A PATTERN OF INFLUENZA VIRUS VARIATION[1]

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It is now generally recognized that the influenza viruses include numerous different but related strains. It is not clear, however, whether those numerous strains are stable in their characteristics and potentialities, or whether the virus is an unstable one which undergoes frequent change, variation, or mutation. The characteristics of the influenza viruses known to be subject to change or variation are many, but the antigenic characteristics are perhaps the most suited to study.

The present paper is a report of a comparative study of antigenic characteristics of 100 strains isolated during the past 18 years. The data indicate that the changes which have occurred during that period of time have followed, with few exceptions, a consistent pattern. Antigenic components which were dominant in previously isolated strains disappeared or were obscured in more recently isolated strains, and components which were not apparent (by the tests employed) in the earlier strains became dominant in the later strains.

The replacement of components which had been dominant in earlier isolated strains was noted in early studies (Francis and Magill, 1938). Moreover, results quite in agreement with those included in the present paper were obtained with somewhat different methods by Hilleman, Mason, and Rogers (1950) and Hilleman, Mason, and Buescher (1950). Thus, the available data suggest that the environment provided by the host may result either in alteration of the virus, or in the biological selection of strains, variants, or mutants as the case may be.

MATERIALS AND METHODS

Strains of virus. Tests were made with 100 strains of virus; 8 of the strains were isolated more than 10 years ago; 2, during the year 1945–46; 2, during the year 1946–47; 3, during the year 1947–48; 36, during 1948–49; 17, during 1949–50; 31, during the winter of 1950–51; and one strain was isolated during the past summer (1951). The 8 strains, 10 years or older, were isolated in ferrets or mice and then adapted to embryonated hen eggs; the remaining 92 strains were isolated directly in eggs.

Antiserums. The antiserums were from rabbits inoculated thrice intraperitoneally at weekly intervals and bled one week following the third inoculation.

Six strains of virus were employed to prepare the antiserums: strain PR8 (Francis, 1934); strain FM-1 (Rasmussen *et al.*, 1948); strain Coamo (Pérez, 1948); strain Nederland 1/49 (Van der Veen and Mulder, 1950); strain Lee

[1] From the influenza virus Strain Study Center, Commission on Influenza, Armed Forces Epidemiological Board; and the Strain Study Center of the Americas, World Health Organization.

(Francis, 1940); and strain Seattle 2/49 (Lazarus, 1949). The first four strains represented the influenza A group; the latter two strains represented the influenza B group. Six antiserums, one of each kind, of high homologous titer were selected for the tests.

The test. All serums were treated with cholera filtrate after the method of Burnet and Stone (1947). Briefly, the method employed was as follows: To one part of undiluted serum was added 4 parts of undiluted filtrate of material pressed from 18 hour infusion agar cultures of *Vibrio comma*; the mixtures were incubated at 37 C overnight and then heated for one hour at 56 C. The treated serums then were diluted 1/5 with saline. Tests were made with equal volumes (0.2 ml) of the resultant 1/25 dilution of serum, serial dilutions of the strain of virus (allantoic fluid) to be tested, and 1/2 per cent chicken erythrocytes. Results were read on the basis of hemagglutinating units of virus inhibited by the constant quantity of the serum.

RESULTS

It is obvious that use of the 6 antiserums made it possible to test for 6 different antigenic complexes, 4 influenza A complexes and 2 influenza B complexes. It is equally obvious that those 6 complexes were selected arbitrarily and doubtless include only a portion of the total antigenic complexes present among the influenza viruses; nevertheless, they were adequate for the intended purpose. The 6 complexes may be referred to as no. A-1934 (PR8), no. A-1947 (FM-1), no. A-1948 (Coamo), no. A-1949 (Ned 1/49), no. B-1940 (Lee), and no. B-1949 (Seattle 2/49).

On the basis of tests employed, the 100 strains of virus fall into 9 fairly clear-cut groups, 5 groups of influenza A strains and 4 groups of influenza B strains. The characteristics of the 9 subgroups are summarized in table 1.

