TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE

HYGIENIC LABORATORY—BULLETIN No. 123
FEBRUARY, 1921

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

III. EXPERIMENTS UPON VOLUNTEERS TO DETERMINE THE CAUSE AND MODE OF SPREAD OF INFLUENZA, BOSTON, FEBRUARY AND MARCH, 1919

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

WASHINGTON
GOVERNMENT PRINTING OFFICE
1921

ORGANIZATION OF HYGIENIC LABORATORY.

H. S. CUMMING, *Surgeon General.*

United States Public Health Service.

ADVISORY BOARD.

Col. Eugene R. Whitmore, Medical Corps, United States Army; Rear Admiral E. R. Stitt, United States Navy; Dr. John R. Mohler, Chief of United States Bureau of Animal Industry; and Surg. George W. McCoy, United States Public Health Service, *ex officio*.

Prof. William H. Welch, Johns Hopkins University, Baltimore, Md.; Prof. Simon Flexner, Rockefeller Institute for Medical Research, New York; Prof. Victor C. Vaughan, University of Michigan, Ann Arbor, Mich.; Prof. Reid Hunt, Howard University, Boston, Mass.; Prof. M. P. Ravenel, University of Missouri, Columbia, Mo.

LABORATORY CORPS.

Director.—Surg. George W. McCoy.
Assistant director.—Surg. A. M. Stimson.
Executive Assistant.—E. B. K. Foltz.
Administrative assistants.—C. O. Sterns, Ph. G., and Claude C. Cannon, Ph. C.
Artist.—Leonard H. Wilder.
Librarian.—M. G. Motter, M. D.

DIVISION OF PATHOLOGY AND BACTERIOLOGY.

In charge of division.—Surg. George W. McCoy.
Assistants.—Surgs. Edward Francis, Arthur M. Stimson, R. M. Grimm, H. E. Hasseltine, James P. Leake; Passed Asst. Surgs. C. W. Chapin, Gleason C. Lake.
Bacteriologists.—T. W. Kemmerer, M. D., Ella M. A. Enlows, M. S.
Sanitary bacteriologists.—Ida A. Bengtson, Ph. D., Alice C. Evans, M. S.
Technical assistant.—Walter D. Cannon, LL. B., A. M., M. D.
Bacteriologic technician.—William Lindgren.

DIVISION OF ZOOLOGY.

Chief of division.—Ch. Wardell Stiles, Ph. D.
Assistant.—Surg. Joseph Goldberger.
Laboratory aids.—J. L. Baer, A. B., Myrtle B. Rhodes, B. S., Gordon Thomson, A. B.

DIVISION OF PHARMACOLOGY.

Chief of division.—Carl Voegtlin, Ph. D.
Technical assistants.—A. G. Du Mez, Ph. D., George B. Roth, M. D.
Physiologist.—Alfred E. Livingston, M. D., Ph. D.
Pathologic physiologist.—Charles W. Hooper, M. D.
Chemist.—James M. Johnson, Ph. D.
Pharmacologist.—M. I. Smith, Ph. D.
Junior chemist.—John W. Thompson, B. S.
Scientific assistant.—K. Dorothy Wright, A. B.

Chief of Division.—Wm. Mansfield Clark, Ph. D.
Chemist.—Elias Elvove, M. S., Pharm. D., Ph. D.
Technical assistant.—Hyman L. Shoub, B. S.
Assistant chemist.—Charles G. Remsburg, B. S.

SPECIAL DETAIL.

Special experts.—Julius Stieglitz, Ph. D., Russell L. Cecil, M. D.
Organic chemist.—W. A. Perlsweig, B. S.
Bacteriologist.——Gustav I. Steffen.

CONTENTS.

I. SERIES OF EXPERIMENTS AT BOSTON, NOVEMBER AND DECEMBER, 1918.[1]

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.

