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Influenzaviruses isolated in South-East Asia during the period of August 1979~January 1981

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Summary

Influenzaviruses isolated from throat swab specimens collected in Thailand and the Philippines in August 1979~January 1981 were studied by the HI test. Eighty-five strains from Thailand comprised 12 AH 1, 69 AH 3 and four B viruses, whereas 14 strains from the Philippines were 12 AH 3 and two B viruses. The AH 1 viruses were isolated before May 1980, the AH 3 during the whole period of this study, and B in May~August 1980. Some AH 1 viruses isolated in Thailand showed antigenic similarity to A/USSR/92/77 which prevailed in Japan a few years before. Most of the AH 3 viruses isolated from Thailand resembled A/Tokyo/1/77, A/Bangkok/1/79 or A/Aichi/1/80, being not so different from Japanese isolates during the same period. However, a similar antigenic drift of AH 3 virus was found in A/Bangkok/138/80, isolated in August, and A/Kyoto/C-1/81, isolated in January. A possibility of the occurrence of the same antigenically drifted variants in Japan and South-East Asia is discussed.

Key words : influenza virus, antigenic drift, South-East Asia, Japan, infection

Introduction

Influenza occurs either in sporadic intermediate form or in periodic epidemics or in pandemic form. It affects people all over the world, because it is ubiquitous. In temperate zones, the epidemic tends to occur in winter, though the virus may survive between epidemics in the form of subclinical, or mild and sporadic cases. In order to understand how the virus survives during the interepidemic season of the temperate zone, a hypothesis has been presented by Fukumi^{1,2)} that the virus may survive through sporadic occurrences in the tropics, where the antibody levels in man are often lower than those in the temperate zone³⁾. In South-East Asian countries, a year is divided into three seasons, i.e., hot (March to May), rainy (June to October), and cool (November to February) seasons. In general, the number of influenza patients increases in the rainy season¹³⁾.

The sequential spread of the disease in the temperate epidemic zone and the tropical virus-reservoir zone on the occurrence of the disease alternately in these two zones will facilitate a circulation of the virus in Far East and South-East Asia. During the circulation, the virus evolves under selective pressure supplied by host immunity in different geographical areas. Within a viral subtype, the strains able to grow and survive are those which have undergone relevant mutations in the gene mainly coding for the hemagglutinin (HA). This is called the antigenic drift.

Two subtypes of influenza A virus, H3N2 and H1N1, and B virus have been cocirculating in the world since 1977, which had not been experienced before 1976. Antigenic drift in Hongkong virus (H3N2) was reported to occur in a high frequency compared to Asian virus (H2N2)¹⁴⁻¹⁶⁾, and the recent antigenic drift of H1N1 virus in Japan was studied with advanced biochemical techniques³¹⁾. History of the outbreak of the disease in Thailand was reviewed by Thongcharoen⁴⁾, and several virological and epidemiological studies in Thailand have been published³⁻¹³⁾. However, there are only few reports in which virus isolates in South-East Asia were immunologically compared with those in Japan.

Which area has the favourable conditions for the virus to undergo the antigenic drift? In order to have the actual evidence, we studied 99 virus strains, isolated in Thailand and the Philippines during the period of August 1979~January 1981, and compared them with viruses which prevailed in Japan. Our present study made use of the HI test with antisera raised in infected ferrets, because it is convenient for the study of many strains of the virus at the present stage of this research. Advanced biochemical and immunological techniques, such as peptide mapping, polyacrylamide gel electrophoresis of viral RNA, RNA hybridization, oligonucleotide mapping³¹⁾, determination of the HA gene nucleotide sequence³²⁾, and use of monoclonal antibodies will be suitable for analysis of limited number of strains. However, adequate number of monoclonal antibodies is not available at present.

Materials and Methods

Throat swabs collected from suspected patients were suspended in nutrient broth containing 800 u of penicillin and 400 μ g of streptomycin sulfate per ml and stored at

—70°C, before 0.2 ml of the suspension was inoculated into a 9~11 days embryonated hen egg via amniotic route¹⁸⁾. Eight eggs were inoculated per specimen, and two or three blind passages were undertaken before a specimen was considered not to contain virus. Sixty virus strains were isolated in the laboratories of the Nippon Medical School and of the National Institute of Health, Tokyo from 555 specimens listed in **Table 1**, and 39 strains were isolated in the laboratories of the Siriraj Hospital, Mahidol University, and of the Virus Research Institute, Bangkok.

