had had experience with influenza A2 antigens responded to administration of influenza vaccine of the current formula with formation of antibody capable of reacting with A2 viruses of diverse antigenic composition. People who had had no previous contact with influenza A2 antigen (*e.g.*, our 1957 study group) could have been anticipated to respond to administration of contemporary vaccine on a single occasion with formation of antibody directed against only the strains contained in the vaccine. Multiple injections, however, would be expected to broaden the immunologic response.

Summary. Outbreaks of influenza in Europe, the Far East and New Zealand and widespread influenza in eastern and central United States in the winters of 1961-1962 and 1962-1963 were found to be caused by influenza type A2 viruses which had undergone considerable antigenic change from the prototype A2 virus recovered in 1957. This conclusion was based on evidence first obtained in virus neutralization tests employing the hemadsorption inhibition technique with contemporary and prototype strains of type A2 virus and serum obtained from mice and man following vaccination with influenza vaccine containing either A2/Japan/305/57 or A2/Japan/170/62 and later confirmed in virus neutralization tests performed in mice with the same reagents. These findings were made in time to incorporate a contemporary influenza type A2 virus into the influenza vaccine to be produced for use in the United States in the winter of 1963-1964.

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Antigenic Variation of Recently Isolated A_2 Influenza Viruses. (28693)

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During January – March 1963 a sharp increase in influenza was observed in certain military populations and veterans resident on the eastern seaboard of the United States. From many of these patients strains of influenza virus, type A_2, were recovered and associated immunologically with illness. Clinical influenza was occurring in a well immunized population; for example, attack rates for influenza for the staff of the Walter Reed Army Institute of Research during February and March in persons who received polyvalent vaccine approximately 5 months earlier (16-18%) were similar to those for non-immunized persons (18-21%), and no significant difference was observed in attack rates be-

TABLE I. A_2 Influenza Strains Studied.

A_2 virus	Source	No. passages in indicated host for: HI antigen	CF antigen	Seed virus*
Jap 305/57	Japan	E6–9		E4, GMK10, MK2
FLW 301/60	Ft. Leonard Wood, Mo.	E3–5		
Jap 170/62	Tokyo, Japan, '62	E7		E7, MK4
DC 301/63	Washington, D. C.	E6		
DC 302/63	*Idem*	E6, 7		E3, GMK2, MK3
DC 327/63	"	E6–8	E7 (CAM)	E7, MK4
DC 317/63	"			GMK4, MK4
DC 342/63	"			MK1, GMK1, MK5
FB 322/63	Ft. Bragg, N. C.	E6		
FD 382/63	Ft. Dix, N. J.	E7		

* E = Embryonated egg. MK = Rhesus monkey kidney cell culture. GMK = African green monkey kidney cell culture.

tween military and civilian personnel. The newly recovered strains were therefore subjected to more detailed antigenic analysis which showed that the 1963 strains could be distinguished from the older Asian viruses (1) and are immunologically similar to the virus recovered by Fukumi in Japan during the winter of 1961-1962(2). This report describes the immunologic reactions of the 1963 variant viruses, some of their biological characteristics, and preliminary observations on the status of immunity in military and veteran populations.

Materials and methods. Patients and specimens. During January – March 1963 ninety-one patients with influenza were sampled by military or veterans administration physicians from Washington, D. C., Ft. Bragg, N. C., Ft. Dix, N. J., Ft. Jackson, S. C., Martinsburg, West Va., Memphis, Tenn., Norfolk, Va., and Temple, Texas. Throat washings for attempts at virus isolation were collected either in Hanks' balanced salt solution (BSS) containing 0.4% bovine plasma albumin (BPA) or in nutrient broth. Specimens other than those collected locally were received in the frozen state; all were stored at —20°C until tested. Paired sera were obtained from each patient. Serial sera were also obtained from laboratory personnel immunized with polyvalent influenza vaccine (military formula)* in August, 1962. All sera were stored at —20°C until tested.

Virus isolations. Throat washings as collected were treated with penicillin and streptomycin (1000 u. and 1000 μg/ml respectively), incubated for 20-30 minutes at room temperature and inoculated into the allantoic and amniotic cavities of 9-10 day embryonated eggs. After incubation of eggs at 35°C for 3 days, hemagglutinins for human red cells, type "O", were sought in extra embryonic fluids by methods routinely used in this laboratory(3). Recovered strains were identified presumptively by hemagglutination-inhibition (HI) tests with rooster antisera prepared against 1957 strains of A_2 virus.