One influenza A group (A-1) included the WS-like strains; those strains effected hemagglutination which was not inhibited by any of the 6 antiserums. The second group (A-2) included the PR8-like strains, characterized by hemagglutination which was inhibited readily (80 or more hemagglutinating units) by the PR8 antiserum, slightly (9 to 27 hemagglutinating units[2]) by FM-1 antiserum, but not by Coamo, Nederland 1/49, Lee, or Seattle 2/49 antiserums. The third subgroup (A-3) included the strains in which hemagglutination was inhibited slightly by PR8, FM-1, and Ned 1/49 antiserums. The fourth subgroup (A-4) included the FM-1-like strains in which the hemagglutination was inhibited readily by the Coamo, FM-1, and Ned 1/49 antiserums, but not by the other 3 antiserums. The fifth group of A strains (A-5) was characterized by marked inhibition of agglutination by Ned 1/49 antiserum, moderate to slight inhibition by the FM-1, and slight or no inhibition by the Coamo. One group of B strains (B-1) was characterized by slight inhibition of hemagglutination by the Lee antiserum but no inhibition by the Seattle 2/49; a second group (B-2), by marked

[2] One hemagglutinating unit is defined as the smallest quantity of virus (highest dilution of unit volume of allantoic fluid) which completely agglutinates the unit volume of erythrocytes in the test system employed.

inhibition by Lee but none by Seattle 2/49 antiserum; a third group (B-3), by marked inhibition by both Lee and Seattle 2/49 antiserums; and a fourth group (B-4), by inhibition by Seattle 2/49 but not by Lee.

Table 2 summarizes the results of the hemagglutination-inhibition tests and includes pertinent data concerning place and year of isolation of each strain, and worker by whom the strain was isolated (or forwarded). An asterisk in the subgroup column denotes that the respective strain does not fit neatly into the subgroup. For instance, hemagglutination by the Melbourne strain (no. 3) was only partially inhibited by the PR8 antiserum; hemagglutination by the Henry strain (no. 4) was inhibited fairly well by the PR8 antiserum, but it was almost as well inhibited by the Ned 1/49 antiserum. In the case of the Rosenfeld strain (no. 50, Cleveland 1949), the FM-1 and Coamo components were less evident

TABLE 1

The nine subgroups of influenza A and B viruses determined by tests with the 100 strains of viruses and six antibody complexes

Degree* of inhibition by each of the six antibody complexes, of hemagglutination by strains of the different subgroups.

ANTIBODY COMPLEX (ANTISERUM)	SUBGROUP								
	A-1	A-2	A-3	A-4	A-5	B-1	B-2	B-3	B-4
No. A-1934 (PR8)	0	++	+	0	0	0	0	0	0
No. A-1947 (FM-1)	0	+	+	++	+	0	0	0	0
No. A-1948 (Coamo)	0	0	0	++	±	0	0	0	0
No. A-1949 (Ned 1/49)	0	0	+	++	++	0	0	0	0
No. B-1940 (Lee)	0	0	0	0	0	±	++	++	0
No. B-1949 (Seattle 2/49)	0	0	0	0	0	0	0	++	++

* ++: Inhibition of 81, or more, hemagglutinating units of virus.
+: Inhibition of 9 to 27 hemagglutinating units of virus.
0: No inhibition of agglutination.
±: Equivocal; some tests +, others 0.

than in other A-4 strains (i.e., hemagglutination was not as well inhibited by the FM-1 and Coamo antiserums). In the case of strain WRU-37/50 the FM-1 component was weak and the Coamo component strong. The asterisked 1950–51 strains effected hemagglutination which was only weakly inhibited by FM-1 antiserum.

The data included in table 2 suggest a chronological order in the prevalence of the different subgroups. For instance, strains included in subgroup A-4 (the so-called A-prime strains) were not recovered haphazardly during the years, but during the period 1946–47 through 1949–50. Moreover, few other influenza A strains occurred during that period of time. Similarly, the A-5 strains were recovered during the year 1950–51, and not during previous years.

