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New Antigenic Variant in Far East Influenza Epidemic, 1957. (23306)

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During the late spring of 1957, epidemic influenza characterized by clinically mild illness and by high attack rates occurred throughout the Far East, following a winter of relatively low influenza prevalence throughout most of the world. The viruses responsible for the Far East epidemic were shown(1) in this laboratory to be type A influenza of strikingly different antigenic composition from strains recovered in previous years. This paper summarizes the clinical and epidemiological reports from the Far East outbreak, presents the results of the detailed antigenic analyses of these viruses and the antibody response to them in patients, and gives information concerning the biological properties of these viruses and the antibody status of U.S. military personnel.

Materials and methods. Far East virus strains designated A-Japan-305-57 and A-Japan-307-57 were recovered in embryonated eggs from American military personnel by Drs. S. E. Grossberg and I. Gresser at U.S. Army 406th Medical General Laboratory, Zama, Japan, and were forwarded to this laboratory. The A-Malaya-309-57, 310-57, and 311-57 strains were recovered in Malaya by Dr. J. H. Hale of the University of Singapore. A-Hong Kong-304-57 virus was isolated in this laboratory from throat washings collected by Dr. C. Kraul of the 406th Medical General Laboratory from a patient in the Hong Kong outbreak. The A-Formosa-313-57 strain, recovered at the Taiwan Serum Vaccine Laboratory, was sent by the U.S. Naval Medical Research Unit #2 to the 406th Medical General Laboratory and forwarded to this institution. *Prototype strains.* Strains selected from previous epidemics for purpose of comparison are referred to as prototype strains. The prototype virus designated A-Japan-301-56 was recovered in Dec. 1956 in Japan and was furnished by Dr. M. Kitaoka, of the Nat. Inst. of Health, Tokyo, Japan. Miss Betty Ransom of the U.S. Army Hawaiian Med. Laboratory furnished the A-Hawaii-303-56 strain. The remaining prototype viruses used to prepare antigens or antisera in this laboratory have been described (2,3). *Hemagglutinating antigens* were allantoic fluids from embryonated eggs infected with influenza virus. The antigens prepared from the new Far East isolates were in egg passage 2 to 5 and titered 1:40 to 1:320 in tests with human "O" red blood cells. Drs. Thomas Francis, Jr., Keith Jensen, Bernice Eddy, or Robert M. Chanock furnished hemagglutinating antigens for the A-AA-1-57 (Mich.), A-Denver-2-57 (Colo.), A-AA-4-56 (Mich.), A-Ned-36-56 (Netherlands), A-Sendai-5-56 (Japan), B-GL-1739-54 (Ill.), A-Swine, and type D-Sendai (Japan)(4,5) viruses. The remaining antigens were standard reagents used by this laboratory and the strains employed in their preparation are described above or elsewhere(2,3). The methods used to prepare the antigens have been described(2,3,6). *Animal antisera.* Sera against the new Far East isolates were prepared in chickens by methods described earlier(2,3,6). Each chicken received 20 ml of undiluted infected allantoic fluid on 2 successive days and the animal was bled 9 or 10 days following the first injection. The ferret