Antigens and seed viruses. HI and complement-fixing (CF) antigens were prepared from representative strains (Table I) by methods described previously(3,4). The A_2/Japan 170/62 virus was received from Dr. J. A. Morris in 6th passage. Four of the 1963 strains as well as A_2/Japan 170/62 and A_2/Japan 305/57 viruses were adapted to growth in Rhesus monkey kidney (MK) monolayer cultures, and used for neutralization tests in MK cultures† at the passage levels indicated in Table I.

Antisera. Rooster antisera were prepared to newly recovered viruses, and certain older strains using a modification of earlier methods (5). Pairs of 8-10 lb roosters were inoculated both intravenously (5.0 ml) and intraperitoneally (5.0 ml) with undiluted infected allantoic fluid. A final 5.0 ml was injected

* Military formula influenza vaccine contained 100 CCA units of strains A/swine/1976/31, A/PR8/34, A_1/Ann Arbor/1/57 and B/Lee/40, 200 CCA units of B/Great Lakes/1739/54, and 400 units of A_2/Japan/305/57 per ml.

† Cell cultures and media were obtained from Microbiological Associates, Bethesda, Md., and Flow Laboratories, Rockville, Md.

intravenously 2 days later; sera were harvested 9-10 days after the second dose. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

Serologic procedures. Hemagglutination-inhibition (HI) and CF tests were performed as reported earlier(4,6). Human sera were treated with receptor destroying enzyme (RDE) as described by Mulder *et al*(7), or with potassium periodate (KIO_4) alone(8), or with potassium periodate and trypsin(9). Treatment with periodate was modified as follows: freshly prepared M/90 potassium periodate solution (0.3 ml) was added to 0.1 ml of human serum which had been heated at 56°C for 30 minutes. After incubation for one hour at room temperature, 0.3 ml of 5% dextrose in saline was added to neutralize the action of periodate; this mixture was then diluted to a final serum concentration of 1:10 with phosphate buffered saline solution, pH 7.2. Further serial 2-fold dilutions were made in 0.85% saline solution. Rooster sera were tested after treatment with RDE, since this was more effective than periodate for production of inhibitor-free sera.

Neutralization tests. Neutralizing antibodies in human and rooster sera were measured in MK monolayer cultures using a modification of the hemadsorption-inhibition technique described by Shelokov *et al*(10). Serial 2-fold dilutions of sera previously heated to 56°C for 30 minutes were made in Hanks' BSS. To 0.2 ml of each dilution was added an estimated 5-50 hemadsorption $doses_{50}$ ($HAdD_{50}$) of influenza virus contained in an equal volume of BSS containing 0.4% bovine plasma albumin. Mixtures were incubated at 25°C for 1 hour; 0.1 ml was then inoculated into each of 2 tube cultures of MK cells. These cultures had been propagated with 10% calf serum, and in the presence of antiserum to SV-5 virus, had been washed twice, and were maintained with M-199 containing penicillin and streptomycin in final concentrations of 100 u. and 100 μg/ml respectively. After stationary incubation at 37°C for 3 days, inoculated cultures were examined for cytopathic effect (CPE), and tested for hemadsorption. Cultures were washed once with BSS; immediately after, 1.0 ml of a 0.1% suspension of fresh, washed guinea pig red cells in Hanks' BSS was added to appropriate tubes. Red cells were allowed to settle upon the cell sheet at 4°C for 20 to 30 minutes. Chilled tubes were examined immediately for hemadsorption. Titers of neutralizing antibody were expressed as the highest dilution of serum which completely inhibited growth of influenza virus.

Results. Recovery of influenza viruses and association with human disease. Throat washings from 42 of 91 patients yielded influenza virus; all but 2 strains were recovered upon initial egg inoculation (first passage). After 2-3 further passages in the allantoic cavity of embryonated eggs, all strains produced sufficient hemagglutinin (titers 1:20 or $>$ /0.25 ml) to permit identification by HI test. Each strain was related presumptively to A_2 virus by HI tests with rooster antisera prepared to A_2/Japan 305/57 virus.

