Etiological and Serological Studies in Epidemic Influenza*

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PRIOR to the onset of the influenza epidemic of 1936–1937, much of the evidence which related influenza virus to epidemic influenza of man was inferential. Attempts to determine the incidence of virus infection in the course of an epidemic had not been thoroughly carried out. Furthermore, while it had been demonstrated that in certain instances an increase in antibodies neutralizing influenza virus followed clinical infection,[1,2] the frequency with which this phenomenon was associated with the actual presence of virus in the throat of the patient was not known. Technics, new or improved, were developing but had not been sufficiently applied to permit their evaluation except in the most marked instances. The significance of negative tests could only be surmised. Thus the failure to recover influenza virus from the epidemic of respiratory disease which occurred throughout the United States in the early months of 1936 [3] and the fact that the convalescents did not develop antibodies to influenza virus could only tentatively be taken as evidence that a different disease had been prevalent.

The recent epidemic afforded the opportunity to test the applicability of different procedures to the diagnosis and study of epidemic influenza. The limitations and variations of results could be observed and the data applied to correlative interpretation of the different procedures.

Accordingly, specimens of blood, throat washings, or both, were obtained from 120 patients suffering with respiratory infections during the epidemic period from December, 1936, to March, 1937. The cases were divided among 7 institutions in Metropolitan New York, 1 institution in Tallahassee, Fla., and 1 satisfactory specimen was obtained from San Francisco, Calif. The results of studies in one group of 28 patients have been presented elsewhere.[4] The present report comprises an analysis and summary of the studies of the entire series together with an

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interpretation of the results of the various tests when viewed in the presence of one another.

METHODS

DEMONSTRATION OF VIRUS IN RESPIRATORY TRACT OF PATIENT

Since the recovery of influenza virus from the respiratory tract of a patient constitutes *prima facie* evidence of the nature of the illness, attempts were made in a high percentage of cases to determine, if possible, the presence or absence of influenza virus in the nasopharyngeal washings. The washings were instilled into the nostrils of an anesthetized ferret and subsequently one or both of the following procedures were employed:

1. Suspensions were made of the lung and nasal scrapings of the inoculated ferret and serial transfers made to normal ferrets, after which the virus was established in mice and identified.

2. The inoculated ferret was allowed to recover, and 10 to 14 days after the instillation of the patient's washings, the ferret was bled from the heart. The serum of the recovered ferret was then tested against a known strain of epidemic influenza virus to determine the presence or absence of specific neutralizing antibodies.

In 4 of 6 instances in which attempts were made, the virus was established directly in mice by nasal instillation of washings from the patient's throat, and subsequent serial passages with suspensions of lungs of the inoculated mice.[4, 5] In certain instances filtered throat washings were introduced into tissue culture medium and successive transfers made. With other samples the filtered material was introduced on the chorio-allantoic membrane of the developing egg to attempt direct propagation of virus.[6] The two latter procedures were discontinued after trial since too long a period ensued before virus could be satisfactorily demonstrated without recourse to animal inoculation.

SEROLOGICAL TESTS FOR DEMONSTRATION OF ANTIBODIES

A. *Neutralization or Mouse Protection Test*—Blood was obtained from patients, so far as possible, in the first 3 days of acute illness and again after 3 to 4 weeks. The acute and convalescent sera were tested at the same time for their capacity to protect mice against fatal infection with 1,000 lethal doses of the known P.R. 8 strain [7] of epidemic influenza virus. Undiluted serum and dilutions of serum 1:5, 1:10, 1:20, 1:40, 1:80 and 1:160 in saline were used. Fresh suspensions of infected mouse lung were made in 10 per cent horse serum-saline. To 0.3 c.c. of the serum dilution was added 0.3 c.c. of 0.2 per cent virus suspension. After thorough mixing, the mixtures were incubated at 37° C. for 30 minutes. Three anesthetized Swiss mice were inoculated intranasally with 0.05 c.c. of a serum-virus mixture from a 0.25 c.c. tuberculin syringe. The mice were observed for a period of 10 days, at which time the experiment was concluded. The day of death of each mouse was recorded and the titer of the serum was considered to be the point at which 50 per cent of the mice in the series would be protected.[8] Thus, if all the mice receiving virus and serum in a dilution of 1:40 survived while those receiving virus and serum in a dilution of 1:80 died, the titer would be considered to be 1:60. All titers were recorded in terms of the final dilution of serum. When mice receiving a mixture of virus and the highest dilution of serum (1:320) survived, an arbitrary end-point of 1:640 was given.

At the time of each test a known positive human serum was titrated so as to determine whether gross aberration from the usual had occurred. There was a surprising constancy in the titer of the control serum in different tests, but no attempt was made to record the test sera in terms of

the control as described by the English investigators.[9]

Titrations of the potency of the virus were made with each test. In general the mice receiving the 10^{-6} dilution of virus died while the 10^{-7} dilution was not fatal. Thus the inoculum (0.1 per cent) in the serum tests was 1,000 lethal doses.

One other point was carefully observed. Mice, 4 to 5 weeks of age, from the same breeder were used throughout. This precaution, together with the comparative uniformity of the virus titer and the simultaneous testing of the sera to be compared, resulted in a test capable of repetition within a comparatively close range of constancy.

B. *Complement-fixation Test* — For the purpose of studying antibody titers to influenza virus by means of the complement-fixation test, a procedure similar to that described by Fairbrother and Hoyle [10] was used. The antigen was prepared in a different manner, however. Lungs were removed from 100 to 200 mice infected with the P.R. 8 strain of epidemic influenza virus at or about the time of death. The lungs were ground in physiological salt solution to make a 10 per cent suspension. After centrifugation for 30 minutes at 3,500 revolutions per minute, the supernatant fluid was removed and 10 c.c. amounts were transferred to tightly stoppered vials. The vials were then placed in a thermos at —80° C.[11] When needed for a test, the material was removed from the freezing mixture, thawed, centrifuged lightly, and then diluted to 2 per cent concentration for use. In this range the virus antigen was never anticomplementary. By the procedure described, antigen of uniform strength can be prepared in sufficient quantities to last over a considerable number of tests.

Serum was diluted with physiological salt solution in twofold dilutions up to 1:128.

Guinea pig complement so diluted that 0.1 c.c. contained 2 units was employed.

The hemolytic system comprised 5 per cent suspension of sheep's washed erythrocytes sensitized with rabbit amboceptor. The amboceptor was titrated against 0.1 c.c. amounts of cell suspension; equal volumes of the dilution of amboceptor containing 2 units per 0.1 c.c. and 5 per cent washed cells were mixed.

The reagents were added in the following order:

0.25 c.c. serum
0.15 c.c. complement (3 units)
0.25 c.c. antigen
0.35 c.c. saline

The mixtures were incubated in the water bath at 37° C. for 60 minutes for fixation; 0.5 c.c. of sensitized cells was added and the mixtures were incubated 45 minutes. Final readings were made at that time. Anticomplementary controls of 1 or more dilutions of each serum were always included. The end point was taken as the last tube in which fixation was present. With the test as employed, the end points were rather sharp, fixation infrequently occurring more than 1 tube beyond that in which +++ or ++++ fixation was last observed. All readings were recorded in terms of final dilutions of serum in order to conform with the neutralization test.

Acute and convalescent sera from the same person were included in the same test and a control serum of known titer was always tested for the sake of comparison with other tests. New antigen was always standardized in a similar manner.

