

## STUDIES ON THE VIRUS OF INFLUENZA

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From the time of its discovery in 1891 until 1918 the bacillus of Pfeiffer was generally regarded as the cause of influenza; as a consequence of this belief nearly all of the huge volume of investigation done at the time of the recent great pandemic concerned itself with this organism. These studies gave rise to many confused and contradictory results, and ended by casting doubt upon the etiological significance of Pfeiffer's bacillus, or any other of the visible microorganisms that could be cultivated from the respiratory tract. As a result of this uncertainty a filtrable virus was sought in connection with influenza, and as early as August, 1918, Selter (1) reported the production of a mild influenza-like disorder in one of 2 volunteers who inhaled the filtered throat secretions from an active case of the disease. A few other reports (2-4) seemed to confirm this observation, but the very small number of experiments, the lack of quarantine procedures, variation in incubation period, and the fact that an epidemic was in progress at the time, surrounded these early essays with an atmosphere of doubt. In addition, it is noteworthy that other observers (5-7) failed to obtain like results in a larger series of attempts, and by 1922 it is safe to say that the evidence against a filtrable virus as the cause of influenza was more convincing than that in favor of it.

Since 1918 there has been no great pandemic of influenza; the existence, however, of so called interpandemic influenza, ordinarily occurring in winter in rather localized outbreaks, in which many of the cases closely resemble the pandemic variety, has tempted investigators in recent years to direct their attention again to this disease. In 1931 Long and his coworkers (8) reported the appearance in chimpanzees of a febrile disease after inoculation with filtered secretions from influenza patients; in 1932, however, Costa-Mandry *et al.* (9) failed to achieve the same result in human volunteers with material obtained during the Puerto Rico epidemic of that year.

Our own studies of the common cold soon led us to the belief that this problem could not be separated from that of influenza. Clinical and epidemiological studies alike suggest that many examples of influenza during a typical outbreak so closely resemble the common cold as to be indistinguishable from it. This has been observed in isolated communities in the far north when infection has been introduced from the outside by a single individual. A study and knowledge of both diseases is therefore essential to the determination of the value of any form

of prophylactic immunization. In an accompanying paper (10) we have reported in detail our investigations of the common cold which convinced us that the primary etiological agent of this disease is a filtrable virus. Having developed a technique for the study of this virus, it has seemed advisable to apply this same technique to the study of interpandemic influenza. In 1933 we reported (11) the cultivation *in vitro* of a virus derived from a patient with influenza, and the cultivation of a second strain was noted in 1934 (12).

In 1933 (13) Smith, Andrewes, and Laidlaw announced that they had succeeded in initiating a virus disease of ferrets, transmissible in series, with filtered throat secretions of influenza patients, and later on (14) observed that the influenza virus after passage through ferrets became also pathogenic for mice. Similar observations have been made by Francis (15). Although it has not yet been possible to produce influenza in human beings with this virus, nevertheless the isolation of a number of strains from different parts of the world and their serological identity as shown by both Andrewes (16) and Francis (17), and the study of the protective power of convalescent serum by the latter, strongly suggest that these workers are dealing with the virus of interpandemic influenza. Recently Francis (18) has announced that the virus obtained from mouse lung can be cultivated in tissue medium under aerobic conditions.

The object of the present paper is to report in detail the experiments we have performed with the virus of influenza from 1931 to 1935.

#### *Methods*

The methods used in isolating the virus, preserving it, cultivating it, and testing for its activity in human volunteers, have been described in complete detail in a previous publication (10).

#### RESULTS

*1. Attempted Transfer of Influenza from Individual to Individual.*—The first experiment was performed in January, 1931, and consisted of the intranasal inoculation of a fresh, bacteria-free filtrate of nose and throat washings from a patient with influenza.

*Case.*—M. S., a student nurse free from upper respiratory symptoms for a period of 2 months, suddenly noted cough, malaise, and moderate sore throat; later on she developed nasal obstruction and discharge. Temperature at onset 101.6° *per os*, W.B.C. 7,300. She remained in bed 4 days, and returned to duty on the 6th day. During this month there was a rather high incidence of influenza in New York City. The washing was taken 24 hours after the onset of the disease.