Initially, HI antibodies in paired patients' sera were measured simultaneously against 4 newly recovered strains in low egg passage, as well as against A_2/Japan 305/57 and A_2/Japan 170/62. This was done to determine quickly which strain was best suited for serologic diagnosis by HI test, and because it was known that further antigenic shift had occurred among viruses infecting man in the Far East in 1962(2). Of the first 21 pairs of sera tested (14 from patients yielding virus), 18 showed 4-fold or greater increases in HI antibody to one or another of the recently recovered viruses; in 16, antibody increases were detected with A_2/Japan 305/57 antigen, and in 15, with A_2/Japan 170/62 antigen. Thus it appeared that there were no significant differences among antigens in their efficiency for detecting HI antibody responses in man. However, no single recently isolated strain detected uniformly all antibody increases. The newly recovered strains appeared to be more inhibitor sensitive than the other two, and subsequent HI tests were performed with only A_2/Japan 305/57 and A_2/Japan 170/62 antigens. Paired sera from all 91 patients were so tested. During study of the HI antibody response to these 2 antigens, an evaluation was made simultaneously

of 4 separate methods of treatment of human serum (Table II). Significant antibody responses were shown more frequently with paired sera treated with periodate alone. Periodate followed by trypsin (Table III) reduced titers of specific antibody considerably below those shown following treatment with periodate alone or with RDE. Based upon these observations (Tables II and III) human sera were routinely treated with periodate alone for serologic tests. Sera from all patients were tested for CF antibody response using an antigen prepared from a recently recovered virus. Serologic evidence of recent infection with influenza virus was obtained in 80 of the 91 patients when both HI and CF tests were used (Table IV).

Antigenic analysis of 1963 strains. Even though the hemagglutinins of recently recovered viruses were inhibited by antisera prepared against A_2/Japan 305/57 virus, detailed antigenic analysis of 5 representative strains of the 1963 virus was made to explain the occurrence of disease in personnel who had received influenza vaccine in 1962. The reactions of rooster antisera prepared to 1963 viruses with the hemagglutinins of both older prototype(3) and recently isolated strains are summarized in Table V. From this interpretive summary it can be seen first, that the

TABLE II. Effect of Treatment of Serum upon Demonstration of Significant Increases in Antibodies to Influenza Viruses.

	Frequency of significant HI antibody rises following indicated treatment of human sera							
	Heat alone*		RDE		KIO_4		KIO_4 + trypsin	
Test antigen	A†	B	A	B	A	B	A	B
A_2 Japan 305/57	3/6	1/4	5/6	1/4	23/32 (.72)	18/33 (.55)	8/29 (.27)	6/24 (.25)
A_2 Japan 170/62	1/6	1/4	4/6	1/4	35/42 (.83)	29/49 (.59)	11/29 (.38)	8/24 (.33)

* 56°C for 30 min. † A = Patients yielding virus. B = Patients not yielding virus.

TABLE III. Influence of Serum Treatment upon Titer of Antibody to A_2 Japan 170/62 Virus.

		Reciprocal HI titers of indicated sera after treatment with							
		Heat		RDE		KIO_4		KIO_4 + trypsin	
Patients		A*	C	A	C	A	C	A	C
Virus recovered	1	160	320	40	80	*20*	*160*	10	10
	2	320	640	*40*	*640*	*40*	*320*	10	40
	3	320	640	*80*	*320*	*40*	*320*	*<10*	*20*
	4	320	640	*10*	*160*	*<10*	*80*	<10	10
	5	*160*	*640*	*<10*	*640*	*<10*	*640*	*<10*	*80*
	6	160	320	20	40	10	20	<10	10
No virus recovered	7	320	640	*20*	*640*	*20*	*320*	*<10*	*40*
	8	320	320	160	160	160	160	20	10
	9	640	640	320	320	320	320	40	40
	10	≧1280	≧1280	≧1280	640	640	640	40	40

* A = acute, C = convalescent serum.

TABLE IV. Frequency of Antibody Responses in Patients with Clinically Diagnosed Influenza.

	No. showing significant increases by indicated tests			
Patient category	HI*	CF	HI and/or CF	Neut
Virus recovered	35/42	31/42	39/42 (.93)	36/41 (.88)
No virus recovered	29/49	32/46	41/49 (.84)	13/16 (.81)

* Virus used for HI test = A_2 Japan 170/62, for CF test = A_2 DC 327/63, for neut test = A_2 DC 302/63.

TABLE V. Interpretive Summary, HI Reactions of Current Influenza Strains with Other Viruses.

