isolated in a different district in a different year, but this strain showed a similar pattern to this antigenic type, and might be included in this type, though this strain showed slightly higher NK values than the strains in 1956 (Fig. 8).

2. "'57-type"

Two of the 4 strains isolated during the epidemic in Yamaguchi in 1957 (Yata and Tamaki) showed quite similar patterns to each other, and the antigenicities of these 2 strains were classified as "'57-type".

The NK values of the Watanabe strain (Ehime, 1957) with anti-Yutani and anti-Ishikura sera resembled those of "'56-type" strains but with other sera, the NK values were similar to the "'57-type" and this strain could be classified neither as a "'56-type" nor as a "'57-type".

The Shiraishi strain isolated in Aomori in 1959 may be classified as a "'57-type".

3. "'58-type"

All strains isolated in Kyushu in 1958 showed antigenically similar patterns and they were classified as "'58-type".

4. "'60-'61 type"

Not only the strains isolated from epidemic cases in Kyushu in 1960 (A-12-60 and M-34-60) but also those isolated from sporadic cases in Osaka (Ogawa, Tanino and Fukuoka) and from epidemic cases in Yamaguchi (Y-12-61) and in Kyushu (S-5-61) in 1961 showed antigenically similar patterns and these were classified as "'60-'61 type" (Fig. 10).

The high NK values of the Kawasaki (Hokkaido, 1959) and Matsushita (Kyushu, 1959) strains with anti-Kawasaki, anti-Ogawa and anti-Y-12-61 sera suggested the antigenic similarity of these strains to the "'60-'61 type", though these strains could not be included in this type, since their NK values with anti-Yutani and anti-Ishikura sera were quite different from those of strains of the "'60-'61 type".

The 1150-1 strain (Osaka, 1959) has not yet been classified.

DISCUSSION

Though WENNER *et al.* (1956, 1959) reported antigenic variation among polioviruses by the

neutralization technique, the relationship between polio-epidemics and antigenicity of the epidemic strains could not be analyzed sufficiently. On the other hand we reported the antigenic analysis of the wild strains of type 1 poliovirus during recent epidemics in Japan by the kinetic neutralization test (TOYOSHIMA *et al.*, 1964). Through the study, the Ogawa (OSAKA, 1961) and KAWASAKI (Hokkaido, 1959) strains were considered to have homologous antigenicities judging by the reciprocal kinetic neutralization test, they showed slightly different patterns of NK values in tests with 6 anti-sera, and were considered to be antigenically different strains.

Although the serum absorption test (FOGEL *et al.*, 1963; TOYOSHIMA *et al.*, 1964) may be useful to determine the antigenicity of a virus, the procedure of the test is troublesome. Hence, the epidemics were analyzed by classification of the patterns of their NK values with 6 or 7 anti-sera.

The strains used for the test were selected on the basis of their distribution in time and place of original isolation, though 25 strains may be too few to form a basis for analysis of all the epidemics. Each epidemic of type 1 poliovirus between 1956 and 1961 in Japan was mainly caused by a single antigenic type as follows; the epidemics in Hokkaido and Ehime in 1956 were caused by the "'56-type", the epidemic in Yamaguchi in 1957 was caused by the "'57-type", the epidemic in Kyushu in 1958 was caused by the "'58-type" and the large epidemic which occurred during 1960 to 1961 was caused by the "'60-'61 type". Most of the other strains isolated in these epidemic years showed similar antigenicity to the strains isolated in the epidemics (Fig. 11). The "'56-type" strains isolated in different place in 1957 and 1959 or from sporadic cases suggest that the vertical relationship between a polio-epidemic and the antigenicity of the strain may be indisputable.

KANAMITSU *et al.* (1965) reported that two antigenically distinct types were isolated in different cities in Hokkaido during the epidemic