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,

[1] 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.
Allan, Robert Andrew.
Anderson, Arthur Raymond.
Bolduc, Joseph Real.
Bullock, Muro Chester.
Calabrese, James Joseph.
Center, Edward Thomas.
Colton, Charles.
Conroy, H. A.
Crist, Bertram.
Crowley, Henry Edward.
Denaard, Arthur Frederick.
Edman, Charles Frederick.
Englert, Henry Joseph.
Felton, James Elwyn.
Fleming, George William.
Foster, John.
Fournier, Ernest Joseph.
Garriott, Simon George.
Gerow, Percy Hector.
Gibson, Edward Molten.
Goodwin, R. E.
Healy, Thomas B.
Hedges, Daniel Judd.
Kearney, Engene Aloysius.
Klient, Thomas.
Malone, Walter James.
Marcum, Charles.
Maas, Paul Alfred.
Morrell, William Francis.
Murphy, Leonard Richard.
Murphy, William Joseph.
McAnneny, John Henry.
McKenna, Joseph Edward.
Nerling, Gustave.
Ortiz, Julius.
O'Toole, Frank Codman.
Peak, George Francis.
Pruett, George.
Reid, Robert Lincoln.
Scott, Robert James.
Slipp, Clarence.
Stanton, Judson Horatio.
Vandermeer, John William.
Vanelli, Arthur Nicholas.
Veteto, Gus Robert.
Vieira, Leopold Joseph.
Wanless, Frank B.
Heine, John Joseph.
Hill, Warren Arthur.
Holmes, Harrison Stephen.
Aimar, Bertram Hillard.
Crews, Millard.
Dawson, Harvey Allen.
Fink, Herbert Jacob.
O'Neill, Nick Persian.
Evans, Hugh John.
Holziner, Carl Peter.
Warren, Robert Flagg.
Whipp, Raymond Calvin.
Walker, E. F.
Hickey, Edward John.
Jones, Orlando Lloyd.
Lang, William Norman.
Myers, Fred.
Balbian, Frederick.
Campbell, Verlin Everett.
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 1.—*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	
2	18	Sept. 29	No	No	No	
3	20	Sept. 24	Yes	No	Doubtful, 1916 and 1917.	On Sept. 7 and Sept. 8 slept with a comrade who was coming down with an attack.
4	20	Aug. 1	No	No	Yes, 1917	Associated with No. 11 who had an attack. Also exposed at Lawrence, Mass., on furlough from Deer Island.
5	19	Sept. 15	No	No	No	Tonsilitis, 1914; sore throat every winter.
6	21	Sept. 13	No	No	No	
7	18	Oct. 24	No	Probably about Oct. 1.	No	Not noted in official medical record, but history very suggestive.
8	21	Sept. 21	At Brooklyn Navy Yard, Sept. 17–20.	No	No	
9	19	July 28	No	No	No	
10	23	Aug. 15	No	No	No	
11	18	July 31	No	Yes [1]	No	Influenza attack Sept. 16.
12	19	June 11	No	No	Yes, 1917	Fairly typical history of attack in 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	
14	20	July 19	No	No	No	History of close contact at Deer Island.
15	20	Sept. 25	At New York receiving ship.	Yes [1]	No	Influenza Sept. 18 at receiving ship, N. Y.
16	31	Oct. 5	Norfolk, Va., Sept. 15–Oct. 1.	No	No	
17	20	June 26	No	No	Doubtful, Apr., 1918.	
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	History of close contact.
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	
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.	Epidemic in New Haven at time.
34	15	Aug. 21	No	Yes, Sept. 15.	No	Not noted in official health record, but history of attack quite clear.
35	19	May 28	No	No	No	
36	19	June 17	No	No	No	
37	19	Aug. 30	No	No	No	Close contact with No. 60.
38	20	July 3	No	No	No	Close contact at Deer Island.
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	
47	18	Sept. 25	At Brooklyn, in brig, Sept.	No	No	Close contact at Brooklyn and Deer Island.
48	20	Aug. 31	No	No	No	
49	18	Sept. 4	No	No	No	
50	20	June 26	No	No	No	
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	Intimate contact at Philadelphia.
54	20	Sept. 6	No	Yes [1]	No	
56	21	Aug. 22	No	Yes, Sept. 23 [1]	No	
57	20	Aug. 22	No	Yes, Sept. 29 [1]	Probable, 1915	
58	21	Aug. 22	No	Yes, Sept. 22 [1]	No	
59	21	Sept. 3	No	Yes, Sept. 9 [1]	No	
60	20	Aug. 24	No	Yes, Sept. 22 [1]	No	
61	22	Sept. 11	No	Yes, Sept. 29 [1]	Probable several attacks.	
62	18	Mar. 28	No	Yes, Sept. 24 [1]	No	
63	20	July 1	No	No	No	