Viruses isolated were typed according to the HI test¹⁹⁾, using antisera from ferrets infected with A/USSR/92/72, A/Brazil/11/78, A/Fukushima/103/78 and A/Kumamoto/37/79 of H1N1; A/Kumamoto/22/76, A/Tokyo/1/77, A/Bangkok/1/79, A/Aichi/1/80, A/Bangkok/211/81 and A/Kyoto/C-1/81 of H3N2; and B/Kanagawa/3/76, B/Amakusa/9/79, B/Singapore/222/79 and B/Yokohama/1/80. Antisera were prepared by the cooperation of Hikari Factory, Takeda Pharmaceutical Co., Yamaguchi.

Results

Frequency of isolation of influenza viruses

Sources and types of viruses studied in this paper are summarized in **Table 1**. In August 1979, AH1 and AH3 viruses were isolated from 25.8% of specimens in Chiang Mai. In May 1980, AH1 virus was isolated from 5.4% of suspected patients in Chiang Mai; and both AH1 and AH3 virus strains were isolated from 9.5% of patients in Sakaeo and 9.7% in Bangkok, and 3 out of 8 strains from Bangkok were from the members of a same family who came back from a trip to Hongkong and Manila a few days ago. On the other hand, AH3 and B viruses were isolated from 38.9% of suspected patients in Manila in May 1980. In August 1980, both AH3 and B viruses were isolated from 20.0% of specimens in Chiang Mai and 18.8% in Bangkok, but no virus was isolated from the specimens obtained in Manila. During the period of December 1980~January 1981, only one strain of AH3 virus was isolated from a patient in Bangkok among 159 cases in Thailand and the Philippines.

Besides these, 38 strains of AH3 virus were isolated during the period of August 1979~September 1980 and one strain of B virus was isolated during the period of June~August 1980 in the Siriraj Hospital and the Virus Research Institute, Bangkok.

In summary, in August and September 1979, six strains of AH1, four of AH3 and none of B were isolated from specimens of Thailand. During the period of May~September 1980, six strains of AH1, 76 of AH3 and six of B were isolated from specimens of Thailand and the Philippines. In January 1981, one strain of AH3 virus was isolated in Bangkok (**Table 2**). Viruses were isolated more frequently in months of May to September than in October to April. **Table 2** describes also the number of virus strains isolated in Japan and the USA during the same period. The scale of the survey is so different from our scale that it is not meaningful to compare simply the absolute number. It is nevertheless recognized that AH3 was prevalent in South-East Asia; AH1 in Japan; and B in the USA.

Table 1 Isolation of viruses in Thailand and the Philippines, August 1979~January 1981

Date	Throat swabs location ^{a)} number		Viruses			rate(%) ^{b)}
			AH 1	AH 3	B	
Aug. 20~23, '79	CM-IMC	9	1	0	0	25.8
	CM-STC	21	5	1	0	
	CM-GST	1	0	1	0	
Aug.~Sep. '79	BK-SIR	—	0	2	0	—
May 13~20, '80	CM-IMC	13	1	0	0	5.4
	CM-STC	4	1	0	0	
	CM-PED	20	0	0	0	
May 11~20, '80	Sakaeo	42	3	1	0	9.5
May 11~22, '80	BK-SIR	55	0	0	0	9.7
	BK-SOM	7	1	5	0	
	BK-PHC	—	0	2	0	
May 27~30, '80	ML-SLH	36	0	12	2	38.9
June~Aug. '80	BK-PHC	—	0	17	1	—
	BK-SIR	—	0	8	0	—
Aug. 18~22, '80	CM-IMC	36	0	7	2	20.0
	CM-STC	16	0	2	0	
	CM-PED	28	0	5	0	
Aug. 12~23, '80	BK-SIR	48	0	8	1	18.8
Aug. 27~Sep. 1, '80	ML-SLH	63	0	0	0	0
Aug.~Sep. '80	Bangkok	—	0	9	0	—
Dec. 29, 30, '80	CM-IMC	11	0	0	0	0
	CM-STC	5	0	0	0	
	CM-PED	10	0	0	0	
Dec. 27, '80	Raming Tea	29	0	0	0	0
Jan. 2, '81	BK-SIR	26	0	0	0	2.2
	BK-PHC	19	0	1	0	
Jan. 6~8, '81	ML-SLH	56	0	0	0	0
total			12	81	6	—

a; CM, Chiang Mai. IMC, internal medicine. STC, students clinic. PED, pediatrics. GST, graduate student. BK, Bangkok. SIR, Siriraj Hospital. SOM, Somboon's clinic. PHC, public health center. ML, Manila. SLH, San Lazaro Hospital. b; (number of virus isolates)/(number of throat swabs)

