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I. EXPERIMENTS UPON VOLUNTEERS TO DETERMINE THE  
CAUSE AND MODE OF SPREAD OF INFLUENZA,  
BOSTON, NOVEMBER AND DECEMBER, 1918

By M. J. ROSENAU, W. J. KEEGAN, JOSEPH GOLDBERGER,  
and G. C. LAKE

II. EXPERIMENTS UPON VOLUNTEERS TO DETERMINE THE  
CAUSE AND MODE OF SPREAD OF INFLUENZA, SAN  
FRANCISCO, NOVEMBER AND DECEMBER, 1918

By G. W. McCOY and DE WAYNE RICHEY

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BOSTON, FEBRUARY AND MARCH, 1919

By M. J. ROSENAU, W. J. KEEGAN, DE WAYNE RICHEY,  
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LEAKE, and G. C. LAKE



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## I. SERIES OF EXPERIMENTS AT BOSTON, NOVEMBER AND DECEMBER, 1918.<sup>1</sup>

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By Lieut. Commander M. J. ROSENAU and Lieut. W. J. KEEGAN, United States Navy, and Surg. JOSEPH GOLDBERGER and Passed Asst. Surg. G. C. LAKE, United States Public Health Service.

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### INTRODUCTION AND ACKNOWLEDGMENTS.

These experiments were carried on jointly by medical officers who were detailed for this purpose from the United States Navy and the United States Public Health Service, at the United States Quarantine Station, Gallups Island, and the United States Naval Hospital, Chelsea, Mass. The experiments were started November 6, and unavoidably discontinued December 23, 1918.

We desire especially to acknowledge the hearty cooperation accorded us by Surg. Gen. W. C. Braisted, United States Navy, and Surg. Gen. Rupert Blue, United States Public Health Service, and the sympathetic understanding of the officers in these bureaus, particularly Lieut. Commander J. R. Phelps, of the Bureau of Medicine and Surgery, United States Navy, and Assistant Surgeon Generals J. W. Schereschewsky and R. H. Creel, United States Public Health Service. We are furthermore particularly indebted to the late Surgeon Donald Currie, United States Public Health Service, in command of the United States Quarantine Station on Gallups Island, for many courtesies and facilities. Toward the close of the study, Dr. Currie contracted influenza, complicated with pneumonia, and died. His assistants, Acting Assistant Surgeons F. X. Crawford and E. M. Looney, helped the work in many direct and practical ways. We are under special obligations to Capt. John M. Edgar, district medical aide, United States Navy, and his able associate, Surgeon W. M. Bryan, United States Public Health Service, sanitary inspector of the first naval district, for practical assistance, which made it possible to carry on many details of the experiments. It is a pleasure also to acknowledge the cooperation we had from Capt. N. S. Blackwood, Medical Corps, United States Navy, in command of the naval hospital at Chelsea, and to his efficient executive surgeon, Commander J. M. Brister, Medical Corps, United States Navy. We were freely given the time and experience of Lieut. Commander L. W. McGuire, Medical Corps,

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<sup>1</sup>Submitted for publication May, 1919.

United States Navy, and Lieut. W. R. Redden, Medical Corps, United States Navy, in helping us select donors and in acting as consultants in the case of one of the volunteers who was taken ill at Gallups Island. Acting Assistant Surgeon C. J. Longstreet, United States Public Health Service, helped in supervising the separation of the experimental groups.

A word of appreciation is due to the men who subjected themselves to experimentation; they were warned of the danger and believed, as did those who conducted the study, that they were risking their lives. The fact that none was harmed does not detract from the fine spirit, splendid courage, and readiness to serve humanity displayed by all of them.

Following is the list of names of those who volunteered to take influenza for the purposes of these experiments:

Abney, Dewey Lavern.*	Nerling, Gustave.
Allan, Robert Andrew.	Ortiz, Julius.
Anderson, Arthur Raymond.	O'Toole, Frank Codman.
Bolduc, Joseph Real.	Peak, George Francis.
Bullock, Muro Chester.	Pruett, George.
Calabrese, James Joseph.	Reid, Robert Lincoln.
Center, Edward Thomas.	Scott, Robert James.
Colton, Charles.	Slipp, Clarence.
Conroy, H. A.	Stanton, Judson Horatio.
Crist, Bertram.	Vandermeer, John William.
Crowley, Henry Edward.	Vanelli, Arthur Nicholas.
Denaard, Arthur Frederick.	Veteto, Gus Robert.
Edman, Charles Frederick.	Vieira, Leopold Joseph.
Englert, Henry Joseph.	Wanless, Frank B.
Felton, James Elwyn.	Heine, John Joseph.
Fleming, George William.	Hill, Warren Arthur.
Foster, John.	Holmes, Harrison Stephen.
Fournier, Ernest Joseph.	Aimar, Bertram Hillard.
Garriott, Simon George.	Crews, Millard.
Gerow, Percy Hector.	Dawson, Harvey Allen.
Gibson, Edward Molten.	Fink, Herbert Jacob.
Goodwin, R. E.	O'Neill, Nick Persian.
Healy, Thomas B.	Evans, Hugh John.
Hedges, Daniel Judd.	Holziner, Carl Peter.
Kearney, Eugene Aloysius.	Warren, Robert Flag.
Klient, Thomas.	Whipp, Raymond Calvin.
Malone, Walter James.	Walker, E. F.
Marcum, Charles.	Hickey, Edward John.
Maas, Paul Alfred.	Jones, Orlando Lloyd.
Morrell, William Francis.	Lang, William Norman.
Murphy, Leonard Richard.	Myers, Fred.
Murphy, William Joseph.	Balbian, Frederick.
McAnneny, John Henry.	Campbell, Verlin Everett.
McKenna, Joseph Edward.	Micks, Albert.

## SUBJECTS OF EXPERIMENT.

The men subjected to these experiments were all volunteers from the United States Naval Training Station, Deer Island, Boston. They numbered 62 in all, and varied in age from 15 to 34 years, 54 of them being 18 to 21 years of age. Aside from the fact that several had more or less enlarged tonsils, all appeared to be in excellent physical condition.

An epidemic of influenza had prevailed at the Deer Island Station, 186 cases having been recorded between September 7 and November 3, 1918, in an average population of 1,058 men (an incidence rate of 176 per 1,000), so that in varying degree all of these men had been exposed to the infection at this station, and in some instances also at preceding stations and places.

From a study of the individual official health records, and from histories elicited by questioning each volunteer, it would appear that 12 of them had an attack of influenza during the recent epidemic, 2 gave a history of illness which was probably this disease, 1 a doubtful history, and 47 appear to have escaped an attack during the epidemic. Of the latter 47, 3 gave histories of influenza-like attacks previous to the present epidemic, 2 of attacks that may be classified as probably influenza, and 3 of attacks of a suggestive but doubtful character. Of our 62 subjects, therefore, 39 were without history of an attack of influenza at any time, 15 with a history of this disease, and 8 with a history of attacks which may or may not have been influenza.

A list of the volunteer subjects with summary of pertinent data is presented in Table I.

TABLE I.—*List of volunteers, Boston experiments, November and December 1918.*

No.	Age.	Possible exposure to influenza during present epidemic, 1918.		History of attack of influenza or "grippe."		Remarks.
		On Deer Island since—	Previous to arrival at Deer Island.	Epidemic, 1918.	Previous to epidemic, 1918.	
1	19	Sept. 15	No.....	Yes, Sept. 23..	No.....	On Sept. 7 and Sept. 8 slept with a comrade who was coming down with an attack. Associated with No. 11 who had an attack. Also exposed at Lawrence, Mass., on furlough from Deer Island.
2	18	Sept. 29	No.....	No.....	No.....	
3	20	Sept. 24	Yes.....	No.....	Doubtful, 1916 and 1917.	
4	20	Aug. 1	No.....	No.....	Yes, 1917.....	Tonsilitis, 1914; sore throat every winter.
5	19	Sept. 15	No.....	No.....	No.....	Not noted in official medical record, but history very suggestive.
6	21	Sept. 13	No.....	No.....	No.....	
7	18	Oct. 24	No.....	Probably about Oct. 1.	No.....	
8	21	Sept. 21	At Brooklyn Navy Yard, Sept. 17-20.	No.....	No.....	Influenza attack Sept. 16. Fairly typical history of attack in 1917.
9	19	July 28	No.....	No.....	No.....	
10	23	Aug. 15	No.....	No.....	No.....	
11	18	July 31	No.....	Yes <sup>1</sup> .....	No.....	
12	19	June 11	No.....	No.....	Yes, 1917.....	



TABLE I.—*List of volunteers, Boston experiments, November and December, 1918—Continued.*

No.	Age.	Possible exposure to influenza during present epidemic, 1918.		History of attack of influenza or "grippe."		Remarks.
		On Deer Island since—	Previous to arrival at Deer Island.	Epidemic, 1918.	Previous to epidemic, 1918.	
13	20	Aug. 10	No.....	No.....	No.....	History of close contact at Deer Island. Influenza Sept. 18 at receiving ship, N. Y.
14	20	July 19	No.....	No.....	No.....	
15	20	Sept. 25	At New York receiving ship	Yes <sup>1</sup> .....	No.....	
16	31	Oct. 5	Norfolk, Va., Sept. 15-Oct. 1.	No.....	No.....	
17	20	June 26	No.....	No.....	Doubtful, Apr., 1918.	History of close contact.
18	19	Sept. 2	No.....	No.....	No.....	
19	21	Sept. 4	No.....	No.....	No.....	
20	19	Oct. 4	No.....	No.....	No.....	
21	19	Oct. 4	Norfolk, Va., in brig, Sept. 25.	No.....	No.....	
22	20	June 26	No.....	No.....	No.....	
23	19	Oct. 4	No.....	No.....	No.....	
24	19	Aug. 31	No.....	No.....	No.....	
25	19	Sept. 3	No.....	No.....	Probable, 1916	
26	18	Sept. 1	No.....	No.....	No.....	
27	19	July 4	No.....	Doubtful, Sept. 30.	Doubtful, 1917	Epidemic in New Haven at time. Not noted in official health record, but history of attack quite clear.
28	17	Sept. 19	At Brooklyn Navy Yard, Sept. 11-18.	No.....	No.....	
29	18	Sept. 15	No.....	No.....	No.....	
30	19	Aug. 17	No.....	No.....	No.....	
31	20	Aug. 15	No.....	No.....	No.....	
32	21	Oct. 4	Norfolk, Va., in brig.	No.....	No.....	
33	19	Aug. 22	No.....	No.....	Probable, 1915.	
34	15	Aug. 21	No.....	Yes, Sept. 15.	No.....	
35	19	May 28	No.....	No.....	No.....	Close contact with No. 60. Close contact at Deer Island.
36	19	June 17	No.....	No.....	No.....	
37	19	Aug. 30	No.....	No.....	No.....	
38	20	July 3	No.....	No.....	No.....	
39	18	Oct. 21	No.....	No.....	No.....	
40	25	Sept. 12	No.....	No.....	No.....	
41	19	Sept. 19	At Brooklyn, in brig, Sept. 14-18.	Probable Aug. 8 on U. S. S. Frank H. Buck.	No.....	
42	19	Sept. 13	No.....	No.....	No.....	Close contact with No. 58.
43	34	Oct. 24	At Brooklyn, in brig, Aug. 25-Oct. 23.	No.....	Doubtful.	
44	21	Sept. 28	No.....	No.....	No.....	
45	29	Sept. 28	No.....	No.....	No.....	
46	20	Oct. 5	No.....	No.....	No.....	Close contact at Brooklyn and Deer Island.
47	18	Sept. 25	At Brooklyn, in brig, Sept.	No.....	No.....	
48	20	Aug. 31	No.....	No.....	No.....	
49	18	Sept. 4	No.....	No.....	No.....	
50	20	June 26	No.....	No.....	No.....	Intimate contact at Philadelphia.
51	19	June 26	No.....	No.....	Doubtful, 1916	
52	19	Aug. 22	No.....	No.....	No.....	
53	18	Nov. 1	At Philadelphia, Apr. 19-Oct. 31.	No.....	No.....	
54	20	Sept. 6	No.....	Yes <sup>1</sup> .....	No.....	
56	21	Aug. 22	No.....	Yes, Sept. 23 <sup>1</sup>	No.....	Probable, 1915
57	20	Aug. 22	No.....	Yes, Sept. 29 <sup>1</sup>	No.....	
58	21	Aug. 22	No.....	Yes, Sept. 22 <sup>1</sup>	No.....	
59	21	Sept. 3	No.....	Yes, Sept. 9 <sup>1</sup>	No.....	
60	20	Aug. 24	No.....	Yes, Sept. 22 <sup>1</sup>	No.....	
61	22	Sept. 11	No.....	Yes, Sept. 29 <sup>1</sup>	Probable several attacks.	
62	18	Mar. 28	No.....	Yes, Sept. 24 <sup>1</sup>	No.....	
63	20	July 1	No.....	No.....	No.....	

<sup>1</sup> Diagnosis in Naval Health Record.

Influenza had burnt itself out on Deer Island, and the possibility that the volunteer subjects of our experiments might be insusceptible was given careful consideration. While planning the program we even doubted the desirability of working with men who had so recently been exposed. In other words, it was logical to assume that these men having passed through the fire might not be burned because they were fireproof.

While this question of the susceptibility of the volunteer subjects has been a matter of concern throughout the work, we hoped to neutralize this factor by using 10 or more men for each experiment, assuming that in so large a group a sufficient number would be susceptible, especially to large amounts of the infecting virus.

Recognizing the drawback presented by the uncertain receptivity of our subjects, it seemed desirable to take advantage of any opportunity to work with subjects not known to have been exposed to the prevailing epidemic, and thus more probably susceptible. Learning of such possible group at the naval training station at Yerba Buena Island, San Francisco, a party of workers was dispatched from Washington jointly by the Public Health Service and the Bureau of Medicine and Surgery of the Navy, to attempt a similar study. The report of this party appears in this bulletin, page 42.

TABLE II.—Summary of Boston experiments, November and December, 1918.

Ex- peri- ment No	Date, 1918.	Material.				Recipients.		Remarks.	
		Kind.	Source.		Quantity.	Mode of inoculation.	Presumably non-immunes.		With doubtful or definite history of pre- vious attack.
			Donor	Stage of illness.					
1	Nov. 13	Pfeiffer's bacillus, saline solution sus- pension.	W. K. ....	Second day .....	Approximately $\frac{1}{2}$ loopful of an 18 - hour cul- ture.	Instilled into nose	Nos. 2, 13, 30....	Nos. 57, 58, 60.	No appreciable effect.
2	Nov 16	A. Secretions from upper air passages in saline solution; unfiltered.	W. W. D. .... K. J. J. .... F. D. E. ....	Third day .....	Not measured...	Instilled into nose and sprayed into nose and throat.	Nos. 5, 24, 26, 29, 31, 32, 35, 63.	Nos. 25, 33....	The inoculations were made 5 to $5\frac{1}{2}$ hours after securing secretions. One of the vol- unteers, No. 29, developed fever 36 hours after inocula- tion, considered as prob- ably due to an inflamed throat, but influenza could not be excluded. The others showed no reaction Nos. 16, 18, 19, 21, and 27 also received instillation in the eyes. Inoculations were made 4 to $4\frac{1}{2}$ hours after securing secretions. No appreciable reactions.
				Fourth day .....					
3	Nov 21	Secretions from up- per air passages in saline solution; unfiltered.	A. B. M. ... C. R. .... G. J. J. ... H. L. W. ... F. H. H. ... B. R. H. ... M. R. J. ... R. E. L. ...	62 hours after onset..	Not measured...	Instilled into nose, eyes and sprayed into nose and throat.	Nos. 8, 10, 20, 22, 40, 45, 46, 49, 53.	No. 43.....	Inoculations made about 1 hour and 40 minutes after securing secretions. No appreciable reactions.
				38 hours after onset..					
4	Nov. 23	Secretions from nose and naso-pharynx.	S. T. J. ... K. L. P. ... McC. J. ... O. A. .... H. M. .... McL. C. F.	58 hours after onset..	Not measured...	Transfer by swab from nose to nose and throat to throat.	Nos. 9, 35..... No. 14..... No. 5..... Nos. 16, 32..... No. 63..... Nos. 18, 19..... No. 21..... Nos. 24, 26..... No. 31.....	No. 33..... No. 4..... Nos. 3, 25..... No. 1..... No. 27.....	The time elapsing between donor and recipient did not exceed 30 seconds in any instance. No appre- ciable reactions.
				44 hours after onset..					
				57 hours after onset..					
				33 hours after onset..					
				70 hours after onset..					
				45 hours after onset..					
				55 hours after onset..					
				57 hours after onset..					
				42 hours after onset..					
				31 hours after onset..					
				57 hours after onset..					
				51 hours after onset..					

5	Nov. 25	Filtered secretions from upper air passages.	R. W. F. . . . . M. V. . . . . B. C. H. . . . . R. F. J. . . . . E. F. J. . . . .	46 hours after onset. 8 hours after onset. 7 hours after onset. 31 hours after onset. 73 hours after onset.	Not measured.	Subcutaneous.	Nos. 20, 28, 36, 37, 38, 42, 44, 52.	Nos. 17, 51. . . .	Interval between securing secretions and inoculation varied between 2 and 5 hours. No appreciable reactions. The donors in this experiment also furnished the blood for the next experiment (No. 6).
6	Nov. 25	Blood from venous circulation.	R. W. F. . . . . M. V. . . . . B. C. H. . . . . R. F. J. . . . . E. F. J. . . . . N. T. C. . . . . F. L. M. . . . . Y. E. . . . . N. W. A. . . . .	50 hours after onset. 12 hours after onset. 11 hours after onset. 35 hours after onset. 77 hours after onset. 21 hours after onset. 10 hours after onset. 27 hours after onset. 56 hours after onset.	1.5 c. c. from each of the 5 donors.	Subcutaneous.	Nos. 2, 6, 13, 23, 30, 39, 47, 48.	Nos. 11, 12. . . .	Interval between drawing blood and inoculation not over 45 minutes. No appreciable reactions.
7	Nov. 26	"Droplet," breath, and close contact.	K. A. L. . . . . F. L. W. . . . . G. W. F. . . . . B. E. C. B. . . . . B. C. L. . . . . M. V. . . . .	30 hours after onset. 72 hours after onset. 24 hours after onset. 84 hours after onset. 34 hours after onset. 34 hours after onset.	-----	Direct exposure in close contact for from 3 to 5 minutes to each donor.	Nos. 8, 10, 20, 22, 40, 45, 46, 49, and 53.	No. 43. . . . .	No appreciable reactions.
8	Dec. 2	Pfeiffer's bacillus, suspension in saline solution of 13 strains. Strains 1 to 13, Table III.		See table. . . . .	0.5 c. c. of suspension representing about one billion organisms.	Sprayed into nose and throat.	Nos. 6, 20, 28, 37, 38, 39, 44, 48, and 52.	Nos. 7, 15, 34, 41, 51, 54, 56, 59, 60, 61.	About 48 hours after inoculation volunteer No. 38 complained of headache and sore throat and temperature rose to 38° C. Next day temperature was normal and he remained well. Otherwise nothing of significance.

## DESCRIPTION OF EXPERIMENTS.

EXPERIMENT NO. 1—WITH A SINGLE CULTURE OF *B. INFLUENZAE*.

On November 13, 1918, we inoculated six men with a saline suspension of a culture of Pfeiffer's bacillus. Three of the men (Nos. 2, 13, and 30) were nonimmunes, i. e., were not known ever to have had an attack of influenza; the other three (Nos. 57, 58, and 62) were presumably immune, having a history of an attack of the disease during the recent epidemic, and were used as controls.

The culture (No. 14) was isolated from the sputum of a case (W. K.)<sup>1</sup> on November 9, the second day of the disease. When used, it was an 18-hour blood-agar culture, the fifth generation on artificial media. Approximately one loopful of the 18-hour culture was rubbed up in 6 c. c. of saline solution and 1 c. c. of this suspension instilled into the nose of each of the six subjects. The instillation was made with the subject on his back, about 0.5 c. c. being instilled into each nostril.

*Results.*—No appreciable effects were observed following this inoculation.

## EXPERIMENT NO. 2—CRUDE AND FILTERED SECRETIONS.

On November 16 secretions were secured from the upper respiratory passages of three cases of influenza at the Peter Bent Brigham Hospital. Two of the cases (W. W. D. and K. J. J.) came from a barracks building of a school in which there was an outbreak of influenza. The third case (F. D. E.) was that of a student from another school at which there was an outbreak of the disease. The first two cases (W. W. D. and K. J. J.) were in the third day of their illness, and the third (F. D. E.) in the fourth day, when the secretions were secured.

At about 12 o'clock noon, mouth, nasal, and pharyngeal washings, bronchial sputum, pharyngeal and nasopharyngeal swabs were collected in sterile physiological saline solution from each of the cases and the three sets of specimens pooled in a single sterile bottle and shaken with beads. Part of these pooled secretions was filtered through Mandler filters, the filtration lasting about 2.4 hours. The secretions were taken to Gallups Island and there used for the following inoculations:

(a) *Unfiltered secretions.*—The crude secretions in saline solution were used for the inoculation of 10 men. This inoculation was made between 5 and 5.30 p. m., or approximately 5 to 5½ hours after the secretions were secured. The recipients were Nos. 5, 24, 25, 26, 29, 31, 32, 33, 35, and 63. None of these men had a history of an attack of the disease during the recent epidemic. One (No. 33), however, gave a history of having had an influenza-like attack in 1915; another (No. 25) gave a history of an illness in 1916 which

<sup>1</sup> See history of cultures for details, Table III; Appendix B, page 28.



may have been such an attack, thus leaving eight of the men without a history of influenza or influenza-like sickness at any time.

The inoculation was made by spraying the nose and throat and by instilling into the nostrils. It was estimated that each man received in these ways, in all, between 5 and 6 c. c. of the mixed unfiltered suspension of the secretions.

(b) *Filtered secretions*.—The filtrate obtained by passing the secretions through Mandler filters was used for the inoculation of 10 men. The inoculation was made between 4.30 and 5 p. m., or 4 to 4½ hours after the secretions were collected. The recipients were Nos. 1, 3, 4, 9, 14, 16, 18, 19, 21, and 27.

Of these 10 men 8 were without a history of an attack of the disease during the recent epidemic. One (No. 1) is reported to have had an attack in September, and one (No. 27) gave a doubtful history of such an attack.

Of 2 of the men who gave no history of influenza during the recent epidemic, one (No. 4) gave a history of an influenza-like attack in 1917; the other (No. 3) gave a doubtful history of such attacks in 1916 and 1917, so that of this group of 10 men, 6 were without history of influenza or influenza-like sickness at any time. The inoculation was made by spraying the nose and throat and by instillation into the nose. In the case of 5 of these men, viz, 16, 18, 19, 21, and 27, a drop or two of the filtrate was also instilled into each eye. In all, each of the 10 men received not less than 5 c. c. of the filtrate.

In both the group of men receiving the crude, and in that receiving the filtered secretions some, if not all, of the men in all probability swallowed some of the material.

*Results*.—With one exception, none of the above two groups of men developed any unpleasant effects. The exception was that of volunteer No. 29, inoculated with unfiltered secretions. About 36 hours after the inoculation, this young man's temperature rose and remained above normal for a week. (See chart 1.) Subjectively, he made almost no complaint; his tonsils, which before the inoculation were noted to be considerably enlarged, became somewhat more swollen and red. The submaxillary glands were slightly enlarged and somewhat tender. The only other physical findings were a few coarse râles, heard posteriorly at lower angle of the right scapula, which persisted for several days. Once or twice he mentioned some indefinite pains in the chest and some soreness of the throat. These poorly defined subjective symptoms were not complained of until about 30 hours after his rise in temperature.

There was no complaint of weakness, nor was there any appearance of prostration. Blood examination showed on November 19, W. B. C. 8,000, on November 20, 6,000, and on November 21, 9,000. Throat culture on November 20 showed hemolytic and also green producing streptococcus colonies.

On November 20 he was seen with us in consultation by Lieut. Commander McGuire, United States Navy, and Lieut. Redden, United States Naval Reserve Force. It was agreed that the manifestations recorded were probably due to the inflamed condition of the throat, but a diagnosis of influenza could not positively be excluded.<sup>1</sup>

#### EXPERIMENT NO. 3—CRUDE SECRETIONS.

On November 21, 1918, secretions were secured from the upper respiratory passages of four cases of influenza at the Chelsea Naval Hospital and used for the inoculation of 10 men. The interval between the collection of the secretions and inoculation was one hour and 40 minutes.

The donors were A. B. M., who furnished the secretions about 62 hours after the onset of his symptoms; C. R., who furnished secretions about 38 hours after the onset; G. J. J., who furnished secretions about 58 hours after the onset; and H. L. W., who furnished secretions about 44 hours after the onset.

The secretions were secured by washing out the nose with physiologic salt solution, by swabbing the pharynx and naso-pharynx, and by having the donors cough and expectorate bronchial and buccal secretions into a sterile receptacle.

The secretions from the four cases were mixed and shaken in a sterile bottle with glass beads. In transit to Gallups Island the bottle containing the secretions was carried in the pocket in order to prevent too great chilling. The interval elapsing between the collection of the material and the completion of the inoculation was 1 hour and 40 minutes.

The inoculations were made by spraying the crude material into the nose and throat, and by instilling some of it into the eyes and nose of each of the 10 volunteers. Approximately 6 c. c. of the saline suspension was given to each volunteer. The recipients, 10 in number, were volunteers Nos. 8, 10, 20, 22, 40, 43, 45, 46, 49, and 53. Of these 10 men none had a record of influenza during the recent epidemic; 1 (No. 43), however, had a doubtful history of a previous influenza-like attack.

*Results.*—None of these men experienced any unpleasant effects following the inoculation.

#### EXPERIMENT NO. 4.—DIRECT TRANSFER OF SECRETIONS FROM NOSE TO NOSE AND THROAT TO THROAT.

On November 23, 1918, 19 of the 20 men used in experiment No. 2, having been under observation for seven days and not having shown

<sup>1</sup> He is reported to have subsequently developed an attack of influenza, lasting from Jan. 28 to Feb. 4, while on furlough in New York City.

any evidence of illness (with the single exception, No. 29, already discussed), were submitted to another test.

It occurred to us that our failure to reproduce the disease thus far might be due to several factors, two of which we decided to eliminate. These two factors were (1) the time which elapsed between collecting the material from the donors and introducing it into the volunteer recipients, and (2) the salt solution. By transferring the secretions directly from nose to nose, and from throat to throat, the time interval was reduced to a minimum, and the salt solution eliminated.

In this experiment, then, cotton applicators, consisting of "diphtheria swabs," were used to transfer the muco-purulent secretions directly from nose to nose; and "West tubes" were used to transfer the material from throat to throat. The time interval between donor and recipient was not over 30 seconds.

In this experiment there were 10 donors, from each of which transfers of secretions were made to each of a pair of recipients, with one exception, in which there was only a single recipient.

In the manner described, nasal and naso-pharyngeal secretions were transferred:

(a) From case F. H. H., 57 hours after onset of illness, to volunteers Nos. 9 and 35, neither of whom had a history of an attack of influenza at any time.

(b) From case B. R. H., 33 hours after the onset, to volunteers Nos. 14 and 33, neither of whom had a history of influenza in the recent epidemic, but one of whom (No. 33) had a history of an influenza-like attack in 1915.

(c) From case M. R. J., 70 hours after the onset, to volunteers Nos. 4 and 5, neither of whom had a history of an attack during the recent epidemic, but one of whom (No. 4) gave a history of an influenza-like attack in 1917.

(d) From case R. E. L., 45 hours after the onset, to volunteers Nos. 3 and 25, neither of whom had a history of influenza during the recent epidemic, but both of whom gave a more or less doubtful history of an influenza-like attack, No. 3 in 1916 and 1917, and No. 25 in 1916.

(e) From case S. T. J., 55 hours after the onset, to volunteers Nos. 16 and 32, neither of whom had a history of influenza at any time.

(f) From case K. L. P., 57 hours after the onset, to volunteers Nos. 1 and 63, the former of whom (No. 1) had a history of an attack during the recent epidemic, while the latter (No. 63) was without history of the disease at any time.

(g) From case McC. J., 42 hours after the onset, to volunteers Nos. 18 and 19, neither of whom had a history of the disease at any time.

(h) From case O. A., 31 hours after the onset, to volunteers Nos. 21 and 27, the former of whom (No. 21) was without a history of

influenza at any time, while the latter (No. 27) gave a doubtful history of a mild attack, both during the recent epidemic and in 1917.

(i) From case H. M., 57 hours after the onset, to volunteers Nos. 24 and 26, neither of whom had a history of influenza at any time.

(j) From case McL. C. F., 51 hours after the onset, to volunteer No. 31, who had no history of ever having had an attack of influenza.

All of the donors above mentioned were from the U. S. S. *Yacona*.<sup>1</sup>

*Results.*—None of the volunteers showed any unpleasant effects following the inoculation.

#### EXPERIMENT NO. 5—SUBCUTANEOUS INJECTION OF FILTERED SECRETIONS.

November 25, 1918. This experiment was designed to test the infectivity of filtered secretions from the upper air passages of cases of influenza when given subcutaneously, following Nicolle and Lebailly.<sup>2</sup>

On November 25 secretions were obtained as nasal, pharyngeal and mouth washings, bronchial sputum, and pharyngeal swabs, in sterile physiological solution from case R. W. F., 46 hours after onset of illness, from case M. V., 8 hours after the onset, and from case B. C. L., 7 hours after the onset, mixed and shaken with beads.

Secretions were similarly secured from case R. F. J., 31 hours after the onset, and from case E. F. J., 73 hours after the onset, likewise mixed and shaken with beads. The two sets of specimens of secretions were then separately filtered through Mandler filters; the first through filters with 11 pounds positive pressure value, the second through a filter of 9 pounds pressure value. After filtration, 2.5 c. c. of the filtrate of the first of the two specimens and about 2 c. c. of the filtrate of the second were subcutaneously inoculated into each of the following 10 volunteers, Nos. 17, 20, 28, 36, 37, 38, 42, 44, 51, and 52.

Of these men, none gave a history of an attack during the recent epidemic. One (No. 17) gave a doubtful history of an influenza-like attack in April, 1918, and one (No. 51) gave a history of such an attack in 1916. Of this group, therefore, eight were without a history of influenza or influenza-like illness at any time.

The time that elapsed between securing the secretions and the inoculation of the men with the filtrate was about 2 to 2.5 hours with respect to the first of the two sets of specimens above mentioned and about 5 hours with respect to the filtrate of the second set.

*Results.*—None of the men developed any appreciable reaction following this inoculation.

<sup>1</sup> Appendix C, page 30.

<sup>2</sup> C. Rend. Acad. d. Sc., 1918, vol. 167, p. 607.



# EXPERIMENT NO. 6—SUBCUTANEOUS INJECTION OF BLOOD FROM INFLUENZA CASES.

November 25, 1918. This experiment was designed to test the infectivity of the blood of cases of influenza, when inoculated subcutaneously, following Nicolle and Lebailly.<sup>1</sup> On November 25 blood was drawn from the venous circulation (arm vein) of each of five cases of influenza; the patients were the same as those furnishing the secretions in the immediately preceding experiment (No. 5) but about 4 hours later, so that when the blood was drawn the patients were from 11 to 77 hours after the onset of their illness. About 20 c. c. of blood was drawn from each patient into a syringe containing about 4 c. c. of sterile 5 per cent sodium citrate solution. The five specimens of blood thus drawn were pooled and 10 c. c. (representing approximately 1.5 c. c. of undiluted blood from each of the five cases) subcutaneously injected into each of the following volunteer subjects: Nos. 2, 6, 11, 12, 13, 23, 30, 39, 47, and 48. Of these men, nine were without history of an attack during the recent epidemic, one (No. 11) had such history, and of the nine, one (No. 12) had a history of an influenza-like attack in 1917, so that of the group, eight were without history of influenza or influenza-like illness at any time.