antisera were obtained from Dr. Fred Davenport and the chicken A-Swine antiserum was furnished by Dr. B. Eddy. Drs. K. Jensen and R. M. Chanock supplied the B-GL-1739-54 and D-Sendai antisera. The Newcastle Disease Virus antiserum was obtained from Miss Audrey Somer of the U.S. Dept. of Agriculture. The remaining sera were standard reagents used in this laboratory and the strains of virus employed in their preparation are described above or earlier(2,3). *Complement-fixing antigens.* Embryonated eggs, incubated for 9 days, were inoculated into the allantoic cavity with 0.2 ml of infected allantoic fluid diluted $10^{-2.0}$. The chorioallantoic membranes, harvested after 36 hours' further incubation, were washed, triturated with 1 ml of physiological saline g weight of membrane, then frozen and thawed 3 times. The supernate obtained following centrifugation at 15,000 rpm in the angle centrifuge for 30 minutes was the antigen and this usually titered 1:64 or 1:128 when tested with 2 units of human influenza A or B convalescent serum antibody by the complement-fixation (CF) technic. *Paired sera from influenza patients.* Acute and convalescent serum specimens were collected by Dr. C. Kraul from both Oriental and Caucasian patients in Hong Kong during late April. The paired sera from U.S. military patients aboard a ship at Yokosuka, Japan, were drawn by Dr. S. E. Grossberg of the U.S. Army 406th Med. General Laboratory. The paired sera from cases of influenza in the outbreak in the Philippines were forwarded by the 406th Med. Gen. Laboratory. The remaining paired sera were from patients with influenza A or B in outbreaks which occurred in military personnel in Hawaii, Md., Kan., D.C., or Va. during the years 1955 (influenza B), 1956, or 1957 (influenza A). *Serological procedures.* The hemagglutination and hemagglutination-inhibition titrations were performed using human "O" cells according to the method of the Committee on Standard Serological Procedures in Influenza Diagnostic Studies(7). Treatment of sera with cholera filtrate to remove non-specific inhibitors was carried out according to Mulder *et al.*(8). The complement-fixation test procedure has been described(9). Primary incubation in the CF tests was carried out at 37°C for 75 minutes. All virus and serum titers are expressed in this report as the initial dilution prior to addition of the other reagents.

Background epidemiological information. Winter of 1956-57 prior to Far East epidemic. According to present information(1,10-12), the first occurrence of influenza for the 1956-57 respiratory disease season was during Nov. and Dec., 1956, in Japan. The attack rates were high in certain outbreaks, especially in children, necessitating the closing of schools. The disease occurred also in the U.S. Armed Forces in Japan. In U.S.A., the disease was first noted at Great Lakes Naval Training Center, Ill. during Dec., 1956 and by Apr. 1957, localized and scattered outbreaks had occurred throughout much of the nation. There was comparatively little influenza in France, England or Wales during the same period. However, epidemic influenza was widespread throughout Central Europe and the Scandinavian countries during March and April. Clinically, the illness in these outbreaks was mild and of 3 to 4 days duration. The attack rates were as high as 15% in certain "closed" populations such as schools or other institutions. These epidemics were caused, predominantly, by type A prime influenza strains which resembled closely the 1956 Dutch prototype strain A-Ned-36-56. Influenza B and C were also reported but the incidence was low. *Far East outbreak, Spring 1957*(1,10-12). The first official report of influenza in the Far East during the spring of 1957 described an epidemic in Hong Kong which began early in April and affected both Oriental and Caucasian populations. The epidemic was reported in Formosa by the end of April and in early May in the Philippines and Malaya. Late in May the epidemic spread to India, South Viet Nam and Japan. Epidemics occurred also aboard many U.S. Navy vessels which had contact with the disease in Far East ports during that period. Unconfirmed reports indicate that influenza epidemics may have been widespread in China prior to the Hong Kong epidemic. Prominent features in the epidemics have been the rapid spread of the disease and the

TABLE I. Hemagglutination-Inhibition and Complement-Fixation Titers of Paired Sera from Cases of Influenza in Far East and in Recent Outbreaks (1955-57) in Tests with Far East and Prototype Influenza Viruses.