Chart 1

Volunteer 25.		Virus: filtered washing from M.S.					
Date, 1931.....	Observation period			Inoculation Jan. 19	20	21	22
	Jan. 16	17	18				
Nasal obstruction.....	0	0	0	0	+	0	+
Sneezing.....	0	0	0	0	+	+	0
Nasal discharge.....	0	0	0	0	+	0	±
Cough.....	0	0	0	0	+	0	+++
Sore throat.....	0	0	0	0	0	0	0
Headache.....	0	0	0	0	+	0	0
Malaise.....	0	0	0	0	0	0	0
Fever.....	0	0	0	0	0	0	0

± very mild, + mild, ++ moderate, +++ marked, ++++ severe.

As will be seen from the foregoing chart, this subject developed very mild symptoms following intranasal inoculation; these were entirely referable to the upper respiratory passages, with the exception that cough became marked on the day of discharge from the hospital. Constitutional symptoms were absent. The type of response manifested by this individual indicated the presence in the filtrate of a virus similar in action to the virus of the common cold.

4 days after discharge he reappeared at the hospital with a temperature of 103° *per os*, complaining of cough, headache, and marked prostration. He stated that his cold became worse immediately after discharge, and that he felt feverish the next day. He was found to have bronchitis and sinusitis, and remained 2 weeks under treatment on the medical wards. The significance of the relapse and intensification of symptoms is not clear. Perhaps outside exposure caused a complication of his infection.

Volunteer 26, who was simultaneously inoculated with the same material, manifested no signs of infection.

On the basis of this single experiment tentative opinion only could be formed that there existed in one patient suffering from interpandemic influenza, a virus similar in action in an experimental individual to the virus of common cold.

2. *Experiments with Virus of Interpandemic Influenza Cultivated in Vitro.*—Strain F was derived from the following case.

In February, 1932, a telephone operator was abruptly seized with nausea, followed by headache, backache, and a shaking chill. Temperature 1 day later was 103°, W.B.C. 7,000. She showed slight cyanosis, injected conjunctivae,

some nasal obstruction, and a dry cough. Fever lasted 4 days, and on the 3rd day the white blood count was 4,800. Influenza was prevalent at the time. This patient had also a very mild chronic cystitis which was not altered by the superimposed respiratory infection.

A tissue culture was initiated with material derived from this patient in the manner already described (10). Transfers were made at 3 to 5 day intervals. In the 19th transfer of the culture, Strain F was tested on 2 volunteers.

Chart 2

Volunteer 55 a. Virus F 19									
Date, 1932.....	Apr. 29	Inoculation May 2	3	4	5	6	7	8	9
Nasal obstruction.....	0	0	++	++	+	++	+++	++	±
Sneezing.....	0	0	+	0	+	0	+++	+	0
Nasal discharge.....	0	0	++	++	++	+	+++	+	+
Cough.....	0	0	+	++	+	++	++	±	0
Sore throat.....	0	0	0	+	0	0	0	0	0
Headache.....	0	0	+	0	±	0	±	0	0
Malaise.....	0	0	+	+	0	0	0	0	0
Fever.....	0	0	0	0	0	0	0	0	0

This patient, as is seen from the above chart, developed a typical head cold of more than average severity. There was an exacerbation of symptoms on the 5th day, but throughout its course the disease had obvious characteristics of a head cold, with mild constitutional reaction and no fever. On the 3rd day the leucocytes were 9,500.

Chart 3

Volunteer 56 a. Virus F 19									
Date, 1932.....	Apr. 29	Inoculation May 2	3	4	5	6	7	8	9
Nasal obstruction.....	0	0	+++	+++	++	++	+	+	±
Sneezing.....	0	0	++	+	+	0	0	0	0
Nasal discharge.....	0	0	++++	++++	++	++	+	±	±
Cough.....	0	0	++++	++++	+	0	±	+	±
Sore throat.....	0	0	±	+	+	±	0	±	±
Headache.....	0	0	++++	++++	++	0	0	0	0
Malaise.....	0	0	+	+	+	+	0	0	0
Fever.....	0	0	0	0	0	0	0	0	0

Like the preceding volunteer, this man developed symptoms characteristic of a severe head cold. There was only a mild degree of malaise, and no fever. On the 3rd day the leucocytes were 10,050.

This experiment indicates that from a typical example of the inter-pandemic form of influenza a filtrable virus was cultivated in tissue medium. This cultivated virus when inoculated intranasally into 2 human volunteers produced in both volunteers the symptoms of a severe common cold. The degree of constitutional reaction was slight and neither of the volunteers experienced a rise in temperature. There was no characteristic alteration in the leucocyte count. In view of the similarity of the symptomatology to that of common cold, an effort was made to determine if infection with the above culture virus gave a cross immunization against the culture virus of common cold. An attempt was first made to test the immunity of the 2 above individuals against infection with the homologous culture virus strain F.