THE RECOVERY OF VIRUS

Throat washings (in 2 cases, postmortem lung specimens) from 75 pa-

TABLE I

Summary of Studies in 120 Patients with Respiratory Disease During Epidemic of Influenza

Group No.	Number of Cases	Classification	Average Serum Antibody Titer			
			Neutralization Test		Complement-fixation Test	
			Acute	Convalescent	Acute	Convalescent
I	41	Virus recovered Rise in serum antibodies	22.4	235.0	14.4	142.5
II	1	Virus recovered No rise in serum antibodies	12.5	15.0	64.0	64.0
III	9	Virus recovered No serum tested				
IV	9	No virus recovered Rise in serum antibodies	20.0	292.0	14.0	309.0
V	18	No test for virus Rise in serum antibodies	14.8	340.0	15	405.3
VI	11	No virus recovered No rise in serum antibodies	50.4	43.2	40.7	24.2
VII	2	No test for virus	80	140	32	32
		No rise in serum antibodies	15	15	32	16
VIII	13	No test or negative test for virus Acute serum obtained late	185.7	231.9	416	357
IX	8	Convalescent serum only	.....	123	...	344
X	5	Incomplete or unclassified				

Total of 117 listed is due to the fact that the throat washings of 3 patients were pooled with others.

tients were tested by ferret inoculation for the presence or absence of influenza virus. Of these, 52 samples were found to contain the virus of epidemic influenza even though many of the specimens had been stored for a considerable period before testing. From 23 of the patients strains of virus were established in mice; 4 by direct transmission, 1 after primary isolation in tissue culture medium, the remainder after intermediate ferret passage. In general, little difficulty was encountered in transferring the disease to mice, much less than had been the case with some of the strains from other epidemics.

The virus recovered was in each instance human influenza virus; the virus was neutralized by serum of animals immune to the P.R. 8 strain but not by swine influenza serum. Thus, it is shown that the primary etiological agent of the epidemic of respiratory disease was the virus of epidemic influenza.

SEROLOGICAL STUDIES

Samples of serum were obtained from 106 patients. From 96 of these patients, serum was secured during the acute period of illness and again in the third week of convalescence. Examination of throat washings for the detection of virus and titrations of antibodies in the patient's acute and convalescent sera were done in 65 cases.

TABLE II

Serological Reactions in Patients with Respiratory Disease

Case No.	Lab. No.	Day of Disease	WBC in 1,000	Past History	Titer of Neutralizing Antibodies: Acute Serum	Titer of Neutralizing Antibodies: Convalescent Serum	Titer of Complement-fixing Antibodies: Acute Serum	Titer of Complement-fixing Antibodies: Convalescent Serum	Diagnosis and Remarks
				Group I—Virus in Throat Washings—Positive Serological Reactions					
1	BL-1	2	5.8		6	280	16	256	Epidemic influenza
5	BL-5	1	9.0	1926	40	70	8	16	"
6	BRK-1	2	10.0	Neg.	0	140	0	256	"
8	BRK-3	2	5.5	Neg.	6	200	0	128	"
11	TF	1	7.3	1923	15	400	..	...	"
12	BRK-7	3	5.4		15	640	8	256	"
26	BRK-28	1	5.2	1918	15	640	8	512	"
28	BRK-34	2	...	1936 ?	30	200	64	128	"
33	BRK-39	2	...	?	40	120	0	8	"
34	BRK-40	2	...	1918, 1936	4	100	8	16	"
38	NYH-3	3	6.9	Neg.	0	15	8	64	"
39	NYH-4	2	5.0	Neg.	6	100	16	64	"
40	NYH-5	3	5.6	Neg.	6	225	0	128	"
45	NYH-10	2	8.6	Pneumonia, 1933	200	640	8	64	"
47	NYH-12	3	7.4	Neg.	50	640	16	128	"
48	NYH-13	1	3.4	1918	50	320	0	64	"
49	NYH-14	2	4.8	Neg.	4	218	0	128	"
50	NYH-15	3	8.5	Neg.	30	100	0	64	"
51	NYH-16	2	6.7	1931, 1933	15	640	0	128	"
54	NYH-19	2	6.1	Neg.	0	35	8	512	"
55	NYH-20	3	6.2	Neg.	6	50	16	64	"
56	NYH-21	2	5.8	Neg.	0	35	32	64	"
57	NYH-22	2	7.8	Neg.	10	60	8	128	"
58	NYH-23	4	3.5	1928	30	206	32	128	"
59	NYH-24	3	3.3	Neg.	4	103	8	128	"
61	NYH-26	3	8.0	1918	15	100	0	16	"
62	NYH-27	3	3.8	1918	6	240	8	128	"
63	NYH-28	3	5.6	Neg.	30	240	8	128	"
65	MET-2	1		1918	15	640	16	512	"
66	MET-3	1		1918, 1935	16	60	0	32	"
67	RIH-1	3	4.2		0	35	32	64	"
72	RIH-6	2	5.5	Neg.	6	140	8	256	"
74	HH-1	4	8.3	1918, 1927	120	200	16	32	"
75	HH-2	4	5.5	Yes	0	25	0	256	"
78	RI-10	3	6.5	1929	12.5	100	8	32	"
79	LTC	1	8.3	Neg.	20	200	8	32	"
80	WC	2			0	15	8	64	"
81	ERR	0		1918, 1933	30	70	8	16	"
88	FLA-7	3	4.0		8	100	64	256	"
93	FLA-12	4	5.4		35	640	0	512	"
99	FLA-18	4?	6.0		30	640	512	1,024	History inaccurate
Average		2.3*	5.9		22.4*	234.7*	14.4*	142.5*	

* (Exclusive of No. 99)

				Group IV—No Virus in Throat Washings—Positive Serological Reactions					
9	BRK-4	3	7.2		30	640	16	256	Epidemic influenza
27	BRK-32	2	7.8	Neg.	8	640	8	512	"
30	BRK-36	1	9.1	1918, 1933	12.5	280	32	512	"
36	NYH-1	3	6.8	1918	6	50	0	32	"
37	NYH-2	2	4.1	Neg.	4	160	32	128	"
42	NYH-7	4	4.0	1918	100	280	16	256	"
44	NYH-9	2	4.5	Neg.	15	120	8	32	"
82	FLA-1	4			6	320	8	1,024	"
105	FLA-24	3			8	640	8	32	"
Average		2.7			20	292	14	309	

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TABLE II—(Continued)