Table 2 Number of influenza virus isolates in South-East Asia, Japan and the USA

	South-East Asia ^{a)}			Japan ^{b)}			USA ^{b)}		
	AH 1	AH 3	B	AH 1	AH 3	B	AH 1	AH 3	B
Aug.~Sep. '79	6	4	0	2	0	0	0	0	25
Oct. '79~Apr. '81	0	0	0	1,412	639	255	24	0	1,334
May '80	6	20	2	6	1	24	0	0	0
June~Sep. '80	0	56	4	0	0	0	—	—	—
Dec. '80~Jan. '81	0	1	0	112	32	21	—	++	—

a; Data from our study, b; Modified from Takeuchi⁸⁹⁾

Table 3 Hemagglutination inhibition reaction of type A Influenza viruses isolated in Thailand in 1979

Antigens	Ferret antisera					Similar to
	A/USSR/92/ 77	A/Brazil/ 11/78	A/Fukushima/ 103/78	A/Kumamoto/ 22/76	A/Tokyo/ 1/77	
A/USSR/92/77	1,024	512	<32	<32	<32	
A/Brazil/11/78	128	1,024	<32	<32	<32	
A/Fukushima/103/78	64	128	1,024	<32	<32	
A/Kumamoto/22/76	<32	<32	<32	1,024	128	
A/Tokyo/1/77	<32	<32	<32	256	256	
A/Chiang Mai/11/79	1,024	256	<32	<32	<32	U
A/Chiang Mai/28/79	1,024	512	<32	<32	<32	U
A/Chiang Mai/32/79	1,024	512	<32	<32	<32	U
A/Chiang Mai/8/79	512	256	<32	<32	<32	U
A/Chiang Mai/20/79	512	512	<32	<32	<32	U
A/Chiang Mai/21/79	512	256	<32	<32	<32	U
A/Chiang Mai/10/79	<32	<32	<32	128	256	T
A/Chiang Mai/25/79	<32	<32	<32	256	256	T
Antigens	Ferret antisera					Similar to
	A/Kumamoto/ 22/76	A/Tokyo/ 1/77	A/Aichi/ 1/80	A/Bangkok/ 1/79	A/Bangkok/ 2/79	
A/Kumamoto/22/76	512	128	64	32	32	
A/Tokyo/1/77	128	512	256	64	64	
A/Aichi/1/80	64	1,024	1,024	256	128	
A/Bangkok/1/79	64	1,024	512	256	64	A
A/Bangkok/2/79	64	512	1,024	32	512	?

Influenza viruses isolated in 1979

Thirty-nine specimens were collected from patients of 18 to 52 years of age at Chiang Mai in August 1979, and eight influenza A virus strains were isolated from individuals of 19 to 25 years of age. Results of HI tests are described in **Table 3**. Six strains were similar to A/USSR/92/77 and the other two were similar to A/Tokyo/1/77¹⁹⁾ which is equivalent of A/Texas/1/77. None of the isolates was similar to either of A/Brazil/11/78, A/Fukushima/103/78 or A/Kumamoto/22/76. A/Kumamoto/22/76¹⁹⁾ is equivalent to A/Victoria/3/75 variant. A/Fukushima/103/78 is a variant of H1N1 which is similar to A/Omachi/1/53, and there have been some arguments as to the origin of the strain²⁰⁾.

Two strains isolated from an outbreak in August and September, A/Bangkok/1/79 and 2/79, were H3N2. One was similar to A/Aichi/1/80, and the other was different from any of the reference strains.

Influenza A viruses isolated in May 1980

Twenty-six strains of influenza A virus were isolated from 141 and 36 specimens from Thailand and Manila, respectively. Sakaeo is located near the Cambodian border of Western Part of Thailand, where about 120,000 refugees from Cambodia were admitted to camps, and throat swabs were collected from refugees and farmers in and around the camps.