The orderly manner in which the dominant antigenic complexes were replaced by complexes which previously had been inconspicuous or absent is particularly striking in the case of the influenza B strains. The B-1940 (Lee) complex was

TABLE 2

Identity of the 100 strains of virus included in the study

STRAIN	YEAR	ISOLATION PLACE	WORKER	SUB-GROUP
1. WS	1933	London	Smith, Andrewes, and Laidlaw	A-1
2. PR8	1934	Puerto Rico	Francis	A-2
3. Melbourne	1935	Australia	Burnet	A-2*
4. Henry	1936	New York	Francis and Magill	A-2*
5. TM	1939–40	New York	Magill	B-1
6. Chilaberto	1939–40	New York	Magill	B-1
7. Lee	1939–40	New York	Francis	B-2
8. Coyle	1940–41	New York	Magill and Sugg	A-3
9. Czeck	May 1945	C. Edwards, Mass.	Magill and Carroll	B-3
10. Mel. B	1945–46	Australia	Burnet	B-3
11. Cam	1946–47	Australia	Burnet	A-4
12. FM-1	1946–47	F. Monmouth, N. J.	Army Med. C.	A-4
13. AZ	1947–48	New York	Horsfall	A-4
14. AJ	1947–48	New York	Magill	A-3
15. L-1	1947–48	Lawrencv., N. J.	Sigel	B-4
16. Coamo	Oct. 1948	Puerto Rico	Pérez	A-4
17. Seattle 1/49	1948–49	Seattle	Lazarus	B-4
18. Seattle 2/49	1948–49	Seattle	Lazarus	B-4
19. Be 1/49	1948–49	Berkeley, Cal.	Meiklejohn	B-4
20. Be 2/49	1948–49	Berkeley, Cal.	Meiklejohn	B-4
21. Cunane	1948–49	Boston	Cheever	B-4
22. Warner	1948–49	Australia	Anderson	B-4
23. SSM1	1948–49	S. St. M., Ontario	Van Rooyen and McClelland	A-4*
24. SSM2	1948–49	S. St. M., Ontario	Van Rooyen and McClelland	A-4
25. SSM3	1948–49	S. St. M., Ontario	Van Rooyen and McClelland	B-2
26. CP 2/49	1948–49	C. P., Ontario	Van Rooyen and McClelland	A-4
27. CP 3/49	1948–49	C. P., Ontario	Van Rooyen and McClelland	A-4
28. CP 5/49	1948–49	C. P., Ontario	Van Rooyen and McClelland	A-4
29. CP 10/49	1948–49	C. P., Ontario	Van Rooyen and McClelland	A-4
30. St. J. 1/49	1948–49	St. John, N. B.	Van Rooyen and McClelland	A-4
31. St. J. 3/49	1948–49	St. John, N. B.	Van Rooyen and McClelland	A-4
32. St. J. 4/49	1948–49	St. John, N. B.	Van Rooyen and McClelland	A-4*
33. St. J. 5/49	1948–49	St. John, N. B.	Van Rooyen and McClelland	A-4
34. St. C. 1/49	1948–49	St. C., Ontario	Van Rooyen and McClelland	A-4

TABLE 2—(*Continued*)