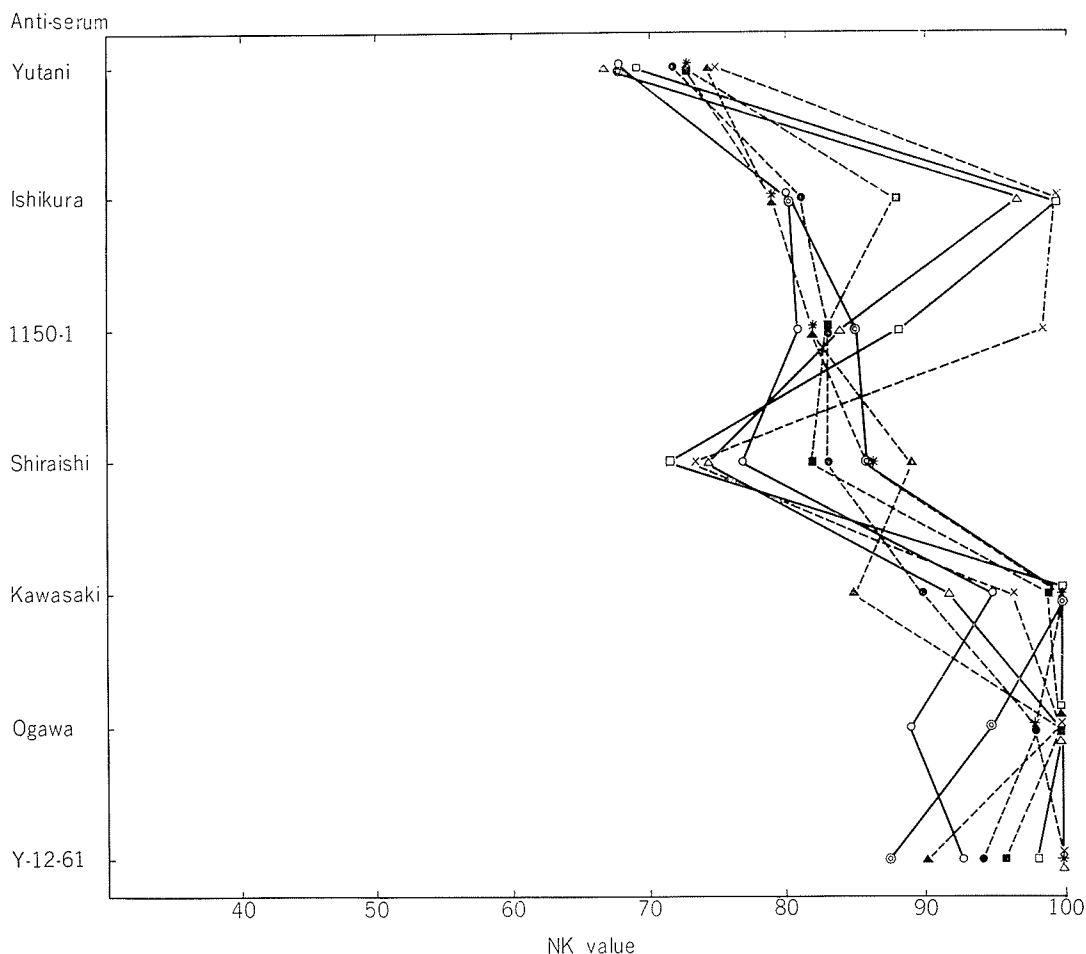


FIGURE 10 Patterns of NK Values with 7 Anti-sera of Ishigaki (○—○), Yudono (◎—◎), A-12-60 (△—△), M-34-60 (□—□), Tanino (●—●), Fukuoka (■—■), Ogawa (×—×), Y-12-61 (*—*) and S-5-61 (▲—▲) Strains Classified as “'60-'61 type”.

in 1960 and one of them had similar antigenicity to the strains isolated in 1959. On the other hand we observed single antigenic strains during the same years, though the test was performed with selected strains. All our strains from Hokkaido in these years were kindly provided by Dr. KANAMITSU, and the antigenicity of the strains classified as “'60-'61 type” could not be accounted for irrespective of which group they were included in. One

of the two antigenic types of their report may belong to the “'60-'61 type”, and the other may be a virus prevalent to the place.

Until this analysis was done, it was considered that the epidemics occurring in Aomori in 1959 might have followed the large epidemic in 1960-61. The vertical relation between the epidemic in Hokkaido in 1956 and the epidemic in Aomori in 1959 was suggested by the fact that the epidemic occurring in Aomori

Strain Isolated in

Year	Hokkaido	Aomori	Osaka	Ehime	Yamaguchi	Kyushu
1956	A A			A A		
1957			A'	B'	B B	
1958						C C C
1959	D''	A B	X			D'
1960	D D					D D
1961			D D D		D	D

FIGURE 11 Classification of 25 Type 1 Polioviruses Isolated from 1956 through 1961

A: "'56-type" strain. A': Strain showed partially similar pattern to "'56-type." B: "'57-type" strain
 B': Strain showed partially similar pattern to "'57-type." C: "'58-type" strain. D: "'60-'61 type" strain.
 D', D'': Strain showed partially similar pattern to "'60-'61 type." X: Could not be classified in any type.
 □: Type 1 polio-epidemic.

was caused by at least 2 different antigenic types and one of these was classified as "'56-type" which appeared in Hokkaido in 1956. No relationship was found between our "'60-'61 type" strains in Hokkaido and 2 antigenic types from Aomori in 1959, but the other antigenic type from Hokkaido in this year reported by KANAMITSU *et al.* (1965) has not been examined.

Further analysis of the epidemics occurring in Hokkaido and Aomori are considered to be useful as a model for analyzing polio-epidemics.

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Antigenic analysis of the strains isolated from sporadic cases may be useful to clarify the interrelationships between epidemics.

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