[1] 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.*

Experiment No	Date, 1918.	Material. Kind.	Material. Source. Donor	Material. Source. Stage of illness.	Material. Quantity.	Material. Mode of inoculation.	Recipients. Presumably non-immunes.	Recipients. With doubtful or definite history of previous attack.	Remarks.
1	Nov. 13	Pfeiffer's bacillus, saline solution suspension.	W. K.	Second day	Approximately ½ loopful of an 18-hour culture.	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 do Fourth 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 5½ hours after securing secretions. One of the volunteers, No. 29, developed fever 36 hours after inoculation, considered as probably due to an inflamed throat, but influenza could not be excluded. The others showed no reaction
		B. Same as A after filtration through Mandler filter.			Not measured	Same as with A (see also remarks).	Nos. 9, 14, 16, 18, 19, 21.	Nos. 1, 3, 4, 27.	Nos. 16, 18, 19, 21, and 27 also received instillation in the eyes. Inoculations were made 4 to 4½ hours after securing secretions. No appreciable reactions.
3	Nov. 21	Secretions from upper 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 38 hours after onset 58 hours after onset 44 hours after onset 57 hours after onset 33 hours after onset 70 hours after onset 45 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. Nos. 9, 35 No. 14 No. 5	No. 43 No. 33 No. 4	Inoculations made about 1 hour and 40 minutes after securing secretions. No appreciable reactions.
4	Nov. 23	Secretions from nose and naso-pharynx.	S. T. J. K. L. P. McC. J. O. A. H. M. McL. C. F.	55 hours after onset 57 hours after onset 42 hours after onset 31 hours after onset 57 hours after onset 51 hours after onset		Transfer by swab from nose to nose and throat to throat.	Nos. 16, 32 No. 63 Nos. 18, 19 No. 21 Nos. 24, 26 No. 31	Nos. 3, 25 No. 1 No. 27	The time elapsing between donor and recipient did not exceed 30 seconds in any instance. No appreciable reactions.
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.	50 hours after onset 12 hours after onset 11 hours after onset 35 hours after onset 77 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.	N. T. C. F. L. M. Y. E. N. W. A. K. A. L. F. L. W. G. W. F. B. E. C. B. B. C. L. M. V.	21 hours after onset 10 hours after onset 27 hours after onset 56 hours after onset 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.

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.)[1] 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 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.

[1] See history of cultures for details, Table III; Appendix B, page 28.

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.[1]

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

[1] 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. 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. 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. 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. 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. 2 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*.

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.[2]

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.

[1] Appendix C, page 30.

[2] 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.[1] 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

[1] 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. Designation.	Culture. Source.	Interval between isolation of culture and inoculations.	Medium.	Transplant used.
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	
12	CD (157)			do	
13	Park (103)			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[14] 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.

[1] Levinthal, W., Influenza. Bakteriologische und serologischen Studien. Berl. klin. Wchnschr. 1918. LIV. 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 passage 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 passage 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 an 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.

DONORS.

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, pain 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.

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

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.

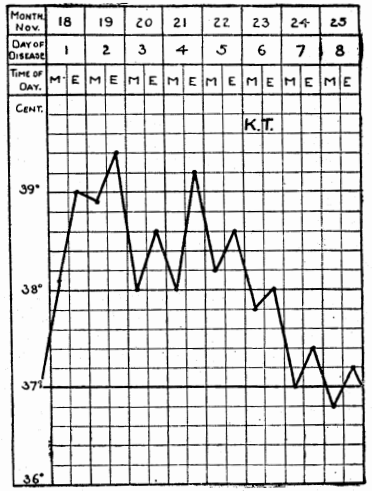


Chart 1.—Temperature curve of Volunteer No. 29 K. T., experiment 2a.

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.

