J. Hyg., Camb. (1966), **64**, 53
Printed in Great Britain

Virological and epidemiological studies on an outbreak of aseptic meningitis caused by echovirus 4 in northern Japan in 1964*

By YORIO HINUMA, KATSUMI URUNO, MORIHIRO MORITA and NAKAO ISHIDA

Department of Bacteriology, Tohoku University School of Medicine, Sendai, Japan

and TOORU NAKAO†

Pediatric Clinic, Aomori Prefectural Central Hospital, Aomori, Japan

(*Received* 12 *October* 1965)

During the summer and autumn of 1964 a number of localized outbreaks of aseptic meningitis occurred in Japan. The main causative agent of most outbreaks was determined as echovirus 4 by virus isolation in regional laboratories. Among these outbreaks, that through northern Japan seemed to be on the largest scale.

The present communication describes virological and epidemiological studies on the epidemic of aseptic meningitis caused by echovirus 4 in northern Japan.

MATERIALS AND METHODS

Subjects and specimens

Patients with aseptic meningitis examined clinically in 1964 by pediatricians in the medical institutions of eight different regions in northern Japan were studied (see Fig. 1). For virus isolation, cerebrospinal fluids (CSF) and faeces were collected from the patients in the acute stage. Serum specimens were taken from the patients in acute and convalescent phases for serological studies. In addition, faecal specimens were obtained from healthy children in Aomori City every month in 1964 for the examination of seeding of enteroviruses. For sero-epidemiological studies, serum specimens were also collected from children and adults residing in Aomori City. Two groups of serum specimens were obtained from May 1963 to March 1964 and from January to April 1965. These two are referred to as the sera before and after the epidemic, respectively. All these specimens were stored at $-25°$ C. until use.

Virus isolation and identification

Primary cultures of cynomolgous monkey kidney cells were used throughout for virus isolation and identification. Procedures were described in a previous paper (Hinuma, Murai & Nakao, 1965). In this study, however, the medium of the cell cultures inoculated with specimens was replaced by fresh medium every 3 or 4 days and the cultures were inspected for 2 weeks.

* Aided in part by a grant from the Ministry of Welfare and Health of Japan.

† Present Address: Department of Pediatrics, Sapporo Medical College, Sapporo, Japan.

https://doi.org/10.1017/S002217240004033X Published online by Cambridge University Press

Neutralizing antibody test

Acute and convalescent serum specimens of one patient were always tested the same day. Sera were inactivated at 50° C. for 30 min. before the test. Fourf serial dilutions of serum were made and tested against approximately 100 TC of test virus. Serum dilution–virus mixtures were allowed to stand at room te perature for 60 min. and 0·2 ml. of these mixtures was inoculated into stationa cell culture tubes. Antibody titres were expressed as the reciprocals of the high

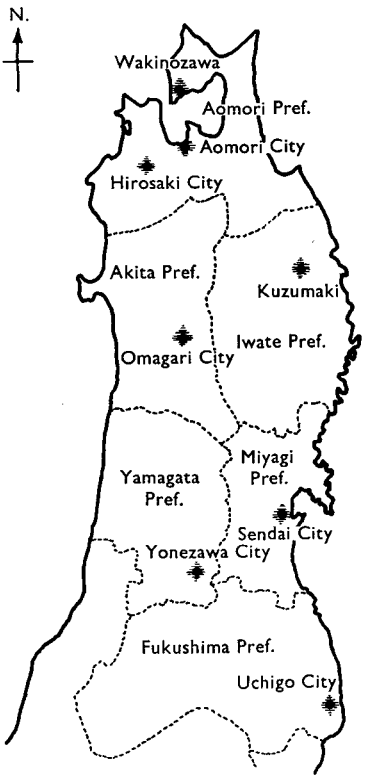

Fig. 1. Regions in northern Japan where outbreaks of aseptic meningitis occurred.

serum dilutions that protected the cells completely after incubation at 36–37° for 5–7 days. For the purpose of serodiagnosis, the DuToit strain (Barron Karzon, 1961) of echovirus 4 was used, unless otherwise stated. For the antibo titration, primary cell cultures of cynomolgous monkey kidney were mainly use but a line of continuous culture of African green-monkey kidney cells, VER established by Dr Y. Yasumura, Department of Bacteriology, Chiba Universi School of Medicine, was also used in part. The culture of VERO cells was sensiti to the DuToit virus as will be described in the text.