Rooster antisera	Antigens to older viruses: A Pr 8	A_1 FM 1	A_1 FW 1/50	A_1 FLW 1/52	A_1 Hawaii 303/56	A_2 Jap. 305/57	A_2 For. 313/57	A_2 FLW 301/60	Current antigens: A_2 Jap. 170/62	A_2 DC 301/63	A_2 DC 302/63	A_2 FB 322/63	A_2 DC 327/63	A_2 FD 382/63
A Pr 8/34	*4+**	0	0	0	0	0	—	0	±	—	0	0	0	—
A_1 FM 1/47	2+	*3+*	3+	1+	1+	0	—	0	0	—	0	0	0	—
A_1 FW 1/50	0	4+	*4+*	2+	2+	0	—	0	0	—	0	0	0	—
A_1 FLW 1/52	±	3+	1+	*4+*	3+	0	—	0	±	—	0	0	0	—
A_1 Hawaii 303/56	0	2+	±	3+	*4+*	0	—	0	0	—	0	0	0	—
A_2 Jap. 305/57	0	±	0	0	2+	*4+*	3+	4+	4+	4+	3+	4+	4+	3+
A_2 For. 313/57	0	0	0	0	0	4+	*4+*	—	3+	2+	2+	2+	3+	—
A_2 Jap. 170/62	0	0	0	0	0	±	0	±	*3+*	3+	3+	3+	3+	2+
A_2 DC 301/63	0	0	0	0	0	±	—	—	3+	*3+*	3+	3+	4+	—
A_2 DC 302/63	0	0	0	0	0	±	±	±	3+	3+	3+	3+	3+	2+
A_2 FB 322/63	—	—	—	—	—	±	—	1+	3+	3+	3+	*4+*	3+	—
A_2 DC 327/63	—	—	—	—	—	0	—	1+	3+	—	3+	3+	*4+*	3+
A_2 FD 382/63	—	—	—	—	—	1+	—	—	3+	—	3+	3+	3+	*3+*

* 4+ = 1:1600-1:6400. 3+ = 1:400-1:800. 2+ = 1:200. 1+ = 1:100. ± = 1:50. 0 = <1:50.
— = Not tested.

antigenic composition of 1963 strains is homogeneous. Second, antisera to the prototype 1957 A_2 viruses readily inhibited the contemporary strains. However, antisera to representative 1963 strains reacted weakly if at all with the older agents, including those A_2 viruses recovered in 1957 and 1960. A_2/Japan 170/62 virus, on the basis of these tests, behaved in a fashion similar to the 1963 viruses. These immunologic relationships between A_2/Japan 170/62 and representative contemporary viruses were confirmed by reciprocal neutralization tests, employing cell culture adapted strains and the same rooster antisera used in HI tests (Table VI). Antisera to 1963 strains neutralized the contemporary strains to significantly higher titers than those observed for the 1957 A_2 viruses; similarly, heterotypic reactions of A_2/Formosa 313/57 antiserum were of significantly lower titer than to the homologous strain. The reactions of rooster antisera, by both HI and neutralization tests, establish that the contemporary influenza viruses are similar to the A_2/Japan 170/62 strain, and that this family of viruses is antigenically different from classical Asian strains.

Neutralizing antibodies in patients' sera. Sera from patients recently convalescent from influenza in 1963 showed neutralization reactions similar to those of rooster antisera. Paired sera from 41 of the 42 persons yielding influenza virus in 1963 were tested for neutralizing antibodies to A_2/Japan 305/57 and A_2/DC 302/63 viruses (Table VII). Titers of neutralizing antibodies in acute phase sera were significantly lower to the 1963 (geometric mean 1:8) than to classical A_2 virus (1:36). Titers in convalescence rose to approximately the same levels for each

TABLE VI. Relationships of Current Strains to Classical A_2 Virus by Neutralization Test.

Rooster sera	Reciprocal titer to indicated virus: A_2 Jap. 305/57	A_2 Jap. 170/62	A_2 DC 327/63	A_2 DC 302/63	A_2 DC 317/63	A_2 DC 342/63
A_2 For. 313/57	*640**	10	20	20	40	20
A_2 Jap. 170/62	10	*160*	80	160	320	160
A_2 DC 327/63	20	20	*160*	320	320	160
A_2 DC 302/63	<5	20	40	*80*	160	80

* Measured against 100-1000 HAd $dose_{50}$ of virus: titers/0.05 ml serum.

TABLE VII. Neutralizing* Antibodies in 41 Patients with Proved Influenza 1963.

Virus	Serum	Number with indicated antibody titer							
		<5	5	10	20	40	80	160	320 or >
A_2 Jap. 305/57	Acute†	5	3	4	4	7	6	10	2
	Convalescent	—	3	—	—	2	4	8	24
A_2 DC 302/63	Acute†	17	4	6	5	6	2	1	—
	Convalescent	—	1	—	2	5	4	15	14

* Measured against 5-60 HAd $doses_{50}$ of A_2 Japan 305/57 and 30-500 HAd $doses_{50}$ of A_2 DC 302/63 viruses. Titer/0.05 ml.