Cases tested			Serum titers obtained with antigens										
			Complement-fixation ("soluble" antigen)				Hemagglutination-inhibition* (viral antigen)						
			Far East viruses		Prototype viruses		Far East viruses			Prototype viruses			
Outbreak	Pt.	Serum sample	A-Japan 305-57	A-Japan 307-57	A-Hawaii 303-56	B-Va, 301-55	A-Japan 307-57	A-Hong Kong 304-57	A-Malaya 309-57	A-Denver 2-57	A-Japan 301-56	A-FLW, 1-52	B-IB1 (1950)
Far East 1957													
Hong Kong	1	A	5	5	5	<5	<5	<5	<5	<5	5	20	10
		C	40	40	80	"	40	40	40	10	10	40	20
	2	A	<5	<5	<5	"	<5	<5	<5	<5	10	20	"
		C	40	40	80	"	40	20	20	5	"	"	"
	3	A	<5	<5	<5	"	<5	<5	<5	<5	"	"	"
		C	40	40	80	"	20	10	20	5	"	"	"
	4	A	<5	<5	5	5	<5	<5	<5	<5	<5	40	40
		C	80	80	80	5	20	10	10	"	5	"	"
Japan (ship)	5	A					<10			20	20	20	
		C					40			"	"	40	
	6	A					<10			5	"	80	
		C					20			10	"	"	
	7	A					<10			<5	5	20	
		C					"			20	20	160	
U. S. A.													
Influenza A (1956-57)	8	A	5		<5	<5	<5	5	<5	5	20	40	40
		C	80		80	"	10	20	10	20	320	320	20
	9	A	<5		<5	"	<5	<5	<5	<5	10	40	80
		C	320		320	5	10	"	10	80	160	640	160
	10	A	<5		<5	<5	<5	"	<5	<5	<5	20	320
		C	20		20	"	"	"	"	10	10	160	"
	11	A	<5		<5	"	"	"	"	<5	5	20	80
		C	20		40	"	"	"	"	10	10	320	"
	12	A					"	"	"	<5	5	20	160
		C					"	"	"	40	40	160	80
Influenza B (1955)	13	A	<5		<5	"	"	"	"			20	40
		C	"		"	160	5	"	5			"	320
	14	A	"		"	<5	<5	"	<5			"	80
		C	5		5	80	"	"	"			40	640

* All sera were treated with cholera filtrate to remove non-specific inhibitor.

high attack rates, varying from 10-30% in both oriental population and Armed Forces. The illness occurred in persons of all ages and was generally mild lasting 3 to 4 days frequently accompanied by fever up to 103°F. Deaths attributable to influenza have been infrequent except in the Philippines where up to 200 children are said to have died as a result of influenza and in India where 21 deaths have been reported to date. The viruses recovered from the recent Far East outbreaks are, as shown in this report, of A type and are strikingly different antigenically from those which were prevalent during the winter of 1956-57 or in preceding years.

Results. One outstanding property of influenza A virus is the change in antigenic constitution over succeeding years(2,3,13-19). Hence, in the detailed analyses reported here, prototype viruses were employed(2,3) which cover the antigenic spectrum of known influenza viruses and represent the various eras

TABLE II. Comparison of Far East Influenza A Isolates with Prototype Strains in Hemagglutination-Inhibition Tests with Chicken Antisera.

Chicken antisera against viruses: (type and strain)	Hemagglutination-inhibition titers obtained in tests with antigens: Far East viruses: A-Japan 305-57	A-Japan 307-57	A-Hong Kong 304-57	A-Malaya 309-57	A-Malaya 310-57	A-Malaya 311-57	Prototype viruses: A-AA, 1-57	A-Denver 2-57	A-Japan 301-56	A-Hawaii 303-56	A-AA, 4-56	A-FLW, 1-52	A-FW, 1-50	A-FM1 (1947)	A-PR8 (1934)	A-WS (1933)	A-Swine	B-Va 301-55	B-IB1 (1950)	Other prototype viruses (homologous titers)
Far East viruses																				
A-Japan-305-57																				
#9488	100	400	400	400	400	200	<25	<25	<25	<50	<25	<25	<25	<25	<25	<25	<25	<25	<25	
#B891	200	"	"	800	"	400														
A-Malaya-311-57	100	800	"	"	"	"	<50	<50	<50	"		<50	<50	<50	<50	<50	<50			
Prototype viruses																				
A-Japan-301-56 (Dec.)	<50		<50	<25	<25	<50			400											
-Hawaii-303-56	<25	<25	"	"	"	"				400										
-AA-4-56	"	"	"	"	"	"					400									
-FLW-1-52	"	"	"	"	"	"						800								
-FW-1-50	"	"	"	<50	"	"							800							
-FM1 (1947)	<50		"	<25	"	"								800						
-PR8 (1934)	"	<50	"	"	"	"									1600					
-WS (1933)	"		"	"	"	"										400				
-Swine	"	<50	"	"	"	"											3200			
B-Va-301-55	"	"	"		"													400		
-GL-1739-54	"	"																		200
-IB1 (1950)	<25	<25	"																400	
-Lee (1940)	<50	<50	"																	"
C-1233 (1947)	<25		"																	1600
D-Sendai	<50		"																	400
NDV	"		"																	1600
Mumps	"		"																	400