https://doi.org/10.1017/S002217240004033X Published online by Cambridge University Press

RESULTS

Incidence of patients with aseptic meningitis

The monthly incidence of aseptic meningitis in six regions is shown in Table 1; reliable data from the other two regions were not available. The peak incidence in each region was observed either in July, August or September. In Aomori City, patients with aseptic meningitis associated with echovirus 4 were found from April to December, as will be shown later. In all the regions examined, the peak inci-

Table 1. *Monthly incidence of aseptic meningitis in* 6 *regions of northern Japan in* 1964

	No. of patients in the following regions:					
	Wakinozawa	Aomori City	Hirosaki City	Kuzumaki	Yonezawa City	Uchigo City
January	0	2	0	0	1	1
February	0	2	0	0	0	0
March	0	5	0	0	1	2
April	0	1	0	0	1	1
May	0	3	10	2	9	2
June	0	12	1	7	9	1
July	45	44	15	30	17	0
August	6	69	47	7	54	14
September	0	48	66	0	22	38
October	0	10	14	0	4	25
November	0	5	0	0	2	9
December	6	7	0	0	2	1
Total	51	208	153	46	122	94

Table 2. *Age and sex distribution of patients with aseptic meningitis in Aomori City and Hirosaki City*

	No. of patients					
	Aomori City			Hirosaki City		
Age (years)	Male	Female	Total	Male	Female	Total
< 1	12	6	18	12	7	19
1	13	6	19	6	7	13
2	14	10	24	9	5	14
3	14	11	25	16	9	25
4	9	13	22	7	4	11
5	13	4	17	13	2	15
6	13	7	20	8	9	17
7	9	4	13	11	5	16
8	11	3	14	8	1	9
9	7	5	12	4	0	4
10	6	5	11	6	2	8
11	4	2	6	0	0	0
12	1	0	1	0	0	0
13	3	1	4	1	0	1
14	2	0	2	1	0	1
Total	131	77	208	102	51	153

https://doi.org/10.1017/S002217240004033X Published online by Cambridge University Press

dence coincided with the peak of echovirus 4 isolations. The age and sex distribution in Aomori City and Hirosaki City is shown in Table 2. In these two regions about 95 % of cases were infants or children under 10 years; this suggests a poor immunity against echovirus 4 in this age group. The incidence in males was about twice that in females; this seemed to be a characteristic feature of the epidemic due to echovirus 4, in comparison with that in the epidemic of coxsackievirus B 5 in Aomori City in 1961 (Nakao *et al.* 1964).

Outline of clinical features

Outstanding symptoms, signs and clinical laboratory findings of the patients in the epidemic were similar to those in many other enterovirus infections.

During the epidemic, one patient with paralysis was observed; this was a 14-year-old boy in Hirosaki City who suffered from flaccid paralysis of upper and lower left extremities on 8 August. Echovirus 4 was recovered from both CSF and faeces obtained 8 days after the onset. The neutralizing antibody titre against the DuToit strain was 16 on both the 8th and the 41st day of disease. No significant rise of antibody titres against three types of poliovirus was found in the paired sera. The results suggest that the paralysis might be associated with echovirus 4 infection.

Among about 800 cases of aseptic meningitis clinically examined in eight regions of northern Japan, nine cases were accompanied by a rash similar to that of rubella. From the faeces of three of them, echovirus 4 was isolated. In the same summer, three strains of echovirus 4, 1 of echovirus 18, 1 of echovirus 25 and 1 of adenovirus were recovered from the faeces of 6 out of 21 patients with rash but without aseptic meningitis. The results may suggest possible aetiological association of echovirus 4 with the rash, although not conclusive.

Virus isolation from the patients

As shown in Table 3, virus was isolated from the CSF of 85 out of 328 patients examined, and 79 (93 %) of the strains were echovirus 4. Viruses were also isolated from the faeces of 152 out of 303 cases. One hundred and thirty-one (86 %) of the strains were identified as echovirus 4. Thus a very large proportion of the strains were found to be echovirus 4, strongly suggesting that this was the main causative agent of the outbreaks in all eight regions.