Of this group of subjects, three—Nos. 2, 13 and 30—had been used previously in experiment No. 1 (inoculation with Pfeiffer's bacillus).

The interval between drawing the blood and inoculating it did not exceed 45 minutes in any case.

*Results.*—Aside from slight soreness at the site of inoculation lasting not over 24 hours, there was no appreciable effect following the inoculation.

# EXPERIMENT NO. 7—DIRECT CONTACT.

November 26, 1918. This experiment was designed to test the transmissibility of influenza by what is assumed to be the natural means, viz, by the expired breath and cough.

The 10 volunteers previously used in Experiment No. 3, in which they were inoculated with mixed unfiltered secretions from the upper respiratory passages from active cases of influenza, were used in the present experiment. They were taken to the naval hospital at Chelsea and in a ward in which 30 cases of influenza were being treated, were exposed to infection from 10 especially selected acute cases, as follows:

Case N. T. C., about 21 hours after onset of illness; case F. L. M., about 10 hours after onset of illness; case Y. E., about 27 hours after

<sup>1</sup> Loc. Cit.



onset of illness; case N. W. A., about 56 hours after onset of illness; case K. A. L., about 30 hours after onset of illness; case F. L. W., about 72 hours after onset of illness; case G. W. F., about 24 hours after onset of illness; case B. E. C. B., about 84 hours after onset of illness; case B. C. L., about 34 hours after onset of illness; case M. V., about 34 hours after onset of illness.

Each volunteer took a position close to the bedside of one of the selected patients and conversed with him for two or three minutes, then the patient was directed to breathe five times and then cough five times directly into the face of the volunteer. After this was done the volunteer proceeded to the bedside of a second patient. In this manner each of the volunteers was exposed in succession to each of the 10 selected cases, the exposure to each being between three and five minutes. The total exposure for each volunteer, therefore, was between 30 and 50 minutes.

*Results.*—None of these volunteers developed any indications of illness following this exposure.

#### EXPERIMENT NO. 8—INSTILLATION OF A MIXTURE OF 13 DIFFERENT STRAINS OF PFEIFFER'S BACILLUS.

On December 2, 1918, we inoculated 19 volunteers with a suspension in a saline solution of 13 strains of pure culture, of Pfeiffer's bacillus. Of the volunteers 10 (Nos. 6, 20, 28, 37, 38, 39, 44, 48, 51, and 52) were nonimmunes, i. e., were without history of an attack of influenza in the recent epidemic, and, with one exception (No. 51) were without a history of an influenza-like attack at any time. In the case of this one man (No. 51) there was a history of what may have been an influenza-like attack in 1916. The other nine volunteers, viz, Nos. 7, 15, 34, 41, 54, 56, 59, 60, and 61, had histories of a definite, or (in two instances, Nos. 7 and 41) a probable attack of the disease during the recent epidemic and served as controls.

A memorandum relative to the origin of the strains of Pfeiffer's bacillus, with certain other pertinent data, is given in Appendix B and a summary is presented in Table III. All 13 strains were isolated from cases of influenza occurring during the recent epidemic. Four of the strains were isolated within five days of the date of inoculation and had been on artificial culture media for not over five generations; two of them, indeed, had been on artificial media for not over 48 hours at the time of inoculation.

TABLE III.—*Cultures used in Boston experiments November and December, 1918.*

No.	Culture.		Interval between isolation of culture and inoculations.	Medium.	Transplant used.
	Designation.	Source.			
1	McC.....	Lungs at necropsy.....	5 days.....	Heated and filtered blood agar.	Fifth.
2	K-OC.....	Nasopharynx, life.....	48 hours.....	do.....	First.
3	K-CF.....	do.....	do.....	do.....	Do.
4	U-W.....	Lungs at necropsy.....	5 days.....	do.....	Fourth.
5	H-E.....	Washed bronchial sputum, life.....	13 days.....	do.....	Seventh.
6	Youngstown.....	Lungs at necropsy.....	12 days.....	do.....	(?)
7	P-BH (123).....	do.....	26 days.....	do.....	Fifteenth (?)
8	Card.....	do.....	38 days.....	do.....	
9	Staizecki.....	do.....	do.....	do.....	
10	Butler.....	Lungs, life (?).....	do.....	do.....	
11	CD (112).....	do.....	do.....	do.....	
12	CD (157).....	do.....	do.....	do.....	
13	Park (103).....	do.....	do.....	do.....	
14	WK.....	Sputum, life, second day..	5 days.....	Whole blood agar.....	Fifth.

See Appendix B, p. 28.

Each of the strains was planted on special blood agar slants<sup>14</sup> on December 1 at 3 p. m. at the laboratory of the Chelsea Naval Hospital. This medium was prepared by adding 10 per cent of defibrinated sheep's blood to melted plain agar, neutral to phenolphthalein, and then boiling and filtering through sterile gauze, the resulting medium being perfectly clear and very favorable to the growth of Pfeiffer's bacillus.

At 11.15 a. m. December 2, the cultures were taken from the incubator, placed in a warm box, and thus transferred to Gallups Island, where they were placed in an incubator at 1 p. m. At 1.45 p. m. a suspension of the growth of each strain on a slant was made in a total of 25 c. c. of warm dextrose beef broth. The growth from but a single slant was used in the case of all strains except Nos. 1, 2, and 3. Of the latter the growth from two slants of each of strains Nos. 1 and 2 and of four from No. 3 was used; thus, in the preparation of the suspension, the growth from eighteen slants in all was used, and the suspension included increased proportions of three of the most recently isolated strains.

A bacterial count of the suspended bacilli by Wright's capillary tube method in comparison with red blood cells showed approximately 2 billion per cubic centimeter.

The inoculation was made between 2.05 and 2.22 p. m. by spraying this suspension into the nose and pharynx, the volunteer taking a deep inhalation when the throat was sprayed. In this manner each man received approximately 0.5 c. c. of the suspension containing about 1 billion bacilli.

<sup>14</sup> Levinthal, W., *Influenza. Bakteriologische und serologischen Studien.* Berl. klin. Wchnschr. 1918, XLIV. 972. Abstracted in J. Am. Med. Association, 1918, LXXI, 1578.

The cultures were carried back to the naval hospital and transplants made from each tube used and also from the remainder of the broth suspension. All transplants gave abundant growths of Pfeiffer's bacillus.

All cultures used were identified morphologically and culturally immediately before and after the experiment.

*Results.*—About six hours after the inoculation volunteer No. 28 had an attack of vomiting and complained of malaise which, however, had begun before the inoculation. His temperature did not rise above normal and he appeared well the next day and remained so.

About 48 hours after the inoculation volunteer No. 38 complained of headache and sore throat and his temperature rose to 38° C. The next day his temperature was normal and he appeared well, and remained so throughout the remainder of the period of close observation of seven days.

Aside from the foregoing developments all of the volunteers remained in good health; none showed any evidence of influenza.

### SUMMARY.

*Subjects.*—Sixty-two volunteers, varying in age from 15 to 34 years, were the subjects of experiment. Of these 39 were without history of an attack of influenza at any time; 14 gave a history of this disease; and 9 had a history of attacks of a doubtful nature. All, however, had been exposed in varying degrees to the epidemic at Deer Island or at a previous station or place.

*Experiments.*—Eight experiments were made: In two, pure cultures of Pfeiffer's bacillus were used, inoculations being respectively by instillations into the nose and spraying of the nose and throat.

In two, unfiltered secretions from the upper respiratory passages were sprayed into the nose and throat; in one of these some of the secretions were also instilled into the eyes.

In one, filtered secretions from the upper respiratory passages were sprayed into the nose and throat and instilled into the eyes, and in another experiment such a filtrate was injected subcutaneously.

In one experiment direct transfers of secretions from nose and nasopharynx by means of swabs were made from nose to nose and from nasopharynx to nasopharynx.

In one experiment freshly drawn citrated blood was injected subcutaneously.

In one experiment there was exposure by close contact to expired breath and "droplet" infection.

*Donors.*—The experimental material was obtained from and exposure made to cases of influenza in various stages of the disease and of different grades of severity. The donors were selected from epidemic groups, thus minimizing the chance of mistake in selection of isolated cases. The crude secretions were obtained from

cases in the second, third, and fourth days of the disease. The secretions in one of the filtration experiments (inoculated subcutaneously) were from cases as early as the eighth and ninth hour after the onset. In the contact and droplet infection experiment the donors were from 10 to 84 hours after the onset of their respective attacks, and in the blood inoculation experiment the donors were from 11 to 77 hours after the beginning of their sickness.

*Results.*—In only one instance (Experiment 2 (a)) was any reaction observed in which a diagnosis of influenza could not be excluded, and here a mildly inflamed throat seemed the more probable cause of the fever and other symptoms. Nothing like influenza developed in the other volunteers.

### DISCUSSION OF RESULTS.

The results of our experiments do not warrant positive conclusions. The negative character of our results is surprising when we call to mind the very high communicability of the disease and the fact that the incidence rate in the recent epidemic was usually 20 per cent, often 30 per cent or more of the population. The incidence of the disease on the U. S. S. *Yacona*, from which we took a number of donors, was 84.2 per cent.

In explanation of our failure to reproduce the disease, many factors naturally suggest themselves for consideration. Among these, the susceptibility of the volunteers and the stage of the disease at which the secretions from the upper respiratory passage were secured stand out as perhaps of the first order.

It is possible that all our volunteers resisted infection because of a natural or an acquired immunity. If this be true, then we have an indication of a much higher degree of immunity to this disease than is generally assumed. The fact that our colleagues in the San Francisco studies (q. v. p. 53) failed to reproduce the disease in volunteers who had not been exposed in the recent pandemic suggests that the immunity of our volunteers was at least not the sole controlling factor.

Epidemiological evidence points to the likelihood that influenza is most communicable during its early stages. Most of our material was obtained during the first, second, or third days of the disease, sometimes as early as the eighth or tenth hour after the beginning of symptoms. In no case, however, did we obtain material during the period of incubation. If our volunteers were susceptible, then it could be argued that the material used did not contain the virus.

Despite our negative results, it is nevertheless probable that the disease is transmitted by the discharges from the mouth and nose. Our failure, however, to reproduce the disease with these discharges suggests that there may be unknown factors involved, either in the discharge of the virus from the body, or its entrance into the victim, or both.



## APPENDIX A.

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### DONORS.

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*A. B. M.* (Sea-1, age 23, U. S. S. *New Jersey*).—The onset of illness was Monday, November 18, at midnight. The patient awoke feeling hot, dizzy, nauseated, and weak. He had a bad headache, and his bones and joints ached. He reported to sick bay Tuesday morning with a temperature of 101° F. He had no sore throat or chest pains; an occasional cough. The leucocyte count November 21 was 7,200, polymorphonuclears 76 per cent, mononuclears 24 per cent. (Chart 2.)

This patient gave no previous history of influenza, although having been in very close contact with it on the ship during an outbreak about October 1. He was perfectly well preceding this attack and recovered without complications.

Furnished secretions from upper respiratory passages on November 21 between 2.20 and 2.35 p. m. for use in Experiment No. 3.

*B. C. L.* (Sea-2, age 19, U. S. S. *Yacona*).—The onset of illness was Monday, November 25, at 6 a. m. The patient awoke with a headache, a slightly sore throat, chilly sensations, and eyes sensitive to light. The leucocyte count November 27 was 4,500, polynuclears 52 per cent, lymphocytes 58 per cent. He recovered without complications. (Chart 3.)

On November 25, at 1 p. m., seven hours after the onset, this patient furnished secretions from the mouth, pharynx, and bronchi, which were used in Experiment No. 5, and four hours later (11 hours after the onset) blood, which was used in Experiment No. 6. He was used a third time, November 26, 34 hours after the onset, in Experiment No. 7, for direct exposure of volunteers.

*B. R. H.* (El-1, age 26, U. S. S. *Yacona*).—The onset of illness was Friday, November 22, at 6 a. m. The patient felt well the night before. He awoke with headache, pains in his back, a slight cough, eyes and nose congested. The leucocyte count November 23 was 8,200, polynuclears 73 per cent, lymphocytes 25 per cent, transitionals 3 per cent. He recovered, with questionable pneumonic complications. (Chart 4.)

On November 23, 1918, 33 hours after the onset, this patient furnished secretions from the nasal fossae and posterior nasopharynx, which were used in Experiment No. 4.

*B. E. C. B.* (Ch. Com. St., age 26, U. S. S. *Yacona*).—The onset of illness was Saturday, November 23, at 6 a. m. The patient felt tired Friday, with a slight headache; Saturday he felt tired all over, with backache. He had no sore throat. The leucocyte count November 27 was 3,600, polynuclears 61 per cent, lymphocytes 38 per cent, transitionals 1 per cent. Recovered, with questionable pneumonic complications. (Chart 5.)

On November 26, 1918, 84 hours after the onset, this patient was used in Experiment No. 7 for direct exposure of volunteers.

*C. R.* (Sea-2, age 28, radio school).—The onset of illness was Tuesday, November 19, at midnight. The patient felt dizzy, with headache, vomiting, and pains in his legs. He first sweat and then felt cold. He had no sore throat. He felt perfectly well Tuesday evening, before midnight. The leucocyte count November 21 was 6,400, polynuclears 55 per cent, lymphocytes 43 per cent, transitionals 1 per cent, basophiles 1 per cent. Recovered, without complications. (Chart 6.)



He gave no previous history of influenza, although he was at the radio school during the first outbreak there. He said the sick bay was full of similar cases the day he reported, November 20.

On November 21, at about 2.20 p. m. (or about 38 hours after the onset) furnished secretions from upper respiratory passages for Experiment No. 3.

*F. F. J.* (Sk-3, age 25, U. S. S. *Yacona*).—The onset of illness was Friday, November 22, at noon, when he felt weak. Friday night he felt sore all over and chilly. He had felt well before this onset. On November 25 the leucocyte count was 3,400, polynuclears 66 per cent, lymphocytes 34 per cent. Developed pneumonia. Recovered. (Chart 7.)

On November 25, 1918, 75 hours after the onset, this patient furnished secretions from the mouth, nose, pharynx, and bronchi, which were used in Experiment No. 5, and, four hours later, blood, which was used in Experiment No. 6.

*F. H. H.* (Qm-1, age 24, U. S. S. *Yacona*).—The onset of illness was Thursday, November 21, at 6 a. m. He awoke with headache, chilliness, pains in his muscles and joints, a dry throat and chest, and an occasional cough. He felt weak the night before. His temperature at sick bay Thursday morning was 102.2°. The leucocyte count November 23 was 9,400, polynuclears 58 per cent, lymphocytes 38 per cent, transitionals 4 per cent. Recovered, with questionable pneumonic complications. (Chart 8.)

On November 23, 1918, 57 hours after the onset, this patient furnished secretions from the nasal passages and posterior nasopharynx, which were used in Experiment No. 4.

*F. D. E.* (student, age 19, female).—The onset of influenza was November 12. Initial symptoms were a severe headache and backache; some cough and fever. Leucocyte count November 15 was 6,400, polynuclears 51 per cent, lymphocytes 48 per cent, eosinophiles 1 per cent. Recovered without complications. (Chart 9.)

On November 16, about 12 m., on the fourth day of illness, furnished secretions for Experiment No. 2.

*F. L. M.* (Sea.-2, age 18, U. S. S. *Yacona*).—The onset of illness was Tuesday morning, November 26; the only symptom was a fever of 101°. Had no aches or pains. November 27 the leucocyte count was 5,400, polynuclears 53 per cent, lymphocytes 41 per cent, transitionals 3 per cent, basophiles 1 per cent, eosinophiles 2 per cent. Recovered without complications. (Chart 10.)

On November 26, 1918, 10 hours after the onset, this patient was used in Experiment No. 7 for direct exposure of volunteers.

*F. L. W.* (Sea.-1, age 20, U. S. S. *Yacona*).—The onset of illness was Saturday, November 23, in the afternoon. It began with headache, weakness, aching in bones and joints. The patient felt dizzy, his throat was dry, and he coughed a little. The leucocyte count on November 27 was 3,400, polynuclears 40 per cent, lymphocytes 60 per cent. Recovered without complications. (Chart 11.)

On November 26, 1918, 72 hours after the onset, this patient was used in Experiment No. 7.

*G. J. J.* (El-R., age 22, radio school).—The patient had been in the radio school, Cambridge, Mass., since the first appearance of pandemic influenza in Boston, and had been in contact with cases of influenza at the radio school and in Boston during the outbreak of the early part of September. He did not contract the disease at that time. A recurrent outbreak occurred at the radio school soon after the Liberty Day celebrations of November 11 and 12. There were about 80 cases in the sick bay at the time G. J. J. entered, mostly of very mild type.

The onset of illness was Tuesday, November 19, at 4 a. m. The initial symptoms were a dizzy headache, aches in the back and legs, and some pain in the stomach. There was no vomiting. The onset was sudden, except that on the preceding day at 5 p. m. the patient had felt a little poorly, and had applied at the sick bay for a

dose of salts. He complained of no sore throat and no previous ailment of any kind. On November 24 the leucocyte count was 6,200, polynuclears 52 per cent, lymphocytes 48 per cent. Recovered without complications. (Chart 12.)

November 21, about 58 hours after the onset, furnished material for Experiment No. 3.

*G. W. F.* (F-2, age 27, U. S. S. *Yacona*).—The onset of illness was Monday, November 25, at 3 p. m., suddenly while on watch. The patient felt weak and ached all over. He had no sore throat or dizziness. The leucocyte count November 27 was 3,300, polynuclears 57 per cent, lymphocytes 43 per cent. Recovered, with questionable pneumonic complications. (Chart 13.)

On November 26, 1918, 24 hours after the onset, this patient was used in Experiment No. 7.

*H. L. W.* (Cqm., age 29, M. I. T.).—The onset of illness was Tuesday, November 19, at 6 p. m. The patient suddenly felt quite ill, feverish, chilly, and with hot and cold flushes, a heavy feeling in his head, and aching pains in eyes and back of eyes. His extremities felt as though they were very heavy, with a mild aching, like fatigue. All day Tuesday he had felt a little ill, but the onset was definite and sudden. He had no sore throat, but had had a cold "in the head" for about three weeks, for which he had been going to sick bay occasionally. The leucocyte count November 21 was 5,400, polynuclears 50 per cent, lymphocytes 40 per cent. Recovered without complications. (Chart 14.)

The previous history showed contact with influenza during the first outbreak in Boston, without contracting it; his two little daughters had influenza at his home where he stayed.

November 21, about 44 hours after the onset, furnished material for Experiment No. 3.

*H. M.* (Sea., age 29, U. S. S. *Yacona*).—The onset of illness was Thursday, November 21, at 6 a. m. When the patient awoke he had pains in his head and chest. The back of his neck ached a little. He coughed considerably and had a raw throat. On November 23 the leucocyte count was 6,000, polynuclears 80 per cent, lymphocytes 14 per cent, transitionals 5 per cent, eosinophiles 1 per cent. Recovered without complications. (Chart 15.)

On November 23, 1918, 57 hours after the onset, this patient furnished secretions from the nasal fossa and posterior nasopharynx, which were used in Experiment No. 4.

*K. A. L.* (El-R-1, age 20, U. S. S. *Yacona*).—The onset of illness was Monday, November 25, in the forenoon. It began with a headache between the eyes. The patient had no sore throat or backache, no chilly or warm sensations. In the afternoon he felt dizzy and coughed a little, and his temperature was 101.6°. He had been well previously, except for a mild cold for about a week. Leucocyte count November 27, 8,400. Recovered without complications. (Chart 16.)

On November 26, 1918, 30 hours after the onset, this patient was used in Experiment No. 7 for direct exposure of volunteers.

*K. J. J.* (S. A. T. C., age 20).—The onset of influenza was November 13, 1918, in the afternoon. The initial symptoms were a fairly severe frontal headache, fever, and hoarseness, backache and general disagreeable feeling. Leucocyte count November 15 was 5,800, polynuclears 78 per cent, lymphocytes 14 per cent, large mononuclears 9 per cent, eosinophiles 2 per cent, mast cells 1 per cent. The urine showed numerous finely granular and coarsely granular casts. Recovered without complications. (Chart 17.)

On November 16, in the third day of illness (about 72 hours after onset), furnished secretions for Experiment No. 2.

*K. L. P.* (Sea., age 22, U. S. S. *Yacona*).—The onset of illness was Thursday, November 21, at 6 a. m. The patient felt slightly ill the night before. In the morning he had fever, headache, and backache. His throat felt a little dry and raw. On

November 23 the leucocyte count was 5,700, polynuclears 54 per cent, lymphocytes 44 per cent, transitionals 2 per cent. Recovered without complications. (Chart 18.)

On November 23, 1918, about 57 hours after the onset this patient furnished secretions from the nasal fossae and posterior nasopharynx which were used in Experiment No. 4.

*McC. J.* (F-2, age 27, U. S. S. *Yacona*).—The onset of illness was Thursday, November 21, between 6 and 12 p. m. The patient came off watch at midnight and was sweating considerably. His throat was sore, head dizzy, he had chilly sensations in his chest, fever, and pains in his back. On November 23 the leucocyte count was 10,000, polynuclears 72 per cent, lymphocytes 22 per cent, transitionals 5 per cent, eosinophiles 1 per cent. Developed pneumonia. Recovered. (Chart 19.)

On November 23, 1918, about 42 hours after the onset, this patient furnished secretions from the nasal fossae and posterior nasopharynx which were used in Experiment No. 4.

*McL. C. F.* (Bm-1, age 24, U. S. S. *Yacona*).—The onset of illness was Thursday, November 21, at noon. It began suddenly with headache, aching in bones and muscles all over. The patient felt chilly, his eyes burned and he had a raw throat, coughing a little. On November 23 the leucocyte count was 5,800, polynuclears 75 per cent, lymphocytes 23 per cent, transitionals 2 per cent. The leucocyte count November 27, during pneumonia, was 5,000, polynuclears 50 per cent, lymphocytes 49 per cent, transitionals 1 per cent. Recovered. (Chart 20.)

On November 23, 1918, 51 hours after the onset, this patient furnished secretions from the nasal fossae and the posterior nasopharynx which were used in Experiment No. 4.

*M. R. J.* (Mm-1, age 22, U. S. S. *Yacona*).—The onset of illness was Wednesday, November 20, at 5 p. m. The patient felt well Wednesday morning. The initial symptoms were chilly sensations, headache, pains in shoulders and back. His eyes burned. He coughed some at night and had pains in his chest. On November 23 the leucocyte count was 4,200, polynuclears 49 per cent, lymphocytes 46 per cent, transitionals 4 per cent, basophiles 1 per cent. Recovered, without complications. (Chart 21.)

On November 23, 1918, 70 hours after the onset, this patient furnished secretions from the nasal fossae and posterior nasopharynx which were used in Experiment No. 4.

*M. V.* (M. Att.-3, age 22, U. S. S. *Yacona*).—The onset of illness was on Monday, November 25, at 5 a. m. It started with a severe headache, shivering, a little cough, and weakness. The patient had had a cough for three to four weeks previously. On November 25 the leucocyte count was 4,800, polynuclears 66 per cent, lymphocytes 33 per cent, transitionals 1 per cent. Recovered, without complications. (Chart 22.)

On November 25, 1918, about 8 hours after the onset, this patient furnished secretions from the mouth, nose, pharynx, and bronchi, which were used in Experiment No. 5, and 4 hours later blood, which was used in Experiment No. 6. He was used a third time on November 26, 34 hours after the onset, in Experiment No. 7.

*N. T. C.* (Bm-2, age 34, U. S. S. *Yacona*).—The onset of illness was Monday, November 25, at 7 p. m. The symptoms were fever, chilliness, pains all over, a slight cough, and a heavy feeling in the chest. The patient had had a slight cough two or three days previously. On November 27 the leucocyte count was 5,600, polynuclears 58 per cent, lymphocytes 38 per cent, transitionals 2 per cent, eosinophiles 1 per cent, basophiles 1 per cent. Recovered, without complications. (Chart 23.)

On November 26, 1918, about 21 hours after the onset, this patient was used in Experiment No. 7.

*N. W. A.* (El-R-2, U. S. S. *Yacona*).—The onset of illness was Sunday, November 24, at 8 a. m. It started with aches all over the body and flashes of heat and cold. The patient had no sore throat, and had felt well previously. On November 27 the leucocyte count was 11,000, polynuclears 79 per cent, lymphocytes 21 per cent, this associated with signs of pneumonia. Recovered. (Chart 24.)

On November 26, 1918, about 56 hours after the onset, this patient was used in Experiment No. 7.

*O. A.* (F-1, age 32, U. S. S. *Yacona*).—The onset of illness was Friday, November 22, at 8 a. m. The patient awoke with a cough, headache, and aching in joints and muscles. He had had a slight cold the preceding three or four days. A severe pneumonia complication appeared November 26, due chiefly to a hemolytic streptococcus. (Chart 25.) The leucocyte count, November 23, was 5,200, polynuclears 69 per cent, lymphocytes 30 per cent, transitionals 1 per cent. A chronic empyema, cavity, with irregular fever, persisting to date, April 7, 1919.

On November 23, 1918, about 31 hours after the onset, this patient furnished secretions from the nasal fossae and posterior nasopharynx, which were used in Experiment No. 4.

*R. E. L.* (El-1, age 28, U. S. S. *Yacona*).—The onset of illness was Thursday, November 21, at 6 p. m. The initial symptoms were headache, chilliness, pains in back and legs. On November 23 the leucocyte count was 4,000, polynuclears 54 per cent, lymphocytes 43 per cent, transitionals 3 per cent. Recovered, with questionable pneumonic complications. (Chart 26.)

On November 23, 1918, 45 hours after the onset, this patient furnished secretions from the nasal fossae and posterior nasopharynx, which were used in Experiment No. 4.

*R. F. J.* (Qm-3, age 20, U. S. S. *Yacona*).—The onset of illness was Sunday, November 24, at 6 a. m. The patient felt well Saturday night at 10 p. m. He awoke with chilly sensations, and later in the morning had a severe headache and backache. His throat was a little dry and he coughed considerably. On November 25 the leucocyte count was 5,900, polynuclears 58 per cent, lymphocytes 42 per cent. Recovered, without complications. (Chart 27.)

On November 25, 1918, 31 hours after the onset, this patient furnished secretions from the mouth, nose, pharynx, and bronchi, which were used in Experiment No. 5, and 4 hours later blood, which was used in Experiment No. 6.

*R. W. F.* (F-2, age 22, U. S. S. *Yacona*).—The onset of illness was Saturday, November 23, at 3 p. m. Symptoms of fever and prostration developed suddenly. The patient complained of no aches, pains, or chills. He had felt well before the onset. Signs of pneumonia developed November 27. On November 25 the leucocyte count was 5,400, polynuclears 84 per cent, lymphocytes 16 per cent. Recovered. (Chart 28.)

On November 25, 1918, 46 hours after the onset, this patient furnished secretions from mouth, nose, pharynx, and bronchi, which were used in Experiment No. 5, and 4 hours later blood, which was used in Experiment No. 6.

*S. T. J.* (Bm-2, age 23, U. S. S. *Yacona*).—The onset of illness was at 8 a. m., November 21. The patient felt well the night before. The disease began with a severe headache and gastric discomfort. There was no sore throat. On November 23 the leucocyte count was 7,200, polynuclears 58 per cent, lymphocytes 40 per cent, transitionals 1 per cent, basophiles 1 per cent. Recovered, without complication. (Chart 29.)

On November 23, 1918, about 55 hours after the onset, this patient furnished secretions from the nasal fossae and posterior nasopharynx, which were used in Experiment No. 4.

*W. W. D.* (S. A. T. C., age 19).—The onset of illness was Tuesday afternoon, November 13. The initial symptoms were cold in the head and chest, cough, headache, and dizziness, but no backache. Leucocyte count, November 14, was 4,100, polynuclears 79 per cent, lymphocytes 16 per cent, large mononuclears 8 per cent, eosinophiles 2 per cent, mast cells 2 per cent. Sputum examination showed pneumococci and influenza bacilli. Questionable pneumonic complications. Recovered. (Chart 30.)

November 16, approximately 72 hours after onset of illness, secretions furnished for Experiment No. 2.



Y. E. (Cbm., age 27, U. S. S. Yacona).—The onset of illness was Monday, November 25, at noon. It started with a headache and a backache. The patient felt warm, his throat was dry, and he had a little cough. He had had no previous sore throat. On November 27 the leucocyte count was 4,800, polynuclears 29 per cent, lymphocytes 59 per cent, transitionals 8 per cent, basophiles 1 per cent, and eosinophiles 3 per cent. Recovery, without complications. (Chart 31.)

On November 26, 1918, about 27 hours after the onset, this patient was used in Experiment No. 7.



## APPENDIX B.

### HISTORY OF CULTURES OF PFEIFFER'S BACILLUS USED IN EXPERIMENTS.

(Table III.)

*No. 1. McC.*—This influenza bacillus was isolated from the lungs at necropsy of one of the cases of the U. S. S. *Yacona*, Tuesday, November 26, 1918, naval hospital, and the culture used in Experiment 8 was the fifth daily transplant. (Chart 32.)

The history of the case indicates a most virulent infection, the disease having lasted only three days. The onset was Saturday morning, November 23. A leucocyte count was not made.

The necropsy showed a coarse, firm, lobular consolidation in both inferior lobes, with beginning larger and more uniformly consolidated areas on both sides, at a site corresponding to the inferior angles of the scapulae.

The cultures from all lobes, except the right middle lobe, which was not involved, gave a predominant staphylococcus aureus, with fairly numerous influenza bacillus colonies.

*No. 2. K-OC.*—This influenza bacillus was obtained by West tube nasopharyngeal culture, Saturday, November 30. (Chart 33.) The patient was from the U. S. S. *Yacona*, and gave a history of onset of typical influenza November 29 at 8 a. m. He had felt well at 4 a. m. The initial symptoms were severe headache, so that he could hardly see, aching across his hips, and alternate warm and chilly sensations. The light hurt his eyes, and his nose bled slightly. The leucocyte count on the third day was 13,600, polynuclears 78 per cent, lymphocytes 20 per cent, transitionals 2 per cent. Signs of pneumonia developed on the third day. Recovered.

The influenza bacillus obtained by nasopharyngeal culture was transplanted once on whole blood agar and on heated blood agar. Pure cultures were obtained with characteristic morphology and cultural qualities on the two media used. These first transplants were used in Experiment 8.

*No. 3. K-CF.*—This influenza bacillus was obtained from nasal and posterior nasopharyngeal cultures on blood agar plates, Saturday, November 30. (Chart 34.) The patient was from the U. S. S. *Yacona*, and gave a history of onset of typical influenza Friday, November 29, at 10 a. m. The initial symptoms were headache, backache, photophobia, but no sore throat. The leucocyte count on the third day of the disease was 32,800, polynuclears 87 per cent, lymphocytes 9 per cent, transitionals 4 per cent. Signs of pneumonia developed on this day. Recovered.

The influenza bacillus obtained by culture November 30 was transplanted December 1 on whole blood agar and heated blood agar slants. Pure cultures were obtained with characteristic morphology and cultural qualities. The first transplants were used in Experiment 8.

*No. 4. U-W.*—This influenza bacillus was obtained from the lungs at necropsy of a case of influenza-pneumonia, Wednesday, November 27. The culture used for inoculation was the fourth transplant.

The history of the case gave an onset of influenza November 12; the patient entered the naval hospital November 19 (Chart 35) with signs of pneumonia. The leucocyte count on entrance into the hospital was 15,000. Hemolytic streptococci were obtained from an empyema developing late in the pneumonia.