All sera were treated with cholera filtrate to remove non-specific inhibitor. Specific antibody titers in the range of 1:400 to 1:1600 were obtained when the untreated Far East sera were tested with Far East viruses.

of their prominence.

Serological tests with paired sera from cases. Table I presents the CF and hemagglutination-inhibition test results with paired sera from cases of influenza among Orientals and Caucasians in the Far East outbreak and from patients in previous outbreaks in the U.S.A. employing, as antigens, Far East viruses and previous prototype strains. The cases in the Far East were clearly established as influenza A by demonstration of significant (4-fold or greater) CF antibody titer increases in paired sera employing CF antigen prepared from prototype A-Hawaii-303-56 virus and by demonstration of significant increases in titer in patients with influenza A in previous outbreaks using, as antigen, Far East viruses. Thus, there was no demonstrable difference between the Far East and previous A viruses insofar as reaction with the "soluble" CF antigen components of the viruses were concerned. This was not the case with the hemagglutinating antigens which measure antibody to the virus itself. In the Far East cases, antibody response was almost exclusively against the Far East viruses and, conversely, the responses in cases from previous epidemics were nearly all against the previous prototypes. In 3 instances, Far East case 1 and USA cases 8 and 9, significant titer increases were noted against both kinds of virus; the rise against the homologous agent was the greatest in each instance. Far East case 7 was unique in that the patient's sera showed an 8-fold titer increase against prototype FLW-1-52 but nothing against Far East virus. It seems most likely, in this instance, that the patient was infected with a 1956 prototype during the epidemic although Far East virus infection cannot be excluded. Far East virus appeared responsible also for the epidemic in the Philippines. Five of 7 paired sera from cases in the Philippines, not included in the table, showed diagnostic titer rises when tested with Far East A-Japan-307-57 virus but not with prototype A-Japan-301-56 virus. There was no evidence of influenza B in the epidemic in the Far East.

Antigenic analysis of Far East viruses with chicken and ferret antisera. Table II summarizes the results of cross-hemagglutination-inhibition tests with chicken antisera between the Far East agents and prototype viruses listed. No demonstrable antigenic relationship was shown in tests between Far East viruses and influenza A prototypes from early 1957 through 1933 and including swine virus. The A-Formosa-313-57 virus, not included in the Table, gave results similar to the other Far East strains. Likewise, there was no antigenic relationship to influenza B, C, D or NDV or mumps viruses. The striking antigenic difference between Far East and 1956-57 influenza A strains was shown further in tests (Table III) with ferret antisera against 1956 and early 1957 influenza A strains recovered in the USA, Europe and Japan. The old 1956-57 prototypes were a remarkably homogeneous group except for minor variation exhibited by strain A-Sendai-5-56. None of these sera showed any detectable reaction with the new Far East agents.

TABLE III. Comparison of Far East Influenza A Isolates with Recent (1956-57) A Type Viruses in Tests with Ferret Antisera.

	Hemagglutination-inhibition titers obtained in tests with antigens												
	Far East viruses					Recent (1956-57) A type viruses							
Ferret antisera* against recent A viruses: (type and strain)	A-Japan 305-57	A-Japan 307-57	A-Hong Kong 304-57	A-Malaya 309-57	A-Malaya 310-57	A-Denver-2-57	A-AA-1-57	A-AA-4-56	A-Ned-36-56	A-Sendai-5-56	A-Japan 301-56	A-Hawaii 303-56	A-FLW-1-52
A-Denver-2-57	<20	<20	<20	<20	<20	640	2560	80	1280	<20	2560	1280	80
-AA-1-57	"	"	"	"	"	160	640	20	320	"	640	320	20
-AA-4-56	"	"	"	"	"	80	160	320	20	20	160	40	320
-Ned-36-56	"	"	"	"	"	160	640	40	320	<20	640	640	40
-Sendai-5-56†	"	"	"	"	"	20	80	80	<20	160	80		

* All ferret antisera were treated with cholera filtrate to remove non-specific inhibitor.
† Not to be confused with type D Sendai virus.