The men were allowed to go home and readmitted to the isolation quarters after the lapse of 3 weeks when they were again inoculated intranasally with strain F transfer 26. The results are shown in the following chart.

Chart 4

Volunteer 55 b. Virus F 26							
Date, 1932.....	May 20	Inoculation May 23	24	25	26	27	28
Nasal obstruction.....	0	0	+++	+	++	++	0
Sneezing.....	0	0	±	0	0	+	0
Nasal discharge.....	0	0	+	++	0	++	0
Cough.....	0	0	+	±	0	+	0
Sore throat.....	0	0	0	0	0	0	0
Headache.....	0	0	0	0	±	0	0
Malaise.....	0	0	0	0	0	0	0
Fever.....	0	0	0	0	0	0	0

  

Volunteer 56 b. Virus F 26							
Date, 1932.....	May 20	23	24	25	26	27	28
Nasal obstruction.....	0	0	++	+	+++	++	±
Sneezing.....	0	0	+	+	0	±	0
Nasal discharge.....	0	0	++	+	0	++	±
Cough.....	0	0	+	++	0	0	0
Sore throat.....	0	0	0	0	0	0	0
Headache.....	0	0	+	++	++++	++	0
Malaise.....	0	0	0	0	0	0	0
Fever.....	0	0	0	0	0	0	0

The above chart shows that both volunteers were susceptible to reinoculation with the F strain of influenza virus 3 weeks after their original infection. In both the disease produced was a moderate head cold without constitutional reaction. Both men declared that the symptoms were quite similar to those produced by the first inoculation, although less than half as severe, an observation borne out by a comparison of the two charts.

This experiment demonstrated that an infection with a strain of culture virus did not confer a solid immunity against reinfection with the same strain. Since little immunity appears under the circumstances of the experiment to develop against the homologous strain, the method is not suitable for cross immunity tests.

Culture strain F was subsequently carried *in vitro* until November, 1933. The total period of cultivation from the time of isolation was approximately 20 months. The intervals of transfer varied from 3 to 5 days. During this period human volunteers were inoculated from time to time and the results of these inoculations are presented in summary in Chart 5.

Chart 5

Date	Volunteer No.	Transfer of culture	Intensity and duration of symptoms		Fever °F., p.o.
			Respiratory days	Constitutional days	
1932					
July	59	37	+2	0	0
"	60	37	0	0	0
Oct.	61	58	±2	±2	0
"	62	58	±2	0	0
"	63	58	±2	±1	0
Nov.	64	65	0	++2	99.2
"	65	65	0	++3	99.0
"	66	65	+5	++2	99.0
1933					
Jan.	67	80	±5	++3	99.4
"	68	80	+5	+4	99.4
"	69	88	+4	+++4	99.0
"	70	88	±3	+3	99.0
Oct.	77	156	0	0	0
"	78	156	0	0	0
"	79	156	0	0	0
Nov.	83	162	0	0	0
"	84	162	0	0	0

The history of culture strain F as depicted in Charts 3, 4, and 5 presents some interesting facts.

The strain of virus was isolated from a patient suffering from a typical attack of influenza of the interpandemic form. There was a moderately high incidence of similar clinical types of influenza at the time. The first series of volunteers responded to inoculation with the symptoms of a severe common cold with marked upper respiratory manifestations and slight or no constitutional reaction. This was followed by a period during which inoculation of the culture produced little or no symptoms of any kind. During this period a strain of the culture was transferred to London where a number of experimental inoculations were made, the majority of which gave no positive evidence of infection. In the series of volunteers 64 to 70 the symptomatology of the experimental disease following inoculation changed markedly. These individuals, after an incubation of 24 hours or longer, all developed rather marked constitutional symptoms consisting of generalized aches and pains, lethargy, and anorexia. In addition to these manifestations they suffered from a mild degree of fever, which up to this time had never been observed in any of the experimentally produced upper respiratory infections. On the other hand the symptoms of irritation of the respiratory tract were very slight. 9 months after these inoculations the culture had entirely lost its capacity to induce symptoms in an inoculated individual.

In January, 1933, a second strain of influenza virus, strain T, was cultivated.