At necropsy there was a massive broncho-pneumonia, with dilated bronchi and purulent exudate on cut surface. Cultures showed a predominant hemolytic streptococcus, associated with pneumococcus in the right upper lobe, and the influenza bacillus in the left lower lobe.

No. 5. *H-E*.—This influenza bacillus was obtained from a specimen of washed bronchial sputum, November 19. (Chart 36.) This culture was transplanted every second day on blood agar, so that the culture used in the experiment was about the seventh transplant.

The history of the case shows an onset of influenza November 13. The patient was admitted to the naval hospital November 17 with signs of pneumonia, leucocyte count 4,200. Tenacious, yellowish-white, purulent bronchial sputum was being coughed up. Smears and cultures of this showed numerous influenza bacilli and a few pneumococci. The patient recovered.

No. 6. *Youngstown*.—This influenza bacillus was obtained at necropsy from the lungs of a patient who died of influenza-pneumonia, about November 20. A subculture was furnished us through the kindness of Dr. G. W. O'Grady.

No. 7. *P-BH (123)*.—This influenza bacillus was obtained from the lungs at necropsy of a case of influenza-pneumonia. The patient had entered the hospital with the history of onset of sickness about a week previously. At that time she had become sick with cough, fever, slight headache and some backache. She had no sore throat. During this time she had been doing her work at intervals. On admission she had signs of pneumonia in her lower right back; in hospital she had a continuous fever of about 101°. After eight days in the hospital, with no alarming symptoms, she suddenly became cyanotic during the night, with difficult respiration, and died within a few hours.

The necropsy findings showed a very discrete broncho-pneumonia in the right lung. The pathological findings hardly explained the sudden death. Cultures from the right lung yielded the influenza bacillus.

The influenza bacillus was obtained from necropsy November 4, 1918, and it was transplanted about every second day. The culture used for inoculation was about the fifteenth transplant on blood agar.

No. 8. *Card*.—This culture was obtained originally at Walter Reed Hospital about October 15 from post-mortem lung puncture of a case of influenza dying of pneumonia. Pneumococcus, Friedlander bacillus, micrococcus catarrhalis and streptococcus viridans were also obtained. A subculture was furnished by the United States Hygienic Laboratory.

No. 9. *Staizecki*.—This culture was originally obtained at Walter Reed Hospital about October 15 from post-mortem lung puncture of a case of influenza dying of pneumonia. Pneumococcus and staphylococcus were also obtained. A subculture was furnished by the United States Hygienic Laboratory.

No. 10. *Butler*.—This culture was originally obtained at Walter Reed Hospital about October 15 from the lung juice of a case of influenza with pneumonia. There were also isolated pneumococcus and staphylococcus. A subculture was furnished by the United States Hygienic Laboratory.

No. 11. *CD (112)*.—No history.

No. 12. *CD (157)*.—No history.

No. 13. *Park (103)*.—Obtained this through the kindness of Dr. W. H. Park.

No. 14. *WK*.—This influenza bacillus was obtained from washed bronchial sputum of a case of pneumonia, not clearly an influenza-pneumonia. The onset was Thursday, November 7, at 9 a. m. (Chart 37.) The patient suddenly felt weak, his bones ached a little, and he had a severe chill, saying his teeth chattered. He had had a "cold" three or four days previously, his head was stopped up, and he coughed some. Sputum examination showed numerous influenza bacilli and pneumococcus Type I. The clinical course corresponded more to a pneumococcus pneumonia, with crisis following antipneumococcus Type I serum therapy. Recovered.

## APPENDIX C.

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### ACCOUNT OF THE INFLUENZA EPIDEMIC ON THE U. S. S. YACONA.

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In view of the fact that many of the donors from whom material was obtained for our experiments came from the epidemic focus on the U. S. S. *Yacona*, a brief account of the salient features of this outbreak follows. The facts were secured from an epidemiologic report furnished by Dr. E. Calloway, the medical officer on board the U. S. S. *Yacona*.

The U. S. S. *Yacona* is a small gunboat of a convoy unit of the United States Navy. There had been no outbreak of influenza on board previously and the crew had remained intact since the pandemic influenza was recognized.

On September 14, 1918, at the admiralty dockyard, Bermuda, an officer from the U. S. S. *Arctic* reported to the sick bay aboard this vessel and was examined and found to have influenza. The U. S. S. *Chicago*, also in port at this time, had several cases of this disease aboard. The same afternoon the *Yacona* went out to an anchorage and had no other contact until September 16, when she put to sea with the *Chicago* and a convoy of tugs and French submarine chasers.

On September 16 Dr. Calloway had chill and temperature 102°. He remained in the stateroom, seeing only the pharmacist's mate and one mess boy, until September 21, when it became necessary to make medical calls to other vessels. Then, as little contact was allowed as possible, and cases brought aboard were isolated. No cases developed among the *Yacona's* crew.

On September 27 the vessel arrived in Ponta del Gada, St. Michaels, Azores. Here influenza existed. All men had liberty in this port.

On October 2 the U. S. S. *Chicago*, with tugs and *Yacona*, got under way for Bermuda. Tugs and *Yacona* were inspected and no cases were aboard. U. S. S. *Arctic* had had two cases on previous trips. A few cases were still aboard the *Chicago*. On October 13 we arrived at the admiralty dockyard, Bermuda. Here an epidemic was flourishing. All ships were quarantined, but this was not effective, as men had to use toilets in dockyard while ship was behind breakwater.

On November 1 two cases were admitted with the diagnosis of influenza; both, however, were normal in 24 hours, and this was probably a wrong diagnosis. On November 2, 10 Hospital Corps men from influenza camp were sent aboard for transportation to the United States, as were men from the U. S. S. *Tallahassee* who had recently had the disease.

On November 5 the vessel left St. Georges, Bermuda, and arrived at New York, N. Y., on November 11. All men had liberty in this port. Left New York on the 14th and arrived at New London November 14. Liberty was granted to all men. On November 17 one case was admitted. This man had had liberty at New London. He was transferred to the naval hospital, New London, on November 18. On November 19 one case was admitted and was transferred on the 20th.

On November 20, at 10 p. m., one case was admitted. On November 21, at 6 p. m., under way for Halifax. At 6 p. m. there were nine cases aboard. The medical officer recommended to the commanding officer that the ship put into Boston to transfer cases. We arrived at Boston November 22, at 1 p. m., and transferred cases to the hospital, as shown below:

Date.	Men transferred.	Officers transferred.
Nov. 22.....	14	1
Nov. 23.....	18	0
Nov. 24.....	20	3
Nov. 25.....	15	2
Nov. 26.....	3	0
Nov. 29.....	2	0
	72	6

Including the two cases of influenza transferred to the United States naval hospital, New London, Conn., during the epidemic of influenza of this ship, there were 80 cases of influenza in an isolated group of 95 men, or 84.2 per cent. This is a very high incidence of the disease, and indicates a high degree of infectiousness of the causative agent or most favorable condition for the transmission of the infection.

Histories and clinical charts were obtained from each of the 78 cases admitted to the United States naval hospital, Chelsea, Mass. The average maximum temperature for the cases during the first two or three days of the disease was 102.7° F. Twenty of the seventy-eight cases developed bronchopneumonia, one of whom died after only 70 hours of sickness. This case was McCormack, from whom staphylococcus and the influenza bacillus (our culture No. 1) were recovered. The remainder recovered, except one, who developed a hemolytic streptococcus empyema, mentioned on page 26 (O. A.). The incidence of pneumonia in the 78 cases is thus seen to be 25.6 per cent, and the total mortality 1.3 per cent. The low mortality of pneumonia cases, 5 per cent, may perhaps be partly accounted for by the fact that all cases, except the one that died, were treated with convalescent human serum. The average duration of temperature in those cases which did not develop pneumonia was five days.

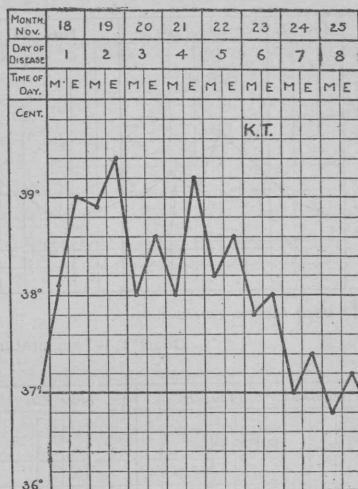
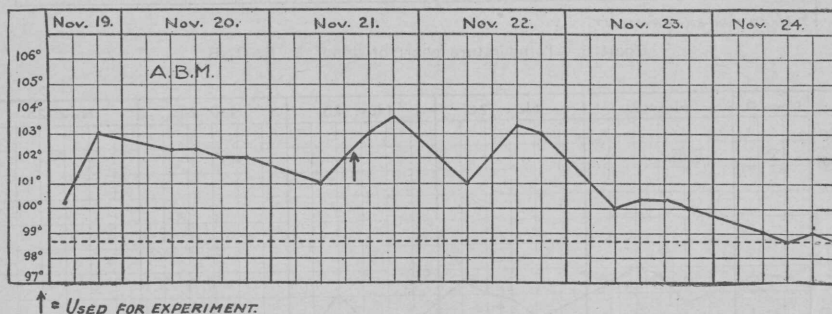
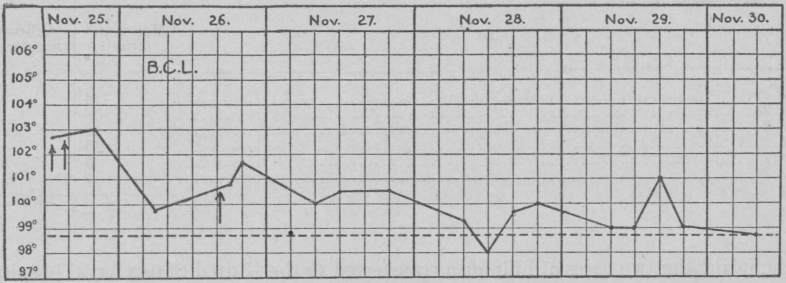


Chart 1.—Temperature curve of Volunteer No. 29 K. T., experiment 2a.



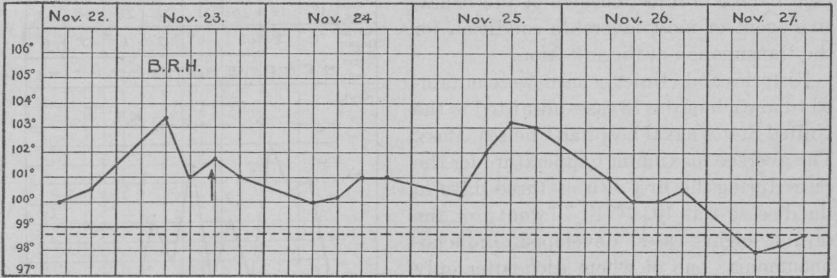
↑ = USED FOR EXPERIMENT.

Chart 2.—Temperature curve of donor A. B. M.



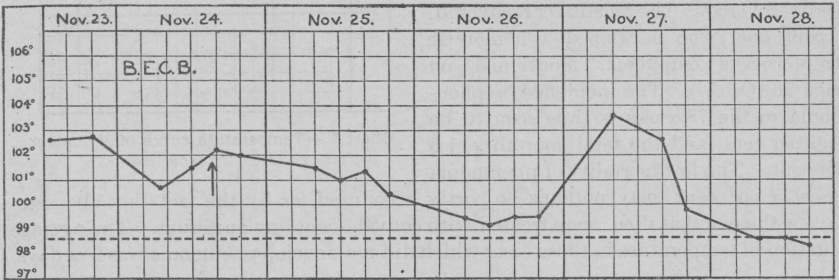
↑ = USED FOR EXPERIMENT.

Chart 3.—Temperature chart of donor B. C. L.



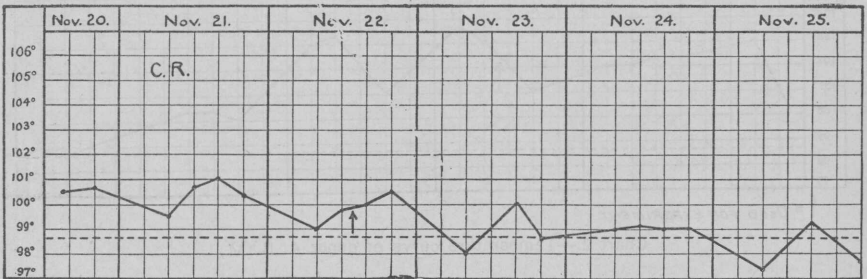
↑ = USED FOR EXPERIMENT.

Chart 4.—Temperature curve of donor B. R. H.



↑ = USED FOR EXPERIMENT

Chart 5.—Temperature chart of donor B. E. C. B.



↑ = USED FOR EXPERIMENT.

Chart 6.—Temperature curve of donor C. R.



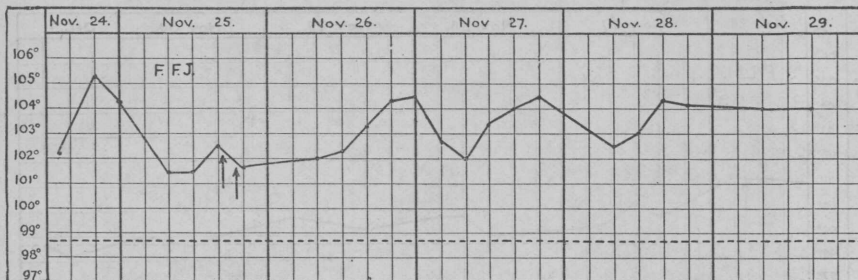


Chart 7.—Temperature curve of donor, F. F. J.

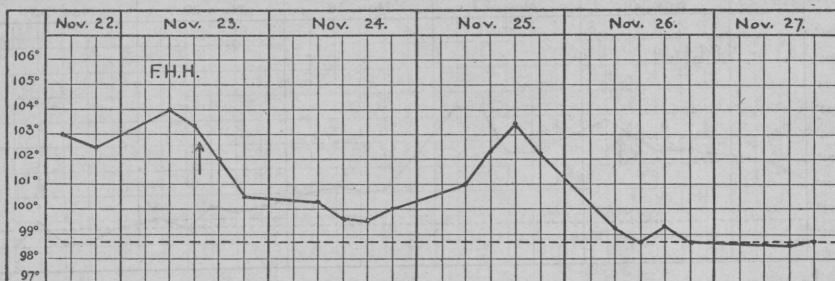


Chart 8.—Temperature curve of donor F. H. H.

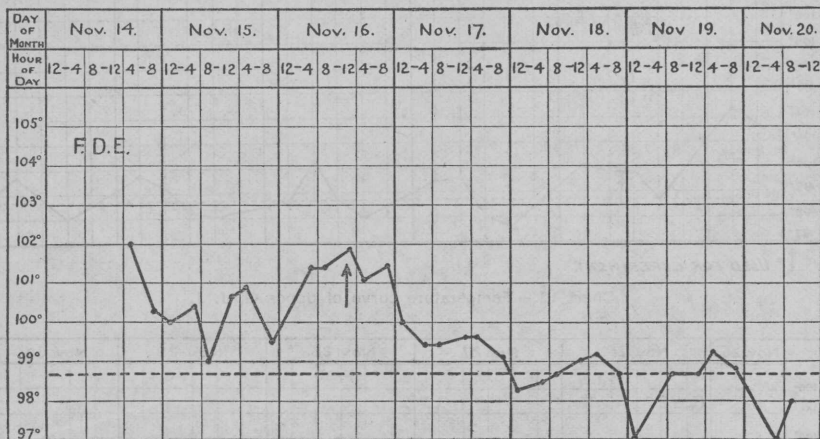


Chart 9.—Temperature curve of donor F. D. E.

181409°—21—3

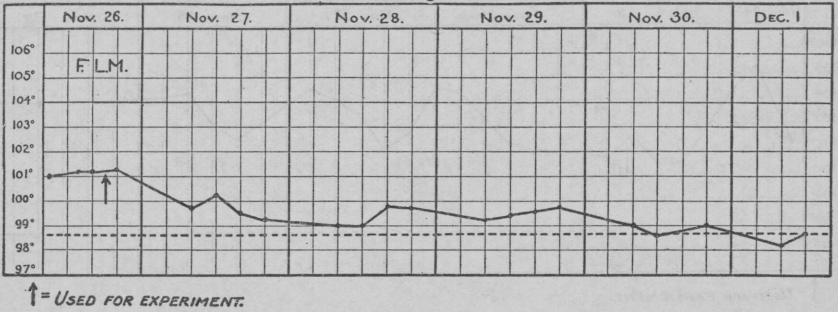


Chart 10.—Temperature curve of donor F. L. M.

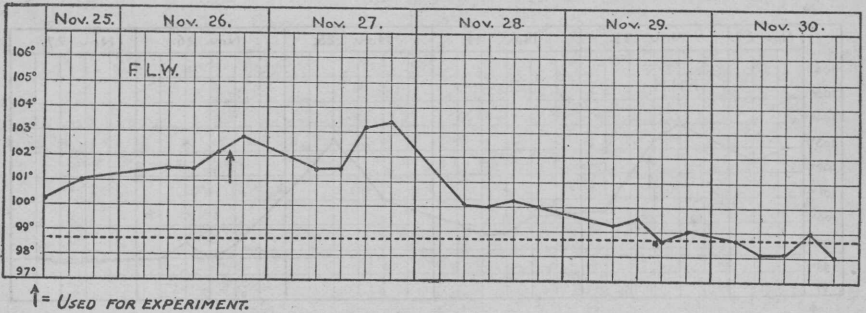


Chart 11.—Temperature curve of donor F. L. W.

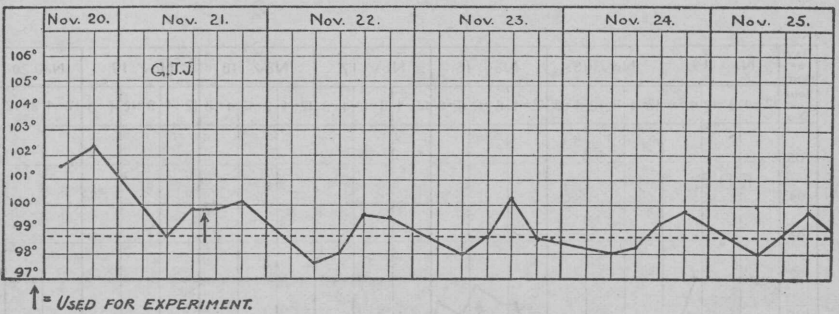


Chart 12.—Temperature curve of donor G. J. J.

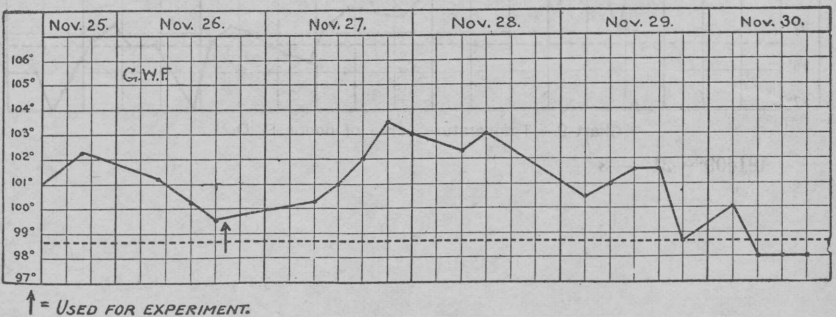


Chart 13.—Temperature curve of donor G. W. F.

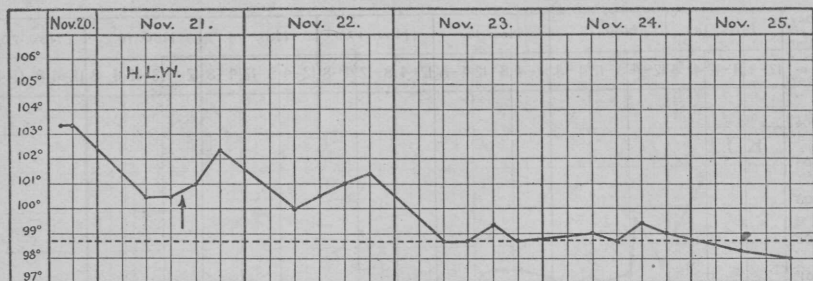


Chart 14.—Temperature curve of donor H. L. W.

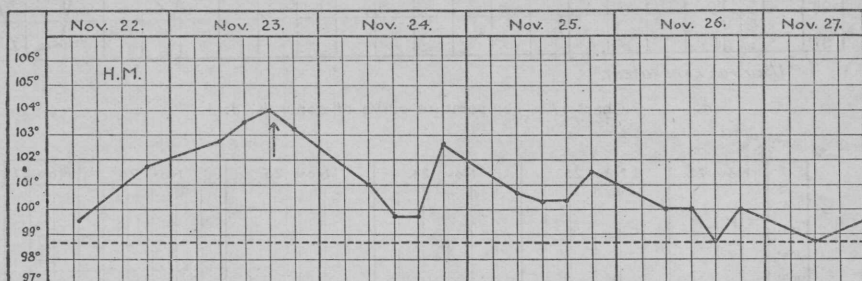


Chart 15.—Temperature curve of donor H. M.

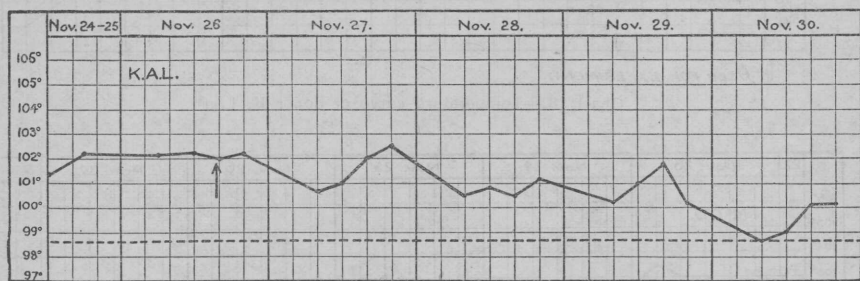


Chart 16.—Temperature curve of donor K. A. L.

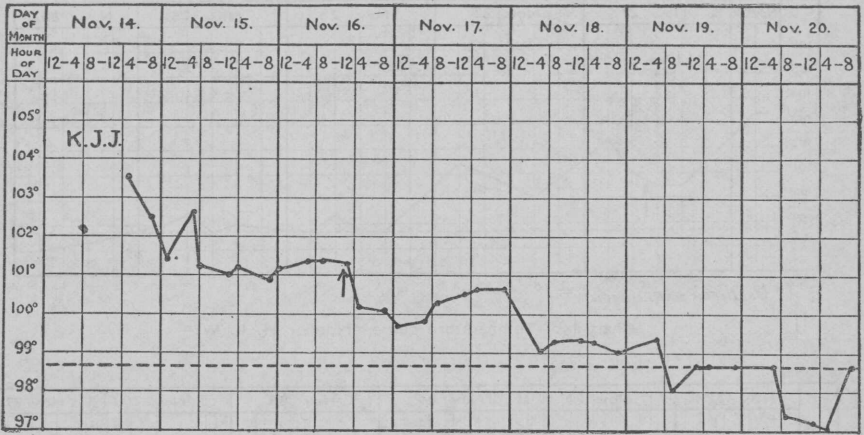


Chart 17.—Temperature curve of donor K. J. J.

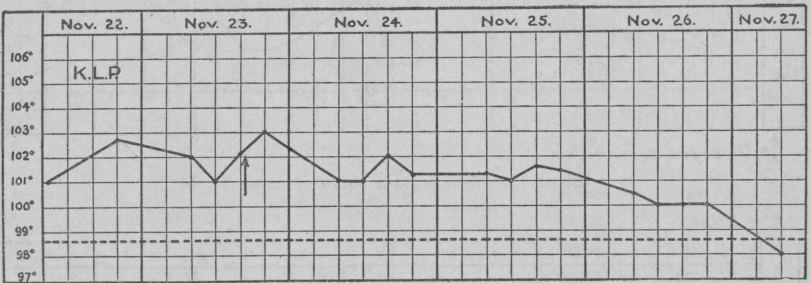


Chart 18.—Temperature curve of donor K. L. P.

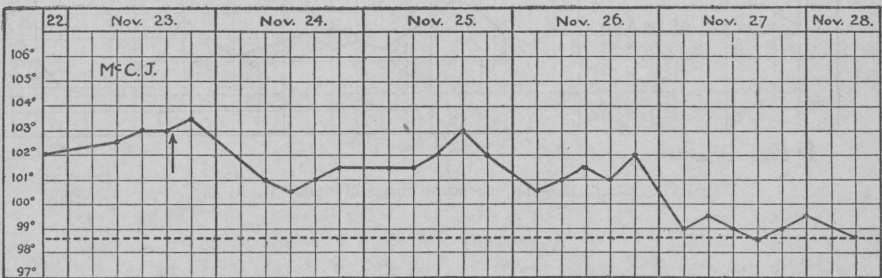
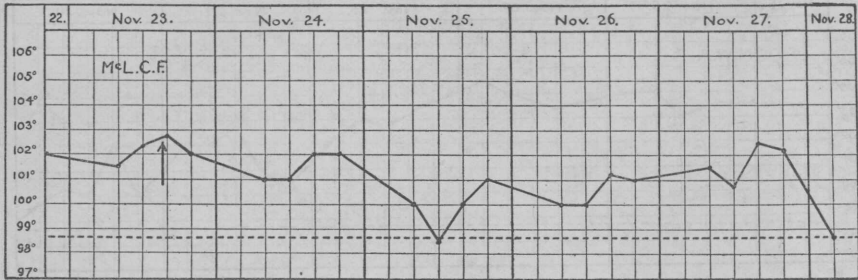
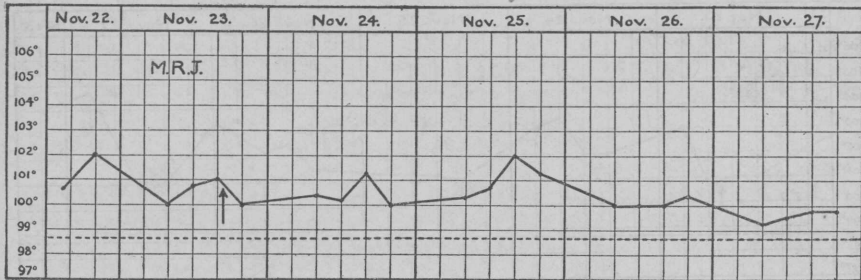


Chart 19.—Temperature curve of donor McC. J.



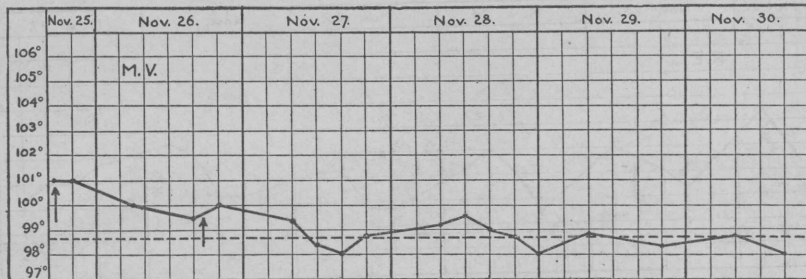
↑ = USED FOR EXPERIMENT.

Chart 20.—Temperature curve of donor McL. C. F.



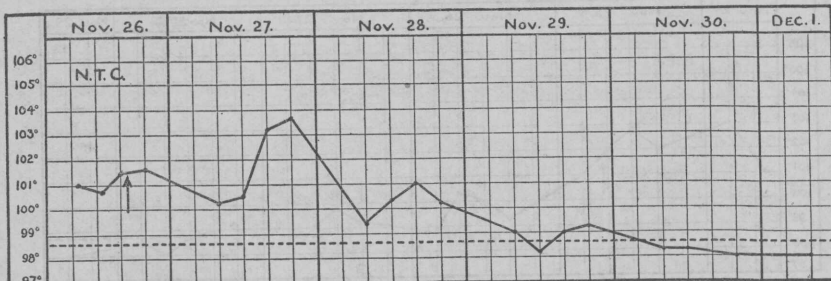
↑ = USED FOR EXPERIMENT.

Chart 21.—Temperature curve of donor M. R. J.



↑ = USED FOR EXPERIMENT.

Chart 22.—Temperature curve of donor M. V.



↑ = USED FOR EXPERIMENT.

Chart 23.—Temperature curve of donor N. T. C.



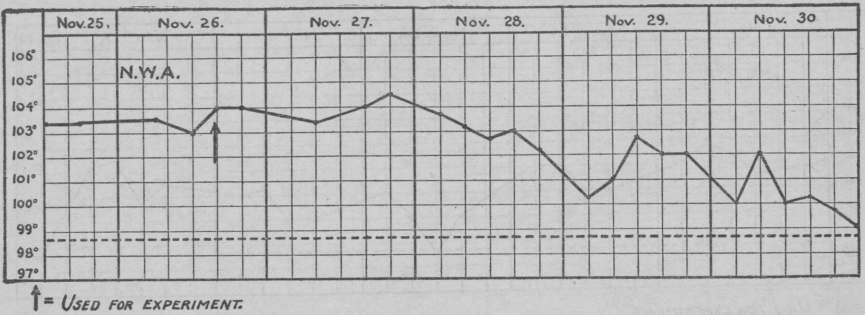


Chart 24.—Temperature curve of donor N. W. A.

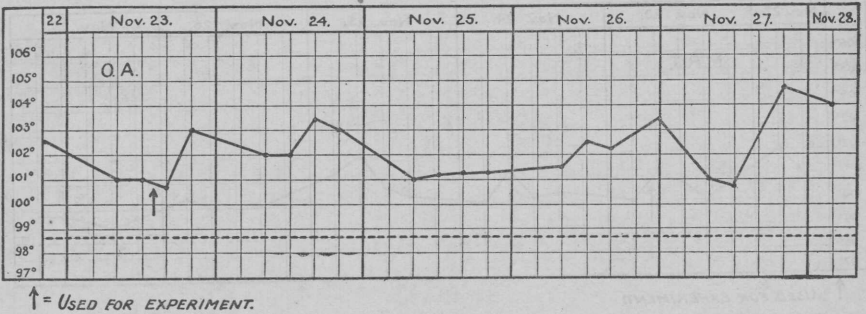


Chart 25.—Temperature curve of donor O. A.

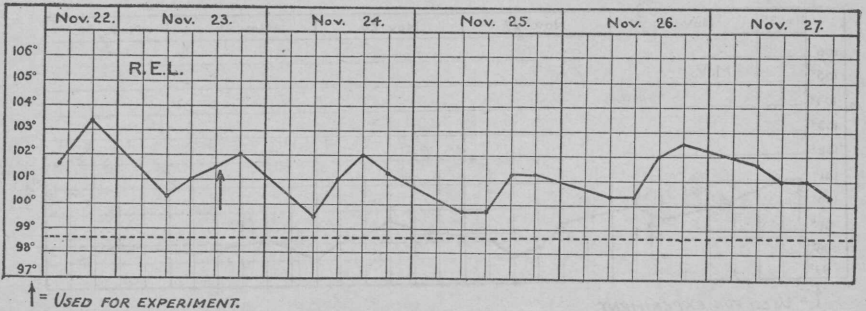


Chart 26.—Temperature curve of donor R. E. L.