TABLE IV. Antibody Status, American Military Cadre Personnel for Far East Influenza Viruses and for Prototype Strains.

	Hemagglutination-inhibition titers						
	Far East viruses				Prototype strains		
Serum* titer	A-Japan 305-57	A-Japan 307-57	A-Malaya 309-57	A-Hong Kong 304-57	A-Japan 301-56	A-FLW 1-52	B-IB1 (1950)
<1:5	30	27	24	29	6	2	0
1:5	0	2	4	1	6	2	1
1:10	0	1	2	0	8	7	4
1:20	0	0	0	0	6	5	12
1:40	0	0	0	0	3	8	9
1:80	0	0	0	0	0	4	3
1:160	0	0	0	0	0	1	1
1:320	0	0	0	0	1	0	0
1:640	0	0	0	0	0	1	0

* All sera treated with cholera filtrate to remove non-specific inhibitor.

Antibody status of selected military personnel. Distribution of hemagglutination-inhibiting antibody against 4 Far East viruses, compared with 3 prototype strains, in tests of 30 sera from enlisted cadre personnel, is shown in Table IV. These persons, in keeping with regulations requiring influenza vaccination in the military, had been vaccinated prior to beginning of 1957. The high levels of antibody against the old prototype strains A-Japan-301-57, A-FLW-1-52 and B-IBl were as expected. However, there was a striking deficiency in antibody against the Far East isolates in these persons. None showed detectable antibody against A-Japan-305-57 virus and 6 showed inhibition against the other Far East strains in very low titers, *i.e.*, 1:5 or 1:10.

Biological characteristics of the Far East viruses. The Far East viruses were readily recovered from patients' throat washings by passage in embryonated eggs inoculated into the amniotic cavity. Following initial recovery, the viruses grew readily in the allantoic cavity giving hemagglutination titers of 1:80 to 1:320 when tested with human "O" or with chicken erythrocytes and incubated at room temperature or at 4°C. Early small volume commercial pools of allantoic fluid harvested from embryonated eggs infected with the A-Japan-305-57 virus gave low titers in the range of 16 to 92 chicken cell agglutination (CCA) units ml as compared with 300 or more units expected when strains well adapted to growth in eggs are employed. The Far East viruses showed the usual hemagglutination pattern and did not elute more rapidly from red blood cells than did the prototype A viruses. All Far East agents studied, except A-Japan-305-57 and possibly A-Formosa-313-57, proved highly susceptible to non-specific inhibitors in human and ferret sera. This inhibitor was destroyed readily by cholera filtrate. Strains A-Japan-305-57 and A-Formosa-313-57, only, were suitable for diagnostic purpose with untreated sera. Certain of the early passage egg fluids of strain A-Japan-305-57 were not readily inhibited by homologous immune serum and appeared to be in Q phase(13). A-Japan-305-57 virus, purified from infected allantoic fluid and examined in the electron microscope, was shown by Dr. R. E. Hartman of this laboratory to consist primarily of virus in filamentous form as is characteristic for newly recovered type A strains(20). This strain of virus was not mouse virulent and induced only minimal lung lesions on primary and second passage in mice inoculated by the nasal route.