A graduate nurse, free from respiratory symptoms for the previous 3 months, suddenly noted general slight malaise, a sensation of swelling in the throat, and some cough. In the evening the malaise deepened, and she had chilly sensations, pain in the back and neck, and prostration. At the end of a restless night her temperature was 101.6°F., W.B.C. 5,800. Aside from some swelling of the lymphoid tissue in the pharynx, there were no significant physical signs. At the end of 48 hours the fever had subsided, and the patient made a rapid recovery. Influenza was prevalent at this time.

A tissue culture was initiated with material from this patient in the usual manner, transfers being made at 3 to 4 day intervals. The strain was first tested on 2 volunteers in the 18th transfer. In this particular experiment an inoculation of filtered nasopharyngeal washings from a normal individual was made 3 days before the actual test with strain T. The object of this inoculation was to ascertain if a virus capable of inducing respiratory symptoms might be carried by a normal person during the winter months. This inoculation was followed by no signs of infection. The results of inoculation with strain T are given in Chart 6.

Chart 6 shows that both men developed head colds, one somewhat more severe than the other. Both had an exacerbation of symptoms on the 4th day. Symptoms were, for the most part, limited to irritation of the upper respiratory tract, and the degree of general malaise was insignificant. Neither man had fever.

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Chart 6

Volunteer 73. Virus T 18									
Date, 1933.....	Mar. 1	Inoculation Mar. 6	7	8	9	10	11	12	13
Nasal obstruction.....	0	0	+	±	0	++	±	0	0
Sneezing.....	0	0	±	0	±	0	0	0	0
Nasal discharge.....	0	0	+	++	+	+++	+	+	+
Cough.....	0	0	0	±	±	++	+	±	0
Sore throat.....	0	0	0	0	0	0	0	0	0
Headache.....	0	0	++	+	±	±	0	0	0
Malaise.....	0	0	0	+	0	0	0	0	0
Fever.....	0	0	0	0	0	0	0	0	0

Volunteer 74. Virus T 18									
Date, 1933.....	Mar. 1	6	7	8	9	10	11	12	13
Nasal obstruction.....	0	0	0	±	±	+	+	±	±
Sneezing.....	0	0	±	++	+	+	+	±	0
Nasal discharge.....	0	0	+	++	++	++	++	++	++
Cough.....	0	0	0	+++	++	+++	+	±	+
Sore throat.....	0	0	0	0	0	+	0	0	0
Headache.....	0	0	±	±	±	±	0	0	0
Malaise.....	0	0	±	0	±	0	0	0	0
Fever.....	0	0	0	0	0	0	0	0	0

Strain T was next tested in the 80th transfer; the results are given in Chart 7.

Chart 7

Volunteer 80. Virus T 80							
Date, 1933.....	Oct. 27	Inoculation Oct. 30	31	Nov. 1	2	3	4
Nasal obstruction.....	0	0	+	±	0	0	0
Sneezing.....	0	0	++	0	0	0	0
Nasal discharge.....	0	0	++	++	0	0	0
Cough.....	0	0	±	+	++	+	0
Sore throat.....	0	0	0	0	0	0	0
Headache.....	0	0	0	+	0	0	0
Malaise.....	0	0	++	0	0	0	0
Fever.....	0	0	0	0	0	0	0



Chart 7—Concluded

Volunteer 82. Virus T 80							
Date, 1933.....	Oct. 27	Inoculation Oct. 30	31	Nov. 1	2	3	4
Nasal obstruction.....	0	0	±	++	++	++	+
Sneezing.....	0	0	0	0	0	0	0
Nasal discharge.....	0	0	++	++	+	+	+
Cough.....	0	0	+	++	++	+++	+
Sore throat.....	0	0	0	+	0	+	0
Headache.....	0	0	0	0	0	0	0
Malaise.....	0	0	±	+	0	0	0
Fever.....	0	0	0	0	0	0	0

  

Volunteer 81. Virus T 80							
Date, 1933.....	Oct. 27	30	31	Nov. 1	2	3	4
Nasal obstruction.....	0	0	++	+++	+++	+	+
Sneezing.....	0	0	+	+	0	0	0
Nasal discharge.....	0	0	++	++	++	+	+
Cough.....	0	0	+	+++	+++	++	+
Sore throat.....	0	0	0	0	0	0	0
Headache.....	0	0	0	++	+	0	0
Malaise.....	0	0	+	+	+	+	+
Fever.....	0	0	0	0	0	0	0