Six strains of AH1 virus listed in **Table 4** were isolated from patients aged 1, 5, 7, 21 and 31 years. Two strains from Chiang Mai and one from Sakaeo were similar to A/Brazil/

Table 4 Hemagglutination inhibition reaction of type A influenza viruses (H1N1) isolated in Thailand in May 1980

Antigens	Ferret antisera			Similar to
	A/USSR/92/77	A/Brazil/11/78	A/Kumamoto/37/79	
A/USSR/92/77	256	128	64	
A/Brazil/11/78	128	256	256	
A/Kumamoto/37/79	32	64	256	
A/Chiang Mai/13/80	256	128	256	B
A/Chiang Mai/st-2/80	256	128	256	B
A/Sakaeo/17/80	256	128	128	U-B
A/Sakaeo/47/80	256	128	128	U-B
A/Sakaeo/22/80	256	128	256	B
A/Bangkok/S-60/80	512	ND	256	?

11/78, and the other two from Sakaeo showed the HI pattern between A/USSR/92/77 and A/Brazil/11/78. One strain from Siriraj, A/Bangkok/S-60/80 was different from either of three reference viruses. No strain was similar to A/Kumamoto/37/79²¹⁾ which is almost equivalent to A/California/10/78. No AH1 virus was isolated from the specimens of Manila.

Eighteen strains of AH3 virus were isolated from the specimens collected from patients of 0 to 32 years of age in Thailand and Manila. Results of HI tests of twenty strains of AH3 virus isolated during this period are described in **Table 5**. One isolate from the San

Table 5 Hemagglutination inhibition reaction of type A influenza viruses (H3N2) isolated in Thailand and the Philippines in May 1980

Antigens	Ferret antisera			Similar to
	A/Tokyo/1/77	A/Bangkok/1/79	A/Aichi/1/80	
A/Tokyo/1/77	512	64	256	
A/Bangkok/1/79	512	256	512	
A/Aichi/1/80	512	128	512	
A/Sakaeo/20/80	512	256	1,024	A-B
A/Bangkok/S-62/80	512	256	1,024	A-B
A/Bangkok/S-63/80	512	256	1,024	A-B
A/Bangkok/S-64/80	512	256	1,024	A-B
A/Bangkok/S-65/80	512	128	512	A(-B-T)
A/Bangkok/S-66/80	512	256	512	A-B
A/Bangkok/1/80	256	128	512	A-B-T
A/Bangkok/2/80	256	128	512	A-B-T
A/Manila/SL-6/80	512	64	256	T-A
A/Manila/SL-25/80	256	32	256	T
A/Manila/SL-29/80	256	64	256	T-A
A/Manila/SL-9/80	512	64	512	T-A
A/Manila/SL-19/80	512	64	512	T-A
A/Manila/SL-27/80	512	128	256	T-A-B
A/Manila/SL-35/80	512	128	256	A-B
A/Manila/SL-8/80	512	128	512	A(-B-T)
A/Manila/SL-13/80	512	128	512	A(-B-T)
A/Manila/SL-16/80	512	128	512	A(-B-T)
A/Manila/SL-18/80	512	128	512	A(-B-T)
A/Manila/SL-34/80	512	128	512	A(-B-T)

Lazaro Hospital, Manila was similar to A/Tokyo/1/77 and three isolates from Bangkok and Manila were intermediate between A/Tokyo/1/77 and A/Aichi/1/80 or A/Bangkok/1/79. Five strains from Bangkok and one from Manila were similar to A/Aichi/1/80 or A/Bangkok/1/79. A/Aichi/1/80 was isolated in Japan in January 1980²²⁾, which is similar to A/Bangkok/1/79²³⁾. A/Bangkok/1/79 virus did not give high HI titer to the homologous antiserum.

Thus, AH 1 and AH 3 viruses seemed to cocirculate in Thailand and AH 3 prevailed in Manila during this period. Three strains of A/Bangkok/S-60/80, S-65/80, and S-66/80 were from the members of a same family who had come back to Bangkok from a trip to Hongkong and the Philippines a few days ago. No influenza virus was isolated from the other 55 specimens from the Siriraj Hospital in May. Twelve strains of AH 3 and two of B virus were isolated from 14 out of 36 specimens from Manila.