Discussion. Continuous change in antigenic composition over succeeding years is a prominent characteristic of influenza viruses (2,3,13-19). This may consist of sudden and marked change such as the shift from classical type A to A prime in 1946-47(21,22) or by more gradual and progressive alteration such as observed in the A prime group since 1947 (3,15,16,23,24). The extent of the antigenic difference between the Far East and the type A prototype viruses recovered in recent years cannot be defined precisely because the difference is so great as to obviate the demonstra-

tion of cross-reactions between these groups in the present tests with the available chicken and ferret antisera. Thus, any similarities which might be present are outside the range of measureability in the tests employed. It may be concluded, from our data, however, that modification of antigenic composition of the Far East viruses represents a greater change from the recent A prime era than occurred in the 1946-1947 shift from the type A to the type A prime viruses. This conclusion is supported by the following: First, an antigenic relationship between the type A and A prime viruses is readily demonstrable(2,13, 16,21) by the hemagglutination-inhibition method using chicken and ferret antisera while such relationship between the A prime and Far East viruses is not shown. Second, paired sera from type A prime cases of influenza which occurred in the 1947 outbreak (21,22) and in later epidemics, showed increases in titer against the old prototype A strains, including 1934 PR8, which were as great as or greater than the rises against the homologous A prime viruses. It cannot be concluded from these data, however, that the Far East viruses consist entirely of antigens created *de novo* with no relationship to viruses of the past. Evidence for some relationship is presented in the occasional small titer increases observed in the tests with paired sera from patients and virus strains from the Far East epidemic and from epidemics of previous years. Additionally, the Far East viruses share the same group-specific "soluble" CF antigen as all the type A viruses.

The outbreak in the Far East was characterized by sudden appearance in epidemic proportions of disease caused by a new or hitherto unrecognized form of influenza A virus which followed, within 4 months, the occurrence of epidemics of classical A prime influenza in Japan, the U.S.A. and parts of Europe. The high attack rates among Orientals and Caucasians, the apparent lack of protection(1) in Service Personnel given the standard vaccine (Formula, A-Swine, A-FMl, and B-GL-1739-54), and the deficiency of Far East virus antibody in selected "normal" persons and in patients recently recovered from influenza A prime infection, all indicate a general low level of immunity against the new agents in the human population and the potential for spread of the virus throughout the U.S.A. and probably much of the world. The reports from the Far East and Asia have indicated a generally mild form of illness, except for deaths reported from the Philippines and India. Whether the Far East viruses will increase in virulence on further rapid passage in the susceptible population and whether the occurrence of the disease during the normal winter respiratory disease season will operate to enhance its severity are important observations for the future.

The epidemic occurrence in the Far East with the new virus serves to reemphasize the importance of the world-wide influenza surveillance carried out by the Armed Forces and by the World Health Organization(25) during the past decade. In the present instance, the virus was recovered and analyzed shortly following the first known epidemic occurrence and the characterization was accomplished soon enough to provide what may prove to be sufficient time for production of vaccine in advance of the next expected influenza season in the U.S.A.

Based on past history, it is not unreasonable to expect that epidemics caused by the Far East virus will occur in the Southern hemisphere during the Northern summer of 1957 and that epidemic prevalence will be experienced in the Northern hemisphere during the late Fall and Winter of 1957-58. In view of the ample time for widespread dissemination of the Far East agent, outbreaks of multicentric origin as well as by contiguous spread may be anticipated. It seems likely also that the A prime virus will not be displaced immediately and that it will occur also during the next epidemic season.

Summary. Viruses responsible for the widespread epidemic of influenza in the Far East during the spring of 1957 were caused by a type A virus which represented a major shift, antigenically, from the type A, A prime and swine influenza viruses of previous years. These conclusions are based on findings in tests of Far East and prototype A viruses with chicken and ferret antisera against Far East and prototype strains and in tests with

paired sera from patients in the Far East and previous epidemics. The marked antigenic shift in Far East viruses, the deficiency of antibody against them in the human population tested, the rapid spread of the disease in the Far East, and the relatively high attack rates, all indicate the potential importance of the new viruses as a health problem in the U.S.A. and probably much of the world. During the present period, the new viruses are being called Far East influenza virus, 1957.*

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* Since the present manuscript was written Dr. Christopher Andrewes, in a personal communication, has also suggested this interim name for the Far East viruses.

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