† Geometric mean titer of prebleed against A_2 Jap. 305/57 = 1:36; against A_2 DC 302/63 = 1:8.

virus. Finally, 4-fold or greater increases in neutralizing antibody were shown more frequently with A_2/DC 302/63 (36/41) than A_2/Japan 305/57 virus (22/41). Paired sera from 22 patients with clinical diagnosis of influenza whose throat washings did not yield virus showed similar patterns of antibody response to the 2 viruses. Thirteen of 16 persons showed 4-fold or greater increases in neutralizing antibody against A_2/DC 302/63 whereas only 10 of 16 showed significant rises to A_2/Japan 305/57 virus. Six persons showed no significant increase in antibody by HI, CF or neutralization test (as tested against either the 1957 or 1963 antigen). Although serial sera were not available for determination of actual pre-infection titers of neutralizing antibody, these data compare favorably with the significant antibody responses obtained in HI and CF tests (Table IV).

Antibody status and responses to immunization. Preliminary tests were made with sera from recruits immunized with military formula vaccine in 1962 before contemporary A_2 variant viruses appeared. These showed that the titers of neutralizing antibody to A_2/Japan 305/57 virus (1:80-1:320 or greater) in sera obtained 3-7 days after immunization were 4- to 32-fold higher than those to A_2/Japan 170/62 virus (1:10-1:40). However, titers of antibodies to both viruses in sera of the same individuals obtained 3 weeks later were approximately the same (1:320 or greater). While this observation could not be interpreted definitively, it suggested that levels of neutralizing anitbody to contemporary strains were lower than those to classical A_2 virus and independent of them, and suggested that vaccines containing 400 CCA units of classical A_2 antigen did evoke significant neutralizing antibody responses to the newer viruses. To evaluate these impressions further, the antibody responses were measured in 7 laboratory personnel immunized on 22 August 1962 with 0.5 ml of influenza vaccine, military formula (Table VIII). Each subject had been given influenza vaccine at least once before 22 August, the last dose being given 6 months to 4 years previously. Titers of neutralizing antibody to classical A_2 viruses before immunization were consistently greater than those to current virus. Except for subject 7, significant antibody increases to A_2/Japan 305/57 virus were not demonstrated, and titers persisted at high levels for at least 7 months following immunization. In contrast, 6 of these 7 individuals responded promptly to immunization by production of antibody to A_2/DC 302/63 virus; in general these antibodies tended to decay over the 7 month interval. However, one subject, #2, showed a significant increase in antibody to the 1963 virus between late September 1962 and March 1963, the interval during which current strains of influenza virus were infecting members of this laboratory. No disease was recognized in this individual; this subclinical infection occurred in the face of high titers of neutralizing antibody to classical A_2 virus.

Discussion. The present experience indicates that the viruses responsible for outbreaks of influenza in the eastern United States during January–March 1963 are antigenic variants of classical A_2 virus. The nature of this variation, however, appears to be unique for influenza A viruses. Hemag-

TABLE VIII. Neutralizing Antibody* Responses to Immunization with Influenza Vaccine (Military Formula—1962).

Test A_2 virus	Subject†	Reciprocal titers at indicated times after immunization					
		0	2	8	14	33 days	7 mo
Jap. 305/57	1	≧320	—	—	≧320	≧320	≧320
	2	160	≧320	≧320	≧320	≧320	≧320
	3	80	—	—	160	80	80
	4	160	—	—	160	≧320	160
	5	≧320	—	—	≧320	≧320	≧320
	6	160	160	≧320	160	160	≧320
	7	*40*	—	—	*160*	80	80
DC 302/63	1	40	—	—	80	80	40
	2	80	*40*	*160*	160	*40*	≧*320*
	3	5	*5*	*80*	80	40	10
	4	40	*40*	*80*	*160*	160	40
	5	20	*10*	*80*	40	—	80
	6	20	*20*	*160*	160	80	40
	7	5	*5*	*80*	80	40	10

* Measured against 16-60 HAd $doses_{50}$ of virus.