The monthly incidence of aseptic meningitis patients from whom viruses were recovered either from CSF or faeces or both in Aomori City is shown in Table 4. It is evident that aseptic meningitis associated with echovirus 4 was found from April to December with a high incidence in July, August and September. The incidence of aseptic meningitis cases associated with other enteroviruses was also concentrated in summer, but the total number of such cases was small.

Properties of echovirus 4 isolated in the epidemic

Strains of echovirus 4 isolated in the epidemic examined so far and the Pesascek strain showed cytopathic effect on the primary culture of cynomolgous monkey kidney cells but not on VERO cells, whereas the DuToit strain was cytopathogenic

https://doi.org/10.1017/S002217240004033X Published online by Cambridge University Press

to both cell cultures. However, all of them, including the Pesascek and DuToit strains, were alike in that they did not show any cytopathic effects on HeLa and FL cells, and were not pathogenic for unweaned mice by intraperitoneal and intracerebral inoculations. The large round plaques of the current strains formed on monkey kidney cells were very similar to those of the Pesascek strain but not to the minute plaques of the DuToit strain, as previously reported by Barron & Karzon (1961).

Table 3. *Virus isolation from the patients with aseptic meningitis*

	CSF							
		No. of virus isolated						
		Coxs		Echo				
Region	No. of specimens tested	A 9	B 3	4	6	25	U*	Total
Wakinozawa	40	—	—	7	—	—	—	7
Aomori City	176	—	1	46	1	1	1	50
Hirosaki City	17	—	—	5	—	—	—	5
Kuzumaki	19	—	—	8	—	—	—	8
Omagari City	0	—	—	—	—	—	—	0
Sendai City	13	—	—	2	—	—	—	2
Yonezawa City	14	—	—	3	—	—	—	3
Uchigo City	49	2	—	8	—	—	—	10
Total	328	2	1	79	1	1	1	85

	Faeces										
		No. of virus isolated								No. of patients	
				Echo							
Region	No. of specimens tested	Polio 3	Coxs B 3	1	4	6	25	U*	Total	Tested	Virus-positive†
Wakinozawa	44	—	—	—	17	—	—	—	17	51	19
Aomori City	188	1	3	3	81	3	4	6	101	202	119
Hirosaki City	11	—	—	—	5	—	—	—	5	17	7
Kuzumaki	18	—	—	—	9	—	—	—	9	23	12
Omagari City	10	—	—	—	8	—	—	—	8	10	8
Sendai City	12	—	—	—	3	—	—	1	4	10	4
Yonezawa City	13	—	—	—	7	—	—	—	7	16	8
Uchigo City	7	—	—	—	1	—	—	—	1	50	12
Total	303	1	3	3	131	3	4	7	152	379	189

* U = unidentified.

† Positive isolation of virus in either CSF or faeces or both.

In order to examine the sensitivity of the current echovirus 4 to human antibody, titres of neutralizing antibodies in the paired sera of seven patients with aseptic meningitis were compared by using KZ-3-64 and W-45-64 strains, selected as representative of the current strains, together with the Pesascek and DuToit strains. The conventional tube-neutralization test with monkey kidney cells was performed. A marked breakthrough phenomenon (Barron & Karzon, 1961) was

https://doi.org/10.1017/S002217240004033X Published online by Cambridge University Press

noticed in tubes with KZ-3-64 and W-45-64 as well as those with Pesascek, but not in those with DuToit. Titres of neutralizing antibody are shown in Table 5.

Low titres were found in all the paired sera by using either of the two current strains or Pesascek, whereas high titres were detected by DuToit so that a significant rise of antibody titre between paired sera was easily demonstrated. The results indicated that the current strains were similar to the Pesascek but not to

Table 4. *Monthly incidence of virus-positive aseptic meningitis in Aomori City*

		No. of patients from whom the following viruses were isolated							
				Echo					
	No. of patients tested	Polio 3	Coxs B3	1	4	6	25	Unidentified	Total
January	2	—	—	—	—	—	—	—	0
February	2	—	—	—	—	—	—	—	0
March	5	—	—	—	—	—	—	—	0
April	1	—	—	—	1	—	—	—	1
May	3	—	—	—	1	—		—	1
June	12	1	2	—	5	—	—	—	8
July	44	—	1	—	21	2	3	2	29
August	67	—	—	3	34	1	1	—	39
September	46	—	—	—	30	—	—	3	33
October	10	—	—	—	6	—	—	—	6
November	5	—	—	—	1	—	—	—	1
December	5	—	—	—	1	—	—	—	1
Total	202	1	3	3	100	3	4	5	119