STRAIN	YEAR	ISOLATION PLACE	WORKER	SUB-GROUP
35. St. C. 2/49	1948–49	St. C., Ontario	Van Rooyen and McClelland	A-4
36. St. C. 3/49	1948–49	St. C., Ontario	Van Rooyen and McClelland	A-4
37. St. C. 5/49	1948–49	St. C., Ontario	Van Rooyen and McClelland	A-4
38. St. C. 6/49	1948–49	St. C., Ontario	Van Rooyen and McClelland	A-4
39. CAP 1/49	1948–49	Canad. Arctic	Nagler	A-2
40. CAP 2/49	1948–49	Canad. Arctic	Nagler	A-2
41. L-4/49	1948–49	Lawrencv., N. J.	Sigel	A-4
42. Conn. 2/49	1948–49	New Haven	Green and Curnen	A-4*
43. Conn. 3/49	1948–49	New Haven	Green and Curnen	A-4
44. Albany 1/49	1948–49	Albany, N. Y.	Gordon	A-4
45. Wilson	1948–49	Pittsburgh	Salk	A-4
46. Pittsburgh 2/49	1948–49	Pittsburgh	Salk	A-4
47. Pittsburgh 3/49	1948–49	Pittsburgh	Salk	A-4
48. Pittsburgh 5/49	1948–49	Pittsburgh	Salk	A-4
49. Morris	1948–49	Pittsburgh	Salk	A-4
50. Rosenfeld	Mar. 1949	Cleveland	Dingle *et al.*	A-4*
51. Ankara	1948–49	Ankara	(Andrewes)	A-1
52. Be 1/50	1949–50	Berkeley, Cal.	Meiklejohn	A-4
53. T. P.	1949–50	New York	Magill	A-4
54. Atlanta Dep.	1949–50	Atlanta	Army Med. C.	A-4
55. WRU-9/50	1949–50	Cleveland	Dingle *et al.*	A-4
56. WRU-37/50	1949–50	Cleveland	Dingle *et al.*	A-4*
57. Woody	1949–50	Cleveland	Dingle *et al.*	A-4
58. WRU-55/50	1949–50	Cleveland	Dingle *et al.*	B-4
59. FW 1/50 (Cuppet)	1949–50	Fort F. Warren	Army Med. C.	A-4
60. Albany 7/50	1949–50	Albany	Gordon	A-4
61. Albany 11/50	1949–50	Albany	Gordon	A-4
62. Th. W. 3	1949–50	Chicago	Loosli	A-4
63. Th. W. 4	1949–50	Chicago	Loosli	A-4
64. Th. W. 6	1949–50	Chicago	Loosli	A-4
65. T-7	1949–50	Tokyo	Kono	B-4
66. MB	1949–50	New York	Horsfall	B-4
67. IB_1	1949–50	New York	Horsfall	B-4
68. IB_2	1949–50	New York	Horsfall	B-4
69. Sweden 3/50	1950–51	Sweden	(Andrewes)	A-5*
70. Ned 1/51	1950–51	Leiden	Mulder	A-5
71. London 1/51	1950–51	London	Andrewes	A-5
72. England 1/51	1950–51	Liverpool	Andrewes	A-5
73. Belfast	1950–51	Belfast	(Andrewes)	A-5
74. Eire 1/51	1950–51	Dublin	(Andrewes)	A-5
75. Whelan	1950–51	Fort Ord (Cal.)	Meiklejohn and Lennette	A-5*
76. Cordova	1950–51	Fort Ord (Cal.)	Meiklejohn and Lennette	A-5*
77. Berkeley 1/51	1950–51	Berkeley, Cal.	Meiklejohn and Lennette	A-5

TABLE 2—(*Continued*)

STRAIN	YEAR	ISOLATION PLACE	WORKER	SUB-GROUP
78. Tokyo 1/51	1950–51	Tokyo	Fukumi	A-5
79. Robert 0	1950–51	S. S. Liberte	Eddy	A-5
80. Saunders	1950–51	S. S. Q. Mary	Eddy	A-5
81. Phil	1950–51	Australia	Burnet (Francis)	A-5*
82. Chom	1950–51	Australia	Burnet (Francis)	A-5*
83. NY 1/51	1950–51	New York	Rose	A-4
84. Benson	1950–51	Pittsburgh	Salk	A-4
85. Ann Arbor 1/51	1950–51	Ann Arbor, Mich.	Francis	A-5*
86. Ann Arbor 2/51	1950–51	Ann Arbor, Mich.	Francis	A-5
87. Albany 1/51	1950–51	Albany, N. Y.	Gordon	A-5
88. Albany 2/51	1950–51	Albany, N. Y.	Gordon	A-5
89. Albany 3/51	1950–51	Albany, N. Y.	Gordon	A-5*
90. Albany 4/51	1950–51	Albany, N. Y.	Gordon	A-5
91. Albany 11/51	1950–51	Albany, N. Y.	Gordon	A-5
92. Phila. 1/51	1950–51	Philadelphia	Sigel	A-5
93. NH 1/51	1950–51	New Haven	Curnen	A-5
94. NH 2/51	1950–51	New Haven	Curnen	A-5
95. NH 3/51	1950–51	New Haven	Curnen	A-5
96. Wallingford	1950–51	Wallingford, Conn.	Curnen	A-5
97. U of C 1/51	1950–51	Chicago	Loosli	A-5
98. U of C 10/51	1950–51	Chicago	Loosli	A-5
99. U of C 12/51	1950–51	Chicago	Loosli	A-5
100. PR 1/51	June 1951	Puerto Rico	Pérez	A-5