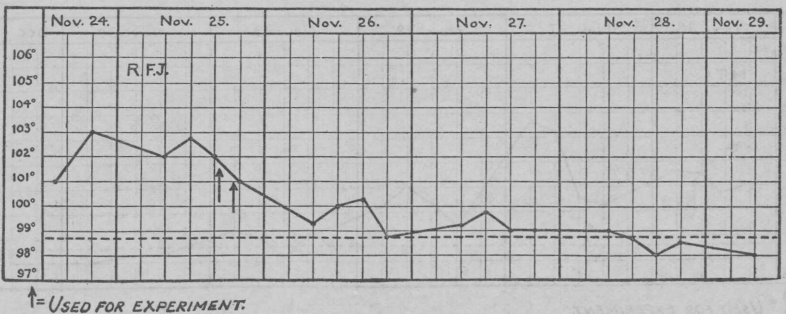


Chart 27.—Temperature curve of donor R. F. J.

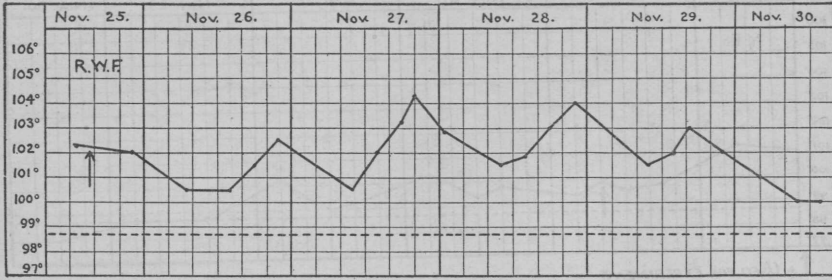


Chart 28.—Temperature curve of donor R. W. F.

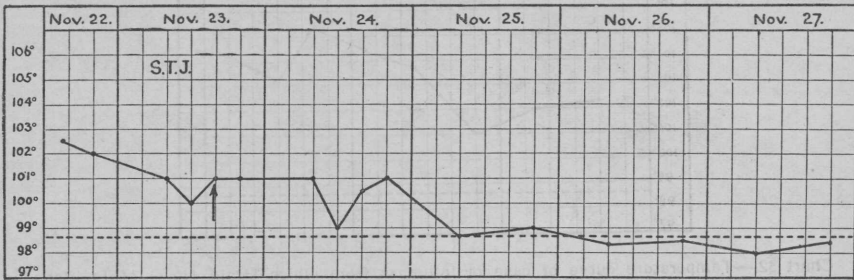


Chart 29.—Temperature curve of donor S. T. J.

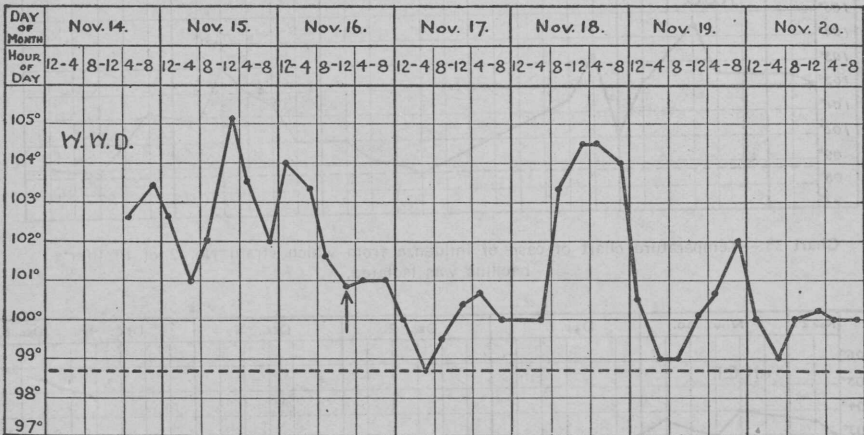


Chart 30.—Temperature curve of donor W. W. D.

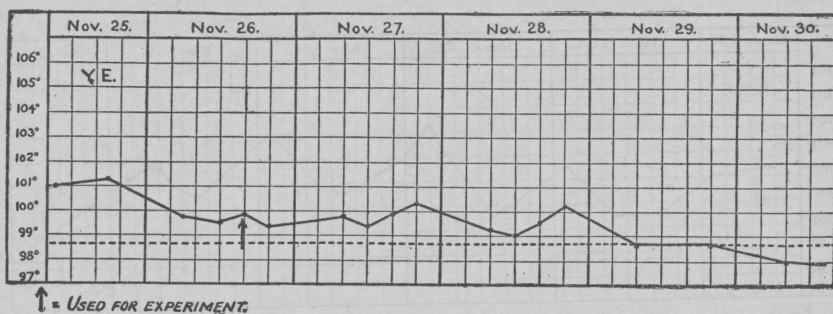


Chart 31.—Temperature curve of donor Y. E.

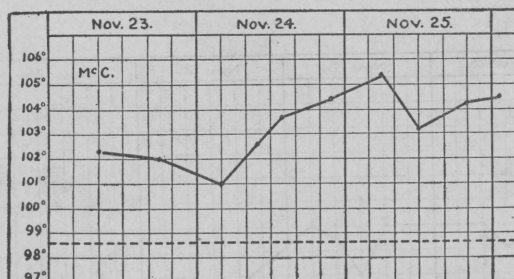


Chart 32.—Temperature curve of case of Influenza from which strain No. 1 of Pfeiffer's bacillus was isolated after death.

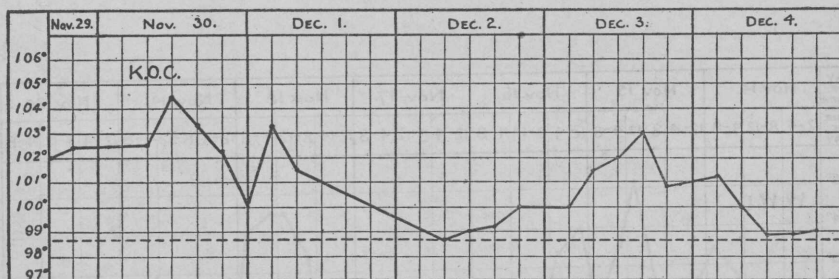


Chart 33.—Temperature chart of case of Influenza from which strain No. 2 of Pfeiffer's bacillus was isolated.

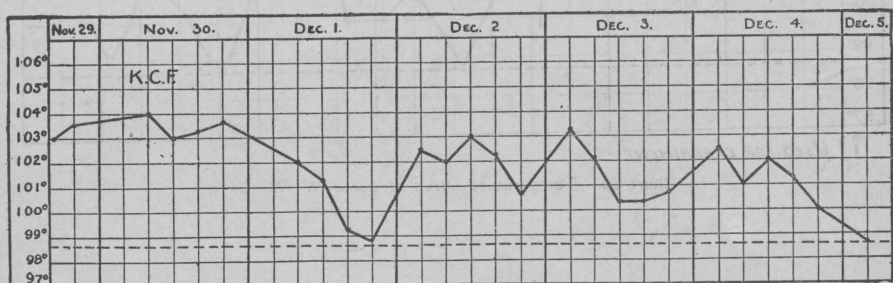


Chart 34.—Temperature curve of case of Influenza from which strain No. 3 of Pfeiffer's bacillus was isolated.

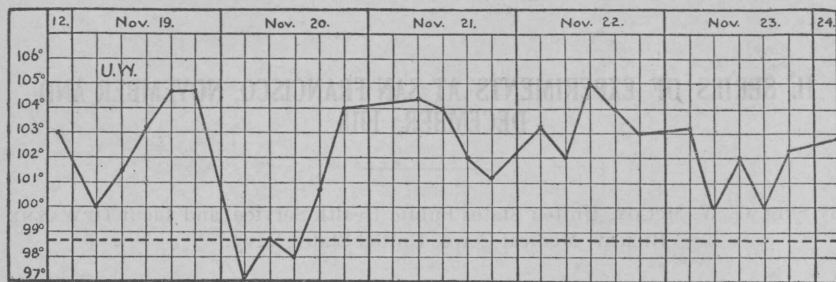


Chart 35.—Temperature curve of case of influenza from which Strain No. 4 of Pfeiffer's bacillus was isolated.

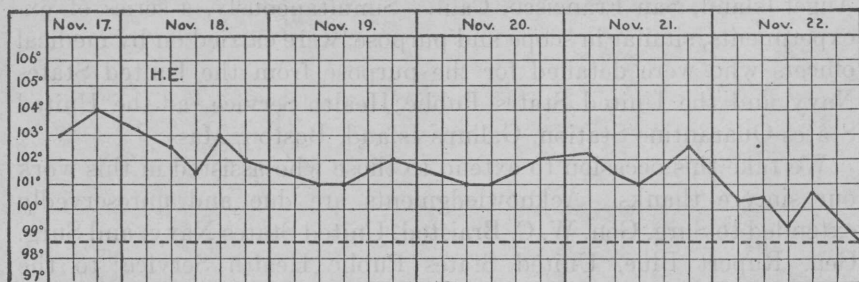


Chart 36.—Temperature curve of case of influenza from which strain No. 5 of Pfeiffer's bacillus was isolated.

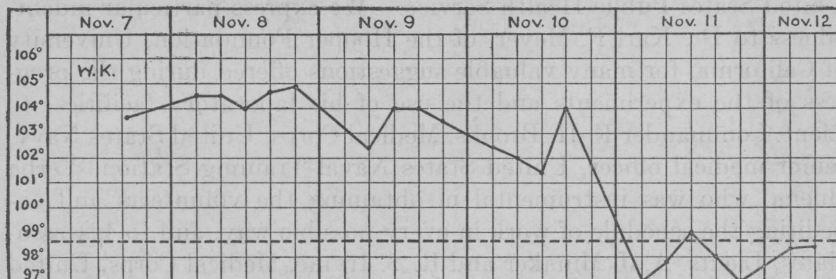


Chart 37.—Temperature curve of case from which strain No. 14 of Pfeiffer's bacillus was isolated.

## II. SERIES OF EXPERIMENTS AT SAN FRANCISCO, NOVEMBER AND DECEMBER, 1918.<sup>1</sup>

By Surg. G. W. McCoy, United States Public Health Service, and Lieut DEWAYNE RICHEY, Medical Corps, United States Navy.

The following experiments designed to add to our knowledge of influenza were conducted at the United States Quarantine Station, Angel Island, San Francisco, Calif. Simultaneously, a series of experiments, similar in scope and purpose, were carried on by medical officers who were detailed for the purpose from the United States Navy and the United States Public Health Service, at the United States Quarantine Station, Gallups Island, Boston, Mass.

We take this occasion to extend to those who assisted in this work our sincere thanks. Acknowledgments are due and unreservedly extended to Surg. Gen. W. C. Braisted, United States Navy, and Surg. Gen. Rupert Blue, United States Public Health Service; to the officers of their respective bureaus, especially Rear Admiral E. R. Stitt and Lieut. Commander J. R. Phelps, Medical Corps, United States Navy, and Assistant Surgeon General J. W. Schereschewsky, United States Public Health Service. We express particular indebtedness to Dr. Karl F. Meyer, of the Hooper Foundation, University of California, for many valuable suggestions offered during the progress of the experiments and the use of his laboratory facilities; to Lieut. Commander F. H. Brooks, Medical Corps, United States Navy, senior medical officer, United States Naval Training Station, Yerba Buena, who was instrumental in obtaining the volunteers and expediting the schedule of work in every possible way, and to his associates, Lieuts. A. J. Minaker and R. S. Irvine, Medical Corps, United States Naval Reserve Force, for their valuable assistance in securing clinical, bacteriological, and serological data on the volunteers; to Surgeons W. A. Korn and W. C. Billings, Passed Assistant Surgeon Joseph Bolton, and Assistant Surgeon W. T. Harrison, United States Public Health Service; to Dr. R. G. Broderick and his staff at the San Francisco Hospital, through whose courtesy donors became available to us.

Too much commendation can not be bestowed upon the volunteers, whose unselfish spirit made the experiments possible. The names are given herewith:

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<sup>1</sup> Submitted for publication, June, 1919.



Leggett, James Verna.  
 Oldham, George W.  
 Eagan, Estis Theodore.  
 Harrell, Lewis Roy Kendall.  
 Toombs, Herbert Edgar Lawrence.  
 Workman, Lester.  
 Thomas, Franklyn Forrest.  
 Bennett, J. C., jr.  
 Combs, Lester Robert.  
 Swan, George.  
 Mulcahey, Daniel Vincent.  
 Taylor, Christopher Anthony.  
 Lester, Roy.  
 Le Duc, Antonio Oliver.  
 Wages, Verne.  
 Wall, Lewis Edward.  
 Lind, Clifford Charles.  
 Crane, Ellis Madison.  
 Thompson, Arthur Eugene.  
 Alsott, Charles Benson.  
 Lipinski, William.  
 Tomlins, Roy Lee.  
 Tegerson, William.  
 Nardoni, A. M.  
 Miller, Frank A.

Burton, Clyde.  
 Dulaney, Floyd Marcue.  
 Eskew, Herman Virgil.  
 Hammer, Adolph.  
 Shankle, John Swanson,  
 Tharp, Robert Herman.  
 Autry, Charlie Lester.  
 Breco, Davis.  
 Casson, Jesse Meredith.  
 Fisher, Earl.  
 McLaughlin, Joseph Francis.  
 Lorenz, Joshua H.  
 Hickson, Samuel Dewey.  
 Morrow, Ernest James.  
 Stephenson, Neato Augusta.  
 Hearing, Elvin.  
 Bertelsen, Hans.  
 Dickenson, Lester William.  
 Bennett, Ray Ernest.  
 Howard, Fred Elmer.  
 Christian, Lester O.  
 McGaughy, Oscar A.  
 Morrison, M. C.  
 Callison, George A.  
 Hosey, R. L.

### SUBJECTS FOR EXPERIMENTATION.

The 50 individuals upon whom the experiments were conducted, were volunteers from the enlisted personnel at the United States Naval Training Station, Yerba Buena, San Francisco, Calif. They had been under quarantine for a month. At no time had influenza occurred in the station. With 4 exceptions, all of the 50 men had experienced one or more of the exanthemata during childhood. Only 5 of them, Nos. 21, 24, 32, 34, and 36 gave a history of a possible influenzal attack before 1918, and 1, No. 41, said he was stricken during the recent pandemic in October, 1918, by a severe coryza from which he completely recovered. Close interrogation as to the exact nature of this illness failed to reveal probability of influenza. This illness had antedated the subject's admission to the training station by several days.

During the second week in October, 1918, the entire personnel of the station, including the volunteers received a vaccine subcutaneously of which 1 c. c. contained—

<i>B. influenzae</i> .....	5,000,000,000
Pneumococcus Type I.....	3,000,000,000
Pneumococcus Type II.....	3,000,000,000
Pneumococcus Type III.....	1,000,000,000
<i>Streptococcus haemolyticus</i> .....	100,000,000

Three doses were given 48 hours apart. The first consisted of 0.5 c. c. and the remaining two of 1 c. c. each. As a rule the reactions, both local and constitutional, were very slight or were absent.

The physical status of the men was very good. Their ages ranged from 18 to 23. They had all spent the greater portion of their lives west of the Mississippi River. Forty-seven yielded negative Wassermann reactions, the other three being doubtfully positive. Their leucocyte counts varied from 6,800 to 13,400. Only five, Nos. 16, 19, 23, 28, and 37, exceeded 11,500. The differential counts on all but two were within the normal limits. These, Nos. 9 and 26, showed a lymphocytosis. The results of the examination of the volunteers, preliminary to the experiments, are summarized in Table II, page 52.

An attempt to ascertain the nasopharyngeal flora was instituted in all instances before experimentation was entered upon. The swabs were streaked on whole and cooked human blood-agar plates (5 per cent), and, when practicable, a second series of plates was inoculated to insure a wider distribution of the organisms. It was found that *B. influenzae* occurred in about 25 per cent of all cases. A gram-negative diplococcus was encountered in all but two cases (96 per cent). A haemolytic streptococcus was seen in 70 per cent and a streptococcus in 36 per cent. Pneumococci occurred in 78 per cent, while staphylococci, corynebacteria, *Micrococcus tetragenus*, *B. subtilis*, *B. pyocyaneus*, *B. proteus vulgaris*, *B. mesentericus*, and members of the *B. mucosus capsulatus* group were noted occasionally. It is worthy of mention that the flora in the several groups tended to become constant, in that either haemolytic or green producing streptococci or pneumococci would be the predominating organism, according to the group examined.

Inasmuch as a rigid quarantine was being maintained at Yerba Buena, the actual work was conducted at the quarantine station, Angel Island. Here the volunteers were sent in contingents of 10. They were immediately separated into groups of five and assigned to separate quarters.

## DESCRIPTION OF EXPERIMENTS.

### EXPERIMENT NO. I—NOVEMBER 11, 1918.

Ten men—Nos. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10.

The donor (No. I) was a girl of 9 years. She had been ill 26 hours and was admitted with the history of contact with influenza patients at home. Clinically, the case was typically one of influenza, with an acute onset, a temperature fluctuating from 101° to 103.2° F., pulse from 110 to 140, a leucocyte count of 3,800 with 61 per cent polymorphonuclears, 37 per cent small lymphocytes, and 2 per cent

large mononuclears. Physical examination of the lungs was negative for pneumonia.

Nasal and pharyngeal swabs and bronchial sputum were introduced into 15 c. c. of sterile plain bouillon. The material was thoroughly shaken and half of it was filtered through a Berkefeld N bougie. Blood-agar cultures of the unfiltered material revealed *B. influenzae*, pneumococci, and staphylococci, while those of the filtrate remained sterile after five days. The secretions were carried to Angel Island, care being taken to keep them warm. The interim between donor and volunteer was 2.5 hours. Three of the men were each given 1 c. c. unfiltered secretions into the nasopharynx, while two were kept as contact controls. Into the nasopharynx of each of three men of a second group was instilled 1 c. c. of the filtrate, the remaining two, being given a few drops of sterile water, were regarded as controls. Instillations into the nares were accomplished by means of a medicine dropper while the subject was reclining, thus permitting the material to flow into the pharynx.

*Results.*—None of the volunteers experienced any inconvenience from the instillations. The temperatures of all the men remained normal and, after a period of observation of seven days, they were allowed to return to their station.

#### EXPERIMENT NO. II—NOVEMBER 14, 1918.

Ten men—Nos. 11, 12, 13, 14, 15, 16, 17, 18, 19, and 20.

The material for inoculation was obtained by swabbing the nasopharynx, pharynx, and tonsillar regions of an infant 1 year old (donor No. II) 24 hours after the onset of the illness. The mother and sister, with whom the child had been in contact, were in the hospital with influenza at the time of his admission. The syndrome presented by the child was that of an uncomplicated attack of influenza. The temperature varied from 100° to 102° F.; the pulse was 126, the respiration 24; the leucocyte count 5,800; the differential count showed polymorphonuclears, 69 per cent; small lymphocytes, 24 per cent; large lymphocytes, 5 per cent; and transitionals, 2 per cent. No pulmonary complications could be demonstrated. The swabs were introduced into 15 c. c. sterile plain bouillon and transported to the laboratory, where the material was thoroughly agitated and cultured on whole and cooked human blood agar-agar. Half of the material was drawn through a Berkefeld N candle by a slight amount of negative pressure. The filtration was allowed to take approximately 15 minutes. Cultural controls of the filtrate were sterile, while *B. influenzae*, *Streptococcus haemolyticus*, pneumococcus, and a gram-negative diplococcus were recovered from the unfiltered secretions after portions of the suspensions had been used for the inoculations. Both the raw and filtered secretions were kept warm

during transportation to Angel Island. The time interval in this instance was 4.5 hours.

The volunteers, having been properly quartered and complying with all the prerequisites, were divided into two groups of five each. Four men of the first group were inoculated with 1 c. c. of the unfiltered nasopharyngeal secretions by instillation into the nose with a medicine dropper while in the recumbent position. Into the noses of four men from the second group the same quantity of the filtrate was similarly introduced. One man in each group was allowed to remain uninoculated to serve as contact control.

*Results.*—In no instance were we able to reproduce the symptom-complex of influenza in those receiving either the unfiltered or the filtered material. One man, No. 11, who received the unfiltered secretions, developed a mild attack of acute lacunar tonsillitis. Upon admission to the quarantine station, Angel Island, it had been noticed that his tonsils were markedly hypertrophic and data were obtained to the effect that he had experienced several similar, though more acute, attacks in recent years. The period between instilling the secretions and the development of symptoms was 50 hours. The temperature was never higher than 100° F., at which time the leucocyte count was 8,880. The pulse fluctuated from 90 to 120 and the respirations were normal. Headache, constipation, and a considerable degree of angina and dysphagia were the chief complaints. Examination of the throat revealed extremely large tonsils, which were of a dusky red color and showed some injection. A few of the crypts contained a small amount of exudate. The temperature reached normal on the fourth day after onset and an uneventful recovery was made. The predominating organism from the cultures of the tonsils was a haemolytic streptococcus of the same type which occurred in the control cultures of the material which the individual received. Although in constant contact, none of the other individuals in this group contracted the disease.

#### EXPERIMENT NO. III—NOVEMBER 19, 1918.

Ten men—Nos. 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30.

In view of the fact that the early, acute, uncomplicated cases of influenza were not available to us at this time, owing to the decline of the epidemic, the following experiment was performed. The material for nasopharyngeal instillation consisted of a plain bouillon suspension of a 24-hour growth on cooked human blood-agar of eight strains of *B. influenzae*. The cultures were obtained through the courtesy of Dr. Karl F. Meyer of the Hooper Foundation, University of California. The various strains were all isolated from the sputum of active early cases of influenza. The exact generation is not known to us, but it was somewhat more distant than the fifth or



sixth. The suspension was a very heavy one. A portion of it was filtered through a Berkefeld N candle. Cultures of the filtrate were negative. The material was carried, at body temperature, to the place of experimentation. The upper respiratory passages of four men of the first group were thoroughly sprayed with the suspension of living *B. influenzae*, while the fifth man was kept as a control. The warm filtrate was introduced in the same manner into the nasopharynges of four men from the second group, there being one control individual for this group. Following the instillation, the unfiltered suspension was taken back to the laboratory, where control cultures yielded an abundant growth of *B. influenzae* in 18 hours. The time interval between the filtration of the suspension and the inoculation of the volunteers was two hours.

The nasopharyngeal cultures, taken before the experiment, of the men who received the suspension of living organisms, failed to show the presence of organisms suggesting Pfeiffer's bacillus. In a second series of cultures, taken from the same individuals three days after the instilling of the living organisms, and spread on uncooked and cooked human blood agar (5 per cent) plates, it was possible to recover the organism in all cases. Cultures from the nasopharynx of the contact control of this group, in which no *B. influenzae* had been found, continued to show an absence of these organisms.

*Results.*—All of the members of this group remained, apparently, perfectly well, and at the end of seven days were permitted to return to their station.

#### EXPERIMENT NO. IV—NOVEMBER 22, 1918.

Ten men—Nos. 31, 32, 33, 34, 35, 36, 37, 38, 39, and 40.

The donor (No. III) for this experiment was a hospital apprentice, aged 22, who had received the three doses of vaccine at Yerba Buena in October. He was taken suddenly ill, after shaving several influenzal patients a day or so previously. The onset was characterized by headache, backache, chills, epistaxis, and photophobia, and had occurred 48 hours previously. The throat was not sore and examination showed the tonsils to be apparently free from any inflammatory involvement. The posterior pharyngeal wall was injected. The chest findings were negative. The temperature ranged from 100 to 102.3° F.; the pulse from 100 to 116; the respirations from 22 to 24. The leucocyte count was 18,500 on one occasion and 19,200 the following day. The differential count revealed 85 per cent polymorphonuclears, 11 per cent small lymphocytes, 3 per cent large mononuclears, and 1 per cent eosinophiles. The urinalysis showed no albumin. The Wassermann reaction and a blood culture were negative.



Forty-eight hours after the onset of the initial symptoms, the patient's upper respiratory passages were thoroughly washed with sterile physiological salt solution. The nasopharynx, pharynx, and tonsillar regions were swabbed, the swabs washed off with the saline solution, and to this was added some freshly expectorated bronchial sputum. The entire bulk was made up to 100 c. c. with additional physiologic salt solution, thoroughly emulsified, and a portion passed through a Berkefeld N candle, with the aid of a vacuum pump. Cultural controls of the unfiltered secretions made before the inoculations of the volunteers showed *Streptococcus haemolyticus*, a green producing streptococcus, a gram-negative diplococcus, and diphtheroids. Cultures from the filtrate showed no growth.

The unfiltered and filtered secretions were taken to Angel Island, and, after an interval of 4.5 hours from the time they had been recovered from the donor, each was sprayed into the nasopharynxes of four volunteers. The remaining two men of this group were, as before, kept with their respective sections as contact controls.

*Results.*—None of the men who received the filtrate presented any untoward symptoms, all remaining quite well during the following week.

Of those who received the unfiltered secretions, two men, Nos. 31 and 32, became ill with a severe attack of acute lacunar tonsillitis. Within 36 hours after the inoculation into the nasopharynx, both complained of headache, malaise, some nausea, chilly sensations, and sore throat. Their temperatures abruptly rose to 103 and 100.2° F., respectively, reaching the fastigium within 72 hours. The pulse ranged from 100 to 120. The leucocyte counts were 18,000 and 14,000, respectively. Examination of the tonsils showed the crypts to be filled with a creamy, yellowish, purulent exudate. The tonsils were swollen and markedly congested. Prostration was not marked and at no time could any abnormal findings be made out over the lung areas. Bacteriological examination of the tonsillar exudate from both cases yielded an apparently pure culture of the same type of haemolytic streptococcus as was encountered in the control cultures of the donor's unfiltered secretions. There was no reason for believing that these were attacks of influenza. Their temperatures reached normal on the fourth day. The other members of the group failed to contract the disease, despite the fact that they were in constant association with the affected volunteers.

#### EXPERIMENT NO. V.—DECEMBER 2, 1918.

Four men—Nos. 41, 42, 43, and 44.

The donor (No. IV) was a nurse 21 years of age. She had become ill 12 hours before the diagnosis was established and the secretions obtained. The onset was sudden and characterized by headache, vertigo, chills, and a cough. The temperature at the time was 101.2°

F., the pulse 104, and respiration 24. The white blood cell count was 8,100, of which 57 per cent were polymorphonuclears, 29 per cent were small lymphocytes, 7 per cent were large mononuclears, 5 per cent transitionals, and 2 per cent eosinophiles. The urinalysis and Wassermann reaction were negative.

Twenty c. c. of sterile, normal saline solution were employed to wash the upper respiratory passages and with this was incorporated the material from nasopharyngeal swabs, as well as freshly expectorated sputum. The entire collections were diluted to 100 c. c. with additional sterile normal saline solution, and after the usual bacteriological controls, a portion was filtered through a Berkefeld N bougie. The filtration consumed about five minutes being facilitated by the negative pressure of a vacuum pump. Cultures from the filtrate showed no growth upon repeated examinations; while those of the unfiltered secretions yielded *B. influenzae*, haemolytic streptococcus, pneumococci, and a gram-negative diplococcus.

The material was collected at 1 p. m. and the filtrate was sprayed into the nasopharynxes of two volunteers, Nos. 41 and 42, at 6.30 p. m., an interval of 5.5 hours. At the same time two men, Nos. 43 and 44, received the same amount, about 3 c. c., of the unfiltered nasal washings. Two other men, Nos. 49 and 50, were not utilized, but were kept segregated as available controls in the event that any of the individuals in this or subsequent experiments contracted any illness.

*Results.*—All of the four individuals remained very well, and at the end of a week were discharged.

#### EXPERIMENT NO. VI.—DECEMBER 2, 1918

Two men—Nos. 45 and 46.

The object of this experiment was an attempt to reproduce influenza by instillation into the conjunctival sac.

The material was from the donor (No. IV) employed in the previous experiment. Only the filtrate was used.

Into both conjunctival sacs of the two men at least 1 c. c. of the filtrate was instilled with a medicine dropper and 1 c. c. sprayed by an atomizer. This occurred 6 hours after the material was secured.

*Results.*—Neither individual showed any indication of illness during the seven days of observation.

#### EXPERIMENT NO. VII.—DECEMBER 2, 1918.

One man—No. 47.

In this experiment an endeavor was made to transmit influenza by means of subcutaneous injection of the filtrate of the nasopharyngeal and bronchial secretion from a patient ill with the disease.

The material was from the same lot utilized in Experiments V and VI.

Two c. c. of the filtrate were injected, subcutaneously, into the deltoid region of the left arm. As was the routine in all the experiments, the volunteer was isolated from any other individuals, or groups of individuals.

*Results.*—The effect of the injection was negative, not even a local reaction being noted.

#### EXPERIMENT NO. VIII—DECEMBER 3, 1918.

One man—No. 48.

This experiment was undertaken to ascertain the effect of subcutaneous injection of whole blood, from a patient ill of influenza.

The donor (No. V) was a nurse 27 years of age. The onset of her illness preceded the withdrawal of the blood by 24 hours, and was attended by intense coryza, headache, general aching, languor, and malaise. The temperature varied from 101.6 to 103.5° F.; the pulse from 100 to 120. The leucocyte count was 10,400, of which 84 per cent were polymorphonuclears, 9 per cent were small lymphocytes, 5 per cent were large lymphocytes, and 2 per cent were eosinophiles. The Wassermann reaction, blood culture, and urinalysis were negative.

Three days after furnishing the blood the patient developed a broncho-pneumonia, from which she recovered.

Ten c. c. of blood were removed from the left median cephalic vein and mixed with an equal amount of sterile 1 per cent sodium citrate solution.

The blood was immediately taken to Angel Island, where, within 1.5 hours, 2.5 c. c. of it were injected into the subcutaneous tissue of the left deltoid region.

*Results.*—The volunteer remained healthy during the week following the injection and was permitted to return to his station.

#### SUMMARY.

Thirteen volunteers received the filtrate of nasopharyngeal secretions into their upper respiratory passages, while 13 were given the unfiltered secretions after a similar fashion. Ten men were used as contact controls. Some of a filtrate was inoculated into the conjunctival sacs of two and injected subcutaneously into a third. Whole blood was administered under the skin of one individual.

Four men were given a pooled suspension of eight living strains of *B. influenzae* into their nasopharynges and four were given the filtrate of the same suspension.

Care was taken to control every step and it is to be regretted that the time interval between donors and volunteers, which varied from two to six hours, could not, under the circumstances, be shortened.

Control cultures of the unfiltered secretions yielded a high percentage of *B. influenzae*, hemolytic streptococci, pneumococci, and Gram-negative diplococci. Cultures of the filtrates were invariably sterile.

In no instance was a clinical case of influenza produced.

Three of the volunteers who received unfiltered nasopharyngeal secretions became ill with acute lacunar tonsillitis.

TABLE I.—Donors, San Francisco experiments.

No.	Age.	Sex.	Occupation.	Onset.	Temperature range.	Pulse.	White count.
I	9	Female....	Child.....	Sudden.....	101 -103.2	110-140	3,800
II	1	Male.....	Infant.....	do.....	100 -102	126	5,800
III	22	do.....	Hospital apprentice.....	do.....	100 -102.3	100-116	18,500
IV	21	Female....	Nurse.....	do.....	101.2-104	104-127	8,100
V	27	do.....	do.....	do.....	101.6-103.5	100-120	10,400

No.	Age.	Sex.	Complications.	Time between onset and collection of material.	History of contact.	Bacteriology of nasopharyngeal washings.
I	9	Female....	None; recovery.....	26 hours.....	Yes.....	<i>B. influenzae</i> , pneumococci, staphylococci.
II	1	Male.....	do.....	24 hours.....	do.....	Gram-negative diplococcus, <i>B. influenzae</i> , pneumococci, streptococcus hemolyticus.
III	22	do.....	do.....	48 hours.....	do.....	<i>Streptococcus hemolyticus</i> , green producing streptococcus, gram-negative diplococcus, diphtheroids.
IV	21	Female....	do.....	12 hours.....	do.....	<i>B. influenzae</i> , streptococcus hemolyticus, pneumococci, gram-negative diplococcus.
V	27	do.....	Post-influenzal broncho-pneumonia; recovery.	24 hours.....	do.....	Secretions not furnished. Supplied blood for subcutaneous inoculation.