Table 5. *Titres of neutralizing antibody tested against 4 strains of echovirus 4 in paired sera of the patients*

		Titre of antibody against the following strains*			
Patient no.	D.D.†	KZ-3-65	W-45-65	Pesascek	DuToit
KZ-3-65	4	< 4	ND‡	< 4	< 4
	23	16		16	256
KZ-12-65	2	< 4	ND	< 4	4
	17	4		4	16
KZ-13-65	2	< 4	ND	< 4	4
	18	16		16	64
W-1-65	1	ND	< 4	ND	4
	17		4		64
W-11-65	1	ND	< 4	ND	4
	15		4		64
W-15-65	6	ND	< 4	ND	< 4
	20		4		64
W-17-65	3	ND	< 4	ND	< 4
	17		16		64

* About 100 TCD_{50}/0·1 ml. of each virus strain was used.
† Days after onset of illness.
‡ ND, Not done.

https://doi.org/10.1017/S002217240004033X Published online by Cambridge University Press

the DuToit strain in their sensitivity to human antibody. It was also shown that about 100 TCD_{50}/0·1 ml. of the strains of echovirus 4 isolated were neutralized by four units of monkey antiserum against Pesascek strain in the conventional tube method.

Neutralizing antibody against echovirus 4 *in the patients*

Table 6 shows the results of neutralizing antibody estimations against the DuToit strain in paired sera from sixty-one patients with aseptic meningitis. A fourfold or greater rise in antibody titre between paired sera, which was considered as significant, was demonstrated in thirty-two (86 %) of thirty-seven

Table 6. *Neutralizing antibody against echovirus* 4 *in the paired sera of patients with aseptic meningitis*

	No. of patients	Antibody present		Antibody absent
		Rise	No rise	
Patients with positive isolation of echovirus 4 from CSF				
Wakinozawa	4	4	0	0
Aomori City	1	1	0	0
Hirosaki City	5	3	2	0
Kuzumaki	5	4	1	0
Omagari City	0	0	0	0
Yonezawa City	2	1	1	0
Total	17	13	4	0
Patients with positive isolation of echovirus 4 from faeces only				
Wakinozawa	8*	8	0	0
Aomori City	2	2	0	0
Hirosaki City	0	0	0	0
Kuzumaki	1	1	0	0
Omagari City	8	7	1	0
Yonezawa City	1	1	0	0
Total	20	19	1	0
Patients from whom no virus was isolated				
Wakinozawa	6	6	0	0
Aomori City	2	1	1	0
Hirosaki City	0	0	0	0
Kuzumaki	3	2	0	1
Omagari City	2	1	1	0
Yonezawa City	11	7	4	0
Total	24	17	6	1

* In three patients from whom faeces were not available the virus was isolated from throat swabs.

patients from whom echovirus 4 was recovered either from CSF or faeces. The remaining five (14 %) of the virus-positive patients showed the presence of antibody without a rise in titre between the paired sera. Out of twenty-four patients with aseptic meningitis from whom virus isolation was unsuccessful, seventeen

https://doi.org/10.1017/S002217240004033X Published online by Cambridge University Press

(71 %) showed a significant rise in antibody titre, 25 % showed antibody without a significant rise in titre, and 4 % showed no detectable antibody titre.

Titres of neutralizing antibody plotted against the days after the onset of illness are shown in Fig. 2. Antibody titre of 4 or lower was found within 6 days after the onset, and a titre of 16 or higher occurred between 10 and 20 days after the onset.

Thus serodiagnostic results, in accordance with results of virus isolation, suggested that almost all the patients with aseptic meningitis were associated with echovirus 4 infection.

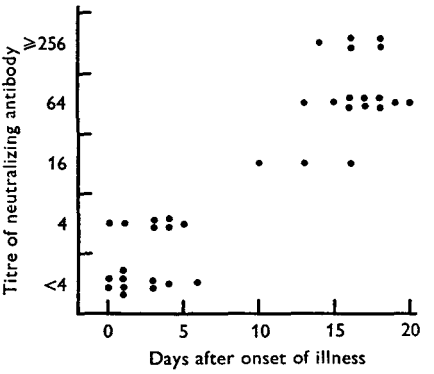

Fig. 2. Titres of neutralizing antibody against echovirus 4 in paired sera of the patients with aseptic meningitis in Wakinozawa.