These results show that strain T was still capable of producing infection in the 80th transfer, the symptoms which it produced being chiefly those of upper respiratory irritation. Volunteer 81 showed a

Chart 8

Volunteer 88. Virus T 113 and 114 mixed							
Date, 1934.....	Feb. 23	Inoculation Feb. 26	27	28	Mar. 1	2	3
Nasal obstruction.....	0	0	+	++	+	++	0
Sneezing.....	0	0	±	±	0	0	0
Nasal discharge.....	0	0	+	±	+	+	0
Cough.....	0	0	+	+	0	0	0
Sore throat.....	0	0	0	0	+	0	0
Headache.....	0	0	0	++	+	+	0
Malaise.....	0	0	0	0	+	0	0
Fever.....	0	0	0	0	0	0	0

moderate degree of malaise. No elevation of temperature occurred in this group.

Strain T was tested for the last time on one volunteer who was inoculated with a mixture of the 113th and 114th transfers of the culture. The result is shown in Chart 8.

Strain T, therefore, showed some infective power after more than 100 transfers of the culture. No subsequent tests were made. To summarize our experience with this strain of virus derived originally from a mild case of influenza, strain T produced in the 6 volunteers inoculated symptoms for the most part referable to infection of the upper respiratory tract and of the type observed in common cold. The degree of constitutional reaction was slight and there was no fever.

In March, 1934, a fourth strain of influenza virus, strain R, was cultivated from a patient suffering from influenza which occurred at that time as a local epidemic in the Pelham Home.

This establishment houses a population of 35 girls, all of whom are rheumatic fever subjects. In March, 1934, a serious epidemic of influenza took place in the Home, apparently introduced by the cook. Coburn and Pauli (19) have reported the clinical aspects of this outbreak in detail, together with a bacteriological and immunological study of the individual cases. The main clinical features of the disease at the Pelham Home were: rather sudden onset, fever of about  $104^{\circ}$  for 3 days, with marked prostration, nausea, vomiting, and insignificant symptoms of upper respiratory irritation. As the epidemic progressed a variation in the type of response took place, so that the intermediate cases showed increasing symptoms of respiratory irritation and decreasing fever. The last cases of the disease were afebrile and resembled the common cold. Leucopenia was not observed during the outbreak. Strain R was derived from a typical example of the disease at the beginning of the epidemic. It was cultivated in tissue medium for 18 transfers and then tested in 3 volunteers. The results are given in Chart 9.

These protocols show that the 18th transfer of strain R of influenza virus produced quite different effects in 3 volunteers inoculated with the same dosage under identical conditions.

Volunteer 93 experienced the symptoms of a common cold of moderate intensity which lasted 3 days. No. 94 had symptoms of a severe cold lasting 5 days, and in addition, on the first 4 days there was a marked constitutional reaction consisting of prostration, dizziness, loss of appetite, and pain in the neck. On the night of May 29 he vomited several times. There was no fever.

Chart 9

Volunteer 93. Virus R 18									
Date, 1934.....	May 25	Inoculation May 28	29	30	31	June 1	2	3	4
Nasal obstruction.	0	0	++	+	0	0	0	0	0
Sneezing.....	0	0	++	±	0	0	0	0	0
Nasal discharge...	0	0	++	++	+	0	0	0	0
Cough.....	0	0	++	+	+	0	0	0	0
Sore throat.....	0	0	+	+	0	0	0	0	0
Headache.....	0	0	+	+	0	0	0	0	0
Malaise.....	0	0	+	0	0	0	0	0	0
Anorexia.....	0	0	0	0	0	0	0	0	0
Vomiting.....	0	0	0	0	0	0	0	0	0
Prostration.....	0	0	0	0	0	0	0	0	0
Dizziness.....	0	0	0	0	0	0	0	0	0
Fever.....	0	0	0	0	0	0	0	0	0

  

Volunteer 94. Virus R 18									
Date, 1934.....	May 25	28	29	30	31	June 1	2	3	4
Nasal obstruction.	0	0	±	+	+	++	+	0	0
Sneezing.....	0	0	0	0	0	+	0	0	0
Nasal discharge...	0	0	++	++	++	++	+	0	0
Cough.....	0	0	±	+	++	+	±	0	0
Sore throat.....	0	0	0	0	0	0	0	0	0
Headache.....	0	0	±	+	+	+	±	+	0
Malaise.....	0	0	++	+++	+	++	0	0	0
Anorexia.....	0	0	0	+	±	±	0	0	0
Vomiting.....	0	0	0	+	0	0	0	0	0
Prostration.....	0	0	0	+	0	0	0	0	0
Dizziness.....	0	0	+	+	+	0	0	0	0
Fever.....	0	0	0	0	0	0	0	0	0