Influenza A viruses isolated in Bangkok during the Period of June~August 1980

Twenty-six strains were isolated in Bangkok during this period. Of these, 25 were type A and the rest one was type B. Results of HI tests of the A viruses are shown in

Table 6 Hemagglutination inhibition reaction of type A influenza viruses (H3) isolated in Thailand from June to Aug. 1980

Antigens	Ferret antisera			Similar to
	A/Tokyo/ 1/77	A/Aichi/ 1/80	A/Bangkok/ 1/79	
A/Tokyo/1/77	1,024	128	64	
A/Aichi/1/80	1,024	512	128	
A/Bangkok/1/79	2,048	512	256	
A/Bangkok/3/80	512	128	32	T
A/Bangkok/4/80	512	256	32	T
A/Bangkok/5/80	512	128	32	T
A/Bangkok/6/80	512	256	32	T
A/Bangkok/7/80	512	256	32	T
A/Bangkok/8/80	512	128	32	T
A/Bangkok/15/80	512	128	64	T
A/Bangkok/19/80	512	128	32	T
A/Bangkok/21/80	512	128	64	T
A/Bangkok/25/80	512	128	64	T
A/Bangkok/27/80	1,024	128	32	T
A/Bangkok/9/80	512	256	64	T-A
A/Bangkok/10/80	512	256	64	T-A
A/Bangkok/29/80	1,024	256	64	T-A
A/Bangkok/23/80	512	256	128	T-A
A/Bangkok/11/80	1,024	256	128	A-T-B
A/Bangkok/16/80	1,024	256	128	A-T-B
A/Bangkok/28/80	1,024	512	128	A-T-B
A/Bangkok/12/80	1,024	512	256	A-B
A/Bangkok/13/80	1,024	512	256	A-B
A/Bangkok/14/80	1,024	512	256	A-B
A/Bangkok/22/80	1,024	512	256	A-B
A/Bangkok/26/80	1,024	512	256	A-B
A/Bangkok/17/80	256	128	64	?
A/Bangkok/20/80	256	128	32	?

Table 6. Eleven strains were similar to A/Tokyo/1/77, 5 were similar to A/Aichi/1/80 or A/Bangkok/1/79, and 7 were between these references. The other two strains were different from either of the reference viruses, but they might be related to A/Tokyo/1/77. AH 1 virus was not isolated.

Influenza A viruses isolated in Thailand in August 1980

Twenty two strains of AH3 virus were isolated from throat swabs collected from 128 suspected patients of 0 to 48 years of age in Thailand. Virus was not found in 63 specimens from Manila. Results of HI tests are shown in **Table 7**. Four strains from Bangkok were similar to A/Tokyo/1/77. One from Bangkok and 8 from Chiang Mai were between A/Tokyo/1/77 and A/Aichi/1/80 or A/Bangkok/1/79. One from Chiang Mai was similar to A/Aichi/1/80 or A/Bangkok/1/79. The other 6 strains were different from either of the three references. No AH 1 virus was isolated from specimens in August 1980.

The remaining 9 strains were isolated in the Siriraj and the Virus Research Institute, Bangkok in an outbreak from August to September 1980. All of them were AH 3. One strain of them, A/Bangkok/38/80 is described in **Table 8**.

Table 7 Hemagglutination inhibition reaction of type A influenza viruses (H3N2) isolated in Thailand in August 1980

Antigens	Ferret antisera			Similar to
	A/Tokyo/ 1/77	A/Aichi/ 1/80	A/Bangkok/ 1/79	
A/Tokyo/1/77	1,024	128	64	
A/Aichi/1/80	1,024	512	128	
A/Bangkok/1/79	2,048	512	256	
A/Bangkok/109/80	512	128	64	T
A/Bangkok/124/80	512	128	64	T
A/Bangkok/128/80	512	64	32	T
A/Bangkok/131/80	512	64	32	T
A/Bangkok/115/80	1,024	256	128	A-B-T
A/Bangkok/132/80	256	64	32	?
A/Bangkok/137/80	256	64	32	?
A/Bangkok/138/80	256	64	32	?
A/Chiang Mai/IMC-102/80	512	256	128	T-A
A/Chiang Mai/IMC-103/80	512	256	64	T-A
A/Chiang Mai/IMC-132/80	1,024	256	64	T-A
A/Chiang Mai/IMC-126/80	1,024	256	128	A-T-B
A/Chiang Mai/IMC-110/80	1,024	512	128	A(-B)
A/Chiang Mai/IMC-128/80	1,024	512	128	A(-B)
A/Chiang Mai/IMC-124/80	512	512	32	?
A/Chiang Mai/PDC-121/80	512	256	64	T-A
A/Chiang Mai/PDC-115/80	1,024	256	128	A-T-B
A/Chiang Mai/PDC-128/80	1,024	256	128	A-T-B
A/Chiang Mai/PDC-104/80	1,024	256	256	A-B
A/Chiang Mai/PDC-101/80	512	512	32	?
A/Chiang Mai/STC-112/80	1,024	256	128	A-T-B
A/Chiang Mai/STC-114/80	512	512	32	?