Serological Reactions in Patients with Respiratory Disease

Case No.	Lab. No.	Day of Disease	WBC in 1,000	Past History	Titer of Neutralizing Antibodies: Acute Serum	Titer of Neutralizing Antibodies: Convalescent Serum	Titer of Complement-fixing Antibodies: Acute Serum	Titer of Complement-fixing Antibodies: Convalescent Serum	Diagnosis and Remarks
				Group V—No Throat Washings Tested—Positive Serological Reactions					
15	BRK-13	2	8.0		17.5	640	0	128	Epidemic influenza
16	BRK-14	2	11.2		15	640	..	512	"
18	BRK-16	3	10.4	1918	6	640	0	128	"
19	BRK-17	3		1918	6	60	8	64	"
20	BRK-18	3	10.2	1918	0	30	0	256	"
21	BRK-18A	4	5.1	?	17	80	32	128	"
24	BRK-20	4	5.4	1918	60	640	8	128	"
25	BRK-21	2	7.6	?	6	640	0	1,024	"
29	BRK-35	2	...	?	0	640	0	1,024	"
41	NYH-6	3	...	1918	6	225	16	256	"
46	NYH-11	4	...	1918	30	240	16	128	"
73	RIH-7	2	7.3	?	6	640	16	1,024	Type III Pneumococcus bronchitis
85	FLA-4	4	...		0	35	32	128	Epidemic influenza
87	FLA-6	5	11.0		4	60	32	512	"
94	FLA-13	4	6.5		70	320	32	256	"
95	FLA-14	4	6.0		15	80	8	128	"
100	FLA-19	4			0	640	32	1,024	"
111	RIH-10	1			6	70	8	64	"
Average		3.1			14.8	340	15	405.3	
				Group VI—No Virus in Throat Washings—Negative Serological Reactions					
32	BRK-38	3		Neg.	8	13	0	8	Not influenza
43	NYH-8	2	12.6	Neg.	30	30	8	8	Strep. throat
52	NYH-17	3	25.0	Neg.	60	50	32	32	"
53	NYH-18	2	12.0	Neg.	100	100	32	32	"
60	NYH-25	5	4.8	Neg.	0	0	0	0	Afebrile sinusitis
76	RIH-8	6	13.1	Multiple	15	17	32	32	Bronchitis
77	RIH-9	3	10.3	1918, 1931, 1936	107	35	64	64	Mild coryza
108	LV-2	5			25	17.5	32	32	Not influenza
112	RIH-11	2	7.5	1934	60	60	16	16	Proven case 1934
70	RIH-4	3	21.9	1918, 1936	120	70	256	64	Strep. tracheitis
89	FLA-8	9	8.5		30	60	8	8	Not influenza
Average		4			50.4	43.2	40.7	24.2	
				Group VII—No Throat Washings Tested—Negative Serological Reactions					
31	BRK-37	1	13.4	?	80	140	32	32	Not influenza
102	FLA-21	5			15	15	32	16	
				Group VIII—Patients Seen Late in Disease—Positive Serological Reactions					
68	RIH-2	9	17.0	Neg.	60	60	512	128	Atypical pneumococcus pneumonia
69	RIH-3	9	10.9	Neg.	200	70	1,024	512	Acute bronchitis
71	RIH-5	7	9.7; 18.0	?	125	125	32	32	Atypical Type V pneumonia
83	FLA-2	6	4.7		30	200	128	256	
84	FLA-3	6	9.0		60	120	128	256	
86	FLA-5	9	6.0		280	240	1,024	512	
90	FLA-9	9	5.6		100	400	128	256	
92	FLA-11	6			0	240	128	512	
96	FLA-15	6	8.0		103	240	128	256	
97	FLA-16	5+			640	640	512	512	Relapse
98	FLA-17	4+	6.2		120	240	128	128	"
101	FLA-20	11			640	240	1,024	256	
104	FLA-23	8			60	200	512	1,024	
Average		8			185.7	231.9	416	357	

(Table continued on next page)

TABLE II—(Continued)

Serological Reactions in Patients with Respiratory Disease

Case No.	Lab. No	Day of Disease	WBC in 1,000	Past History	Titer of Neutralizing Antibodies: Acute Serum	Titer of Neutralizing Antibodies: Convalescent Serum	Titer of Complement-fixing Antibodies: Acute Serum	Titer of Complement-fixing Antibodies: Convalescent Serum	Diagnosis and Remarks	Titer 3 Months Later: Neutralization Test	Titer 3 Months Later: Complement-fixation Test
Group IX—Only Convalescent Serum Obtained—Positive Serological Reactions											
91	FLA-10	8	4.8					512			
103	FLA-22	5						1,024			
115	BRK-26	35	6.2			120		128		120	16
116	BRK-23	30	6.7			240		256		240	128
117	BRK-25	35	16.7			35		256		35	64
118	BRK-24	40	8.4			240		256		240	64
119	BRK-22	33	11.2			35		64		35	0
120	BRK-33	18	9.6			70		256		50	128
Average						123		344		120	66.6

In the great majority of instances the first sample of serum was taken before the 5th day after onset, but in 13 the first serum was not obtained until the 6th day or later. So far as possible, with each specimen of serum parallel titrations of antibody content were made by means of the protection test in mice and by the complement-fixation test. Since a large percentage of the cases studied serologically were also investigated for the presence of influenza virus in the throat, the significance of the various results was more readily established. The results are summarized in Table I and presented in detail in Table II.

RESULTS OF SEROLOGICAL TESTS IN RELATION TO THE RECOVERY OF VIRUS

Titrations were made with the acute and convalescent sera of 42 patients in whose throat washings influenza virus was found. In 41 instances (Group I) a rise in the antibody content of the convalescent serum over that of the acute serum was observed. The average titer of neutralizing antibodies in the acute sera was 22.4 and in the convalescent sera, 235. The average titer of complement-fixing antibodies was 14.4 for the acute sera, 142.5 for the convalescent. With both tests the average increase in titer was approximately tenfold. In only one case, a patient who experienced characteristic symptoms but had no fever (Group II), was virus recovered without a corresponding rise in antibodies. Since the first group of 41 patients comprises only individuals in whom the virus was clearly shown to be present, tests made with the serum of these subjects represent the serological reactions of established cases of epidemic influenza. The uniform rise in antibodies in convalescence and the individual variations observed afford a satisfactory basis for the interpretation of the results obtained with material from other patients.

From throat washings of 9 patients (Group III), virus was recovered but no serum was obtained for serological studies.

In 9 cases (Group IV), virus was not recovered from the throat wash-

ings but the serological tests yielded results which conformed with those observed in patients whose throat washings contained influenza virus. The average titer of neutralizing antibodies in the acute sera was 20, in the convalescent sera 292. With the complement-fixation test, the average acute titer was 14.2, the average convalescent titer, 309.3. In each instance a sharp rise in titer occurred. Seven of the specimens of throat washings from these 9 patients had been kept in storage for 6 weeks to 4 months while the other 2 arrived by mail and contained heavy bacterial growth. Despite the fact that no virus was demonstrated, the striking increase which occurred in antibody titer appears to warrant the diagnosis of epidemic influenza in these cases.

From a series of 18 patients (Group V), throat washings were not available for study. Samples of acute and convalescent serum from each patient were examined serologically, however. The average titers by neutralization test for the acute and convalescent sera were 14.8 and 340, respectively; by the complement-fixation test, 15 and 405.3, respectively. In this series again the rise in antibodies is of sufficient degree to permit the assumption that the responses were the result of infection with the virus of epidemic influenza.

In the first group of patients presented above a diagnosis of epidemic influenza was based primarily upon a demonstration of the influenza virus in the patients' throats. When the antibody titers of the same patients were determined, a distinct sequence of events was noted. Following recovery from the virus infection the antibody titer of the convalescent serum had increased on the average to 10 times the height observed in the serum obtained during the acute illness. Applying the standards afforded by those studies, it was possible to interpret the results of serological tests made with the serum of certain patients whose throat washings failed to yield virus or from whom no throat washings were available. Because of the similarity of the serological findings in both groups, it was concluded that the latter individuals had also suffered from epidemic influenza.