  

Volunteer 95. Virus R 18									
Date, 1934.....	May 25	28	29	30	31	June 1	2	3	4
Nasal obstruction.	0	0	+++	++	±	±	+	0	0
Sneezing.....	0	0	+	0	0	0	0	0	0
Nasal discharge...	0	0	+++	++	+	+	+	+	0
Cough.....	0	0	+	++++	+++	+++	++	±	+
Sore throat.....	0	0	0	+	++	0	±	0	0
Headache.....	0	0	+++	+	++	+++	±	±	±
Malaise.....	0	0	+++	++++	++	+++	±	±	0
Anorexia.....	0	0	+	++	+	+++	±	0	0
Vomiting.....	0	0	0	+++	Nausea	+++	0	0	0
Prostration.....	0	0	0	++	+	+	0	0	Weakness
Dizziness.....	0	0	+	+	+	0	0	+	+
Fever.....	0	0	0	101° p.o.	0	0	0	0	0

The effect of inoculation in volunteer 95 was striking; while coryzal symptoms were not prominent after the first day, severe constitutional manifestations developed which did not disappear entirely for 6 days, and a cough which was still present on discharge from the hospital a week after inoculation.

On the 2nd day of his disease he presented an appearance like that of influenza; he was prostrated and complained of aching pains, chilly sensations and sweating, marked dizziness, nausea, and vomiting. His eyes were suffused, face flushed, throat diffusely red. Lungs were clear. The mouth temperature was 101°F. Leucocytes were 10,900. Except for the fact that his fever lasted only a day, the clinical picture was like that seen in interpandemic influenza of average severity. The volunteer declared that the experimental disease was very similar to, though somewhat less severe than, an attack of influenza he had experienced in 1918 while in the army.

Strain R was tested for activity in 5 volunteers in later transfers of the culture; the results of these tests are briefly summarized in Chart 10.

*Chart 10*

Date	Volunteer No.	Transfer of culture	Intensity and duration of symptoms		Fever
			Respiratory	Constitutional	
1934					
Sept.	96	R 53	0	0	0
"	97	R 53	0	0	0
"	98	R 63	+3 days	0	0
Oct.	99	R 63	±2 "	0	0
"	100	R 63	+3 "	0	0

Chart 10 shows that of 5 inoculations into human volunteers of later transfers of the culture strain R a mild coryza resulted in 3 instances.

In summary, strain R was isolated from a patient during an outbreak of influenza which occurred in a small semi-isolated population. When tested in the 18th transfer this culture virus gave rise to one infection resembling clinical influenza, to one "grippy" cold, and to one coryzal type of cold. Subsequent inoculations of the 53rd and 63rd transfers resulted in mild colds of the coryzal type in which there were no constitutional symptoms.

In October and November, 1935, two additional strains, Ra and To,

were cultivated from two examples of what appeared to be the sporadic type of influenza.

Strain Ra was obtained from a 41 year old man with a past history of maxillary sinusitis. 4 days before admission he caught cold and felt feverish; he had nasal discharge, dry cough, and increasing malaise. The day before admission his temperature was said to have been 104°. On admission it was 101°, and became normal 2 days later. W.B.C. 5,900. X-ray examination was suggestive of sinusitis, and 6 days after admission the right antrum was irrigated and an improvement of the patient's condition resulted.

A nasopharyngeal washing was obtained on the day of admission, and a culture initiated in the usual manner. The final diagnosis of this patient's condition was acute sinusitis.

Strain To was obtained from a patient with the following history: A 45 year old American housewife, after exposure at the house of a friend to a case of what was called influenzal pneumonia, suddenly noticed chilly sensations, and extreme prostration. Her temperature was found to be 103°F. The next day she was admitted to the hospital. There were no symptoms of respiratory irritation. Her only complaints were feverishness, great prostration, and generalized aching pains. Physical examination showed an acutely ill woman with some heliotrope cyanosis and a slightly red throat. W.B.C. 3,250. Fever lasted 3 days, the highest temperature being 104°. W.B.C. remained below 7,000 for 2 weeks and the patient remained in the hospital 27 days on account of extreme asthenia which disappeared very slowly. During most of this period the blood pressure was low, and for the first week of her hospital stay capillary fragility was increased as was indicated by a positive tourniquet test.