Table 8 Hemagglutination inhibition reaction of antigenically drifted type A influenza viruses (H3N2) after 1980

Antigens	Ferret antisera				
	A/Tokyo/ 1/77	A/Bangkok/ 1/79	A/Aichi/ 1/80	A/Bangkok/ 211/81	A/Kyoto/ C-1/81
A/Tokyo/1/77	2,048	512	512	512	2,048
A/Bangkok/1/79	4,096	2,048	2,048	1,024	4,096
A/Aichi/1/80	2,048	1,024	2,048	1,024	2,048
A/Bangkok/211/81	128	64	64	1,024	256
A/Kyoto/C-1/81	256	128	128	512	1,024
A/Bangkok/137/80	256	128	128	128	2,048
A/Bangkok/138/80	512	64	128	256	2,048
A/Chiang Mai/IMC-124/80	512	2,048	2,048	4,096	1,024
A/Chiang Mai/PDC-101/80	512	2,048	2,048	4,096	2,048
A/Chiang Mai/STC-114/80	512	2,048	2,048	4,096	1,024
A/Bangkok/20/80	512	2,048	2,048	4,096	2,048
A/Bangkok/38/80	1,024	512	512	1,024	2,048
A/Shanghai/31/80	1,024	512	512	512	2,048

Antigenically drifted influenza A viruses after 1980

In January 1981, two AH3 viruses were isolated, HI tests of which showed significant differences from either of A/Tokyo/1/77, A/Bangkok/1/79, or A/Aichi/1/80. One is A/Bangkok/211/81 which was isolated from one out of 19 specimens collected at a Public Health Center in Bangkok, and the other is A/Kyoto/C-1/81 which was isolated in Japan²⁴). Six isolates, which showed different HI patterns from the reference viruses in experiments described in **Tables 6** and **7**, were reexamined together with A/Bangkok/38/80, 211/81, A/Kyoto/C-1/81 and A/Shanghai/31/80, as shown in **Table 8**. A/Shanghai/31/80 is a new variant according to the information given to the NIH, Tokyo, by the CDC, USA.

A/Bangkok/138/80 isolated in August 1980 was antigenically related to A/Kyoto/C-1/81. A/Bangkok/38/80, isolated in September 1980, and A/Shanghai/31/80 were similar to A/

Table 9 Hemagglutination inhibition reaction of type B influenza viruses isolated in Philippines (May) and Thailand (August) in 1980

Antigens	Ferret antisera			Similar to
	B/Kanagawa/3/76	B/Amakusa/9/79	B/Yokohama/1/80	
B/Kanagawa/3/76	128	64	128	
B/Amakusa/9/79	64	256	128	
B/Yokohama/1/80	64	128	256	
B/Manila/SL-31/80	128	64	128	K-Y
B/Manila/SL-33/80	128	64	256	K-Y
Antigens	B/Kanagawa/3/76	B/Singapore/222/79	B/Yokohama/1/80	
B/Kanagawa/3/76	512	256	128	
B/Singapore/222/79	256	512	128	
B/Yokohama/1/80	256	256	256	
B/Bangkok/18/80	256	512	128	S(-K-Y)
B/Chiang Mai/IMC-114/80	128	256	128	S-Y
B/Chiang Mai/IMC-118/80	256	256	128	K-S-Y
B/Bangkok/S-4/80	128	256	128	S-Y

Tokyo/1/77. The other 5 strains from Chiang Mai and Bangkok, isolated before August 1980, differed from any reference strains.

Influenza B viruses isolated in months of May~August 1980

Two strains of influenza B virus were isolated from 2 out of 36 specimens collected in Manila in May 1980. They were similar to B/Kanagawa/3/76¹⁹⁾ and B/Yokohama/1/80²²⁾, as shown in **Table 9**. Type B virus was not isolated from Thailand in May 1980.