In contrast to the preceding groups is a series of 11 patients (Group VI) from whose throat washings virus was not recovered and in whom, moreover, the serological tests gave quite different results. The average acute and convalescent titers as determined by the neutralization test were 50 and 43, respectively, and with complement-fixation test, 40 and 24, respectively. In only 1 patient (No. 32) was an increase noted by both tests and this was well within the limit of error. In the majority of the others the acute and convalescent titers were almost identical. Of this group, 4 suffered from hemolytic streptococcus infections. One of these had been ill 3 weeks previously and possessed a high titer of antibodies when observed in the present illness, suggesting that the earlier illness had been influenza. Three of the patients suffered from rather prolonged respiratory affections with low grade fever. One of them (No. 112) had a proven case of epidemic influenza in 1934; 2 additional attacks of mild respiratory disease had occurred in the interval. The antibody titer of the serum showed a marked rise after the first illness but when tested before and after the other episodes remained constant. Nor was it influenced by the present illness.

It appears then that these patients from whom no virus was recovered did not respond serologically in a manner similar to that of established cases of epidemic influenza. The fact that the comparatively low titers of the acute

sera remained low and, in most instances, identical in convalescence is significant. The completely negative evidence appears to justify the conclusion that the causative agent of illness in these patients was not the virus of epidemic influenza. Quite striking is the fact that 4 of them were hemolytic streptococcus infections which occurred in the midst of the influenza epidemic but with no evidence that they were etiologically related in any way to the epidemic. The entire group represents cases of respiratory infection occurring at the same time as an influenza epidemic which etiological and serological studies combine to identify as of a different nature. Similar conclusions were drawn regarding 2 patients (Group VII) in whom only serological tests were done. No significant differences were observed between acute and convalescent antibody titers.

In contrast to the patients observed early in the disease, whose sera showed a marked rise in antibodies following recovery, is another series of 13 patients (Group VIII) who were first seen 6 to 11 days after onset. From 3 of them throat washings were tested, but no virus could be demonstrated. The outstanding difference between the results obtained in these cases and those previously mentioned lay in the fact that the antibody titer of the first serum sample was in general as high as that of the usual convalescent serum. Thus, by neutralization test the average acute and convalescent titers were 186 and 232, respectively, and by complement-fixation test, 416 and 357, respectively. The high titers observed in the supposedly "acute stage" serum indicate that these subjects were in fact serologically convalescent from epidemic influenza. It is impossible to state whether the illness from which the patient was suffering at the time of observation was a prolongation of the attack of influenza although this is suggested in 3 cases which were diagnosed tentatively to be cases of atypical pneumonia. Two other patients were suffering from what were termed relapses. Among the remainder, the first serum specimens were obviously obtained after recovery had begun and they should probably, therefore, be considered to represent early convalescence. This is further emphasized by the demonstration that the second specimen of serum obtained 3 to 4 weeks later from the same cases showed no prominent increase in complement-fixation titer, which in 7 of the 13 remained unchanged at a high level or had decreased. The titer of neutralizing antibodies increased to some extent in 7 instances, and the results suggest that in general a high titer of antibodies is detected earlier with complement-fixation than with the neutralization test.

Finally 12 cases are included concerning which the data are incomplete. Two of the specimens were of lung obtained at autopsy from cardiac patients who suddenly developed fever and exaggerated pulmonary symptoms during the epidemic and died. From one of the lung specimens, after repeated ferret passage, virus was recovered. From 2 patients the only specimen was serum obtained during the acute illness. One patient (No. 35) is unclassified although considered negative. From the remaining 8 patients (Group IX) only convalescent serum was obtained. The average titer of neutralizing antibodies in these sera was 123, of complement-fixing antibodies, 344. While it is impossible to be conclusive, the antibody titers of the sera were sufficiently high, but for 1 doubtful case, to permit their inclusion with the specific convalescent sera.

EVALUATION OF SEROLOGICAL TESTS

While on the average the antibody titer of the convalescent serum was

10 to 20 times greater than that of serum taken during the acute illness considerable variation occurred in the antibody responses of different individuals to infection. The tests in a group of 62 cases from which the acute specimens of serum were definitely obtained in the first 4 days of the disease were analyzed with the assistance of Dr. Hugo Muench to determine what correlations could be drawn between the original and convalescent titers as measured by the neutralization test in mice and by complement-fixation technic. The distribution of the results is shown in Figures I and II. The extent to which the titer will increase following infection is not predictable by either method. Of the 62 acute sera, however, only 7 possessed neutralizing antibodies in a concentration greater than 1:40. The titers of these latter sera were 50, 50, 50, 80, 100, 120, and 200, respectively. In fact, only 20 of the 62 acute sera possessed neutralizing titers higher than 1:15. On the other hand, among the convalescent sera all but 8 had titers higher than 1:40 (15, 16, 17.5, 20, 30, 35, 35, 35) and 7 of these 8 results were in patients whose acute serum possessed no neutralizing capacity. The convalescent titer in 35 instances was greater than 1:128 and in 15 the limit of titer, 1:640, was reached.

FIGURE I

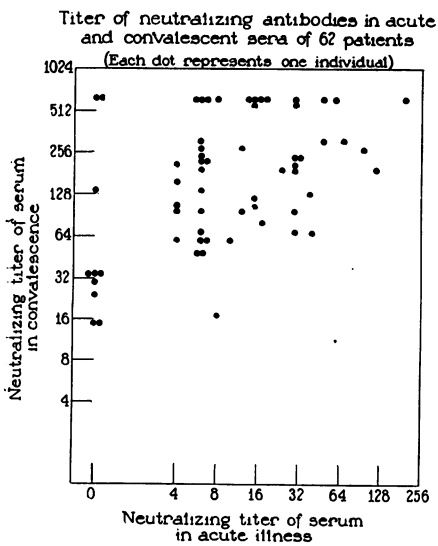
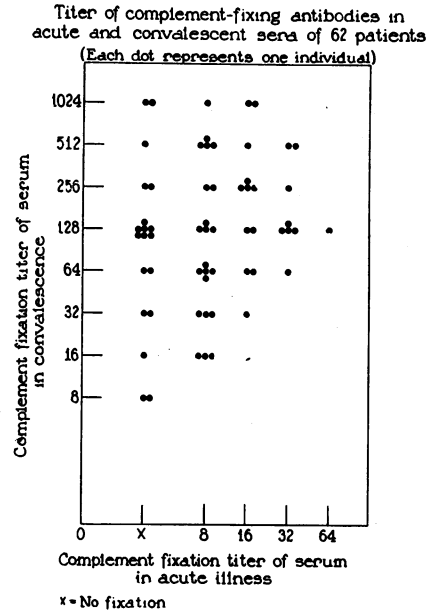


FIGURE II



Similar results were obtained by means of the complement-fixation test. The titers of 19 of the 62 acute sera were negative (less than 1:8), 22 possessed titers of 1:8, while only 1 reached a titer of 1:64. In convalescence 2 of the sera titered 1:8, 4 titered 1:16, 6 titered 1:32. The remaining 50 sera all possessed complement-fixing properties in the presence of influenza virus to a titer of 1:64 or greater, 5 of them reaching titers of 1:1,024.

It appears from the results of the parallel tests that a critical antibody level is reached somewhere in the range of 30–40. Over 85 per cent of the acute sera fall below this level, while of the convalescent sera approximately the same percentage has titers higher than 1:30. With either test the convalescent sera of low titer were from

patients whose antibody titer was at the lower limits of mensuration during the acute illness.