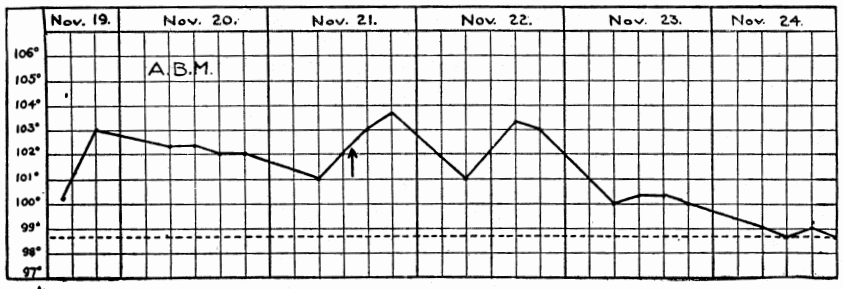


Chart 2.—Temperature curve of donor A. B. M.

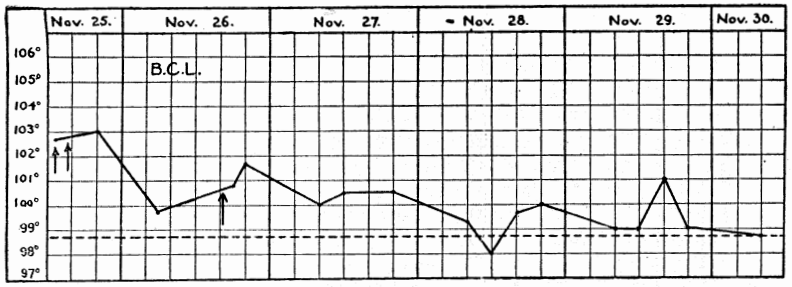


Chart 3.—Temperature chart of donor B. C. L.

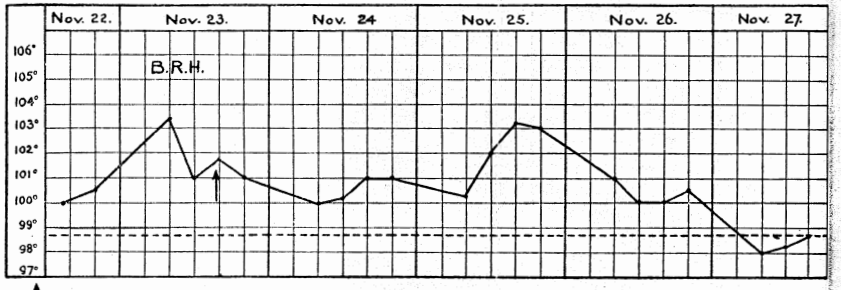


Chart 4.—Temperature curve of donor B. R. H.

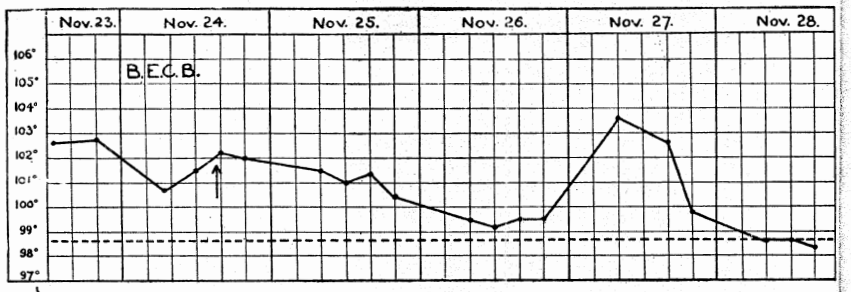


Chart 5.—Temperature chart of donor B. E. C. B.

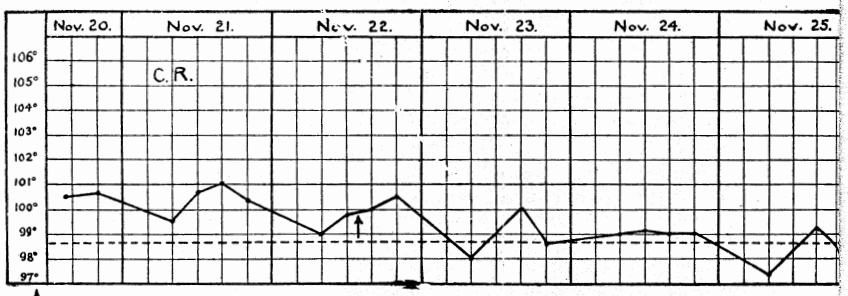


Chart 6.—Temperature curve of donor C. R.