Although no epidemic was in progress at the time, the symptoms from which this individual suffered exactly corresponded to those of uncomplicated influenza of the pandemic variety. A nasopharyngeal washing was obtained 48 hours after the onset of the disease and cultivated in tissue medium for 13 transfers before inoculation of human volunteers. The volunteers used in the test of culture To had a few days previously been inoculated with culture Strain Ra, which had proven inactive. The results of inoculation with both Strain Ra and To are presented in Chart 11.

These protocols show that inoculation of strain Ra produced no infection in either of the 2 volunteers. However, both were found to be susceptible a few days later to infection with strain To. In view of the fact that the final diagnosis of patient Ra's condition was acute sinusitis, it is possible that no filtrable virus was present in the respiratory passages.

The type of infection produced by inoculation of strain To was different in the 2 volunteers tested. In volunteer 120, constitutional symptoms—pains about

Chart 11

Volunteer 120													
Date, 1935.....	Virus Ra 8 and 9						Virus To 13						
	Nov. 23	24	Inoculation Nov. 25	26	27	28	Inoculation Nov. 29	30	Dec. 1	2	3	4	5
Nasal obstruction..	0	0	0	0	0	0	0	0	++	++	++	++++	+
Sneezing....	0	0	0	0	0	0	0	0	0	0	0	0	0
Nasal discharge....	0	0	0	0	0	0	0	0	+	++	++	++	+
Cough.....	0	0	0	0	0	0	0	0	+++	+++	++	++	±
Sore throat..	0	0	0	0	0	0	0	0	0	0	0	0	0
Headache...	0	0	0	0	0	0	0	0	++++	+	+	++	±
Malaise.....	0	0	0	0	0	0	0	+	++	++	++	±	0
Fever.....	0	0	0	99.4°	0	0	0	0	0	0	0	0	0

  

Volunteer 121													
Date, 1935.....	Virus Ra 8 and 9						Virus To 13						
	Nov. 23	24	25	26	27	28	29	30	Dec. 1	2	3	4	5
Nasal obstruction..	0	0	0	0	0	0	0	+	+	+	+	+	±
Sneezing....	0	0	0	0	0	0	0	0	0	0	0	0	0
Nasal discharge....	0	0	0	0	0	0	0	++	++	++	+	+	±
Cough.....	0	0	0	0	0	0	0	++	+++	+++	++	±	±
Sore throat..	0	0	0	0	0	0	0	+	+++	+	+	0	0
Headache...	0	0	0	0	0	0	0	0	0	0	0	0	0
Malaise.....	0	0	0	0	0	0	0	0	+	±	0	0	0
Fever.....	0	0	0	0	0	0	0	0	0	0	0	0	0

the head and ears, dizziness, insomnia, and anorexia—were prominent features of the experimental disease. In addition, symptoms of upper respiratory irritation and severe cough were present. There was no fever. In volunteer 121 the symptoms resulting from inoculation were of the coryzal type accompanied by moderately severe cough and sore throat, and there was little or no constitutional reaction.

Throughout the course of these studies careful observation of the bacterial flora of the nasopharynx of inoculated volunteers was maintained. In accordance with our usual procedure individuals harbor-

ing Pneumococcus Types I, II, or III, or *S. hemolyticus* were not used for experimental infection. However, certain individuals throughout the period of an experiment harbored in their throats pneumococci of types other than those mentioned and frequently considerable numbers of *H. influenzae*. The presence of such organisms during the course of an experimental infection did not seem, as far as could be determined by observation, to alter the severity or character of the clinical manifestations when compared with the experimental disease in individuals free from pneumococci or *H. influenzae*. We have previously observed that during a spontaneous or experimental cold in chimpanzees (20), when *H. influenzae* is present in the nasopharynx of these animals a shift from the R form of this organism to the S form may take place. No such change of type of *H. influenzae*, though carefully looked for, was observed in human beings. In a number of instances the virulence of the pneumococcus present was tested by mouse inoculation both before and after inoculation. No change in virulence was observed. It is true, however, that complications such as purulent sinusitis and severe bronchitis were not observed and it is possible that the conditions under which the experiments were performed diminished the liability to such complications.