The mean proportionate increase in antibody titer measured by the two serological tests has a positive statistical correlation, emphasizing the tendency to parallelism of the results. The individual variation is pronounced, however, and a marked rise in antibodies by the protection test may occur with only a mild or moderate rise in complement-fixing antibodies. There is, nevertheless, a tendency for the sera of the individuals having high original titers to have a smaller logarithmic increment of antibody development in convalescence.

In certain instances the classification of the serum as positive might be questioned if the results of only one test had been relied upon, but with the additional data at hand it has been possible more clearly to evaluate the variations in the individual cases than it would have been had only one test been used. The results demonstrate as with all other immune processes that while in general a sharp response to the immunogenic agent occurs, certain individuals respond meagerly. The extent of the response appears to be related more to the peculiarity of the individual than to any measurable factor such as the severity of disease or a preëxisting antibody level.

SEROLOGICAL STUDIES IN NORMAL CONTACTS

Two cases (Nos. 79 and 81) have been recorded [4] in which the patients exhibited such minimal evidence of disease as to constitute instances of subclinical infection. Furthermore, it was of interest to determine whether individuals who escaped clinical infection possessed a characteristic level of serum antibodies. In order to secure information bearing upon these points, serum was obtained in one institution from a group of persons who had multiple exposures to the disease, frequently

FIGURE III

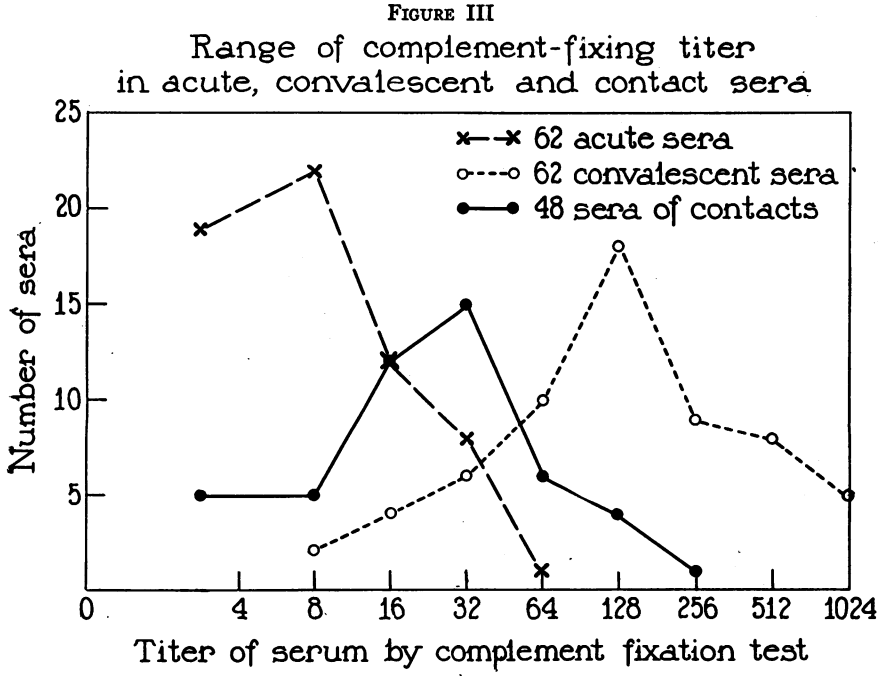
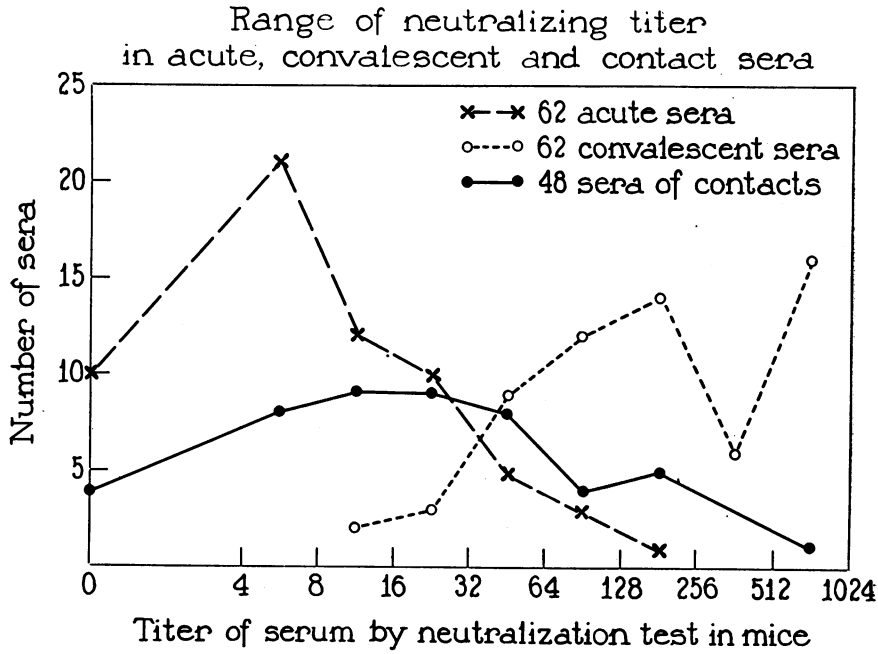


FIGURE IV



by close contact in the same wards, but who developed no clinical evidence of infection. At the time the first specimens of serum were taken from the contacts (January 21, 1937), the epidemic had been in progress for over a month, so that considerable opportunity for infection had occurred. In 5 of the 53 contacts (Nos. 6, 9, 30, 40, and 43), however, histories of illness during the current epidemic period required that these 5 patients be considered probable cases of influenza.

All sera were tested for neutralizing and complement-fixing antibodies to influenza virus. The average titers of the 48 sera were 58.5 by neutralization test and 38.5 by complement-fixation test. The results of the titrations were then compared with the acute and convalescent titers of sera from influenza patients. The comparison is presented graphically in Figures III and IV. The titers of acute and convalescent sera from the same 62 patients previously discussed, together with the serum titers of 48 contacts, are shown in the form of distribution curves.

With complement-fixation 41, or 66 per cent, of the acute sera have titers of 1:8 or less, while of the convalescent sera 40, or 64.4 per cent, have titers of 1:128 or more. The sera from the 48 contact cases, however, occupy an intermediate position since the titers of 33, or 69 per cent, of them ranged from 1:16 to 1:64. Only 10, or 21 per cent, of the contact sera fall in the zone of antibody titer in which two-thirds of the acute sera congregate, while 5, or 10.4 per cent, reach the height to which two-thirds of the convalescent sera attain. Thus the titers of the great majority of the contact sera lie in the range of the upper limit of the acute titers and the lower limits of the convalescent titers. The chance of probability that the titers of the sera from contacts belong in either the acute or conva-

lescent group is of the order of 0.000.000.1, which indicates that these results comprise a statistically distinct group rather than a mixed group of individuals in the acute and convalescent periods. In the latter case the distribution should be represented by a diphasic curve with its dip in the zone where the highest point actually occurred.

With the neutralization test the same tendency prevails although the differential is not so strikingly apparent. In fact, the distribution curve has more of a plateau which does, however, tend to occupy a mid-position. An analysis of the neutralizing antibody titers similar to that made of the complement-fixation results reveals that 31, or 50 per cent, of the acute sera and 12, or 25 per cent, of the contact sera have titers of 1:8 or less. In the upper range 36, or 58 per cent, of the convalescent and 6, or 12.5 per cent, of the contact sera have titers greater than 128. In the intermediate zone of titers between 8 and 128 fall 30, or 62.5 per cent, of the contact sera, an equal number (30, or 48.3 per cent) of the acute sera, and 26, or 42 per cent, of the convalescent sera. In any case the greatest frequency in the titers of the contact sera lies in the range beyond the greatest frequency of the acute titers and below that of the convalescent sera. The chance that the titers of the contact group represent individuals similar to those in the acute group is 0.003, and that they belong in the convalescent group is .000.000.01. These tests confirm the impression gained from the graphic representations.