TABLE II.—*Volunteers, San Francisco experiments.*

No.	Age.	State.	Doses of vaccine.	Previous attacks possibly influenza.	Wassermann.	White count.	Polymorpho-nuclears.	Small lym-phocytes.	Large lym-phocytes.	Large mono-nuclears.	Transitionals.	Eosinophiles.	Basophiles.
1	21	Oklahoma.....	3	.....	Negative.....	8,800	66	21	7	4	1	1	0
2	18	do.....	3	.....	do.....	9,200	67	25	3	1	4	0	0
3	22	Missouri.....	3	.....	do.....	8,200	65	26	5	2	2	0	0
4	19	Texas.....	3	.....	do.....	9,000	59	36	0	5	0	0	0
5	20	do.....	3	.....	do.....	9,000	48	34	7	0	2	9	0
6	18	Oklahoma.....	3	.....	do.....	9,400	56	34	4	2	4	0	0
7	23	do.....	3	.....	do.....	8,800	57	35	6	1	0	1	0
8	18	Texas.....	3	.....	do.....	9,600	71	21	4	1	3	0	0
9	18	Oklahoma.....	3	.....	do.....	7,900	52	43	2	1	2	0	0
10	19	do.....	3	.....	do.....	9,400	60	28	7	1	0	4	0
11	19	do.....	3	.....	do.....	7,200	58	35	5	1	1	0	0
12	21	do.....	3	.....	do.....	10,200	62	27	2	2	5	1	1
13	21	Texas.....	3	.....	do.....	6,900	71	20	5	3	1	0	0
14	19	Oklahoma.....	3	.....	do.....	8,200	51	35	9	2	1	2	0
15	19	do.....	3	.....	do.....	10,250	60	24	9	4	3	0	0
16	19	do.....	3	.....	Positive (†).....	13,400	77	16	4	1	2	0	0
17	18	do.....	3	.....	Negative.....	7,600	62	28	7	1	2	0	0
18	19	do.....	3	.....	do.....	10,300	62	24	10	3	1	0	0
19	21	do.....	3	.....	do.....	11,850	69	21	6	3	0	1	0
20	21	do.....	3	.....	do.....	10,300	65	24	5	3	2	0	1
21	19	Nebraska.....	3	<sup>1</sup> 1	do.....	10,600	75	15	7	1	2	0	0
22	19	do.....	3	.....	do.....	9,800	65	23	6	3	1	1	1
23	22	Oklahoma.....	3	.....	do.....	13,350	66	21	8	4	0	1	0
24	18	Iowa.....	3	<sup>1</sup> 1	do.....	10,000	70	20	3	4	1	1	1
25	18	Utah.....	3	.....	Positive (†).....	11,500	58	22	11	5	1	2	1
26	18	Mississippi.....	3	.....	Negative.....	7,800	35	48	10	4	1	0	2
27	22	Utah.....	3	.....	do.....	7,300	67	24	4	4	0	1	0
28	19	Oklahoma.....	3	.....	do.....	12,000	60	25	7	5	1	2	0
29	19	Arkansas.....	3	.....	do.....	9,500	67	22	5	3	2	0	1
30	20	Michigan.....	3	.....	do.....	11,500	57	23	10	6	3	1	0
31	18	Washington.....	3	.....	do.....	8,800	64	24	7	1	4	0	0
32	18	California.....	3	<sup>2</sup> 1	Positive (†).....	10,700	65	20	8	5	1	1	0
33	19	Minnesota.....	3	.....	Negative.....	8,000	66	24	8	1	0	1	0
34	22	Oregon.....	3	<sup>2</sup> 1	do.....	7,200	61	30	7	2	0	0	0
35	19	Washington.....	3	.....	do.....	8,400	55	30	14	0	0	0	1
36	21	Idaho.....	3	<sup>1</sup> 1	do.....	7,800	66	18	12	3	1	0	0
37	19	California.....	3	.....	do.....	11,800	70	20	6	1	1	2	0
38	18	Oklahoma.....	3	.....	do.....	7,300	70	22	6	1	0	1	0
39	18	Colorado.....	3	.....	do.....	8,400	70	20	6	3	1	0	0
40	18	do.....	3	.....	do.....	8,200	64	25	9	1	1	0	0
41	19	California.....	3	<sup>3</sup> 1	do.....	7,400	72	23	2	0	3	0	0
42	21	Colorado.....	3	.....	do.....	9,300	69	26	3	0	1	1	0
43	18	Minnesota.....	3	.....	do.....	8,800	70	26	1	0	1	0	2
44	20	Oklahoma.....	3	.....	do.....	7,300	74	25	3	0	2	0	0
45	21	Missouri.....	3	.....	do.....	7,100	75	20	4	1	0	0	0
46	19	Colorado.....	3	.....	do.....	8,100	68	27	2	0	2	1	0
47	20	California.....	3	.....	do.....	8,400	69	25	4	0	1	1	0
48	20	Oklahoma.....	3	.....	do.....	9,100	64	31	2	0	1	1	1
49	18	do.....	3	.....	do.....	6,800	77	18	3	0	2	0	0
50	21	do.....	3	.....	do.....	9,000	72	24	3	0	0	1	0

<sup>1</sup> 1916.<sup>2</sup> 1917.<sup>3</sup> 1918.



TABLE III.—San Francisco experiments.

No.	Material.	Site or mode of inoculation.	Date.	Result.
1	Unfiltered nasopharyngeal washings.....	Nasopharynx.	Nov. 11, 1918	Negative.
2	do.....	do.....	do.....	Do.
3	do.....	do.....	do.....	Do.
4	None (contact control).....	do.....	do.....	Do.
5	do.....	do.....	do.....	Do.
6	Filtrate of nasopharyngeal washings.....	Nasopharynx.	do.....	Do.
7	do.....	do.....	do.....	Do.
8	do.....	do.....	do.....	Do.
9	None (contact control).....	do.....	do.....	Do.
10	do.....	do.....	do.....	Do.
11	Unfiltered nasopharyngeal washings.....	Nasopharynx.	Nov. 14, 1918	Acute lacunar tonsillitis.
12	do.....	do.....	do.....	Negative.
13	do.....	do.....	do.....	Do.
14	do.....	do.....	do.....	Do.
15	None (contact control).....	do.....	do.....	Do.
16	Filtrate of nasopharyngeal washings.....	do.....	do.....	Do.
17	do.....	do.....	do.....	Do.
18	do.....	do.....	do.....	Do.
19	do.....	do.....	do.....	Do.
20	None (contact control).....	do.....	do.....	Do.
21	Unfiltered suspension ( <i>B. influenzae</i> ).....	do.....	Nov. 19, 1918	Do.
22	do.....	do.....	do.....	Do.
23	do.....	do.....	do.....	Do.
24	do.....	do.....	do.....	Do.
25	None (contact control).....	do.....	do.....	Do.
26	Filtrate of suspension ( <i>B. influenzae</i> ).....	Nasopharynx.	do.....	Do.
27	do.....	do.....	do.....	Do.
28	do.....	do.....	do.....	Do.
29	do.....	do.....	do.....	Do.
30	None (contact control).....	do.....	do.....	Do.
31	Unfiltered nasopharyngeal washings.....	Nasopharynx.	Nov. 22, 1918	Acute lacunar tonsillitis.
32	do.....	do.....	do.....	Do.
33	do.....	do.....	do.....	Negative.
34	do.....	do.....	do.....	Do.
35	None (contact control).....	do.....	do.....	Do.
36	Filtrate of nasopharyngeal washings.....	Nasopharynx.	do.....	Do.
37	do.....	do.....	do.....	Do.
38	do.....	do.....	do.....	Do.
39	do.....	do.....	do.....	Do.
40	None (contact control).....	do.....	do.....	Do.
41	Filtrate of nasopharyngeal washings.....	Nasopharynx.	Dec. 2, 1918	Do.
42	do.....	do.....	do.....	Do.
43	Unfiltered nasopharyngeal washings.....	do.....	do.....	Do.
44	do.....	do.....	do.....	Do.
45	Filtrate of nasopharyngeal washings.....	Conjunctiva.	do.....	Do.
46	do.....	do.....	do.....	Do.
47	do.....	Subcutaneous.	do.....	Do.
48	Whole, citrated, human blood.....	do.....	Dec. 3, 1918	Do.
49	None.....	do.....	do.....	Do.
50	do.....	do.....	do.....	Do.

In considering the results of these experiments, which, to our surprise, resulted uniformly negatively so far as transmission of influenza is concerned, it must be borne in mind that we were so situated that a considerable time always elapsed between the taking of the material from the donor and its application to the recipient. This interval may be sufficient to account for the negative results secured.

### III. SERIES OF EXPERIMENTS AT BOSTON, FEBRUARY AND MARCH, 1919.<sup>1</sup>

By Lieut. Commander M. J. ROSENAU, Lieut. W. J. KEEGAN, and Lieut. DE WAYNE RICHEY, United States Navy; and Surg. JOSEPH GOLDBERGER, Surg. G. W. MCCOY, Passed Asst. Surg. J. P. LEAKE, and Passed Asst. Surg. G. C. LAKE, United States Public Health Service.

#### GENERAL CONSIDERATIONS.

These experiments were conducted at the United States Quarantine Station, Gallups Island, Boston, Mass., upon volunteers from the United States Naval Detention Training Camp, Deer Island, Mass., by medical officers detailed for the purpose from the United States Public Health Service and United States Navy. They can be regarded as a continuation of the previous series of experiments at the same place under the auspices of the same services and with the same objects, to ascertain the cause and mode of spread of influenza.

Cooperation and assistance, without which these experiments could not have gone forward, were received from Surg. Gen. W. C. Braisted, United States Navy; Surg. Gen. Rupert Blue, United States Public Health Service; Rear Admiral E. R. Stitt, and Commander J. R. Phelps, Medical Corps, United States Navy; Asst. Surg. Gens. J. W. Schereschewsky and R. H. Creel, United States Public Health Service; Capt. John M. Edgar, Commander F. M. Furlong, Lieut. Commander L. W. McGuire, Lieut. W. R. Redden, Lieut. A. L. Grant, Lieut. J. W. Parsons, and Lieut. T. J. Kennedy, Medical Corps, United States Navy; Lieut. J. W. Flannery and Chaplain J. M. J. Quinn, of the Deer Island Training Station, United States Navy; Surg. W. M. Bryan, Act. Asst. Surgs. F. X. Crawford and E. M. Looney, United States Public Health Service; Dr. Harry Linenthal, Prof. Reid Hunt, and Prof. Worth Hale, of the Harvard Medical School; and the donors and recipients of the experimental material. The volunteers particularly deserve credit; their names are given in Table I.

*Time.*—The experiments began with the advent of the volunteers to the island on February 4-6, 1919, and were concluded March 10, 1919.

*Place.*—Gallups Island is a small island of about 16 acres, lying 6 miles down Boston Harbor. It is one-fourth mile from the nearest land adjacent, Fort Standish, on Lovells Island. Its topography is

<sup>1</sup> Submitted for publication July, 1919.

hilly; the hygienic conditions are very good and its buildings, about 30 in number, including quarters, barracks, mess halls, galleys and hospitals, are equipped with modern heating, lighting and sanitation facilities.

*Climatic conditions.*—Gallups Island shared in the unusually mild winter of the Atlantic seaboard. During the time the experiments were in progress the maximum temperature was 50° F., and the minimum 18° F., with a mean temperature of from 38° to 43° F. As a rule the days were clear, and plenty of sunshine prevailed. There was always a brisk breeze which sometimes became accelerated to a gale of about 40 miles per hour. Occasionally it rained and less frequently snow fell. No one, at any time, experienced any inconvenience, much less hardship, from the weather during the sojourn on this station.

*Volunteers.*—The entire contingent consisted of 49 men, 30 of whom arrived on February 4, 1919, and 19 on February 6, 1919. Of these, 6 did not come under experimentation, leaving 43 on whom 82 inoculations were made. These are accounted for as follows: 1 man received 3 inoculations, 37 received 2, and 5 received 1.

The men were from 19 to 36 years of age. Two were nineteen; 30 were from 20 to 25; 9 were from 26 to 30; and 2 were 33 and 36, respectively.

Physically, the men were in very good condition. Eleven showed rather large tonsils, with some congestion of the pharynx. The weights ranged from 125 to 182 pounds. The mean weight on admission was 157 pounds, and on discharge 157.6 pounds. Sixteen men gained from 1 to 12 pounds, 15 lost from 1 to 12 pounds, and the weight of 12 remained stationary.

The leucocyte counts varied from 5,600 to 11,200. Care was taken to obtain all blood counts at approximately 1 hour before mealtime.

At Deer Island, from which place the volunteers came, cases of influenza since January 1, 1919, are recorded as follows:

January 2.....	1	January 24.....	1
January 3.....	1	January 25.....	1
January 4.....	1	January 27.....	1
January 6.....	1	January 29.....	1
January 8.....	1	January 30.....	1
January 11.....	1	January 31.....	1
January 18.....	1	February 3.....	2
January 20.....	1	February 4.....	1

A careful history was taken of each man prior to the beginning of actual experimentation. Stress was laid upon data pertinent to previous health, and, more especially, upon their activities and ailments during the recent pandemic of influenza. It was found that

the men had always enjoyed very good health, some of them having never been ill, to their knowledge, in their lives. As to contact with influenza patients since the early autumn, 18 men (42 per cent) had not been exposed; 12 men (28 per cent) had experienced the casual contact of the ordinary walks of life, while 11 men (26 per cent) had had close contact with patients ill with influenza. Volunteer No. 24 gave a history of an attack of influenza while at Deer Island in September, 1918. Another, No. 40, probably had an attack while at Portsmouth, N. H., in October, 1918.

The names, numbers, and ages of the men, with their history as regards exposure to influenza and the result of examination for susceptibility to diphtheria by the Schick test, are given in Table I.

During the first week of their sojourn on the island, the men were quartered in large barracks, ate at the same mess and were allowed to congregate at will. They entered into out-of-doors sports and did light chores about the station. For five days before the first experiment was inaugurated their temperatures were taken at 8.30 in the morning and at 6.30 in the evening.

During this period of observation, from February 5, 1919, to February 10, 1919, 12 men reported at sick call with varying degrees of tonsillitis. Of these, three were admitted to the hospital, complaining of sore throat, headache and malaise. One, No. 44, had a fever ( $38.6^{\circ}\text{C.}$ ) for the first evening only; the temperature of the others did not reach  $37.8^{\circ}\text{C.}$ , and all were discharged in 72 hours or less, having completely recovered from their complaints.

Another man, F. K. E., No. 18, presented a more perplexing syndrome. He became ill the day of his arrival on the island, having felt perfectly well before this. This volunteer, and No. 44, who had badly involved tonsils and fever of one afternoon's duration, were not accepted as fit subjects for experimentation due to physical disabilities. The clinical data of this case are herewith given:

*F. K. E.* (age 24, No. 18).—Not used in experiment.

*Diagnosis.*—Daily intermittent fever of unknown origin and paroxysmal tachycardia.

The patient said that he had always been healthy, with no serious illness except an attack of pleurisy and arthritis in February, 1918. He stated that he had had no exposure to influenza. He came to Gallups Island February 4, 1919, feeling well.

On the afternoon of February 5, 1919, the day after his arrival, the patient's temperature was  $38.2^{\circ}\text{C.}$  but he had no complaint. He turned into his bunk early and the following morning his temperature was  $36.9^{\circ}\text{C.}$  The same evening, the temperature was  $38.3^{\circ}\text{C.}$ , with the patient still feeling well, but he was admitted to the hospital for observation. He had been constipated for the three previous days.



The next morning (Feb. 7) the patient's temperature was  $37^{\circ}$  C. and he complained of some headache and vague pains in the epigastrium and chest. The headache was frontal, temporal, and occipital in distribution and was worse when the temperature was highest. There was but little lassitude, weakness, or depression at any time, and all subjective symptoms disappeared each morning with the subsidence of the fever. No vertigo, photophobia, cough, dyspnea, hemoptysis, vomiting, diarrhea, jaundice, nor any symptoms pointing to genito-urinary involvement developed. The patient never complained of sore throat.

Physical examination on admission was negative.

During his stay in the hospital, the patient's temperature intermitted daily, varying from  $36.2^{\circ}$  in the morning to  $39.2^{\circ}$  C. in the evening and gradually coming down on the seventh day to normal, but rising to  $37.6^{\circ}$  on the ninth day and to  $38^{\circ}$  on the twelfth. The pulse (except as noted below) ranged from 72 to 100; the respirations from 18 to 24. The leucocyte count of February 9, was 15,800, dropping to 7,800 four days later. Urine analyses were negative.

On February 13, 1919, a careful examination of the patient was made by Drs. Leake, Lake, and Richey. It was decided that, in view of the leucocytosis, the intermitting fever, continuing for a week or more without severe symptoms, and the absence of prostration, back pains, photophobia, flushing, or cough, the case could not be diagnosed as influenza, though the possibility of an atypical attack could not be entirely ruled out.

On the evening of February 16, 1919, after the temperature had been normal for five days, while the patient was lying quietly in bed, he became conscious of palpitation. On examination at this time it was found that the apex beat was 220 and quite regular. There were no signs of cardiac decompensation. In the course of 20 or 30 minutes, immediately after the application of an ice bag to the precordium, the heart rate returned to 72 as rapidly as it had increased. He said that he has had at least three such attacks, the last occurring four months ago. In the absence of gross irregularity and a pulse deficit, this attack was considered one of paroxysmal tachycardia.

The patient was discharged from the hospital on February 17, 1919, having quite recovered. His nasopharyngeal flora at the time was pneumococcus, staphylococcus, a gram negative diplococcus and *B. influenzae*.

In view of the bare possibility that this might have been an anomalous case of influenza and on account of the presence of a cardiac arrhythmia, the patient was considered as not a fit subject for experimentation.



The routine procedure preparatory to any of the experiments consisted in a careful examination of each volunteer's nasopharynx, a leucocyte count, and a nasopharyngeal culture. All inoculations were made by instilling the material into the nares and mouth by

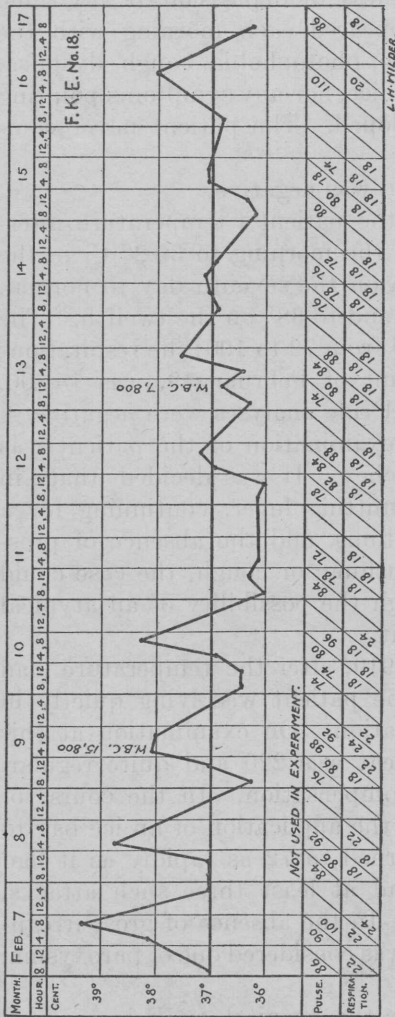


Chart No. 38.

both spray and dropper. The total amounts of material given to each volunteer varied from 1.5 c. c. to 10 c. c., according to the quantity available. The recipient was made to lie flat on his back during the time the material was being instilled, and for several minutes afterwards, to insure the maximum effect.

The men were turned into previously prepared quarters and were isolated from the other volunteers. Precautions were taken to prevent any contact with those not having to do with the experiment. Food was dispensed through a common galley in some cases, while in others the men were permitted to prepare their own food. Temperatures were taken two or three times a day on each group under experimentation. The men were allowed certain hours for routine exercise and remained very happy and contented.

Especial care was taken in the collection of the presumably infectious material from donors. The nose and pharynx were syringed with from 50 to 60 c. c. of sterile, physiologic salt solu-

tion or Locke's solution. Locke's solution was used in all transfers from human sources except from donor 1. This material for flushing was carried in a well-stoppered, sterile bottle containing glass beads. A separate autoclaved syringe was used for each donor. The patient was made to cough into the previously collected material so that very fresh bronchial secretions were obtained. The material, having been collected, was transported as rapidly as possible to the volun-

teers. The time elapsing between the donor and the volunteer was never longer than two hours, and, when the donor was on the station, the interim was within 15 minutes. By means of the glass beads, the secretions and washings were thoroughly emulsified and cultures made before they were finally distributed among the recipients of the particular group. Each group was kept under surveillance for six or seven days subsequent to the inoculations. Upon discharge another nasopharyngeal culture was taken.

Those who became ill were immediately admitted to the hospital, where they were attended by the medical officers in charge of the experiment and one or two nurses.

The details of the various experiments, with the flora of the nasopharynx before and after inoculation, are shown in Table II. The predominant organism in the nasopharyngeal examination is indicated in each instance by black-faced type.

## EXPERIMENT I.

FEBRUARY 11, 1919—3 P. M.

Attempt to produce influenza by inoculation with Mathers's coccus.

*Recipients.*—The 10 recipients, Nos. 1, 2, 3, 6, 7, 8, 9, 10, 15, and 42, employed in this experiment, were all in good physical condition. Their ages ranged from 20 to 30—the average age being 24.4 years. Two, Nos. 3 and 10, showed some enlargement of the tonsils. None of the men gave a history of a previous attack of influenza. Four, Nos. 1, 2, 3, 6, had been in close contact with influenza patients; four, Nos. 7, 10, 15, 42, had had a casual contact, two, Nos. 8 and 9, said they had not been exposed to the disease.

*Material.*—The material consisted of four strains of cocci—63 AT, 40 AN<sub>6</sub>, 65 CT<sub>2</sub>, 6 BNP<sub>4</sub>—somewhat similar to, or identical with, those obtained by Dr. Mathers from cases of influenza at Camp Meade. These were available to us through the courtesy of Dr. Hektoen. Subcultures—29 hours old—in dextrose bouillon, were used. Macroscopically, there was a wide difference in the character of growth: Strain 65 CT<sub>2</sub> showed a very scanty growth, while strain 6 BNP<sub>4</sub> grew as a heavy, white, flocculent precipitate. The others formed a heavy uniform cloud in the medium. Smears taken at the time of instillation showed gram positive, pleomorphic organisms, occurring in pairs and short chains. Some tended to a lanceolate shape. The control cultures, on blood agar plates, varied considerably, ranging from green colonies to gray colonies with a greenish halo. All hemolyzed the blood agar after the third day.

*Procedure.*—A heavy pooled suspension in broth was administered into the nasopharynx by spray and dropper, so that each man

received 1.5 c. c. while reclining. Nos. 1, 2, 3 received a spray of 4 per cent solution of sodium bicarbonate previous to the inoculation—in sufficient quantity to make the nasal secretions alkaline to litmus paper. In a similar manner, Nos. 6, 7, 8, were given a 0.5 per cent solution of acetic acid until the reactions were distinctly acid. Nos. 9, 10, 15, 42, who received no preliminary treatment, showed a slightly acid reaction.

*Results.*—The results were entirely negative during a period of seven days observation; the men remained without fever or other disturbance of their health. Unfortunately, the predominating organism of the nasopharyngeal flora was a green-producing one before inoculation except No. 15, where staphylococcus played the leading rôle. After seven days' isolation, the predominating bacterium had been maintained in all individuals, and the flora was not materially altered. It is of interest that the incidence of *B. influenzae* increased from 40 per cent before inoculation to 80 per cent seven days afterwards.

## EXPERIMENT II.

FEBRUARY 13, 1919—5.30 P. M.

Attempt to transmit influenza via respiratory tract from secretions of acute case.

*Recipients.*—These 10 men, Nos. 5, 11, 13, 14, 16, 17, 19, 20, 21, and 40, were in good physical condition. Nos. 13 and 14 had enlarged tonsils, while No. 5 showed a somewhat congested throat. Their ages were from 19 to 26, the average age being 22. Two, Nos. 5 and 17, gave a history of no exposure to influenza; five, of casual contact, Nos. 11, 13, 16, 19, and 20; two, Nos. 14 and 21, had been in close contact, and one, No. 40, probably had influenza while at Portsmouth, N. H., in October, 1918.

*Donor.*—The donor was Dr. A. C., who treated influenza patients during the autumn and winter, but had not previously contracted the disease. On February 12, 1919, at 5 p. m., he had slight malaise and chilly sensations; by 8.30 p. m. these, and especially a pain in his back, had become so severe that he left a banquet, and on reaching home at 9.30 p. m. his temperature was 100.8° F.; at midnight it was 102.2°, and at 8 o'clock the next morning 102.4°; at 1 p. m. the temperature was 103.4°. When the nasopharyngeal washings were obtained, at 3.25 p. m., the white count was 6,900; he had a continuous headache and pain in his back, was chilly, with a slight coryza—a tenacious mucoid discharge partially blocking the nares. This coryza did not persist and was never prominent. There was no soreness of the throat nor tenderness of the neck, nor evidence of tonsillar infection, but the fauces were reddened, the face was flushed, and the conjunctivae suffused. There was an occasional cough with an increased

pharyngeal secretion, though the throat felt dry. There were no urinary symptoms and the chest examination was negative. During the two following days there was a slight sore throat and some muscular tenderness on the right side of the neck, without glandular enlargement. The temperature fell rapidly and convalescence progressed satisfactorily without complications.

*Material.*—The material consisted of nasopharyngeal washings and bronchial secretions from a patient acutely ill of influenza (Dr. C.), 22 hours after the onset of his illness. The material was collected in 30 c. c. sterile physiologic salt solution; this was thoroughly emulsified and control cultures were made, which revealed the presence of a pneumococcus, *B. influenzae*, *Streptococcus hemolyticus*, *Staphylococcus albus* and *Staphylococcus citreus*, a gram-negative diplococcus, and an organism similar to *B. mucosus capsulatus*.

*Procedure.*—Two hours after its recovery from the patient, 3 c. c. of the material were instilled into the nasopharynx of each of the 10 men by spray and dropper. All men were in the recumbent position at the time of inoculation.

*Results.*—Two men from this group became ill. One, J. J. C., No. 14, after an incubation period of 72 hours, developed a mild attack of acute follicular tonsillitis. His history follows:

J. J. C. (age 22, No. 14).—Experiment II.

*Diagnosis.*—Acute lacunar tonsillitis (mild).

The patient says he has had gripe-like attacks every year for several years, none of which have necessitated going to bed. His last attack occurred over a year ago. During the recent epidemic, he was in close contact with influenza patients.

Seventy-two hours after receiving the material in this experiment the patient's temperature rose to 38° C. and he complained of a sore throat. Examination at this time showed his tonsils to be enlarged, inflamed, and the crypts, particularly the left, to be filled with a purulent exudate. A blowing, systolic murmur at the apex was the only other positive finding. It was noted that a moderate amount of exercise increased the pulse rate.

A few hours after being in bed the temperature returned to normal. The tonsillar condition was readily amenable to treatment and in three days the patient made a good recovery from this mild attack. Hemolytic streptococci were found in the flora, but the predominating colony remained a pneumococcus.

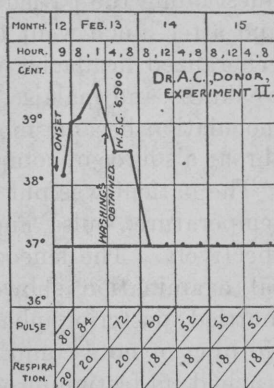


Chart No. 39.



The other man, F. W. B., No. 5, after an incubation period of five days, developed a syndrome which was very suggestive of influenza, as follows:

*F. W. B.* (age 23, No. 5).—Experiment II.

*Diagnosis.*—Influenza (?).

*Incubation period.*—Five days (?).

The patient says he has always been quite well, never having been in bed on account of sickness. During the last few weeks he has had an anterior urethritis, which is discharging at the present time.

On the evening of February 18, 1919, five days after he had received the nasopharyngeal washings, the temperature was found to be 38.2° C. The morning temperature had been normal. Upon questioning the patient, he said he had some anorexia that morning and after lunch went to bed. Toward evening he complained of a generalized headache, chilly sensations over entire body, backache, weakness, and malaise. A cough which had been present before the inoculation became more intense on this day. At no time was sore throat a source of complaint.

The patient was put to bed. At 7 o'clock of the same evening the temperature, pulse, and respiration were 38.2° C., 82, and 20, respectively. The leucocytes (during digestion) were 13,000. Physical examination showed a flushed face, a congested and rather mottled posterior pharyngeal wall, but no tonsillar involvement. Nothing of note could be made out in the chest. The temperature reached its fastigium on the third day, when it rose to 39.2° C. At this time the pulse was 100, and the respirations 26. The leucocyte count was 10,500, his normal being 8,400. Urinalysis was negative. Other than diminished breath sounds over the left base, posteriorly, and a faint blowing systolic murmur at the apex, the physical signs of the chest were negative. The cough still persisted, with no sore throat. The case so closely simulated one of influenza that a passage experiment (No. IV) was done, using this patient's secretions as the source of the material.

The following day, February 22, the temperature dropped to 37.2° C., returning to normal on the fourth day after onset, but rose to 38° for a single observation on the sixth day. One week after the onset the white cell count was 9,000. He made an uneventful recovery, being discharged well February 27, 1919, nine days after the onset.

On February 19 the bacteriological examination of the nasopharynx showed a pneumococcus and a slightly hemolytic streptococcus to be the predominating organisms. *B. influenzae* and a gram-negative diplococcus were also found. Before inoculation his flora consisted of a green-producing coccus, a hemolytic streptococcus, *B. influenzae*, staphylococcus, and a member of the *B. mucosus capsulatus* group.



In the absence of any definite involvement of the tonsils the diagnosis in this case would seem to rest between influenza and a streptococic sore throat. In favor of influenza is the presence of headache, backache, depression, and exacerbated cough, while against it is a relatively high leucocyte count.

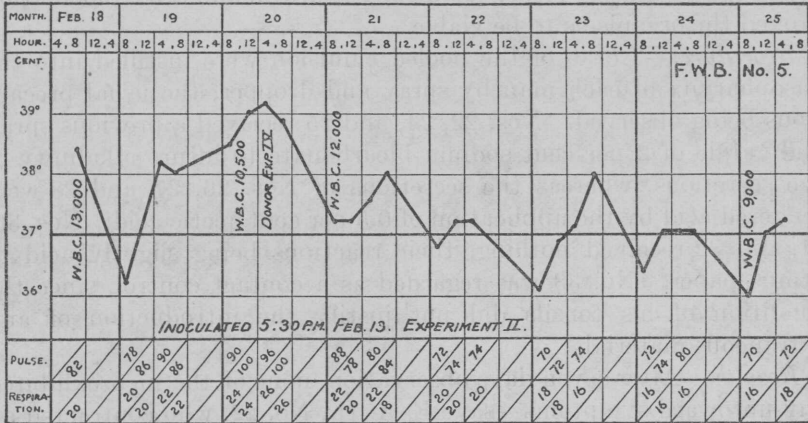


Chart No. 40.

The other men, after six days' observation, were discharged. They had not become ill. The flora of these individuals was not essentially altered. Of the two men in this experiment who gave no history of prior contact with influenza, one suffered a probable attack five days after inoculation with material from a case in the first 24 hours of his illness.

### EXPERIMENT III.

FEBRUARY 17, 1919—4 P. M.