One strain of influenza B virus was isolated in Bangkok, during the period of June~August, and 3 strains were from 3 out of 128 specimens from Thailand in August 1980. They were similar to B/Singapore/222/79 as shown in **Table 9**. No influenzavirus was isolated in Manila during the period of August 27~September 1, 1980.

Discussion

In order to study the viruses prevailing in South-East Asia and to know the relation to Japanese isolates, we tested 99 influenza virus strains isolated from patients in Thailand and the Philippines during the period of August 1979 to January 1981 for the HI reactions with ferret antisera raised to a number of reference strains. This study will provide also a clue to select virus strains for elavolating the influenza vaccine. One actual example to support this idea is that A/Bangkok/1/79 and B/Singapore/222/79 strains were advised by WHO to be contained in the vaccine in 1980¹⁷⁾.

According to the analysis of the Thai Government Statistics^{13, 25)}, reported case number of influenza increased every year during the period of June to November, that is, the rainy season, except in the year 1978, in which the increase occurred in February due to the outbreak of the H1N1 influenza¹¹⁾. In each year, the smallest monthly case number is 10 to 29% of the largest monthly case number, in contrast to 0 to 0.004% in Japan during the period of 1975~1979²⁶⁾. This means that influenza tends to occur with less preference as to the season in Thailand than in the temperate zone where the epidemic occurs predominantly in winter season.

This seasonal difference of influenza outbreaks between Japan and Thailand will support the concept of the temperate epidemic zone and the tropical virus-reservoir zone proposed by Fukumi^{1, 2)}. Further evidence that supports his hypothesis can be cited from past records :

① Three or more different variants of type A virus were isolated in the Philippines without any predominance of a particular variant in 1975²⁷⁾. Presumably the antibody level among people in the area was not high enough to eliminate the variant which had prevailed before.
② New variant viruses were isolated in the Philippines and South China, prior to their isolation in Japan, in 1972 (B/England/874/73-like variant), in 1973 (A/Tokyo/1 F/73 and 2 F/73)²⁸⁾, and in 1975 (A/Victoria/3/75-like variant)²⁹⁾.

According to the report from WHO³⁰⁾, during the period of October 1978~September 1979, H1N1 virus continued to appear in all regions of the globe. Whereas the epidemic due to the H1N1 virus that occurred in 1977~1978 had mainly affected individuals aged from 13 to 20, the victims of the 1978~1979 outbreaks were mainly young children. Many

of the H1N1 viruses circulating during the period of October 1978~September 1979 belonged to the variant A/USSR/90/77. However, the large proportion of the H1N1 strains prevalent in some parts of the world, including Japan²²⁾, belonged to the new variant A/Brazil/11/78 in the season of 1978~1979; and between April and August 1979, it was mainly A/Brazil strains that were isolated in Southern Hemisphere. Six out of 8 influenza virus strains isolated in Chiang Mai in August 1979 were AH1 viruses similar to the A/USSR/92/77-like variant and they were isolated from individuals aged from 19 to 25. A similar situation occurred in the season of 1977~1978 in some parts of the world including Japan. This time lag between 1977~1978 and 1979 for the occurrence of the similar situation means that the A/USSR/92/77-like variant survived and circulated for a longer time in Chiang Mai than in Japan as well as in some parts of the world. Consequently, it could be concluded that Chiang Mai is a favourable candidate for one of the influenza virus reservoirs.

During the period of October 1978~September 1979, the H3N2 virus was isolated only in a few countries in the world, and it was not reported in Japan^{22,23)}. Two strains isolated in Chiang Mai in August 1979 were similar to A/Tokyo/1/77 or A/Texas/1/77-like virus. Two strains isolated in the outbreak of August~September 1979, A/Bangkok/1/79 and 2/79, exhibited significant antigenic drift from A/Tokyo/1/77 and the former strain was similar to A/Aichi/1/80²²⁾. According to antigenic analysis by WHO, A/Bangkok/1/79 was advised to be included in the vaccine¹⁷⁾. The results of our study show not only that AH3 virus survived in Thailand and not in Japan, but also that a new variant was isolated in Bangkok about five months prior to its isolation in Japan.