The intrepretation of these results is not entirely obvious. While statistically the contact group represents a significant difference from the other two, it is apparent that since the titers lie in the intermediate position between acute and convalescent sera three explanations may be offered. The first is that these subjects represent individuals who have had higher antibody levels and are gradually reverting to a lower level. The second possible explanation is that the majority had suffered from sub-clinical infections resulting in comparatively low antibody titers. The third is that the majority of these patients had stabilized at this level and had actually escaped recent infection. The average titer of neutralizing antibodies in the group was 58.5 and of complement-fixing antibodies 38.5, not strikingly different from the patients of Group VI in whom no evidence of virus infection was obtained, but higher than the average acute serum. That the titers in the upper extremes, which comprise 10 cases (Nos. 2, 4, 12, 17, 20, 26, 31, 33, 34, 37), may represent recent infections cannot be denied. One of them (No. 17) most probably was, and in a similar manner those of the lowest titers might well under proper conditions become typical cases.

In an attempt to gain information for interpreting these points, serum from 39 of the contacts after an interval of 3 months was again obtained and tested, and histories were carefully analyzed. The results are shown in Table III. One patient (No. 27) had suffered from influenza beginning 4 days after the first bleeding, with a distinct rise in the antibody content of the second bleeding. In 3 other instances (Nos. 5, 21, 53) an increase in antibodies by both tests was noted. In 1 instance (No. 17) which had the highest titer of the group previously, a definite decline was observed by both procedures.

In 2 instances (Nos. 4 and 33) it is suggested that the patients were actually in the process of building up their antibodies at the time of the first bleeding since the complement-fixation value was high and a sharp

TABLE III

Serological Reactions in Normal Human Contacts

Contact No.	Date of Bleeding	Titer of Neutralizing Antibodies	Titer of Complement-Fixing Antibodies	History Prior to First Bleeding	History Subsequent to First Bleeding	Comment
1	1-21-37	4	8	Grippe Nov., 1936	Negative	
	4-22-37	6	8	Influenza 1918, 1933		
2	1-21-37	200	128	Negative	Negative	Probably sub-clinical infection
	4-22-37	200	64			
3	1-21-37	8	32	Grippe Nov., 1936 Influenza 1918, 1935		
4	1-21-37	30	128	Negative	Negative	"
	4-22-37	120	128			
5	1-21-37	6	8	Influenza 1917; none since	Negative	"
	4-22-37	30	32			
6	1-21-37	240	64	Severe cold Dec., 1936	Negative	Considered to be clinical case; not contact
	4-22-37	160	64			
7	1-21-37	30	32	Negative	Upper resp. inf. Mar. 1937	
	5- 7-37	100	16		Sore throat, temp. 104° 4 ds.	
8	1-21-37	60	64	Influenza 1931	Negative	
	5- 7-37	70	16			
9	1-21-37	50	64	Influenza few weeks before; no fever	Negative	Considered to be clinical case; not contact
	5- 7-37	640	32			
10	1-21-37	35	16	Influenza 1927	Negative	No evidence of disease
	4-22-37	50	16			
11	1-21-37	50	16	Negative	Negative	"
	5- 7-37	70	16			
12	1-21-37	240	64	Negative	Negative to 3-1-37	Sub-clinical infection?
13	1-21-37	60	32	Negative	Negative	
	4-22-37	100	32			
14	1-21-37	6	8	Negative	Negative	
	4-22-37	4	0			
15	1-21-37	17.5	8	Negative	Negative	
	4-22-37	30	8			
16	1-21-37	25	64	Negative	Negative	
	4-22-37	30	32			
17	1-21-37	640	256	Influenza 1918	Negative	Certainly sub-clinical infection
	4-22-37	120	64			
18	1-21-37	8	0	Influenza 1918	Apr. 1, 1937, sore throat, cough, no fever, 5 days	No evidence of influenza
	4-22-37	6	0			
19	1-21-37	30	16	Influenza 1918	Negative	
	4-22-37	30	0			
20	1-21-37	100	32	Influenza 1933	Negative	
	4-22-37	50	32			
21	1-21-37	27	16	Negative	Negative	Questionable antibody rise suggests infection
	4-22-37	60	32			
22	1-21-37	6	16	Negative	Negative	
	4-22-37	4	8			
23	1-21-37	8	32	Negative	Negative	
	4-22-37	15	32			
24	1-21-37	8	16	Negative	Negative	
25	1-21-37	0	32	Influenza 1919	Negative	
	4-22-37	0	16			
26	1-21-37	240	128	Negative	Negative	High antibody suggests sub-clinical infection
	4-22-37	200	64			
27	1-21-37	0	0	Negative	Influenza beginning 1-25-37	Typical case during course of observation
	4-22-37	30	32			
28	1-21-37	80	64	Negative	Negative	
	5- 6-37	50	8			
29	1-21-37	25	32	Negative	Negative	
	4-22-37	25	16			
30	1-21-37	70	128	1-12-37 upper resp. inf. temp. 103.6°		Eliminated as contact, considered clinical case
	4-22-37	30	64			
31	1-21-37	140	16	Influenza 1919	Died 4-4-37	Cardiac death

TABLE III—(Continued)

Serological Reactions in Normal Human Contacts

Contact No.	Date of Bleeding	Titer of Neutralizing Antibodies	Titer of Complement-Fixing Antibodies	History Prior to First Bleeding	History Subsequent to First Bleeding	Comment
32	1-21-37	0	0	Influenza 1922		
	4-22-37	6	0			
33	1-21-37	60	128	Negative	Negative	Probably sub-clinical infection
	4-22-37	240	128			
34	1-21-37	100	64	Influenza 1923	Negative	Sub-clinical infection?
	4-22-37	60	16			
35	1-21-37	35	16	Influenza 1918	2-17-37 ill 2 days malaise	Probably not influenza
	5- 7-37	60	8			
36	1-21-37	6	16	12-12-36 2 days temp. 100° ?		Probably not influenza
37	1-21-37	240	64	Negative	Negative	High antibody suggests sub-clinical infection
	4-22-37	200	64			
38	1-21-37	17.5	32	Negative	Afebrile cold 3-10-37	
	4-22-37	30	32			
39	1-21-37	50	32	Negative		
40	1-21-37	60	64	Influenza 1918	Negative	Eliminated as contact; considered clinical case
	4-22-37	60	32	Influenza 1 day 1937		
41	1-21-37	60	32	Influenza 1932	Cold March, 1937	
	4-22-37	100	64			
42	1-21-37	8	16	Negative	Cold in March	
	4-22-37	6	8			
43	1-21-37	240	64	Influenza Jan. 1937	Negative	Eliminated as contact; considered clinical case
	4-22-37	120	16			
44	1-21-37	4	8	Influenza 1918		
45	1-21-37	12.5	16	Negative	Negative	
	4-22-37	17.5	8			
46	1-21-37	25	32	Negative	Cold in March, 1937	
	4-22-37	60	32			
47	1-21-37	60	32	Negative	Negative	
	4-22-37	120	32			
48	1-21-37	6	0	Negative	Cold April 1, 1937	
	4-22-37	15	8			
49	1-21-37	8	16	Negative		
50	1-21-37	0	32	Negative		
51	1-21-37	15	0	Negative	Cold in Jan. 1937	
	4-22-37	10	0			
52	1-21-37	6	16	Influenza 1918	Strep. throat 4-1-37	
	4-22-37	16	0			
53	1-21-37	15	32	Frequent colds; no definite influenza	Colds and bronchitis; no suggestion of influenza	Probable case of influenza
	4-22-37	120	64			
Average (eliminating 5 clinical cases):						
Total of 48 1st sera		58.5	38.5			
39 1st "		60.2	39.8			
39 2d "		63.7	28.9			