Table 7. *Enteroviruses isolated from healthy children and infants in Aomori City in* 1964*

	No. of persons tested	No. of persons of positive isolation of the following virus types: Coxs A9	Coxs B3	Echo 1	Echo 4	Echo 14	Echo 23	Unidentified	Total
January	20	—	—	—	—	—	—	1	1
February	128	—	—	—	—	—	—	—	0
March	74	—	—	—	—	—	—	—	0
April	44	—	—	—	—	—	—	—	0
May	67	—	—	—	—	—	—	—	0
June	101	—	1	—	—	—	1	—	2
July	119	—	2	—	1	—	1	—	4
August	103	—	1	2	4	2	—	1	10
September	65	1	—	1	—	1	—	2	5
October	80	—	—	2	—	—	—	2	4
November	16	—	—	—	—	—	—	—	0
December	101	—	1	—	—	—	—	—	1
Total	918	1	5	5	5	3	2	6	27

* Between March and May, 5, 3 and 5 strains of poliovirus type 1, 2 and 3 respectively were isolated. They were considered to be vaccine virus, because Sabin's oral vaccine was given to a number of infants in the city during March and April. These polioviruses are therefore not included in this Table.

https://doi.org/10.1017/S002217240004033X Published online by Cambridge University Press

Enteroviruses isolated from healthy persons in Aomori City

During 1964 faecal specimens were collected every month from healthy children and infants under 6 years of age in Aomori City, and were tested for the presence of virus using monkey kidney cells. The results are shown in Table 7. Of 918 specimens tested, twenty-seven enteroviruses were isolated, most of them during the summer and fall. Among these viruses, five (19%) were echovirus 4, and the isolation rate of this virus was the same as that of coxsackievirus B3 or echovirus 1, viruses which showed very little association with aseptic meningitis in Aomori City in 1964 (see Table 3).

Table 8. *Incidence of neutralizing antibodies against echovirus* 4 *in Aomori City before and after the* 1964 *epidemic*

	Before epidemic			After epidemic		
		Positive antibody*			Positive antibody	
Age (years)	No. tested	No.	%	No. tested	No.	%
0–½	15	1	6·7	27	7	25·9
½–1	10	0	0	24	3	12·5
1	12	0	0	23	3	13·0
2	11	1	9·1	25	12	48·0
3	8	0	0	13	2	15·4
4	10	0	0	12	4	33·3
5–6	21	1	4·8	25	4	16·0
7–9	19	1	5·3	24	2	8·3
10–14	22	5	22·7	20	10	50·0
15–19	11	8	72·7	14	11	78·6
20–29	13	6	46·2	15	12	80·0
30–39	10	6	60·0	12	6	50·0
40–49	6	4	66·7	14	10	71·4
⩾ 50	8	5	62·5	16	12	75·0

* Antibody titre of 4 or higher was considered as positive and below 4 negative.

Sero-immunity against echovirus 4 *of residents in Aomori City*

Serum samples from children and adults in Aomori City, which were obtained before and after the epidemic of aseptic meningitis in 1964, were examined for antibodies against echovirus 4. The sera were from persons free from aseptic meningitis during the past year. The DuToit strain was used for the neutralization test. As seen in Table 8, almost all the children under 10 had no detectable antibody before the epidemic, whereas a much higher proportion of antibody-positive persons was found among children over 10 and adults. This suggested that echovirus 4 had once spread in this region about 10 years ago. After the 1964 epidemic, the proportion of sero-positive persons increased in almost all age groups, suggesting that many persons suffered from an inapparent infection with echovirus 4 during the epidemic. Figure 3 shows actual titres of neutralizing antibody in the sero-positive individuals before and after the epidemic. Before the

https://doi.org/10.1017/S002217240004033X Published online by Cambridge University Press

epidemic, antibody titres of three children under 10 were only 4 units and those above 10 were between 4 and 64 units. However, much higher titres were found even among children under 10, after the epidemic.