#### DISCUSSION

In this paper are presented the results of one attempt to transfer influenza from one human being to another by direct inoculation of a bacteria-free filtrate of nasopharyngeal washings and of five attempts to cultivate in tissue medium a filtrable virus from human cases of influenza both of the epidemic and sporadic type. As a consequence of this study the conclusion can be drawn that there is present in filtered nasopharyngeal washings derived from an acute case of influenza a non-bacterial filtrable agent which is capable of producing in human volunteers infection of the upper respiratory tract and that this agent can be successfully propagated in a medium containing minced chicken embryo. The cultivated agent produces no visible change in the medium in which it grows nor can visible bacterial forms be demonstrated by subculture or by microscopic study. It belongs to the class of filtrable viruses.

In all, 39 human volunteers have been experimentally inoculated

with this filtrable agent. The outstanding outcome of these inoculations has been that in every instance but one the inoculation, when positive, whether of fresh filtered washings or of the cultivated virus, has resulted in the production of an infection more nearly resembling the common cold than human influenza. A significant explanation of these results cannot be given at the present time. It is, of course, well known that a large number of upper respiratory infections occurring during a typical epidemic of influenza resemble in character the common cold. Furthermore, in outbreaks of respiratory infection in colonies in the far north which originate from a single source, types of infection resembling both influenza and common cold are present at the same time.

Other explanations of the predominant occurrence of infections resembling common cold in the experimentally inoculated individuals may be offered. It is not unlikely that the conditions of cultivation of the virus result in a diminution of virulence so that the infection following inoculation manifests symptoms of less severity. Moreover, the importance of virulent secondary organisms in the production of the typical picture of human influenza is not understood at the present time, and the absence of such organisms from the nasopharynx of the inoculated volunteers may have influenced the character of the symptomatology. In addition, the fact that all volunteers during the period of infection were maintained in the favorable and protected environment of a private hospital suite may also have influenced the character of the experimental disease. Finally, there is no way at the present time of differentiating conclusively the virus of common cold from that of influenza. That these two viruses are closely related in their manifest activity is extremely likely and that they are close to one another in biological nature seems probable.

On the whole, experimental infections induced in human beings by inoculation of the filtrable virus cultivated from influenza have been more severe in their symptomatology than a comparable series of infections arising from inoculation of the cultivated virus of the common cold. Furthermore, the tendency to exhibit a more pronounced constitutional reaction after inoculation of influenza virus is more conspicuous. These phenomena appear in connection with three of the viruses studied—designated strains F, R, and To. A study of the



case histories of the patients from which these three were derived indicates that they approached more closely the epidemic type of influenza than did the two (strain T and the filtrate experiment) in which the virus obtained was indistinguishable from the common cold virus. In only one (volunteer 121) of 39 volunteers inoculated has the clinical picture of human influenza been reproduced. This was effected by the virus (R) from the Pelham Home outbreak—an outbreak which aside from its purely local character conformed to the epidemic pattern. It is interesting that of 2 additional volunteers inoculated at the same time and with the same material and maintained under identical conditions, one developed a mild coryzal type of common cold and the other a cold of the so called grippy variety.

Lastly, one attempt was made to cultivate a virus from a case of upper respiratory disease with high fever which appeared to be acute sinusitis rather than influenza. No virus was obtained from this case in an attempt made 4 days after the onset of symptoms.

#### SUMMARY AND CONCLUSIONS

1. Evidence is presented indicating the presence of a filtrable virus in the nasopharyngeal secretions of individuals suffering from influenza.
2. An attempt to transfer influenza from one human being to another by means of filtered nasopharyngeal washings resulted in the production in the inoculated volunteer of a common cold.
3. A filtrable agent has been cultivated in tissue medium from the filtered nasopharyngeal washings of patients with influenza.
4. Inoculation of the cultivated virus into human volunteers results for the most part in the production of a severe common cold with a tendency to pronounced constitutional reaction.
5. In one instance following inoculation of culture virus an infection clinically resembling influenza has been produced.
6. The more closely the source of the virus approached the type of epidemic influenza, the more likely the virus was to provoke constitutional symptoms.
7. The presence of certain pathogenic bacteria in the upper respiratory tract of inoculated individuals was not observed to modify the course or character of the experimental infection.

8. On prolonged cultivation the virus loses the capacity to infect human volunteers.

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