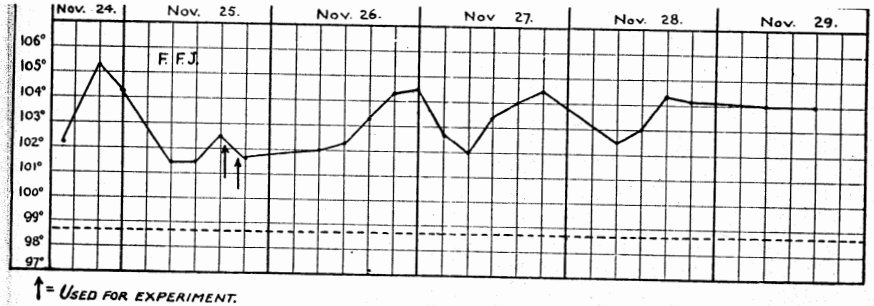


Chart 7.—Temperature curve of donor, F. F. J.

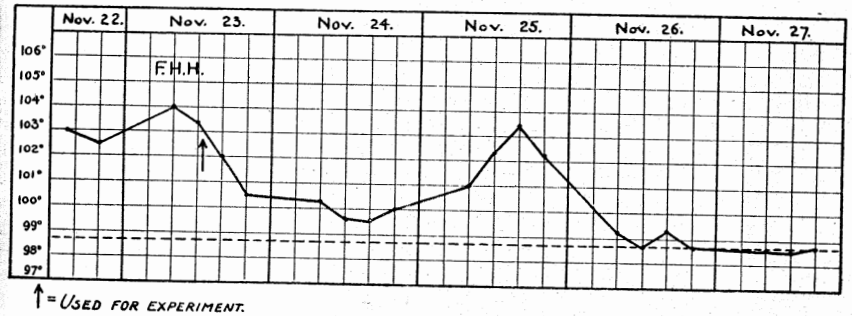


Chart 8.—Temperature curve of donor F. H. H.

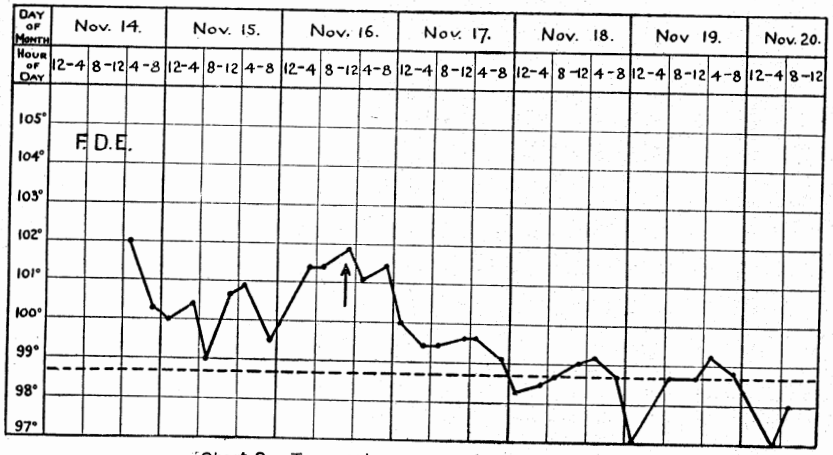
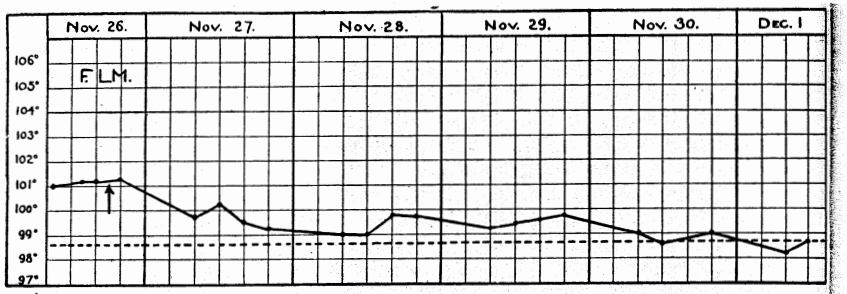
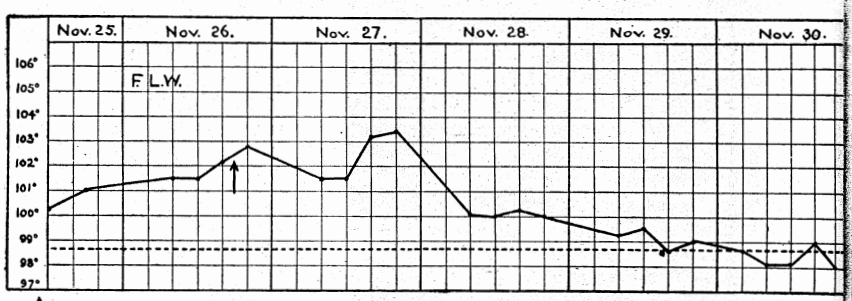