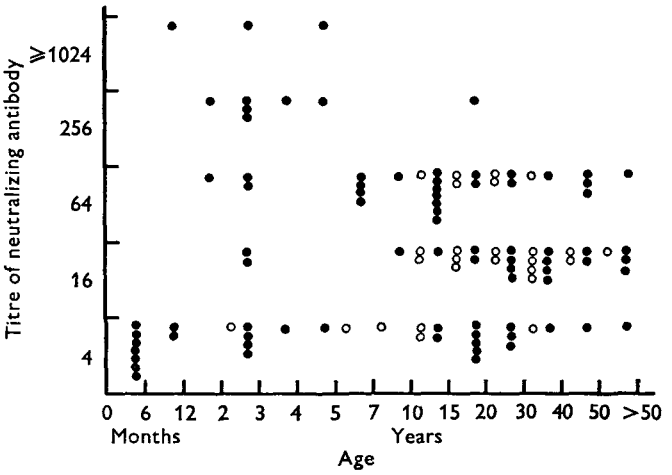

Fig. 3. Titres of neutralizing antibody against echovirus 4 in sera obtained from healthy persons in Aomori City before (○) and after (●) the 1964 epidemic.

DISCUSSION

The main causative virus of the 1964 epidemic of aseptic meningitis which occurred in many regions of Japan, including northern Japan reported here, was determined as echovirus 4 except in one outbreak of aseptic meningitis associated with coxsackievirus B4 in Shizuoka (personal communication from Dr R. Kono, Central Virus Diagnostic Laboratory, National Institute of Health of Japan). Isolation of echovirus 4 in Japan has been described in only one report (Hayakawa, 1964) that the virus was isolated from two sporadic cases of aseptic meningitis in Kyoto in 1963. In northern Japan echovirus 4 had never been isolated before 1964 (Hinuma *et al.* 1964; Hinuma *et al.* 1965). There have been no other reports of epidemics of echovirus 4 in Japan. However, studies of age-specific sero-immunity against echovirus 4 here described strongly suggested that the virus had spread in Aomori about 10 years ago, in 1954. This might be extended to the other regions in Japan, since the range of ages of the patients with echovirus 4 aseptic meningitis in many other regions in 1964 was similarly under 10 years. During 1954–64 there might have been no extensive seeding of the virus, as is suggested by the lack of immunity in the children and infants under 10 in 1964. The 1964 epidemic of echovirus 4 might not have covered most parts of Japan, because several outbreaks of aseptic meningitis due to echovirus 4 have been observed in the summer of 1965 in regions of southern Japan where outbreaks of echovirus 4 were not found in 1964 (unpublished observation). The scale of the epidemic of aseptic meningitis due to echovirus 4 in 1964 and 1965 appeared to be similar to or larger than that caused by coxsackievirus B5 in Japan in 1960 and 1961 (Kono *et al.* 1960; Hinuma *et al.* 1964).

https://doi.org/10.1017/S002217240004033X Published online by Cambridge University Press

Outbreaks of aseptic meningitis associated with echovirus 4 have been reported from many parts of the world, as listed in Table 9. Thus the accumulated reports emphasize that echovirus 4 is one of a few important viruses in the aetiology of large epidemics of aseptic meningitis.

Table 9. *Epidemics of aseptic meningitis associated with echovirus* 4

Year	Place	Publication
1955	U.S.A.	Levan *et al.* (1957)
1956	U.S.A.	Karzon *et al.* (1961)
1956	Sweden	Johnsson, Böttiger & Löfdahl (1958)
1956	Switzerland	Krech (1957)
1956	Australia	Forbes (1958)
1957	South Africa	Malherbe, Harwin & Smith (1957)
1958	Germany	Munk & Nasemann (1959)
1963	Scotland	Bell (1964)

Echovirus 4 strains isolated in the 1964 epidemic were characteristically insensitive to human antibody: this was similar to the Pesascek strain but unlike the DuToit strain. Tissue-culture spectrum and plaque morphology of the current strains were also similar to those of the Pesascek but different from the DuToit. However, a preliminary examination showed that there was some difference between the antigenic structure of the current strains and the Pesascek (unpublished data).