† Last previous immunization preceded test injection of vaccine by 6 mo, subject #1; 7-12 mo, subject #2; 1-4 yr, all others.

glutination by newly recovered strains was inhibited by rooster antisera to prototype A_2 virus. From previous experience, these reactions suggested that the 1963 viruses were similar to 1957 A_2 strains. The finding that antisera to contemporary viruses failed to inhibit older A_2 viruses, however, was unexpected. In extensive antigenic analysis of Type A and A_1 strains occurring between 1933 and 1952(4,5), it was observed that antisera to older viruses reacted with new variants weakly, if at all, whereas antisera to the variants themselves inhibited the older strains. The opposite has been found for the relationship between current A_2 strains and the classical viruses. When first observed, it was thought that the 1963 strains might actually be intermediate to the A_1 viruses of the early 1950's, and A_2 virus. This hypothesis was discarded when no antigenic relationship between current strains and A or A_1 viruses could be demonstrated (Table V). Current strains obviously share some antigens with A_2 virus. However, antisera to 1963 strains neither inhibit hemagglutination nor neutralize infectivity of classical A_2 virus. Therefore it is suggested that the current strains lack one or more antigens originally present in classical A_2 viruses.

The test of significance of antigenic variation in influenza viruses lies in critical evaluation of the effect of the change upon occurrence of disease in man. For the current variants, such evaluation is not possible since controlled studies of vaccine prophylaxis were not made. However, our data and those of Morris *et al*(2) show that normal young adults between 1957 and 1962 possessed little neutralizing antibody to contemporary strains, even though they exhibited high titers of antibody to classical A_2 virus. Further, persons acquiring influenza in 1963 showed essentially this same antibody pattern. It seems unlikely that these differences in titers are due to varying avidities of antibody for the 2 viruses for the following reasons: 1) antibody titers were low when measured against either of 2 strains (A_2/Japan 170/62 or A_2/DC 302/63), 2) following infection, antibody to both current and classical strains reached the same relative titers, and 3) current strains are readily neutralized by homologous rooster antisera. Thus it is likely that previous experience with classical A_2 virus *per se* does not protect uniformly against infection with current strains. The frequency with which this phenomenon occurs in the population at large, however, is unknown, and no accurate evaluation of the protective effect against current viruses afforded by earlier experience with classical A_2 strains can be made. Attack rates of 16-20%, observed here, and in Baltimore(11), would suggest that protection if it occurs, is not absolute.

In our experience, as in that of Morris *et al*(2), the cell culture neutralization test has proved to be a reliable, simple method for measuring specificity and development of neutralizing antibody to influenza virus. With rooster antisera, this test appears to be more specific than the HI test, and in the present study, confirmed the differences between current and older A_2 viruses.

It is noteworthy that methods used for removal of normal inhibitors from human sera profoundly influenced the titers of HI antibody for the contemporary viruses. Thus, for the present work, like that of Horvath (12), digestion of human sera with periodate alone was found to destroy less HI antibody. While differences in titer resulting from serum treatment are of little consequence in the routine serodiagnosis of influenza, they may exert striking influence on the interpretation of quantitative serologic studies, such as are required for antigenic analysis. Since strains of influenza virus vary in their sensitivity to serum inhibitors, it becomes important to use methods to remove normal inhibitor without reducing titers of specific antibody. This is often difficult, and introduces variations between laboratories which make it difficult, if not impossible to compare results. Recognizing this problem, the techniques used in the present circumstances were those which in our hands were most reproducible, and sparing of antibody. Use of the cell culture neutralization test, while more time consuming than the HI test, might obviate the necessity for considering these factors in comparison of data from different laboratories.

Summary. An antigenic variant of Type A_2 influenza virus appeared between 1960 and 1962. Although it was associated with human disease in the Far East in 1962, outbreaks were not noted in the United States until January 1963. This variant appears to lack one or more antigens present in the older A_2 virus, in that rooster antisera prepared to the 1957 strains readily inhibit current strains by HI test, whereas similarly prepared antisera to 1962-1963 viruses react weakly if at all with older strains. Antibody to the current strains in young adults' sera drawn in 1962 was found to be present at a significantly lower level than that to older A_2 viruses. No adequate evaluation can be made of the effect of the present shift upon disease in man. Controlled studies of vaccine prophylaxis hopefully will yield this information. The tissue culture neutralization test (hemadsorption-inhibition) was found to be a reliable method for measuring neutralizing antibody to influenza virus. This method is even more specific than the HI with rooster sera in demonstrating differences among strains. It has the further advantage of obviating the need for preliminary chemical or enzymatic treatment of human sera, and gives a better measure of antibody than other serologic tests.

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