Attempt to produce influenza by nasopharyngeal inoculation of *B. influenzae* (Pfeiffer) and *Staphylococcus aureus*.

Ten men, Nos. 22, 24, 25, 26, 27, 28, 30, 31, 32, and 33, were subjects of this experiment.

*Recipients.*—Their physical status was good. Nos. 27, 32, and 33 had hypertrophied tonsils. Their ages were from 20 to 36 with a mean of 24.3 years. Seven men, Nos. 22, 25, 26, 27, 28, 31, and 32, gave a history of no exposure during the recent epidemic of influenza; one, No. 33, of casual contact; one, No. 30, of close contact, and one, No. 24, had a typical attack of influenza while at Deer Island in September, 1918.

*Material.*—Ten 30-hour-old cooked blood agar slants, on which were luxuriant growths of a virulent strain of *B. influenzae* (200,000,000 being fatal to white mice), were scraped and the organisms were suspended in 20 c. c. nutrient bouillon.

Heavy growths of *Staphylococcus aureus* on three agar slants were scraped into 10 c. c. nutrient bouillon and pooled with a suspension of *B. influenzae*. The suspension was very turbid and an attempt to count it was futile on account of marked clumping of the organisms. It was estimated that there were from three to five billion of each type of organism in 1 c. c. Control cultures made after inoculation proved the organisms to be viable.

*Procedure.*—3 c. c. of the pooled emulsion were instilled into the nasopharynx of each man by spray and dropper, the usual precautions being observed. Nos. 22, 24, and 25 received a previous spray and gargle of 2 per cent sodium bicarbonate to insure alkalinity of the secretions, whereas the secretions of Nos. 26, 27, and 28 were rendered acid by the application of 0.5 per cent acetic acid. Nos. 30, 31, and 32 received nothing, their reactions being slightly acid to litmus paper. No. 33 was regarded as a contact control, since the condition of his tonsils did not justify the introduction of any extraneous material.

*Results.*—After seven days observation, none of the men exhibited any untoward symptoms—save F. A. H., No. 22, who contracted an attack of acute lacunar tonsillitis on the fifth day after inoculation.

F. A. H. (No. 22, age 22).—Experiment III.

*Diagnosis.*—Acute tonsillitis:

The past history of this patient indicates that he has always enjoyed very good health and was not exposed to influenza during recent months.

Five days after receiving the suspension of *B. influenzae* and *Staphylococcus aureus* the patient developed a sore throat. He complained of some anorexia, malaise, and headache. Examination of the throat revealed hypertrophic, dusky red, rather edematous tonsils. No exudate could be made out in the crypts. There was no cough, photophobia, nor general aching. His temperature showed daily variations from 38.6° C. to 36.4° C., gradually coming down on the fifth day to normal. The pulse was never higher than 88. The white count was 6,800, his normal being 5,600. Urine analysis showed no albumen or casts. The patient made a good recovery.

Despite the fact that *B. influenzae* and staphylococci were instilled into his nasopharynx the predominating organisms at the onset of his present illness remained a green-producing organism and *Streptococcus hemolyticus*. The instilled organisms were also present, but in fewer numbers.

It was thought that the throat condition was ample to account for the syndrome which he presented.

The "before" and "after" findings in the nasopharynx were interesting. Prior to inoculation, 6 of the men showed *B. influenzae* to be present in their throats, whereas 7 days after instillation all the

10 members of this group were found to harbor this organism. On the other hand, staphylococcus was encountered in seven instances before and in the same number after the experiment. In four of these, staphylococcus became the predominant organism subsequent

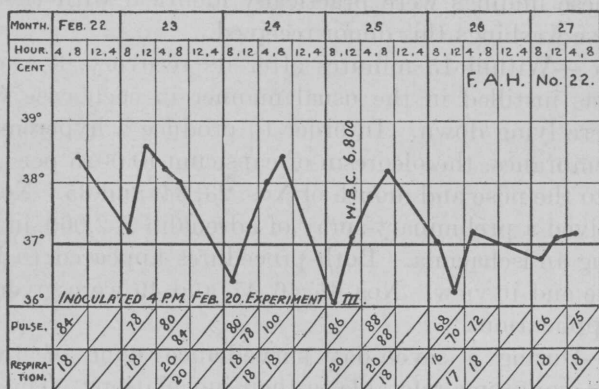


Chart No. 41.

to the inoculation, while it had been such in only one case beforehand. *B. influenzae* was the predominating colony in no instance before the experiment, and in one afterwards. This man, No. 24, was the only one in the whole contingent who was known to have had an attack of influenza prior to these experiments. Before this artificial inoculation with the Pfeiffer bacillus and staphylococcus, a green-producing coccus was the predominating organism, but after the inoculation the Pfeiffer bacillus persisted in its predominance for at least 16 days.

#### EXPERIMENT IV.

FEBRUARY 20, 1919—4 P. M.

Attempt to transmit influenza through upper respiratory tract from secretions of an apparent case (experimental) of influenza on the third day. Passage experiment.

*Recipients.*—Ten men: Nos. 23, 34, 35, 38, 39, 41, 45, 46, 47, and 49 constituted the volunteers. Except for Nos. 38 and 49, who had moderately hypertrophic tonsils, none of these volunteers presented any physical defects. Their ages ranged from 19 to 26; the average was 22.9 years. Five men, Nos. 34, 41, 46, 47, and 49 gave a history of no exposure to influenza; two, Nos. 38 and 45, gave a history of casual contact; and three, Nos. 23, 35, and 39, of close contact.

*Donor.*—F. W. B. No. 5. (See Experiment II.)

*Material.*—Nasopharyngeal washings and bronchial secretions were collected in Locke's solution from 45 to 50 hours after the appearance

of the initial symptoms. After thoroughly shaking with glass beads, a control culture was made, which showed a pneumococcus (Type II) *B. influenzae*, *Streptococcus hemolyticus*, staphylococcus, a gram-negative diplococcus, and a member of the *B. mucosus capsulatus* group. These findings were practically identical with those of the donor, whose washings this donor received.

*Procedure.*—Within 15 minutes after its recovery, 3 c. c. of the material was instilled in the usual manner in each case while the subjects were lying down. In order to produce a hyperemia of the mucous membranes, the oleoresin of capsicum (0.0025 per cent) was sprayed into the nose and mouth of Nos. 23, 34, and 35. Nos. 38, 39, and 41 received a preliminary spray of adrenalin 1–2,000, in the hope of producing an ischaemia. Both procedures appeared to be efficacious for the end in view. Nos. 45, 46, 47, and 49 were given no preparatory applications.

*Results.*—During a seven-day surveillance none of these men showed any symptoms referable to their inoculations. The bacteriological findings of the nasopharynx at the end of a week were practically the same as they were prior to the instillation.

## EXPERIMENT V.

FEBRUARY 22, 1919—6.30 P. M.

Attempt to transmit influenza via upper respiratory tract from pooled secretions of four typical, very early cases.

*Recipients.*—Four men, Nos. 1, 2, 3, and 7, received this inoculation. These men had emerged from experiments in good condition. Their ages were 21, 27, 22, and 24 years, respectively. No. 3 showed some enlargement of the right tonsil. Three of them, Nos. 1, 2, and 3, gave a history of close contact with influenza patients in recent months and the other, No. 7, of casual contact.

*Donors.*—The donors, four in number, were carefully selected from an epidemic which was occurring at the time at the United States Naval Prison, Portsmouth, N. H.

The naval prison, with a population of about 2,200 inmates, is located within the navy yard at Kittery, Me., across the Piscataqua River from Portsmouth, N. H. The influenza epidemic of September, 1918, appeared in the prison earlier than among the personnel of the navy yard or the population of Portsmouth, N. H. The intercommunication among the inmates of the prison is very free, and constant accessions are being received, but there is little communication between the prison and the navy yard, or the city of Portsmouth. The September epidemic in the prison began September 12 and comprised about 400 cases with 30 deaths, the height of the epidemic occurring on September 16. From February 16 to 21, 1919, about 6 influenza cases per day are on record, but, on February 22, 34 new



cases were reported. In this second outbreak there were, in all, 215 cases and 2 deaths, the largest number of cases (38) occurring on February 25. Except in its somewhat lessened extent and its markedly lessened mortality, this epidemic resembled the September outbreak. The inmates had meanwhile changed, in part, and in general other individuals were attacked than those who were sick in September. The same rapidity of spread through the institution to a maximum a few days after the beginning, with a succeeding diminution almost as sudden in number of cases, the same spread throughout the whole institution without marked localization, and the same symptoms were observed as in the first outbreak. At the time of the second outbreak a considerable number of cases of tonsillitis also appeared. The four donors for this experiment were selected as having had their first symptoms only a few hours previously. Many of the other very recent admissions to the sick bay stated that they had had premonitory symptoms as much as 24 hours before reporting sick. These four cases all had a rather sudden onset, with headache, backache, photophobia, prostration, and presented a flushed face with suffused conjunctivae, fauces and palate reddened, but no apparent tonsillar involvement or enlargement of the cervical glands. One other man selected as a donor, with similar symptoms and signs, had a nasal hemorrhage while his pharynx and nose were being washed out, and his washings consequently were not used; this patient later had a very severe but nonfatal broncho-pneumonia. The four donors whose washings were transferred to the volunteers in Experiment V were as follows:

S. M., age 19, entered the prison April 17, 1918, but during the September outbreak of influenza was on a ship which was moored at the prison and which was little affected; he stated that he had never had influenza previously, was not subject to cold, but had frequent attacks of sore throat. He knew of no definite exposure to influenza. At noon on the day of the experiment, six hours before the washings were obtained, he was suddenly and completely prostrated and had to be carried into the sick bay, having been in his usual health during the forenoon. He complained of severe headache and backache, his conjunctivae were suffused and his face and fauces were flushed. There was no tonsillar exudate. On the day following the experiment his white cells were 7,600 per cubic millimeter, and the throat culture showed hemolytic streptococcus, pneumococcus, *Micrococcus catarrhalis*, and staphylococcus, but no streptococcus viridans or influenza bacillus. On the third day in the sick bay, when his temperature was reaching normal, he complained of a slight sore throat, but had no exudate or other evidence of local infection. His heart and lungs were normal on examination, also his urine. He recovered promptly without complications, having had fever only three days, 103° F. at the highest, and was discharged from sick bay after eight days.



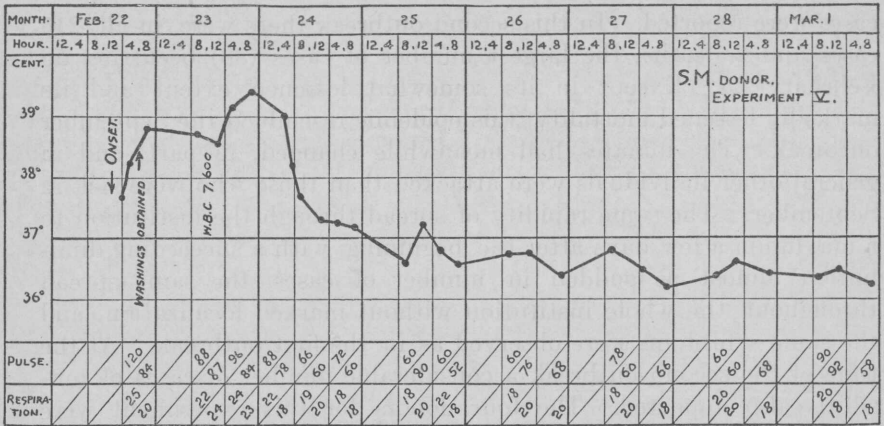


Chart No. 42.

W. L., age 21, entered the naval prison January 24, 1918, but during the September outbreak was on the same prison ship as S. M. No exposure to, nor prior attack of influenza is known. He states that he is not subject to colds. At noon on the day of the experiment, six hours before his nasopharyngeal washings were obtained, he had a sudden onset of severe headache, backache, and pains in chest, with extreme prostration. His face was slightly flushed, his fauces were reddened, and his conjunctivae were suffused. He had a slight cough with muco-purulent sputum, but examination of his chest and of his urine were negative. His leucocytes were 8,800 on the day after the experiment, and pneumococci, staphylococci, and *Micrococcus catarrhalis*, but no influenza bacilli or streptococci, appeared on throat culture. Malaise and weakness continued for several days, but his temperature,  $103.5^{\circ}$  at the highest, reached normal on the fifth day and did not go above normal after that day.

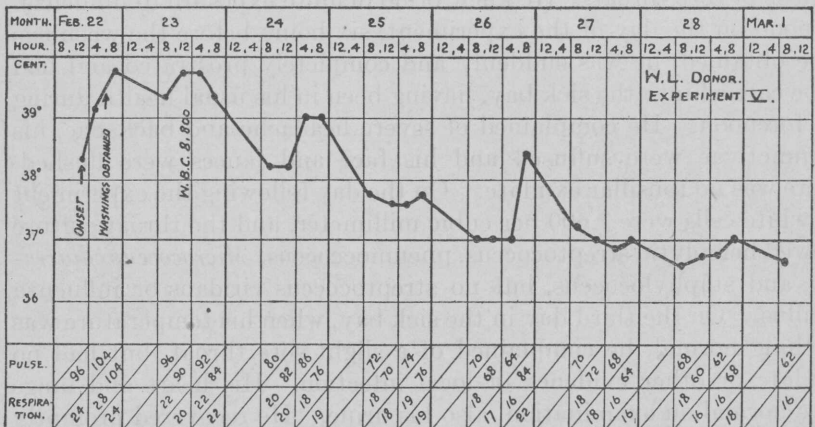


Chart No. 43.

O. J. B., aged 20, had been in the prison since May, 1918, but had had no previous attack of epidemic influenza. On July 4, 1917, he had a slight rise in temperature with cough, mild malaise, and muscular pains, diagnosed as influenza, but he returned to duty in two days. At 4 o'clock on the afternoon preceding the experiment, 26 hours before his washings were obtained, he had an onset of very severe prostration, backache, headache, photophobia, and cramps in abdomen. He had no sore throat at any time. When his washings were taken his temperature was 104.3°. It reached normal on the fourth day, but showed some elevation for three days thereafter, though no complications were observed. His white blood cells were 14,200 on the day after the experiment, and his throat culture showed *Streptococcus viridans*, *Micrococcus catarrhalis*, and staphylococcus, but no hemolytic streptococcus, pneumococcus, or influenza bacillus.

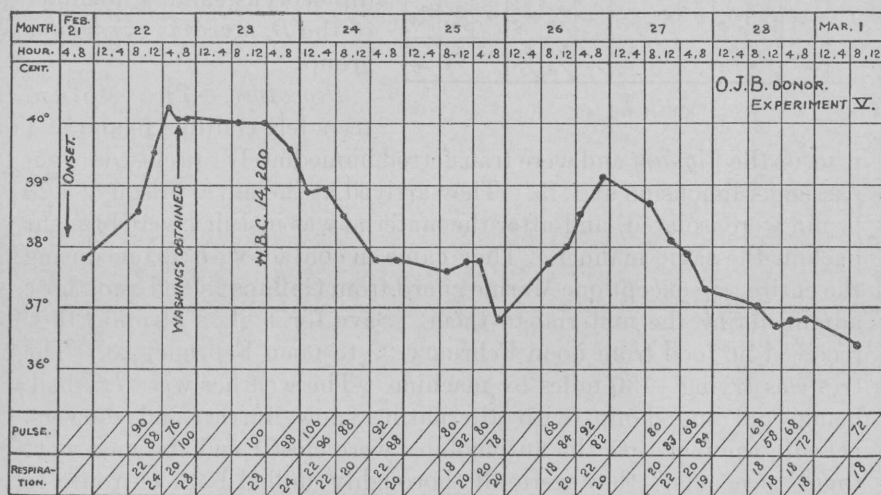


Chart No. 44.

J. P. K., aged 21, had been in the prison since December, 1918. During the autumn outbreak he was at the New York receiving ship and the Deer Island detention camp, but had had no influenza. He stated that he was subject to frequent attacks of sore throat and coryza. An hour after midnight preceding the experiment, 17 hours before his throat and nose were washed out, he was taken sick with a very severe backache, headache, and pain in his legs. He had a slight sore throat, but physical examination revealed only a pharyngitis. His white cells were 14,000 per cubic millimeter on the day after the experiment, and throat culture showed *Streptococcus viridans* and *Micrococcus catarrhalis*, no influenza bacilli, hemolytic streptococci, or staphylococci. He had fever for only 24 hours (maximum 101.2° F.), but was sick for four days and made an uneventful recovery.

**Material.**—The material consisted of a mixture of the crude nasopharyngeal washings and bronchial secretions from the four donors in Locke's solution. The suspension was thoroughly shaken in a

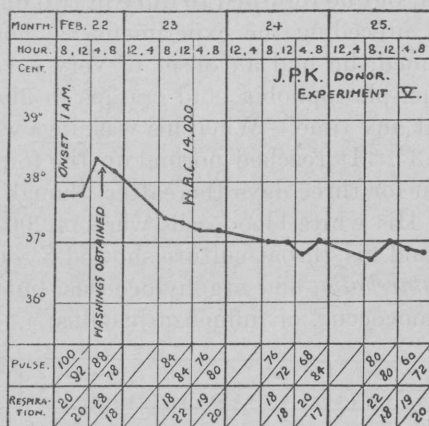


Chart No. 45.

p. m. on the *Vigilant* and were transferred immediately to a closed seven-passenger limousine at 1.35. They arrived at the naval prison at 4.20 p. m., were isolated, and after the material was instilled were brought back in the same manner. They came in contact with no one during the entire trip except one Marine guard from Gallups Island and those administering the material to them. Save for 1 glass of milk, they received no food from noon February 22 to noon February 23. The trip was trying—130 miles by machine. The weather was very bad, being cold and damp, with alternating snow flurries and showers. Despite the fact that the automobiles were closed and the men wore their "peacoats," they were all thoroughly chilled both en route to the prison and on their return. Upon their return to Gallups Island after midnight, they were thoroughly tired. Within 15 minutes after its recovery from the donors, 10 c. c. of the pooled washings were instilled, by spray and dropper, into the nose and throat of each volunteer. Considerable of the material was swallowed. In addition, 100 c. c. of the crude washings from six donors, including the above-mentioned four, were well mixed in 900 c. c. fresh milk. Each man drank 250 c. c. of the milk, or 25 c. c. of the washings.

Two additional donors, originally intended for Experiment VI as healthy inmates of the prison during the epidemic, were found to be somewhat abnormal when their washings were obtained. Being thus excluded from Experiment VI, they were counted as possible atypical or early cases of influenza, and their washings were mixed with those of S. M., W. L., O. J. B., and J. P. K., for the ingestion part of Experiment V. Their histories follow:

sterile container with glass beads. The control cultures showed a green-producing organism, presumably the pneumococcus, to be the predominating one, and, in addition, *Staphylococcus albus* and *aureus*, *Streptococcus hemolyticus* (alpha and beta), *M. pharyngis siccus*, *B. influenzae*, a gram-negative diplococcus, and a member of the *B. mucosus capsulatus* group.

**Procedure.**—The volunteers left Gallups Island at 1

J. J. B. had a flushed face, with reddened fauces and pharynx, and when questioned complained of slight headache and malaise of a few hours' duration; his temperature was normal, but on the following day he reported at sick call with a headache and malaise, and did not go to work that day, though he remained up and about. A week later he was discharged from the Navy. His leucocyte count on the day after the experiment was 14,000, and his throat culture showed hemolytic streptococci, *Micrococcus catarrhalis*, and staphylococci, but no influenza bacilli, *Streptococcus viridans*, or pneumococci.

L. C. H., age 22, complained of headache on the afternoon of the experiment. His temperature was normal, and a later inspection of his medical history showed that he had been a frequent visitor at sick bay during his nine months in the Navy. He had been operated on for chronic appendicitis in June, 1918. At the Deer Island detention camp he was listed as having had influenza with the usual symptoms for the week following September 27, 1918, and again at the Portsmouth Prison on November 25, 1918. He has frequently complained of sore throat and of lame back. On the day following the experiment his white cells numbered 7,400 per cubic millimeter, and a throat culture showed hemolytic streptococci, pneumococci, and staphylococci, but no influenza bacilli, *Streptococcus viridans*, or *Micrococcus catarrhalis*. Ten days after the experiment he was again in sick bay for two days with the diagnosis of influenza, but the symptoms were atypical.

The washings from these two men were used only for mixing with the milk which was taken by the volunteers of Experiment V. The washings from the other four were used both for instillation and for ingestion.

*Results.*—Two of the four men, H. A., No. 1, and W. S. B., No. 7, within 40 hours became ill with attacks of acute follicular tonsillitis, due to *Streptococcus hemolyticus* of the beta variety.

In this regard it is of note that the predominating organism of the nasal pharyngeal flora changed in all four men from a green-producing bacterium before the inoculation to an intensely hemolytic streptococcus after the inoculation. Morphologically, the colonies from the four men were identical. This was true even as late as seven days after the introduction of the material within their nasal pharynges.

H. A. (age 21, No. 1).—Experiment V.

*Diagnosis.*—Acute lacunar tonsillitis.

The patient had always been quite well. Although in close contact with influenza patients during the recent epidemic, he had not been taken ill himself.

Within 40 hours after receiving the material in this experiment and 34 hours after the end of a cold night ride, the patient began to feel ill, complaining of sore throat, headache, anorexia, and malaise.





the one isolated from the tonsil of No. 1, was by far the predominating colony in this instance.

## EXPERIMENT VI.

FEBRUARY 22, 1919—7 P. M.

Attempt to transmit influenza via upper respiratory tract by instillation of pooled nasopharyngeal secretions from persons in contact with early cases of influenza.

*Recipients.*—These four volunteers (Nos. 8, 36, 37, and 43) were in good physical condition. Their ages were 30, 22, 25, and 24, respectively. The histories of Nos. 8, 36, and 37 showed that they had not been exposed during the recent epidemic, while No. 43 had experienced a casual contact.

*Donors.*—The 10 donors employed in this experiment were selected by reason of the fact that they were in a dormitory at the Portsmouth Naval Prison from which two cases of influenza had been removed within 24 hours. All at the time of securing the material were apparently in good health, the intent being to obtain the washings in the incubation period, in the event that one or more of these men would subsequently develop influenza.

Their initials, ages, leucocyte count, and the throat organism found on culture the day after the experiment, were as follows:

Initials.	Age.	White count.	Hemolytic strep.	<i>Strep. viridans.</i>	Pneumococcus.	<i>M. catarrhalis.</i>	Influenza bacillus.	Staphylococcus.
H. J. D.....	18	8,000	+	0	0	+	0	+
F. P. F.....	20	8,400	+	0	0	+	+	+
P. A. J.....	21	11,200	+	+	+	0	+	0
H. V. L.....	26	6,800	+	0	0	+	+	+
A. J. M.....	22	6,400	+	0	+	+	0	0
E. J. M.....	27	9,200	+	+	0	+	0	0
W. H. P.....	20	7,800	+	+	0	0	0	+
C. H. S. <sup>1</sup> .....	26	.....	.....	.....	.....	.....	.....	.....
P. R. S.....	18	6,500	0	0	0	+	0	+
J. J. W. <sup>1</sup> .....	22	.....	.....	.....	.....	.....	.....	.....

<sup>1</sup> Examination not made.

Of these, only H. J. D. had had influenza during the autumn epidemic. P. A. J. and E. J. M. had been on the same ship, comparatively free from influenza, with donors S. M. and W. L. of Experiment V, during the 1918 outbreak.

Following Experiment V, two days after serving as donor, W. H. P. had an indisposition lasting only a day, with headache, a temperature of 101°, and no throat symptoms. On the possibility of this being influenza, he was kept in bed for five days.

Seven days after serving as donor, H. V. L. had a mild pharyngitis lasting about a week, but none of the 10 donors developed the typical symptoms of influenza.

*Material.*—The material consisted of the pooled nasopharyngeal washings and bronchial secretions from the 10 donors, which had been collected in Locke's solution. It was well shaken in a sterile flask containing glass beads. The control cultures showed the presence of a green-producing organism, resembling a pneumococcus, *Staphylococcus aureus* and *albus*, *Streptococcus hemolyticus* (alpha and beta) *B. influenzae*, *M. pharyngis siccus*, and a moist gram negative diplococcus, diphtheroids, *Pneumococcus mucosus*, and one of the *B. mucosus capsulatus* group, presumably *B. Friedlaender*.

*Procedure.*—The same itinerary was followed by this group as described in Experiment V., but the volunteers were carried in another limousine and kept entirely separate from the volunteers of Experiment V. Within 20 minutes after its recovery, 10 c. c. of the material was administered into the nose and throat by spray and dropper. In addition, enough of the secretions were added to milk, so that when 250 c. c. was ingested, 60 c. c. of the nasopharyngeal secretions were taken into the stomach.

*Results.*—In none of these men, during a week's observation, were any untoward symptoms noticed. Save for an increased incidence of *Streptococcus hemolyticus* (beta) the flora of these men was not particularly altered by the inoculation.

## EXPERIMENT VII.

FEBRUARY 24, 1919—7 P. M.

Attempt to transmit influenza via upper respiratory tract by inoculation of nasopharyngeal secretions from a supposed early case. Passage experiment.

*Recipients.*—Ten volunteers, Nos. 9, 10, 11, 13, 15, 16, 17, 20, 21, and 42 were used in this experiment. Nos. 9, 10, 15, and 42 had been left without result from Experiment I, while the other six were not affected by the inoculations in Experiment II. Nos. 10 and 13 had moderately enlarged tonsils. The remaining eight were apparently physically fit. Their average age was 22.1 years, the extremes being 19 and 27 years. Nos. 9 and 17 had had no exposure to influenza; Nos. 11, 13, 15, 16, 20, and 42 had had casual contact and Nos. 10 and 21 close contact.

*Donor.*—The source of the inoculated material in this experiment was W. S. B., No. 7, who was ill following the inoculations made in Experiment V. In the desire to secure material in the very early hours of the disease, and, in this case, to demonstrate infectivity by passage, material was obtained in the ninth hour after the onset of symptoms. Unfortunately, on the following day, it was realized that the presumptive diagnosis of influenza was in reality probably incorrect, inasmuch as a definite lacunar tonsillitis developed. (See clinical data on W. S. B., Volunteer No. 7, under experiment V.)

**Material.**—Nasopharyngeal washings and bronchial secretions were collected as in the previous experiments. The bacteriological controls showed two types of *Streptococcus hemolyticus* to be present in large numbers. The more common appeared on the blood agar plate as a rather large, gray colony with a moderate zone of hemolysis, whereas the other type grew as a pin-point, gray colony, with a much wider and more intense hemolytic halo. In addition, a green producing bacterium, *Staphylococcus aureus*, and *B. influenzae* were noted.

**Procedure.**—In accordance with the method previously described, 5 c. c. of the material were instilled into each of the volunteers within 15 minutes after its recovery from the donor.

**Result.**—Of the 10 men in this group, one, No. 20, left within 48 hours after inoculation, at which time he was in good health. Five of the remaining 9, Nos. 9, 15, 16, 17, and 21, developed attacks, apparently, of tonsillitis, due to *Streptococcus hemolyticus* (beta), of whom three, U. L. C., No. 15, E. W. D., No. 16, and C. D., No. 17, became so ill that they were put to bed in the hospital. The other two, with visible tonsillar inflammation, Nos. 9 and 21, experienced practically no constitutional symptoms. The apparent incubation period of all these cases varied from 36 to 144 hours. Their recovery was complete. One man, P. J. S., No. 42, 66 to 120 hours subsequent to the nasopharyngeal instillation, developed symptoms similar to those of influenza. The remaining three, out of the nine completely observed, developed no symptoms.

The clinical data on Nos. 16, 17, 15, and 42 are as follows:

E. W. D. (age 21, No. 16).—Experiment VII.

**Diagnosis.**—Acute lacunar tonsillitis.

**Incubation period.**—Thirty-six to forty-two hours.

Save for an attack of pneumonia in 1911, the previous health of the patient has been very good. He had a casual contact with influenza patients during the epidemic.

Forty-two hours subsequent to his inoculation, after mild symptoms lasting six hours, the patient was suddenly seized with

headache, chilliness, stiffness in joints, and weakness. The throat was sore, but on examination showed nothing more than a moderate con-

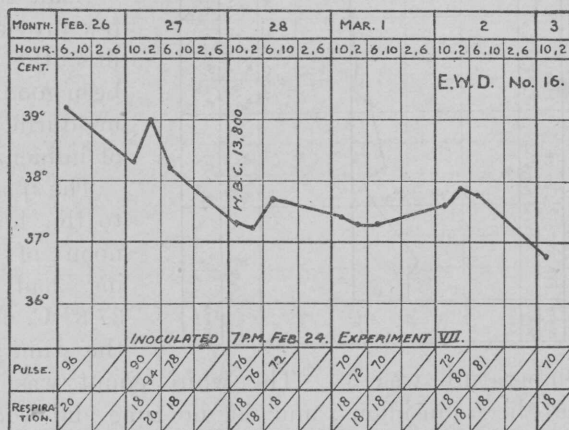


Chart. No. 48

gestion. At this time his temperature was 39.2° C., his pulse 96, and his respirations 20. The white cell count was 13,800, his normal being 6,500. The prostration was slight. The next day the crypts

of his tonsils contained a purulent exudate, which yielded an almost pure culture of streptococcus hemolyticus, growing in small colonies with a wide zone of hemolysis. On the third day the temperature dropped to 37.2° C. and the patient felt much better. On the fifth day the temperature was normal, a good recovery ensuing.

The hemolytic streptococcus had supplanted a green-producing organism as the predominating one. This streptococcus was morphologically similar to one isolated from the secretions of the donor.

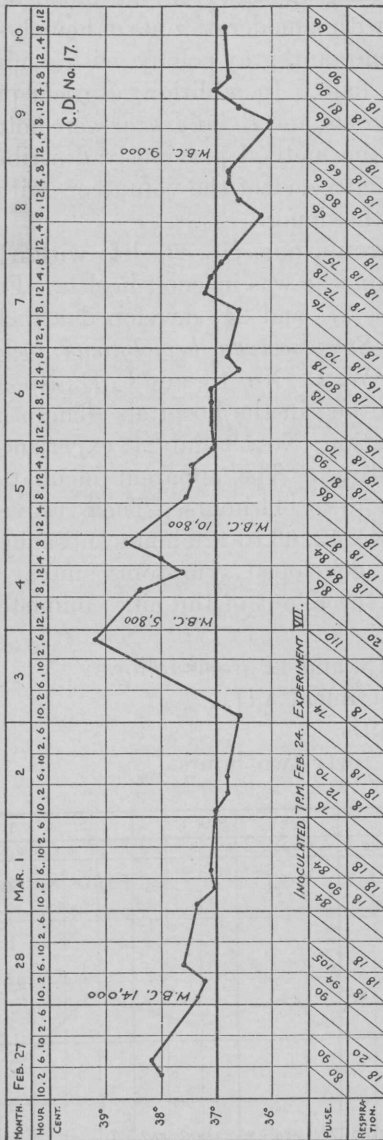
*C. D.* (age 21. No. 17).—Experiment VIII.

*Diagnosis.*—Acute lacunar tonsillitis.

*Incubation period.*—Forty-six to sixty-six hours.

Aside from the history of a few previous attacks of tonsillitis, the patient's health had been good. He had no exposure during the recent outbreak of influenza.

The patient was admitted to the hospital on the afternoon of February 27, having had a temperature of 37.8° C. the day before. At the time of admission the



temperature was 38. The white count was 14,000. The onset had been insidious, and at no time did the patient complain of anything but a sore throat and a slight headache. A small patch of exudate on the right tonsil on the day of admission rapidly spread in a membranous-like fashion. Repeated smears

and cultures were negative for *B. diphtheriae*. The temperature reached normal on the third day and the throat cleared up. On the fifth day the patient was allowed to get up and in a few hours his temperature rose rapidly to 39.2° C. At this time the white cells were 5,800, increasing to 10,800 the next day. His average count before the illness was 8,300. Upon being put to bed, the temperature reached normal again in three days, and the patient was discharged on the twelfth day of his illness after his temperature had remained below 37° C. for five successive days. The white count on discharge was 9,000.