* Strain does not fit as neatly as do other strains into the respective subgroup. See text.

present in the earliest strains isolated (groups B-1 and B-2); it was present also in group B-3, which was the next (in chronological order) included in the study. That latter and more recently isolated group (B-3) differed from the earlier groups (B-1 and B-2) in that it possessed a new complex which had not been apparent in the B-1 and B-2 groups. In still more recently isolated groups, the new complex (B-1949) became the dominant complex (of those tested for) and the B-1940 complex disappeared.

In order to make more apparent the seeming chronologic order of occurrence of groups of strains, data already presented in tables 1 and 2 are summarized in more convenient form in table 3. It has been assumed that complete inhibition of hemagglutination (++, tables 1, 2, 3) is acceptable evidence that much, if not all, of the antigen complex which was dominant in the strain employed to prepare the inhibiting antiserum was present in the hemagglutinating virus; that partial inhibition of hemagglutination (+, tables 1, 2, 3) is evidence that the respective strains possessed some, but not other, antigen complexes in common; and that failure to inhibit hemagglutination (0, tables 1, 2, 3) is acceptable evidence that the strains had no important complexes in common.

It is impressive (table 3) that of the 100 strains of influenza A and influenza B viruses included in the study, there are but 4 major exceptions to the rule that each subgroup was prevalent for a period of time and then was replaced by

another subgroup. In general, antigenic complexes which had been dominant in previous groups were lacking, or obscured, in the more recently prevalent subgroup, and antigenic complexes which had not been apparent in the earlier subgroup were dominant in the more recent subgroup.

TABLE 3

Distribution by years of the antigenic complexes determinable by the six antiserums employed in the tests

YEAR	ANTIGENIC COMPLEX						SUBGROUP	TOTAL STRAINS
	Influenza A				Influenza B			
	1934 (PR8)	1947 (FM-1)	1948 (Coamo)	1949 (Ned-1)	1940 (Lee)	1949 (Seatt. 2)		
1933	0	0	0	0			A-1	1
1934	++	+	0	0			A-2	1
1935	+	0	0	0			A-2*	1
1936	++	0	0	+			A-2*	1
1939–40					±	0	B-1	2
					++	0	B-2	1
1940–41	±	+	0	+			A-3	1
1945–46					++	++	B-3	2
1946–47	0	++	++	++			A-4	2
1947–48	±	±	0	±			A-3	1
					0	++	B-4	1
	0	++	++	++			A-4	2
1948–49	0	0	0	0			A-1	1
	++	+	0	0			A-2	2
	0	++	++	++			A-4	25
					++	0	B-1	1
					0	++	B-4	6
1949–50	0	++	++	++			A-4	12
					0	++	B-4	5
1950–51	0	++	++	++			A-4	2
	0	+	±	++			A-5	29
1951	0	+	±	++			A-5	1

DISCUSSION

The data suggest that a progressive change has occurred in the antigenic characteristics of the influenza viruses during the past eighteen years. Antigenic complexes which were dominant in previously isolated strains disappeared or were obscured in more recently isolated strains, and antigenic complexes which were not evident in previously isolated strains became dominant in more recently isolated strains. Those data are similar to the results obtained with somewhat different methods by Hilleman, Mason, and Rogers (1950), and are in agreement with early data which showed that strains isolated during the same outbreak were closely related, although not necessarily identical, but that they differed as a group from strains which had been isolated in previous years (Magill and Francis, 1938); and that antigenic components appeared in recently isolated

strains which had been inapparent in previously isolated strains (Francis and Magill, 1938).