increase in neutralizing antibody titer was noted in the second test. In general, however, the titers were little altered from those of 3 months previous. The average titer of neutralizing antibodies in these 39 cases was 63.7 and the average complement-fixation titer was 28.9, in comparison with titers of 60.2 and 39.8, respectively, in their earlier tests. A number of minor changes in the range of a single dilution were observed, but 31 of the sera remained essentially unchanged in neutralizing titer, 15 in complement-fixation titer, and an additional 18 increased or decreased to the extent of

one dilution in complement-fixation titer. Furthermore, 9 instances of colds or other upper respiratory infection occurred in the month preceding the second bleeding but in no instance did these experiences cause significant deviation in the titer of antibodies to influenza virus.

The additional tests add little in the way of information except to show that perhaps 10 of these patients had undergone asymptomatic infection with the virus of epidemic influenza and that in 2 patients (Nos. 27 and 53) actual clinical infection ensued. In Contact No. 17 the high original titer and the sharp fall clearly indicate that this individual had been infected. The majority of the patients, however, appear not to have suffered from the disease despite the absence of high antibody titers. This field of inapparent infection and resistance will require much further study.

THE PERSISTENCE OF ANTIBODIES

In a previous report [1] was detailed the persistence of a comparatively high level of neutralizing antibodies in 3 patients for a period of 5 months after recovery. The serum of 1 of these subjects has been studied at intervals for a period of 2 years without showing an essential fall in titer. Nevertheless, little is known regarding the maintenance of antibody levels following infection. To study the rate of decline in antibody titer, serum was obtained from 32 of the patients who had previously shown increased antibody formation in early convalescence. Tests were made of the third serum samples, using the second sample of serum of the patients for comparative controls. In 11 instances the third bleeding was secured 2½ to 3 months after the original sample, the remainder 3½ to 5 months after the original.

It was found that after this interval a decline occurred in the average titers of neutralizing and complement-fixing antibodies to about half the level of early convalescence. The average titers of neutralizing antibodies in the first, second, and third samples were 21.2, 305.5, 162.3, respectively; of complement-fixing antibodies 11.5, 230.4, and 113.3, respectively. Little difference was observed in the extent of antibody decline between the sera tested at 2½ to 3 months and those of 3½ to 5 months.

With either test and at either time interval the average decrease was approximately 50 per cent. Twice, an increase of one dilution level was observed with complement-fixation, but none with the neutralization test. Otherwise a surprising parallelism exists within certain limits in the percentage decline by both methods. A decline in titer of neutralizing antibodies is usually associated with a parallel fall in complement-fixation titer. On the other hand, the complement-fixation titer may decrease without an accompanying change in neutralizing antibodies. Thus, in 17 instances no essential difference was observed in the titer of neutralizing antibodies of the early and late convalescent sera while all but 5 of the complement-fixation determinations showed decrease in titer. In 12 instances of the 27 in which complement-fixation titer decreased, it was only to the extent of one dilution level.

In the neutralization tests one other point stands out: Of the 11 patients whose neutralizing titers in early convalescence were 120 or below, only one showed a significant decrease in circulating antibodies in late convalescence. The relative decrease in the entire group was much more at the expense of those individuals who reached high antibody levels in early convalescence. This comparative stability of the lower titers seems to indicate

TABLE IV

Titer of Antibodies in Serum 2½ to 5 Months After Recovery

Case No.	Interval Between 1st and 3d Bleedings (Months)	Titer of Neutralizing Antibodies			Titer of Complement-fixing Antibodies		
		Acute	Early Convalescence	Late Convalescence	Acute	Early Convalescence	Late Convalescence
6	4	0	140	70	0	256	128
8	4	6	200	140	0	128	256
11	5	15	400	60	..	...	...
12	4	15	640	200	8	256	64
15	3½	17.6	640	160	0	128	32
16	3½	15	640	640	..	512	256
18	2½	6	640	140	0	128	32
19	2½	6	60	70	8	64	64
20	2½	0	30	25	0	256	256
21	2½	17	80	50	32	128	256
25	2½	6	640	280	0	1,024	512
26	3	15	640	200	64	512	128
27	3	8	140	50	8	512	128
28	4	30	200	240	64	128	64
29	2½	0	640	240	0	1,024	512
30	2½	12	280	100	32	512	256
33	3	40	120	120	0	8	0
34	2½	4	100	60	8	16	0
38	5	0	15	15	8	64	32
44	5	15	120	60	8	32	16
45	5	200	640	640	8	64	32
47	5	50	640	280	16	128	64
48	5	50	320	240	0	64	16
50	5	30	100	100	0	64	16
51	5	15	640	120	0	128	32
54	5	0	35	30	8	512	128
55	5	6	50	35	16	64	16
58	5	30	206	240	32	128	64
61	4½	15	100	100	0	16	16
62	4	6	240	120	8	128	64
63	4	30	240	320	8	128	64
79	4	20	200	50	8	32	8
Average		21.2	305.5	162.3	11.5	230.4	113.3

that the high titers represent an overproduction of antibody. The fact that many of the numerically lower titers in convalescent serum were from those individuals without antibody originally suggests that perhaps the possession of a certain amount of antibody before infection leads in general to higher titers in convalescence. The tendency of the neutralizing antibodies to decrease less rapidly than those of the complement-fixing type is of interest since the latter appear to reach their maximum titer earlier than the former, frequently before a significant increase in neutralizing antibodies is detectable.

The observations in this group of patients reveal that an average decrease of 50 per cent in the antibody titer occurs in 2 to 5 months, but this is more uniform in complement-fixing titer than in the neutralizing titer. Moreover, the decline in titer by neutralization test appears to be more common in individuals whose early convalescent sera reached the higher ranges while titers below 120 are more persistent.

DISCUSSION

The results of studies in the present series of 120 patients suffering from respiratory disease during the influenza epidemic of 1936–1937 serve to place the diagnosis of epidemic influenza on a firm basis. Thus, the diagnosis of epidemic influenza was made in 100 of 113 cases concerning which sufficient data were available for interpretation. Throat washings were obtained from 64 of the influenza patients, and in 52 (81 per cent) of them influenza virus was demonstrated. In 12 patients from whose throat washings virus was not recovered and in 36 additional patients from whom no throat washings were obtained, a diagnosis of influenza was made on the basis of serological reactions.

Serum was obtained early in the acute illness and in convalescence from 41 patients whose throat washings were found to contain the virus of epidemic influenza. Parallel titrations of the antibody content of the sera were made by the neutralization test in mice and by complement-fixation. It was found that the titer of the convalescent serum had increased, on the average, to a level 10 times as high as that of the acute serum. Consequently, a similar sequence of events in the serum of other patients even in the absence of virus recovery was considered diagnostic. On the other hand, in 11 patients whose throat washings failed to yield virus and in 2 from whom throat washings were not available, the absence of antibody response indicated that these patients had not suffered from epidemic influenza but from respiratory infections of different etiology.