The secondary rise of temperature was not attended by any sore throat, and examination of the pharynx failed to demonstrate anything other than enlarged tonsils. There was no photophobia, cough, nor particular depression. The leucocyte count was 5,800, rising to 10,800 the next day.

The throat cultures on the second day of his illness as well as after the recrudescence showed an intensely hemolytic streptococcus to be the predominating colony. It resembled the pin-point colony described in the donor's secretions.

The second pyrexia presented a somewhat different picture from the first, which was that of a very definite case of tonsillitis. However, in the absence of any more definite evidence, it is fair to assume that the condition might be attributed to the hemolytic streptococci overwhelmingly predominant in the throat on both occasions.

*J. L. C.* (age 20, No. 15).—Experiment VII.

*Diagnosis*.—Acute lacunar tonsillitis.

*Incubation period*.—Six days.

Other than appendicitis with operation in 1917, the patient has always had good health. Since autumn he has only experienced a casual contact with influenza patients.

About 144 hours after his nasopharyngeal instillations, having felt exceptionally well during the preceding day, the patient began to complain of headache, stiff neck, dryness of the throat, photophobia, and chilly sensations. His throat was sore for one morning only. The subsequent day the temperature rose from 37.4° C. in the morning to 38.8° C. in the evening. The pulse varied from 90 to 96, the respirations were 18, and the leucocyte count was 6,000, the count before the experiment, 8,900. At this time the throat was distinctly sore, though physical examination was quite negative. The following day, which was the second day of his illness, the temperature dropped suddenly to 37° C. and did not go above 37.6 until the fifth day, when, upon getting out of bed it rose to 38.2 in the evening. The white cell count was never above 6,600. The tonsils became moderately enlarged on the third day and showed two small patches of exudate, culture of which gave an almost pure growth of intensely



hemolytic streptococcus. On the seventh day the patient felt so well and his throat had apparently cleared up to such an extent that he was allowed to accompany his shipmates to Deer Island.

The case, in spite of the low leucocyte count and the lack of correlation in time between the symptoms and the throat findings, may be assumed to be tonsillitis. The finding of the streptococcus in the tonsillar cultures in such numbers was more than suggestive of a process similar to that in the other members of this group who were taken ill.

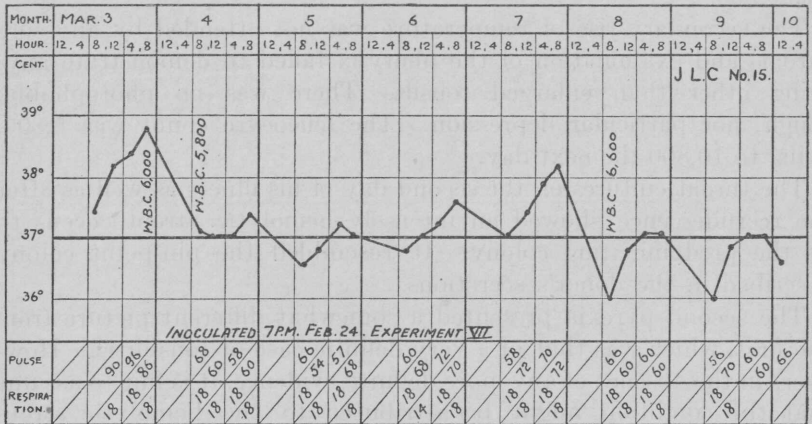


Chart No. 50.

*P. J. S.* (age 21, No. 42).—Experiment VII.

*Diagnosis.*—Influenza.

*Incubation period.*—Sixty-six to one hundred and twenty hours.

The patient had always enjoyed very good health, though he had never been robust and was inclined to be timid and introspective. He had been ill for two months when 10 years old with typhoid and again at 15 with pneumonia. He had never had influenza and only a casual contact with influenza patients in recent months.

By his own statement the patient had not felt well since he received his inoculation, five days previously. He reported sick two days after inoculation, but he had no fever and examination was negative. Four days after inoculation the temperature was 37.3° C. and the next evening it was 37.8°. He was then admitted to the hospital with frontal headache, an annoying cough which had developed suddenly, and pains of moderate intensity in chest, back, and abdomen. He slept poorly. The following day his temperature rose abruptly to 39.4° C. in the morning, and to 39.9° in the evening. The pulse was 105–118 and the respirations from 24 to 26. The patient now was conscious of a fever, had a “splitting” headache, backache, and pains in chest, abdomen, and extremities, with photophobia.

The cough which persisted throughout the course of his illness was accompanied by a tough, mucous expectoration. The leucocyte count was 6,800, his normal being 8,200. Physical examination showed a flushed face with injected conjunctivae, a dusky red throat, with no exudate, a rather rapid heart rate, and negative findings over the pulmonary area. Culture of the nasopharynx yielded a green-producing organism with the characteristics of a pneumococcus, also a hemolytic streptococcus, a gram negative diplococcus, *Staphylococcus aureus* and *B. influenzae*.

The following day, March 3, 1918, the patient was seen by Lieut. Commander McGuire, United States Navy Medical Corps, who found râles posteriorly over both lower lobes and said that in an epidemic the case would surely be called influenza. The urine analysis was negative and the white cell count had fallen to 4,200. The temperature, pulse, and respiration were, on March 4, 38° C., 78 and 18.

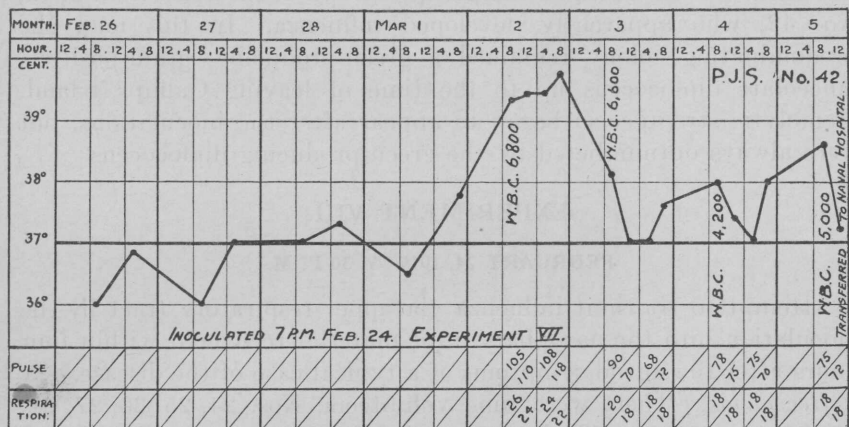


Chart No. 51.

A distressing cough prevailed, but the patient had lost, to a considerable degree, the depression and lethargy of the earlier hours of his illness. On the fifth day of his hospitalization, March 5, 1919, the temperature became 38.6 at 8 a. m., the cough was worse and quite productive, and, while no consolidation could be elicited, numerous transitory râles occurred over the bases of both lungs. The leucocyte count was 5,000. In view of the continuation of the pulmonary findings, however vague, and a slight increase of cough, temperature, and white count, it was deemed advisable to transfer the patient to the United States Naval Hospital, Chelsea. Fortunately, he did not develop a bronchopneumonia. His sputum continued abundant and contained influenza bacilli, and micrococcus catarrhalis during the first three days at the naval hospital. On March 10, besides the influenza bacilli, *Streptococcus viridans* and Type IV pneumococcus were

found. His cough was severe, with pulmonary râles, subnormal temperature, and pain in the side. On March 11 he had a chill and his temperature rose to 38.3, leucocytes to 12,000, with headache, general pains, and injected conjunctivae. The temperature returned to normal in 12 hours. The sputum continued to give about the same bacteriological picture with influenza bacilli in great numbers. He progressed thereafter to a satisfactory recovery. The syndrome presented by this individual was not comparable in any way to the illnesses of Nos. 16 and 17.

The bacteriological findings of the nasopharynges of this group are striking. Prior to inoculation, a green-producing organism was the predominating one, while only three showed the presence of a hemolytic streptococcus. The cultures taken for periods varying from two to seven days after the instillations showed the predominating organism to be a hemolytic streptococcus with intense hemolytic properties in all ten members of the group, except in the case of P. J. S., No. 42, who apparently developed influenza. In this man the predominating colony remained a green-producing, gram-positive, lanceolate diplococcus up to the time of leaving Gallups Island. Hemolytic streptococci began to appear after the inoculations, but were always outnumbered by the green-producing diplococcus.

## EXPERIMENT VIII.

FEBRUARY 24, 1919—7. 30 P. M.

Attempt to transmit influenza via upper respiratory tract by the inoculation into the nasopharynx of material recovered within four hours after the initial symptoms of a typical case of the disease.

*Recipients.*—There were nine volunteers, Nos. 24, 25, 26, 27, 28, 30, 31, 32, and 33. Their ages varied from 20 to 36. These men were used seven days previously in Experiment III, with negative results throughout, having been discharged seven hours before this experiment was begun. All were in good physical trim, and their throats were healthy in appearance. Nos. 27, 32, and 33 had moderately enlarged tonsils. Six men, Nos. 25, 26, 27, 28, 31, and 32, had had no exposure to influenza; one, No. 33, a casual contact; one, No. 30, close contact and one, No. 24, had a typical attack of influenza while at Deer Island in September, 1918.

*Donor.*—The donor, having had no prior attack of influenza, in spite of repeated exposure, was in close contact with the donors of Experiment V, going to Portsmouth by automobile, and returning in the same way during a severe storm, reaching Boston at 5 a. m.; 44 hours after the exposure the onset occurred with headache, backache, pain in thighs, prostration, a temperature of 38.4°, reaching 39.9° in 6 hours, a dry throat with reddened fauces but no tonsillitis.

There were lachrymation and injection of the conjunctivæ for 48 hours, and photophobia for 4 days. An infrequent, paroxysmal cough with moderate mucopurulent expectoration began 24 hours after onset, but began to diminish after 3 days. The leucocyte count was 7,000. Anorexia and slight nausea were present during the first 48 hours, but there was no vomiting. The fever lasted only 60 hours, and convalescence was uninterrupted.

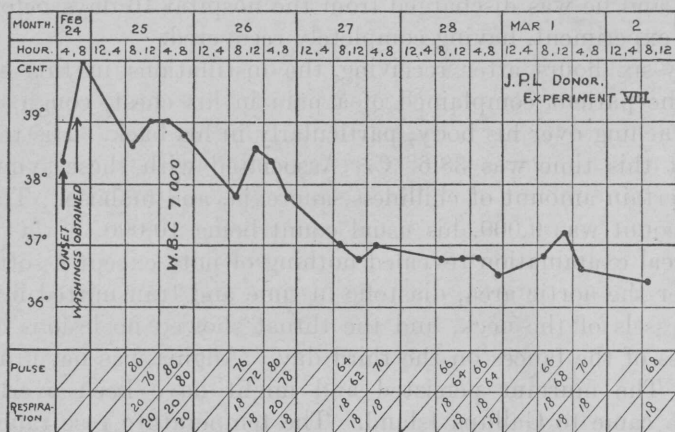


Chart No. 52.

**Material.**—In the prescribed fashion, nasopharyngeal washings and bronchial secretions were collected in Locke's solution, four hours after the onset of the initial symptoms. Bacteriological examination of the emulsified secretions at the time of inoculation yielded a green-producing organism (with characteristics of a pneumococcus), a gram-negative diplococcus, *B. influenzae*, and a few faintly hemolytic streptococci. The presence of *B. proteus vulgaris* prevented isolation of the streptococcus. Another culture, taken from the donor's nasopharynx nine days after the onset of his illness, showed the same types of organisms to be present except for the *B. proteus vulgaris*.

**Procedure.**—In 1 hour and 45 minutes after the collection of the secretions from the donor, 4 c. c. were given by the nose and throat to each of Nos. 26, 28, 30, 31, and 33 by spray and dropper, while Nos. 24, 25, 27, and 32 received 5 c. c. each, in the same manner.

**Results.**—One volunteer, L. F. J., No. 25, after an incubation of 36 hours, pursued a symptom-complex identical with that encountered in influenza. He gave a history of no other exposure to influenza.

*L. F. J.* (age 20, No. 25).—Experiment VIII.

**Diagnosis.**—Influenza.

**Incubation period.**—Thirty-six hours.

The patient states that he had an attack of diphtheria at the age of 7. During the recent epidemic of influenza he was not exposed to any cases, as far as known. He arrived on Gallups Island February 6, on which day he complained of a sore throat. He said he had never been troubled with tonsillitis prior to this attack. His temperature was  $37.5^{\circ}\text{C}$ . and the crypts of his tonsils contained a purulent exudate. The temperature dropped to normal the ensuing day and he was discharged from the hospital 16 days before the present experiment, having completely recovered.

Thirty-six hours after receiving the instillations in this experiment, the patient complained of a pain in his chest, cough, and a general aching over his body, particularly in his back. His temperature at this time was  $38.6^{\circ}\text{C}$ . Associated with these symptoms was a certain amount of chilliness, anorexia, and malaise. The leucocyte count was 9,000, his usual count being 10,000.

Physical examination revealed nothing of note except a soft murmur over the aortic area, diastolic in time and transmitted into the great vessels of the neck, and the throat showed no lesions except a redness of the fauces on the third day. Angina was never a complaint. The murmur persisted and might have been overlooked when he came to Gallups Island. The temperature rose rapidly to  $39.4^{\circ}\text{C}$ ., remaining above  $38^{\circ}\text{C}$ . for about 48 hours and then dropping rapidly to  $37.4^{\circ}\text{C}$ . The pulse was never higher than 126. The leucocyte count at this time was 4,400. Two days later it was 4,600. On the second day of his illness, the patient developed considerable photophobia and postorbital pain, with general headache. Backache and generalized pains persisted until the third day after the onset. The urine analysis was negative. The bacteriological examination of the nasopharynx yielded *B. influenzae* as the predominating colony; a gram negative diplococcus, diphtheroids, a few pneumococci, and *Streptococcus hemolyticus* of both alpha and beta types were also found. Following three days of normal temperature the patient was allowed out of bed.

On March 3, five days after onset, examination by Lieut. Commander McGuire showed some degree of cardiac hypertrophy and a few râles at the apex of the left lung, anteriorly. There was nothing in the previous history of the patient which would lead one to suspect incipient tuberculosis. The patient made a speedy recovery.

By way of résumé, it will be seen that 36 hours after inoculation from an early, typical, uncomplicated case of influenza, the patient suddenly developed a cough, general pains and later photophobia and postorbital aching. The temperature went abruptly to  $39.4^{\circ}\text{C}$ . and came down as suddenly within 48 hours. There was no sore throat. A marked leucopenia was present—4,400. The predominating colony in the flora of the nasopharynx changed from a hemo-



lytic streptococcus before the inoculation to *B. influenzae* shortly after the onset of his illness.

The case was apparently one of influenza, though rather gradual in development. On the third day, after the temperature had reached normal, his secretions were used to inoculate volunteers in Experiment IX.

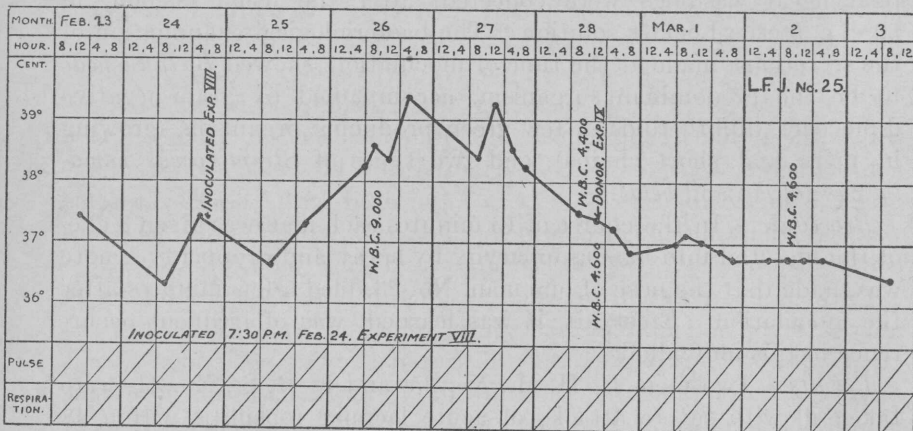


Chart No. 53.

The other volunteers of this group remained quite well during the nine days of observation.

### EXPERIMENT IX.

FEBRUARY 28, 1919—3 P. M.

Attempt to transmit influenza via upper respiratory tract by inoculation with nasopharyngeal washings obtained 54 hours after onset. Passage experiment.

*Recipients.*—Fifteen men, Nos. 5, 8, 23, 34, 35, 36, 37, 38, 39, 41, 43, 45, 46, 47, and 49, were the volunteers in this experiment. No. 5 had experienced an influenza-like attack after inoculation in Experiment II, so was not inoculated on this occasion, being considered as a contact control. No. 8 had been used in Experiments I and VI. Nos. 23, 34, 35, 38, 39, 41, 45, 46, 47, and 49 were the recipients in Experiment IV, while Nos. 36, 37, and 43 were recipients in Experiment VI. All had been recently released from the several previous experiments and were in good physical condition. It was noted that Nos. 38 and 39 had rather large tonsils with prominent crypts. Their ages ranged from 19 to 30, the average age being 23.5 years. Eight men, Nos. 8, 34, 36, 37, 41, 46, 47, and 49, had never been exposed to influenza, according to their history; three, Nos. 38, 43, and 45, had had a casual contact; three, Nos. 23, 35, and 39, had had a close contact.

*Donor.*—The source of the material in this experiment was L. F. J., No. 25, who developed symptoms of influenza apparently as a result of the instillation he received in Experiment VIII. An account of the clinical course of his illness has been given under the results of Experiment VIII.

*Material.*—Fifty-four hours after the onset of his illness, nasopharyngeal washings were collected, after the usual fashion, in 50 c. c. sterile Locke's solution. The bacteriological examination of the secretions, made at the time of inoculation, showed *B. influenzae* to be the predominant organism, accompanied by gram-negative diplococci, diphtheroids, a few green producing organisms (growing in pairs and short chains), and two types of *Streptococcus hemolyticus* (*alpha* and *beta*).

*Procedure.*—In the course of 15 minutes each man was given 3 c. c. of the material into his nasopharynx by spray and dropper. A note was made that the nose of one man, No. 23, bled a few minutes after the inoculation. Epistaxis, it was learned, was of frequent occurrence in this individual.

*Results.*—Two men, H. H. M., No. 35, and E. R. S., No. 41, were taken ill with severe attacks of acute lacunar tonsillitis within 48 hours after inoculation, and a third, T. J. S., No. 43, developed the same condition in 72 hours. The cultures from their throats showed almost pure cultures of a markedly hemolytic streptococcus. H. H. M. and T. J. S. made a good recovery in 10 days. E. R. S. developed a right-sided otitis media due to *Staphylococcus aureus* on the seventh day of his illness, after his tonsils had apparently returned to normal. The tympanic membrane ruptured spontaneously 15 hours after the first slight pain in the ear was experienced. The clinical data of these three cases is herewith appended.

*H. H. M.* (age 23, No. 35).—Experiment IX.

*Diagnosis.*—Acute lacunar tonsillitis.

*Incubation period.*—Forty-six hours.

The previous health of the patient had always been good. During the recent epidemic he had been in close contact with influenza cases, but never contracted the disease. In September, 1918, he was given two inoculations at 48-hour intervals, of a vaccine made from Pfoiffer bacillus, while on duty at Gallups Island.

Forty-six hours after the nasopharyngeal instillations the patient complained of a sore throat, chills, anorexia, headache, backache, and malaise. The temperature was 38° C. A few hours later it rose to 39.4° C. and then to 39.6° C. The leucocyte count was 12,000, becoming 13,000 the next day, when the temperature dropped to 38.2° C. The normal white count was 7,500. The urine analysis was negative. Examination of the throat showed hypertrophy of tonsils with considerable congestion. There was marked swelling

and tenderness of the cervical lymph nodes. On the third day after the onset of the initial symptoms there was a large quantity of exudate in the crypts of both tonsils, particularly the left. Cultures yielded many hemolytic streptococci, and a few gram negative diplococci, *B. influenzae* and pneumococci. The streptococci were very similar to the beta type encountered in the donor's secretions.

Aside from a rather distressing glandular involvement, the patient made a good recovery. The temperature returned to normal on the fifth day, and four days later the patient was allowed to return to Deer Island.

The tonsillar involvement in this case was fully adequate to account for the symptoms presented.

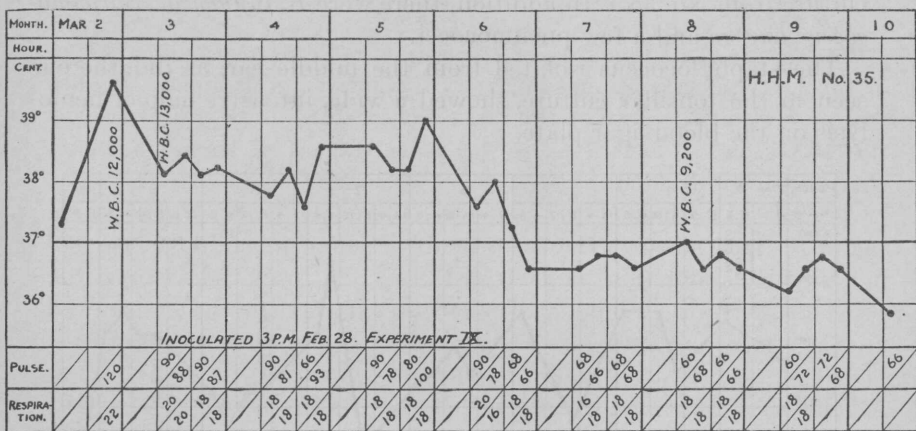


Chart No. 54.

*E. R. S.* (age 25, No. 41).—Experiment IX.

*Diagnosis.*—Acute lacunar tonsillitis, complicated by otitis media.

*Incubation period (tonsillitis).*—Forty-eight hours.

This patient had always been well and had not been exposed to any cases of influenza during the recent outbreak. He received one inoculation of influenza vaccine while at Deer Island, in August, 1918.

Forty-eight hours after the introduction of the washings from No. 25, the patient developed a sore throat and some stiffness in his neck. His temperature was 38.4°C., pulse 96, respirations 18. The leucocyte count was 14,200, his normal being 7,300. Examination of the throat showed the crypts of both tonsils to contain a purulent exudate. On the fourth day, when the temperature was slowly receding, the patient complained of some pain in the right neck and palpation revealed the presence of enlarged tender glands. The following morning the temperature, pulse, and respiration were 36.4°C., 84, and 18 respectively. That evening the temperature rose abruptly to 39.4°C. and the pulse to 104. The patient had some headache and soreness in the neck, but otherwise felt quite comfortable.

The next day, which was the sixth day of his illness, the patient's temperature returned to normal and he felt well. The leucocyte count was 10,600. That night, however, an otitis media began, which caused rupture of the tympanic membrane.

The thick, hemorrhagic, purulent exudate yielded a pure strain of *Staphylococcus aureus*. Immediately after the rupture of the membrane, all subjective symptoms subsided. There was no tenderness over the mastoid process. The patient was sent to the Naval Hospital at Chelsea.

At the height of the tonsillitis a culture from the throat showed the predominating colony to be a hemolytic streptococcus, morphologically similar to a strain seen in the donor's secretions and in the culture from No. 35. In addition, there were *B. influenzae*, *Staphylococcus aureus* and a few pneumococci.

The staphylococcus isolated from the middle ear, as did the one seen in the tonsillar culture, showed a wide, intensive halo of hemolysis on the blood agar plate.

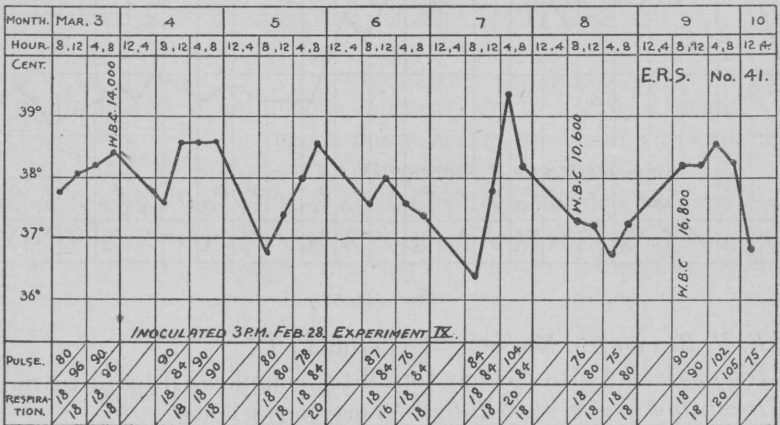


Chart No. 55.

*T. J. S.* (age 24, No. 43).—Experiment IX.

*Diagnosis.*—Acute lacunar tonsillitis.

*Incubation.*—Seventy-two hours.

The patient had always enjoyed good health. He had a casual contact with cases of influenza during the present epidemic. Seventy-two hours after inoculation the patient complained of headache, angina, dysphagia and malaise. His temperature was 37.6° C. and the tonsils were markedly congested and swollen. The following morning he felt quite ill and several patches of exudate were noted over both tonsils. The white cell count was 20,000. The temperature rose to 39.4° C., the pulse to 104, and the respirations were 18. Within three days the temperature returned to normal and he was discharged in a week in good condition.

The bacteriological findings of the throat culture were hemolytic streptococci, gram negative diplococci, *Staphylococcus aureus* and pneumococcus. The first mentioned was by far the most numerous and corresponded, morphologically, with those isolated from the donor and Nos. 35 and 41.

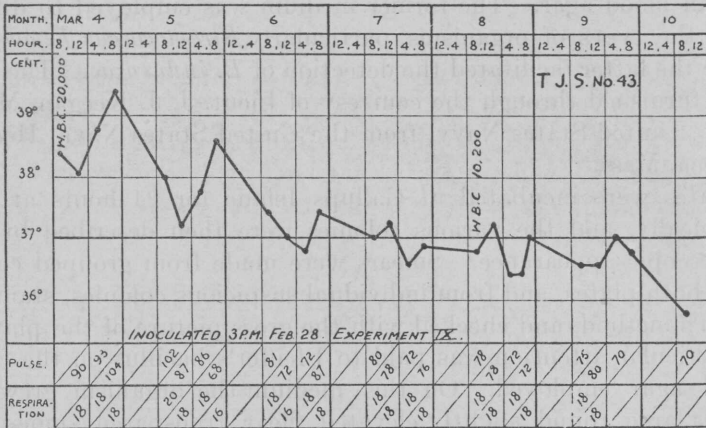


Chart No. 56.

During a period of observation, continuing over eight days, none of the other members of this squad became ill.

The bacteriological findings of the nasopharynx from this group are worthy of mention. Before inoculation a green-producing bacterium was the predominating one in 73 per cent and hemolytic streptococcus in one case (7 per cent), although this type of organism was noted in 20 per cent. Several days after the instillation, hemolytic streptococcus was the predominating organism in 40 per cent and occurred in 86 per cent, whereas the green-producing organism predominated in 46 per cent. The predominating organism of the individual who received no material but remained in the same room with the others changed from a green-producing to a hemolytic streptococcus.

### BACTERIOLOGY.

Bacteriological examinations were made of the nasopharynx of each volunteer before inoculation in the several experiments and upon discharge from the experiment, as well as on those occasions where there was some indication for further investigation, and in the event any individual was taken ill. In addition, cultural controls were made of the material inoculated to determine the type and viability of the organisms inoculated where cultures were used; and to determine the bacteriologic content of nasopharyngeal and bronchial secretions where this was the material inoculated.



The method of procuring the nasopharyngeal cultures consisted in swabbing the posterior pharyngeal wall, high in the vault, by a sterile cotton applicator or West tube. This was then inoculated on a portion of a whole, fresh, human blood agar plate, and a Petri dish containing a thin film of Levinthal's medium—cleared, cooked, human blood agar. The former medium was employed to differentiate the types of organisms, particularly *Streptococcus hemolyticus*, while the latter facilitated the detection of *B. influenzae*. The media were furnished through the courtesy of Lieut. J. J. Keegan, Medical Corps, United States Navy, from the United States Naval Hospital, Chelsea, Mass.

Plates were incubated at Gallups Island for 24 hours at 37.2°, aerobically, and the various colonies were then described by their microscopic appearance. Smears were made from grouped colonies from both plates, and from individual suspicious colonies, stained by Gram's method, and checked with the gross picture of the plate.

Particular attention was paid to Pfeiffer's bacillus on the cooked blood agar employed. On this medium the organism grew as a rather large, round, slightly elevated, clear, transparent, moist, lens-like or tear-drop colony, looking much the same as meningococcus on fresh blood agar, only more transparent. In smear, the appearance of a tiny, short, gram negative bacillus, with a distinct tendency to clumping was deemed necessary for the positive diagnosis of this bacterium.

By the very nature of the procedure, and by virtue of the fact that these examinations were carried out according to the experiment, each volunteer was as a rule cultured more than once. It has been found in reviewing the data that usually the results of the various floral examinations in one individual corresponded quite closely, and, if they did not, the discrepancy could be attributed, for the most part, to the character of a previous instillation.

In the interpretation of results, it is to be remembered that they are based on the 62 inoculations of human secretions made in seven experiments and not on the 43 volunteers as single individuals on whom these inoculations were made.

The total incidence of *B. influenzae* before inoculation was 41 out of 62 or 66 per cent, whereas the incidence in the unused 43 volunteers—that is, before any inoculation—was 48 per cent. After inoculation, 45 of 62, or 73 per cent, gave positive cultures.

It occurred as the predominating colony in one, No. 24, before inoculation with human material (but subsequent to an inoculation with Pfeiffer's bacillus itself), and in two, Nos. 24 and 25, after inoculation. It is of further interest that No. 24, the only man who gave a history of an attack during the recent epidemic, showed this as the predominating organism only after these bacteria had been

instilled into his nasopharynx; on the other hand, L. F. J., No. 25, who was in the same group, showed *B. influenzae* before and after Experiment III, but it was only after he had apparently passed through an attack of the disease, contracted in experiment 8, that they became the predominating colony, supplanting a hemolytic streptococcus.

In Experiment III, where living *B. influenzae* and *Staphylococcus aureus* were given, the former organism was isolated in 60 per cent of the 10 men before instillation, and 100 per cent seven days after instillation. It was the predominating colony in no instance before, and in one case after. On the other hand, *Staphylococcus aureus* occurred in 70 per cent before and after, but it was the predominant colony in 10 per cent before experimentation, and 40 per cent seven days subsequent to the inoculation.

In so far as it was impracticable to determine by routine sugar reactions and bile solubility, and make agglutination and complement fixation tests, all green pigment producing organisms were included under one head. By far the two most frequent components of this group were pneumococcus and *Streptococcus viridans*. The more usual one of these two was a gram positive, lanceolate, capsulated diplococcus, showing umbilication of the small, round, green, colony on a blood agar plate. Prior to the inoculations with human material, this group of organisms occurred as the predominant colony in 77 per cent of cases, and was found in 94 per cent. After inoculation, it predominated in but 60 per cent and was noted in 95 per cent. The discrepancy in the proportion of the predominating colony, before and after, can be explained, in part, by the fact that in 13 instances—in Experiments V, VII, IX—it was supplanted by *Streptococcus hemolyticus* subsequent to the instillation.