The mechanism by which antigenic variation is effected is a subject of obvious importance. It had been suspected that antigenic changes might occur during passage of the virus in laboratory animals, but early experimental evidence indicated that strains which had been established in mice or in tissue culture had not changed (by tests then available) during additional prolonged passage (Magill and Francis, 1938). Francis (1947) reported antigenic differences after change of host from mouse to egg. At about the same time Hirst (1947) demonstrated antigenic changes during early adaptation in mice; and his data, together with those presented by Sugg (1949*a*), indicate that the enhanced virulence of influenza virus for mice following repeated passage in that species involves a process of biological selection of a variant which has an affinity for mouse lung. Sugg's data (1949*b*) show also that the characteristics of mouse virulence and antigenic variation may occur independently of one another.

Questions were raised early as to whether a large number of antigenically stable strains of influenza virus exist or whether the virus is an unstable one giving rise to mutants during epidemics (Smith and Andrewes, 1938), and the antigenic characteristics of which may be altered by the peculiar immunological state of the host (Magill and Sugg, 1943; Taylor, 1949). The obvious experiment of attempting to alter the virus by cultivation in the presence of heterologous antiserum had been attempted, but the results of the early rather extensive experiments with tissue culture methods failed to show antigenic changes (Magill and Francis, 1936–38). However, Archetti and Horsfall (1950) have reported successful experiments along those lines; they obtained changes in antigenic characteristics of influenza A strains after partial neutralization with heterologous immune serum and cultivation in eggs containing developing chick embryos, and suggested that the phenomenon was one of selection of variants which were unaffected by the antiserums.

The present data, in general, are compatible with either the concept of biological selection of variants or the concept of alteration of virus by the host environment. With but several exceptions, the data indicate that a progressive replacement of one group of strains by other groups has occurred. It is particularly striking that the earliest isolated strains possessed dominant antigen complexes which have not recurred among large groups of more recently isolated strains.[3] The several exceptions were strains closely related to, or identical with, the WS, PR8, and Lee strains. Hilleman, Mason, and Buescher (1950) interpreted the exceptions in their studies as an indication of the recurrence of earlier prototypes. However, Isaacs and Andrewes (1951) have contended that most of the strains concerned should be regarded as laboratory pick-ups. That point is one which cannot be discussed profitably because any expression is but

[3] Perhaps it is better to speak of strain populations in order to stress the point that virus suspensions are mixtures of individual particles, each particle, possibly, with its own peculiar characteristics, and that the antigenic structure of the population is the sum of the antigenic structures of all the individuals.

an opinion, but it focuses attention on the need for better facilities and techniques in all laboratories engaged in virus and rickettsia studies. However, in support of the contention of Isaacs and Andrewes are the two strains AJ (no. 14, table 2) and NY 1/51 (no. 83, table 2). The former was isolated in this laboratory from a laboratory worker with clinical influenza, but who had been working with the Coyle strain (no. 8, table 3) which seems indistinguishable from the AJ. The NY 1/51 was isolated by Dr. Harry M. Rose, to whom we are deeply indebted both for the strain and for the quotable information that it had been isolated from a worker in his laboratory who had been spending a large portion of his time with the FM-1 strain which closely resembles the NY 1/51 strain.

SUMMARY AND CONCLUSIONS

One hundred strains of influenza A and influenza B viruses isolated at various times during the past 18 years were tested by hemagglutination-inhibition tests with 6 antisera representative of 6 different antigen complexes. The strains fall into 5 influenza A subgroups and 4 influenza B subgroups.

The prevalence of the various subgroups appears not to have been haphazard but to have been orderly and chronological. That is, antigenic complexes which were dominant in previously isolated strains were obscured, or lacking, in more recently isolated strains, and complexes which were not apparent in the earlier strains were dominant in the more recent strains. Complexes which were dominant among the early strains have not recurred as dominant complexes among large groups of recently isolated strains.

The compatibility of the data with the idea of biological selection in the immune host is discussed.

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