In analyzing the results of the serological tests, it was interesting to note that when tested by either the neutralization or complement-fixation test the titers in the great majority of acute sera were less than 1:40 and the titers in the great majority of convalescent sera from the same patients were higher than 1:40. These observations suggested that a critical level between susceptibility and immunity occurred in that zone of antibody concentration. Hoyle and Fairbrother,[12] basing their opinion on differences in the antibody titers in the serum of the general population before and after an epidemic have concluded that such a level does exist. Moreover, Smorodintseff, *et al.*[13] reported that normal subjects with little or no circulating antibody were readily infected experimentally, while subjects with relatively high antibody titers were resistant. The sera of 48 individuals who had been freely exposed but who had not been ill were tested, therefore, to determine whether their resistance was reflected in any characteristic antibody titer. Interestingly enough, it was found that while a certain number of the results were in the usual range of the acute sera and others in the range of convalescent sera, the greater number of the contact sera congregated in a zone between the acute and convalescent sera and comprised a statistically distinct group. Subsequent study of sera obtained 3 months later from the same individuals suggested that perhaps 10 of them had undergone infection of sub-clinical severity while 2 had actually experienced clinical influenza. Furthermore, it is not unlikely that the higher titers originally observed in this group were a result of inapparent infection. Despite the fact that individuals with little or no circulating antibody escaped infection and that in certain clearly established cases a high titer of antibodies was present at the time of infection, the evidence that a rather distinctive antibody level obtained in the serum of non-infected contacts lends credence to the impression that within flexible limits the antibody titer may be related to the state of resistance.

How long the immunity developed

as a result of an attack of influenza persists is still unknown. In the present series of cases a surprisingly large number of patients, most of whom were adults, had no recollection of a similar illness and comparatively few gave histories of frequent, repeated attacks. Reference has already been made to an individual observed for a period of 2 years after a proved attack of epidemic influenza, who maintained a constant antibody level. From 32 of the patients whose sera were tested in the acute and early convalescent phases of the disease, serum was again obtained and tested 2½ to 5 months later. The average titer by both complement-fixation and neutralization tests had declined about 50 per cent below the level attained in early convalescence. This decline was most regular in the complement-fixation results but in both tests the most marked, and usually parallel, drop was observed in the sera of those individuals whose sera reached the highest titers originally. On the other hand, little change was noted in the titer of neutralizing antibodies among individuals whose titers had not exceeded 1:100 in early convalescence. It appears that these moderate titers are maintained more consistently than the extremely high titers which probably represent immunological over-production. Final opinion regarding the duration of immunity and the persistence of antibodies awaits, however, a much longer period of observation and study.

SUMMARY

A final diagnosis of epidemic influenza was made in 100 patients suffering from respiratory disease during the epidemic period of December, 1936, to March, 1937. From 64 of these patients throat washings were obtained, and in 52 instances the throat washings were shown to contain the virus of epidemic influenza.

In 48 patients the diagnosis of epidemic influenza was made on the basis of the neutralization test in mice and the complement-fixation reaction. Using the same procedures, it was also possible to demonstrate that certain cases of respiratory disease occurring at the same time as the epidemic were of non-influenzal origin. It was also shown that in patients with prolonged illnesses or relapses the continuation of disease was most probably due to intercurrent infection since the patients when first seen were already convalescent, serologically, from influenza and virus was not recovered from the respiratory tract.

During convalescence the antibody concentration of the serum rises to approximately 10 times the height of that observed in the serum early in the disease. The antibody titers of a group of contacts who experienced no clinical evidence of infection occupy a position midway between the usual acute and convalescent zones.

Extremely mild or sub-clinical infections which may play a definite rôle in dissemination of the disease and in immunity are shown to occur.

The duration of immunity and the relation of antibodies to immunity are briefly discussed.

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REFERENCES

1. Francis, T., Jr., and Magill, T. P. *J. Exper. Med.*, 62:505, 1935.
2. Smorodintseff, A. A., Drobyshevskaya, A. I., and Shishkina, O. I. *Lancet*, 2:1383, 1936.

3. Francis, T., Jr. *A.J.P.H.*, 27:211, 1937.
4. Francis, T., Jr., Magill, T. P., Beck, M.D., and Rickard, E. R. *J.A.M.A.*, 109:566, 1937.
5. Francis, T., Jr., and Magill, T. P. *Proc. Soc. Exper. Biol. & Med.*, 36:132, 1937.
6. Francis, T., Jr., and Magill, T. P. *Ibid.*, 36:134, 1937.
7. Francis, T., Jr. *Science*, 80:457, 1934.
8. Muench, H. Unpublished data.
9. Andrewes, C. H., Laidlaw, P. P., and Smith, W. *Brit. J. Exper. Path.*, 16:566, 1935.
10. Fairbrother, R. W., and Hoyle, L. *J. Path. & Bact.*, 44:213, 1937.
11. Turner, T. B. In press.
12. Hoyle, L., and Fairbrother, R. W. *Brit. Med. J.*, 1:655, 1937.
13. Smorodintseff, A. A., Tushinsky, M.D., Drobyshevskaya, A. I., Korovin, A. A., and Osetroff, A. I. *Am. J. Med. Sci.*, 194:159, 1937.

Two other papers from the Symposium on Virus Diseases will be published later: Experiments on Antirabic Vaccination with Tissue Culture Virus, by Leslie T. Webster, M.D., and Lymphocytic Choriomeningitis, by Thomas M. Rivers, M.D., Sc.D., and Robert D. Baird, M.D.

On the Nature of Virus Agents*

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THERE is little that discussion can add to the foregoing papers. They are reports of clinical and experimental observations made by a group of the foremost investigators engaged in these problems and represent notable additions to the knowledge of their respective subjects. Comment on such work without correlated experimentation is time wasted. In listening to this symposium, however, one is impressed with the progress in method and scope which has taken place in virus study during the short space of 10 years. Not only has the field expanded until it is as broad, in its clinical and public health importance, as bacteriology and protozoölogy—but there has been a development of technic by which clinical observation, cultural study, animal experiment and pathology have begun to acquire much of the precision and flexibility of that used in the older fields.

We have begun to emerge from the purely speculative phases of this subject and have arrived at a point at which enough facts have accumulated to permit experimentation to take off from a few solid premises. We are through, among other things, with the elaborate regimentation of questionable evidence strained to interpret virus agents as phases in the life cycles of well known bacteria. Also the temptation to give addresses on "Are Virus Agents Dead or Alive?" is growing more feeble as we recognize that this question is not only futile but leads to utterly sterile metaphysical efforts to define life.

In segregating the so-called virus agents from known infectious microorganisms such as bacteria many features have been stressed, notably those of size and filterability, inclusion bodies, extreme specificity, flexibility of tissue specificity and immunological phenomena. As far as the last named are concerned much of the immunological difference between virus infection and bacterial invasions may be eventually explained by the intracellular position of the former. And since we know that virus agents are definitely antigenic and many serological phenomena, such as precipitation and agglutination, have been performed with virus systems, it may well be that eventually the immunological peculiarities may prove to be dependent upon such secondary attributes as size, surface exposures, etc. Even the apparent

* This paper was presented as a Discussion of the foregoing papers.