In May 1980, 28 strains of influenza virus were isolated from throat swabs collected in Thailand and Manila. HI test showed that six strains were AH1, 20 were AH3 and two were B virus. The AH1 viruses were isolated from individuals aged from 1 to 21 years in Thailand, and 3 out of 6 strains were similar to A/Brazil, two were between A/USSR and A/Brazil, and the other one was not similar to either of the two variants. In Japan, a new variant, A/Kumamoto/37/79 (H1N1) prevailed in the winter of 1979~1980, and 84% of the H1N1 isolates during the months of January~March 1980 belonged to the variant²²⁾. This means that particular variants can circulate for a longer period in Thailand than in Japan.

Among 20 AH3 isolates in Thailand in May 1980, 6 strains were similar to A/Aichi/1/80 or A/Bangkok/1/79, one was A/Tokyo/1/77, 13 were between the two reference viruses. In the winter of 1979~1980, most of Japanese isolates of H3N2 virus were similar to A/Tokyo/1/77²²⁾.

During the period of June to September 1980, 60 virus strains were isolated in Thailand, i.e., 56 AH3 and four B viruses. But AH1 virus was not discovered. HI tests of 43 strains of AH3 viruses showed that A/Tokyo/1/77-like viruses were 31.9%, A/Aichi/1/80 or A/Bangkok/1/79-like viruses were 17.0%, between them were 34.0%, and 17.0% were not similar to either of them. AH3 virus strains, which were not similar to either of A/Tokyo or A/Aichi virus, were not found in May 1980, but they occupied 8.0% of the

AH3 isolates during the months of June~August, and 27.3% in August 1980. These data suggest that AH1 virus disappeared or greatly reduced but AH3 and B viruses cocirculated in Thailand after June, and antigenic drift of AH3 virus advanced in season of 1980.

Six strains of B virus were isolated in Manila and in Thailand in 1980. In Japan, most of B virus isolated during the period of January through March 1980 were similar to B/Kanagawa/3/76, and the majority of the B viruses isolated in the winter of 1980~1981 were B/Singapore/222/79, B/Sendai/46/81 and B/Shiga/75/81²⁴⁾. B viruses isolated in Manila in May were similar to Japanese isolates in the winter of early 1980. B viruses isolated in Thailand from June to August showed antigenic similarity to B/Singapore/222/79 and B/Yokohama/1/80 which was isolated in Japan in October 1980.

During the period of October 1980~May 1981, 1,100 strains of influenza virus were isolated by the Japan Influenza Center Collaborating Prefectural Laboratories, 56% of which were obtained in February 1981. They comprised 718 strains (65.3%) of H1N1, 150 (13.6%) of H3N2 and 232 (21.1%) of B virus²⁴⁾. Among 83 strains of H1N1 tested for HI reactions, about 90% were similar to A/Brazil/11/78 and A/Kumamoto/37/79 variants, and the remaining strains were not similar to any reference strains including A/USSR/92/77, also²⁴⁾. These findings in Japan are different from our results obtained in South-East Asia in 1980 as far as AH1 virus concerns, because AH1 viruses isolated in South-East Asia were similar to A/USSR/92/77 or A/Brazil/11/78 before May, and AH1 virus was not isolated after June.

Results of HI tests of 40 out of 150 strains of H3N2 viruses isolated in Japan during the period of October 1980~May 1981 showed that about 90% were similar to A/Tokyo/1/77 and A/Bangkok/1/79 and the remaining strains were not similar to either of them²⁴⁾. These findings are not so different from our results in South-East Asia in 1980, except that AH3 composed type A viruses isolated in Thailand after June of the year.

The purpose of this kind of research is not only to know the past prevailing viruses, but also to find antigenically drifted viruses. Some drifted viruses disappear and do not become a major pathogen of an epidemic, but some will cause an epidemic in the next season. The important practical problem is to find out the drifted strains, which could be a major potential pathogen in the next season, as soon as possible. Study of the influenza virus isolates in Japan and South-East Asia will satisfy the practical need, because the disease occurs alternately in these two areas every year. Interesting are the following results of this report. A AH3 virus, A/Bangkok/138/80, which was isolated in August, was similar to A/Kyoto/C-1/81, isolated in Japan in February. The A/Kyoto/C-1/81-like virus has been isolated in Thailand in August 1981³³⁾. We isolated another drifted AH3 virus, A/Bangkok/211/81, in January. Several strains of A/Bangkok/211/81-like virus have been isolated in Japan in 1981³³⁾. These variants could be expected to prevail in Japan in the winter of 1982, since antibody levels to these strains were quite low among Japanese people²⁴⁾. These variants should be taken into consideration when the influenza vaccine is elaborated in 1982.

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