SUMMARY

During the summer and autumn of 1964, echovirus 4 was found widely distributed in northern Japan; this virus was associated with localized outbreaks of aseptic meningitis in many regions. The evidence that the echovirus was aetiologically related to aseptic meningitis was shown by the virus isolation from cerebrospinal fluids and faeces and the serodiagnosis of paired sera in many patients. Age-specific sero-immunity and morbidity rate suggested that echovirus 4 had spread extensively in about 1954. The strains of echovirus 4 isolated in the 1964 epidemic were similar to the prototype strain, Pesascek, in biological characteristics.

The authors are indebted to the following pediatricians for their co-operation in these studies: Drs N. Ohi, Y. Izumi, T. Oyamada, A. Konn, N. Kikuchi and N. Yano.

REFERENCES

Barron, A. L. & Karzon, D. T. (1961). Characteristics of ECHO 4 (Shropshire) virus isolated during epidemic of aseptic meningitis. *J. Immun.* **87**, 608–15.

Bell, E. J. (1964). An outbreak of meningitis due to ECHO virus type 4 in Scotland, 1963. *Lancet*, i, 1195–6.

Forbes, J. A. (1958). Meningitis in Melbourne due to ECHO virus. *Med. J. Aust.* i, 246–8

Hayakawa, Y. (1964). Epidemiological and serological studies on aseptic meningitis and encephalitis of viral etiology. *Shonika Kiyo*, **10**, 109–31. (In Japanese with English abstract.)

Hinuma, Y., Murai, Y., Fukuda, M., Numazaki, Y., Ishida, N. & Nakao, T. (1964). An outbreak of aseptic meningitis associated with Coxsackie B5 and A9 viruses in Northern Japan, 1961. Virological and serological studies. *J. Hyg., Camb.*, **62**, 159–70.

https://doi.org/10.1017/S002217240004033X Published online by Cambridge University Press

HINUMA, Y., MURAI, Y. & NAKAO, T. (1965). Two outbreaks of echovirus 14 infection: a possible interference with oral poliovirus vaccine and a probable association with aseptic meningitis. *J. Hyg., Camb.*, **63**, 277–84.

JOHNSSON, T., BÖTTIGER, M. & LÖFDAHL, A. (1958). An outbreak of aseptic meningitis with rubella-like rash probably caused by ECHO virus type 4. *Arch. ges. Virusforsch.* **8**, 306–17.

KARZON, D. T., ECKERT, G. L., BARRON, A. L., HYNER, N. S. & WINKELSTEIN, W. JR. (1961). Aseptic meningitis epidemic due to ECHO 4 virus. *Am. J. Dis. Child.* **101**, 610–22.

KONO, R., ASHIHARA, Y., HAMADA, C., KAWAKAMI, K., AKAGI, N., FURUKAWA, M. & MIYAO, M. (1960). Studies on the epidemiology of Coxsackie B 5 virus meningitis. *Rep. Inst. Virus Res. Kyoto*, **3**, 99–113.

KRECH, U. (1957). Über das Vorkommen von Echo-Virus in der Schweitz. *Schweiz. med. Wschr.* **87**, 558–60.

LEVAN, P. H., CHICK, E. W., KOTO, I. L., CHIN, T. D. Y., HERREN, R. H. & FURCOLOW, M. L. (1957). An epidemic illness associated with a recently recognized enteric virus (ECHO virus type 4). I. Epidemiologic and clinical features. *Am. J. Hyg.* **66**, 63–75.

MALHERBE, H., HARWIN, R. & SMITH, A. H. (1957). An outbreak of aseptic meningitis associated with ECHO virus type 4. *S. Afr. med. J.* **31**, 1261–4.

MUNK, K. & NASEMANN, T. (1959). Untersuchungen über die Ätiolgie der in Süddeutschland beobachteten Fälle des variablen infektiösen Exanthems; Isolierung eines ECHO-virus. *Klin. Wschr.* **37**, 371–4.

NAKAO, T., NITTA, T., MIURA, R., OGATA, K., KUME, T., NOBUTA, K. & HINUMA, Y. (1964). Clinical and epidemiological studies on an outbreak of aseptic meningitis caused by Coxsackie B 5 and A 9 viruses in Aomori in 1961. Tohoku. *J. exp. Med.* **83**, 94–102.

https://doi.org/10.1017/S002217240004033X Published online by Cambridge University Press