Hemolytic streptococci were encountered in 25 instances (40 per cent) before inoculation and in 47 or 76 per cent seven days after inoculation. The beta type (Smith and Brown) was the more frequent, occurring 21 times (34 per cent) before and 46 times (74 per cent) subsequent to inoculation. It formed the predominating colony in but 8 per cent prior to instillation and was predominant in four times as many men (32 per cent) after inoculation. This increase (Table II) is most evident in Experiments V, VII, and IX, in which 10 out of the 12 cases of acute lacunar tonsillitis occurred. In each instance alpha and beta types were found in the donor's secretions but the alpha type was, in no case, the predominant factor. In only one donor—that of Experiment VII—did streptococci outnumber the other bacteria. In only two cases where the beta variety dominated after experimentation was it dominant prior to that event; in one instance it occurred as the most frequent bacterium before instillation and was surpassed in number subsequently, by

the influenza bacillus. This was evident in the case of No. 25, Experiment VIII, who apparently contracted influenza as a result of the inoculation.

The alpha type occurred alone in 4 cases (6 per cent) before, and in 1 case after inoculation. It was never seen as the predominating colony. Both types were noted together four times (6 per cent) prior to inoculation and five times (8 per cent) after the experiment.

No attempt was made to distinguish between *M. catarrhalis* and meningococcus. It was assumed that, in the vast majority of cases, *M. catarrhalis* occurred more frequently than meningococci, from the macroscopic appearance of the colonies on the fresh blood agar plate. Gram negative diplococci, including the two just mentioned organisms and *M. pharyngis siccus*, were found in 46 cases (74 per cent) before inoculation and in the same number seven days after inoculation. This type of bacterium predominated in only one case before and in none after instillation.

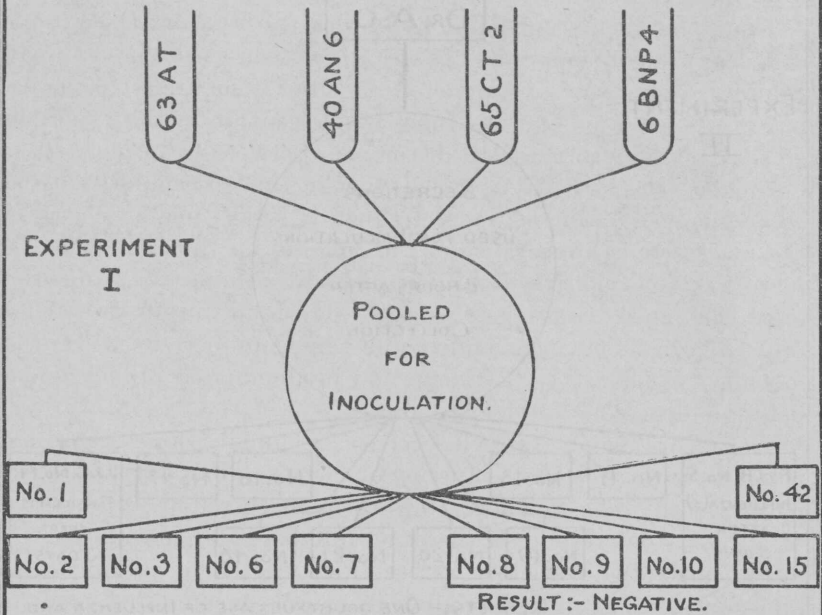
The recording of staphylococci was originally done according to whether they were albus, aureus, or citreus. For purposes of comparison with other organisms, these types occurred in 36 (57 per cent) of all cases before and in the same number after inoculation. Not infrequently two varieties were seen in the same culture—usually albus and aureus. Occasionally, citreus was encountered, alone or in conjunction with one of the other organisms. They were found to predominate in 10 per cent before experimentation; in only one case did they maintain the dominant place after inoculation as well.

Other types of organisms, diphtheroid bacilli, and the groups of the Friedlaender bacillus, *B. proteus*, and *B. subtilis*, were not found in many cases.

### SUMMARY.

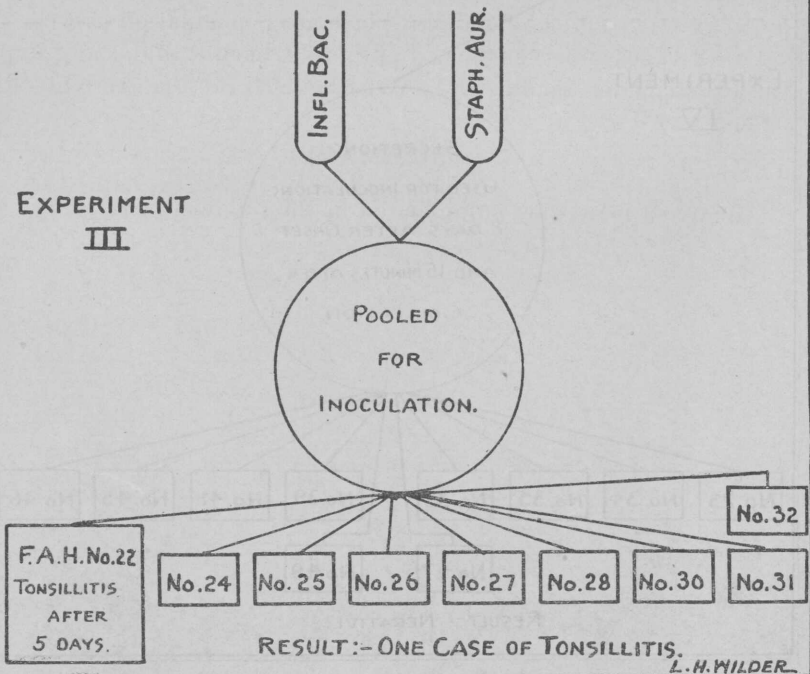
The experiments are summarized in the following charts:

## FOUR STRAINS OF MATHER'S COCCUS.



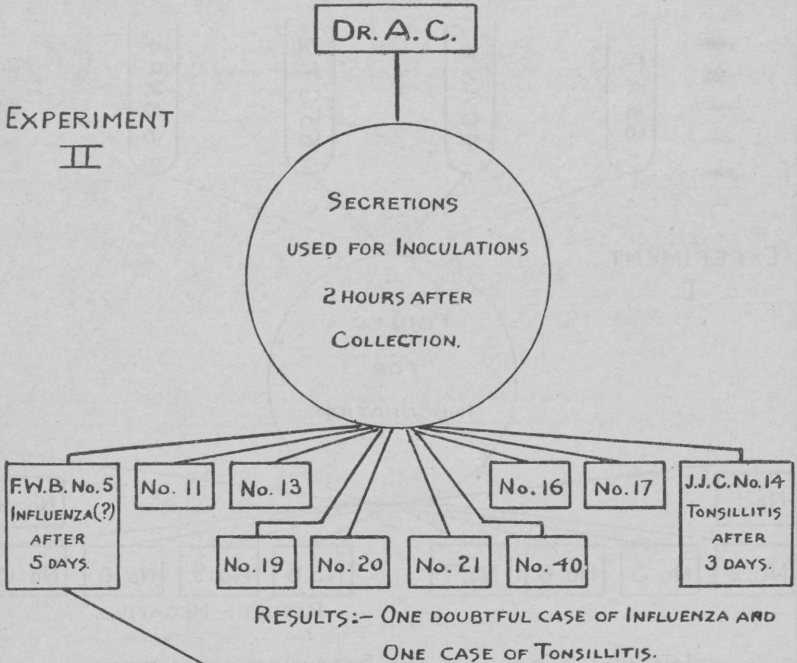
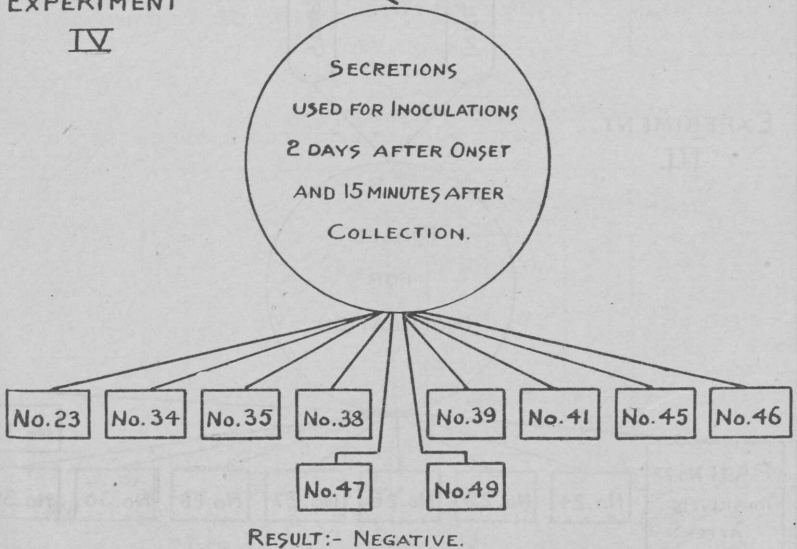
## INFLUENZA BACILLUS AND STAPHYLOCOCCUS AUREUS.

## EXPERIMENT III



L. H. WILDER.

## CASE OF INFLUENZA 22 HOURS AFTER ONSET.

EXPERIMENT  
IIEXPERIMENT  
IV



# CASES OF INFLUENZA IN AN EPIDEMIC.

S.M.  
6 HOURS  
AFTER ONSET.

W.L.  
6 HOURS AFTER  
ONSET

O.J.B.  
26 HOURS  
AFTER ONSET.

J.P.K.  
17 HOURS  
AFTER ONSET.

CASES OF TRANSIENT  
INDISPOSITION DURING  
AN EPIDEMIC OF  
INFLUENZA

J.J.B.

L.C.H.

## EXPERIMENT V

SECRECTIONS POOLED  
FOR INOCULATION BY  
NOSE AND THROAT AND  
MIXED WITH → SECRETION POOLED  
FOR SWALLOWING 15 MINUTES AFTER  
COLLECTION

W.S.B., No. 7.  
ILLNESS AFTER  
40 HOURS,  
LATER APPARENTLY  
TONSILLITIS.

H.A., No. 1.  
TONSILLITIS  
AFTER  
40 HOURS.

No. 2

No. 3

RESULTS:- ONE CASE OF FRANK  
TONSILLITIS, ONE CASE APPARENTLY  
TONSILLITIS.

SECRECTIONS USED FOR INOCULATION 8 HOURS  
AFTER ONSET, 15 MINUTES AFTER COLLECTION.

P.J.S., No. 42  
INFLUENZA  
DEVELOPING  
66 TO 120 HOURS  
AFTER  
INOCULATION.

E.W.D., No. 16  
TONSILLITIS  
36 HOURS  
AFTER

No. 10

No. 11

No. 13

No. 20

J.L.C. No. 15  
TONSILLITIS  
6 DAYS  
AFTER.

C.D. No. 17  
TONSILLITIS  
2 DAYS AFTER  
WITH  
SECONDARY  
FEBRILE  
ATTACK.

No. 9, MILD  
TONSILLITIS  
3 DAYS  
AFTER.

No. 21, MILD  
TONSILLITIS  
2 DAYS  
AFTER.

## EXPERIMENT VII

RESULTS:- APPARENTLY ONE CASE OF  
INFLUENZA AND FIVE CASES OF TONSILLITIS.

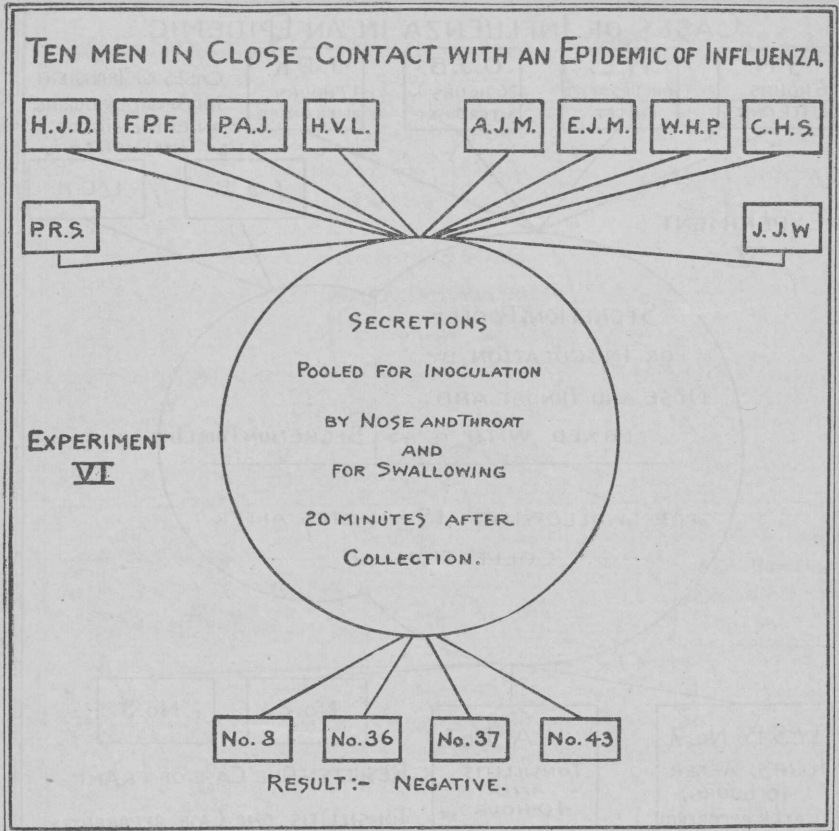
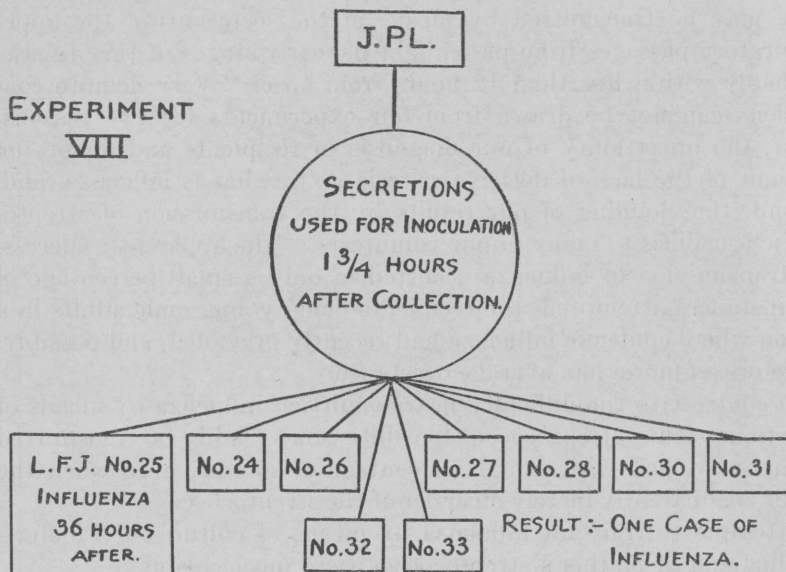
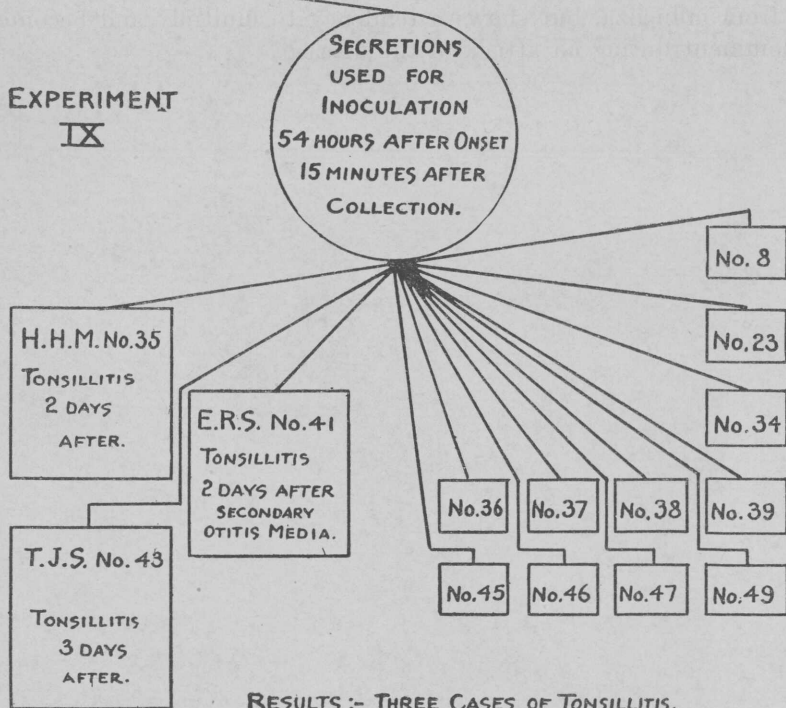


Chart No. 60.

## CASE OF INFLUENZA 4 HOURS AFTER ONSET.

EXPERIMENT  
VIIIEXPERIMENT  
IX

## CONCLUSIONS.

The results of these experiments indicate presumptively that influenza may be transmitted by means of the secretions of the upper respiratory passages from patients in the early stages of this disease, probably within less than 12 hours from onset. Very definite conclusions can not be drawn from our experiments for two reasons: First, the uncertainty of our diagnosis in recipients and donors on account of the lack of decisive criteria as to what is influenza, and, second, the clouding of our results by the transmission of streptococcic tonsillitis to many of our volunteers. The apparently successful transmission of influenza occurred in only a small percentage of the instances attempted, the recipients being young male adults in a region where epidemic influenza had recently prevailed, and possibly, therefore, of more than average resistance.

In contrast to the difficulty in transmitting influenza by means of secretions, acute streptococcic tonsillitis may readily be transmitted in this way, and with a high percentage of success, even when the donor is apparently merely a carrier of the streptococcus.

Attempts to transmit influenza by means of cultures of Pfeiffer's bacillus and of Mather's streptococcus were unsuccessful.

Pfeiffer's bacillus is found in the throats of many people who are free from influenza, but shows a tendency to multiply and become predominant during an attack of the disease.

TABLE I.—*Volunteers, Boston experiments, January and February, 1919.*

No.	Name.	Age.	History referable to recent pandemic.				Weight.		No. of experiments used in.	Remarks.	Illness caused by experiment.	Schick test (read in 72 hours)
			Previous attacks of influenza.	Close contact.	Casual contact.	No exposure.	Before experiment.	After experiment.				
							<i>Pounds.</i>	<i>Pounds.</i>				
1	Alberts, H. ....	21	0.	+	—	—	143	142	1 and 5..		Tonsillitis.....	0
2	Belcher, H. A. ....	27	0	+	—	—	142	144	1 and 5..		0.....	++
3	Bentley, F. E. ....	22	0	+	—	—	144	142	1 and 5..		0.....	++
5	Bolle, F. W. ....	23	0	—	—	+	170	172	2 and 9..		Influenza (?).....	0
6	Brown, Wm. R. ....	29	0	+	—	—	158	158	1.....		0.....	0
7	Burns, Wm. S. ....	24	0	—	+	—	155	156	1 and 5..		Tonsillitis.....	+
8	Caine, S. H. ....	30	0	—	—	+	172	172	1,6, and 9		0.....	++
9	Call, G. W. ....	27	0	—	—	+	147	143	1 and 7..		Tonsillitis.....	Pseudo.
10	Cataldo, C. ....	21	0	+	—	—	135	127	1 and 7..		0.....	+
11	Clancy, R. R. ....	19	0	—	+	—	138	143	2 and 7..		0.....	0
13	Corbett, Hawley..	26	0	—	+	—	156	160	2 and 9..		0.....	0
14	Corbett, J. J. ....	22	0	+	—	—	137	132	2.....	Tachycardia....	Tonsillitis.....	0
15	Corbett, J. L. ....	20	0	—	+	—	135	134	1 and 7..		do.....	0
16	Daniels, E. W. ....	21	0	—	+	—	159	159	2 and 7..		do.....	Pseudo.
17	Deming, C. ....	21	0	—	—	+	165	165	2 and 7..		do.....	+
18	Erwin, F. K. ....	24	0	—	—	+	162	-----		Possible attack of influenza before experiment.	Not used in experiment.	0
19	Fox, J. J. ....	21	0	—	+	—	145	145	2.....		0.....	0
20	Greatrix, G. A. ....	23	0	—	+	—	142	142	2 and 7..		0.....	0
21	Harmon, R. A. ....	22	0	+	—	—	150	150	2 and 7..		Tonsillitis.....	0
22	Hill, F. A. ....	22	0	—	—	—	152	145	3.....		do.....	++++
23	Humphreys, P. F., jr.	19	0	+	—	—	182	184	4 and 9..		0.....	0
24	Isaacson, J. ....	36	1	—	—	—	140	146	3 and 8..		0.....	0
25	Jankowski, L. F. ....	20	0	—	—	+	133	140	3 and 8..		0.....	0
26	Kaplan, Louis. ....	21	0	—	—	—	152	152	3 and 8..		Influenza.....	0
27	Killebrew, Otis ..	26	0	—	—	+	165	166	3 and 8..		0.....	+
28	Kronberg, E. G. ....	21	0	—	—	+	165	176	3 and 8..		0.....	+
30	Louden, A. A. ....	22	0	+	—	—	165	153	3 and 8..	Faint mitral murmur....	0.....	0
31	Maddox, A. W. ....	33	0	—	—	+	155	166	3 and 8..		0.....	0
32	Matthews, A. J. ....	21	0	—	—	+	165	164	3 and 8..		0.....	0
33	Mercurio, J. ....	21	0	—	+	—	156	152	3 and 8..	Contact control in experiment No. 3.	0.....	0
34	Moor, H. W. ....	20	0	—	—	+	155	158	4 and 9..		0.....	0
35	Mulvey, H. H. ....	23	0	+	—	—	151	148	4 and 9..	Influenza vaccine, September, 1918.	Tonsillitis.....	0
36	McKeefray, A. W. ....	22	0	—	—	+	168	174	6 and 9..		0.....	0

<sup>1</sup> Sept. 18, 1918.



TABLE I.—*Volunteers, Boston experiments, January and February, 1919—Continued.*

No.	Name.	Age.	History referable to recent pandemic.				Weight.		No. of experiments, used in.	Remarks.	Illness caused by experiments.	Schick test (read in 72 hours).
			Previous attacks of influenza.	Close contact.	Casual contact.	No exposure.	Before experiment.	After experiment.				
							<i>Pounds.</i>	<i>Pounds.</i>				
37	Nearey, J. J.....	25	0	—	—	+	148	142	6 and 9..	.....	0.....	0
38	O'Hara, T. F.....	23	0	—	—	—	137	146	4 and 9..	.....	0.....	+
39	Reidy, W. D.....	26	0	+	—	—	161	169	4 and 9..	.....	0.....	+
40	Roe, E. F.....	22	2 1?	—	—	—	180	180	2.....	.....	0.....	0
41	Sanford, E. R.....	25	0	—	—	+	143	140	4 and 9..	One dose influenza vaccine in July.	Tonsillitis.....	++
42	Sliney, P. J.....	21	0	—	+	—	140	135	1 and 7..	.....	Influenza.....	0
43	Smith, T. J.....	24	0	—	+	—	152	147	6 and 9..	.....	Tonsillitis.....	0
45	Sullivan, D. J.....	21	0	—	+	—	132	137	4 and 9..	.....	0.....	++++
46	Taylor, H.....	20	0	—	—	+	125	118	4 and 9..	.....	0.....	+
47	Tully, A. P.....	26	0	—	—	+	165	176	4 and 9..	.....	0.....	0
49	Wright, H. D.....	26	0	—	—	+	153	154	4 and 9..	.....	0.....	++

\* Oct. 20, 1918.



TABLE II.—Boston experiments, January and February, 1919.

Experiment No.	Date.	Source of material.	Material inoculated.	Recipients.								Results.				Flora nasopharynx.														White cell count previous to experiment.					
				Character.	Age.	Amount.	Total number.	History of contact with influenza.			Treatment of nasopharynx prior to inoculation.				No. of patients ill.	Incubation period.	Duration of illness.	Diagnosis and remarks.	No. of volunteer.	Before.	After.	Green producing organism (pneumococcus or Streptococcus viridans).	Streptococcus hemolyticus (A or B).	Gram-negative diplococcus (M. catarrhalis, etc.).	Staphylococcus (albus, aureus, citreus).	Corynebacteria (diphtheroids).	B. mucosus capsulatus group.	Pneumococcus mucosus.	B. subtilis and B. mesentericus.						
								Close.	Casual.	None.	Sodium bicarbonate 4 per cent.	Acetic acid 0.5 per cent.	Adrenalin 1-2,000.	Oleoresin capsici 0.0025 per cent.																	Before.	After.	Before.	After.	Before.
I	Feb. 11, 3 p. m.	Dr. L. Hektoen (Chicago).	Mather's coccus, strains 63 AT, 40 AN <sub>6</sub> , 65 CT <sub>2</sub> , 6 BNP <sub>4</sub> ; dextrose broth.	29 hours.....	1.5 c. c.	10	No. 1 2 3 6	No. 7 10 15 42	No. 8 9	No. 1 2 3	No. 6 7 8						No symptoms, whatever, noted during 7 days' observation.	1 2 3 6 7 8 9 10 15 42	+	+	+	+	+	+	+	+	+	+	+	+	+	10,000 8,600 11,200 6,200 7,600 5,600 8,000 10,900 7,900 6,600 8,400 6,600 7,700 8,200 7,000 7,200 8,900 9,600 9,800 6,000 5,600 8,600 10,000 7,000 7,400 6,400 7,200 6,000 8,200			
II	Feb. 13, 5.30 p. m.	Donor; Dr. A. C.; nasopharyngeal and bronchial secretions 22 hours after onset.	Nasopharyngeal washings and bronchial secretions containing green-producing coccus, <i>B. mucosus capsulatus</i> , gram-negative diplococcus, <i>B. influenzae</i> , <i>Streptococcus hemolyticus</i> , <i>Staphylococcus albus</i> and <i>citreus</i> .	2 hours.....	3 c. c.	10	14 21 40	11 13 16 19 20	5 17					5 14	5 days..... 3 days.....	7 days. 3 days.	Influenza (?)..... Tonsillitis, acute follicular.....	5 14 11 13 14 16 17 19 20	+	+	+	+	+	+	+	+	+	+	+	+	+	8,400 6,600 7,700 8,200 7,000 7,200 8,900 9,600 9,800 6,000 5,600 8,600 10,000 7,000 7,400 6,400 7,200 6,000 8,200			
III	Feb. 17, 4 p. m.	Hygienic laboratory, Washington, D. C.	<i>B. influenzae</i> , <i>Staphylococcus aureus</i> ; washings from agar slants in nutrient broth 3 to 5 billion per c. c.	30 hours.....	3 c. c.	10	24 30	33	22 25 26 27 28 31 32	22 24 25	26 27 28			22	5 days.....	5 days.	Tonsillitis, acute follicular..... No. 53 (J. M.) was not inoculated but was left as a contact control.	24 25 26 27 28 30 31 32 33 34 35	+	+	+	+	+	+	+	+	+	+	+	+	+	9,800 6,000 5,600 8,600 10,000 7,000 7,400 6,400 7,200 6,000 8,200			
IV	Feb. 20, 4 p. m.	Donor No. 5 (Bolle) (Exp. II); secretions recovered 45 to 50 hours after onset.	Nasopharyngeal washings and bronchial secretions; pneumococcus II, <i>B. influenzae</i> , <i>Streptococcus hemolyticus</i> beta, <i>staphylococcus</i> , gram-positive diplococcus, <i>B. mucosus capsulatus</i> .	15 minutes....	3 c. c.	10	23 35 39	38 45	34 41 46 47 49			38 39 41	23 34 35				No symptoms noted during 7 days' observation.	23 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49	+	+	+	+	+	+	+	+	+	+	+	+	+	6,100 6,500 8,200 8,400 7,000 6,800 6,000 9,000 7,800 9,800 8,800 6,800 10,400 7,600			
V	Feb. 22, 6.30 p. m.	4 donors (Portsmouth), III 3-24 hours.	Nasopharyngeal washings and bronchial secretions; green colonies (Pnc.), <i>Staphylococcus albus</i> and <i>aureus</i> , <i>Streptococcus hemolyticus</i> (A and B), gram-negative diplococcus, <i>M. pharyngis sicus</i> , <i>B. influenzae</i> , <i>B. proteus vulgaris</i> , diphtheroids.	.....do.....	10 c. c.	4	1 2 3	7						1 7	40 hours..... .....do.....	3 days..... 3 days.	Tonsillitis, acute follicular..... do. Other 2 men developed no symptoms. Men drank 25 c. c. secretions in milk, in addition—6 donors.	1 2 3 7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8,800 6,800 10,400 7,600		
VI	Feb. 22, 7 p. m.	10 donors (Portsmouth), without symptoms, but exposed to epidemic.	As in Experiment V, green colonies (Pnc.), <i>Staphylococcus albus</i> and <i>aureus</i> , <i>Streptococcus hemolyticus</i> (A and B), gram-negative diplococcus, <i>M. pharyngis sicus</i> , <i>B. influenzae</i> , diphtheroids, <i>Pneumococcus mucosus</i> .	.....do.....	10 c. c.	4		43	8 36 37								No symptoms noted during 7 days' observation. Each man drank 60 c. c. secretions in 250 c. c. milk.	8 36 37 43	+	+	+	+	+	+	+	+	+	+	+	+	+	6,600 9,000 6,000 10,400			
VII	Feb. 24, 7 p. m.	Donor No. 7 (Burns) (Exp. V); secretions recovered 9 hours after onset.	Nasopharyngeal washings, <i>Streptococcus hemolyticus</i> (A and B), green colonies (Pnc.), <i>Staphylococcus aureus</i> , <i>B. influenzae</i> .	.....do.....	5 c. c.	10	10 21	11 13 15 16 20 42	9 17					9 15 16 17 21 42	2 days..... 6 days. 36 hours. 2 days. do. 3 days.	2 days. 7 days. 5 days. 12 days. 2 days. See text.	Tonsillitis, acute follicular, not in bed. Tonsillitis, acute follicular, in bed. do. Tonsillitis, acute follicular, not in bed. Influenza, in bed. No. 20 left island at end of 48 hours. Nos. 10, 11, 13 showed no symptoms.	9 10 11 13 15 16 17 20 21 42	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8,000 9,600 8,700 9,900 10,000 6,000 9,400 8,800 10,200 10,000
VIII	Feb. 24, 7.30 p. m.	Donor, J. P. L.; secretions recovered 4 hours after onset.	Nasopharyngeal washings and bronchial secretions, green colonies (Pnc.), gram-negative diplococcus, <i>B. influenzae</i> , <i>Streptococcus hemolyticus</i> , (A), (3 colonies).	1 hour 45 minutes.	4 c. c.	9	24 30	33	25 26 27 28 31 32					25	36 hours.....	5 days.	Influenza..... Other men showed no symptoms during 7 days' observation.	24 25 26 27 28 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8,700 10,000 8,200 7,500 6,600 7,000 6,400 8,400 9,800 9,200 6,800 7,400 8,600 7,800 8,200 9,000 7,600 6,600 8,600	
IX	Feb. 28, 3 p. m.	Donor No. 25; Jankowski. Experiment VIII; secretions recovered 54 hours after onset.	Nasopharyngeal washings, <i>B. influenzae</i> , gram-negative diplococcus, diphtheroids, pneumococcus, <i>Streptococcus hemolyticus</i> (A and B) (few).	14 minutes....	3 c. c.	15	23 35 39 5	38 43 45	8 34 36 37 41 46 47 49					35 41 43	46 hours..... 48 hours. 72 hours.	9 days..... 20 days. 7 days.	Tonsillitis, acute follicular..... do. do. No. 5 (F. W. B.) was not inoculated but was left as a contact control.	23 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	8,000 9,200 6,800 7,400 8,600 7,800 8,200 9,000 7,600 6,600 8,600	



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