Chart 9.—Temperature curve of donor F. D. E.



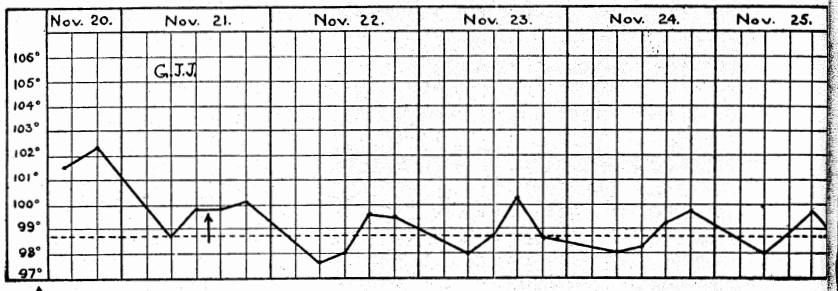
↑ = USED FOR EXPERIMENT.

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



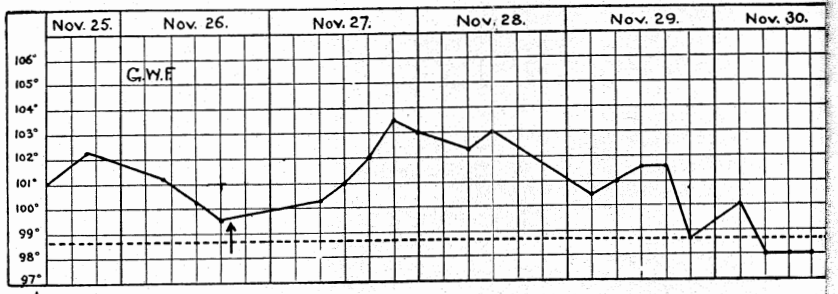
↑ = USED FOR EXPERIMENT.

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



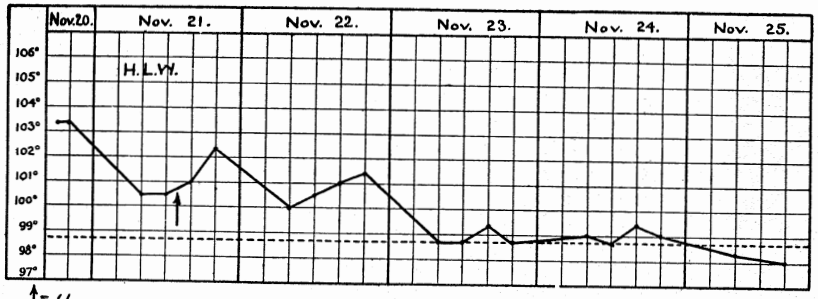
↑ = USED FOR EXPERIMENT.

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



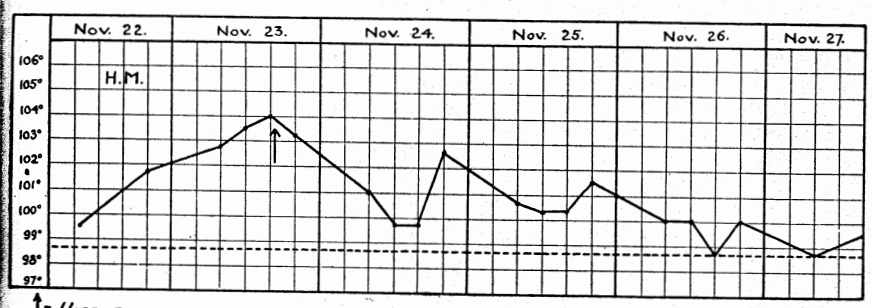
↑ = USED FOR EXPERIMENT.

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



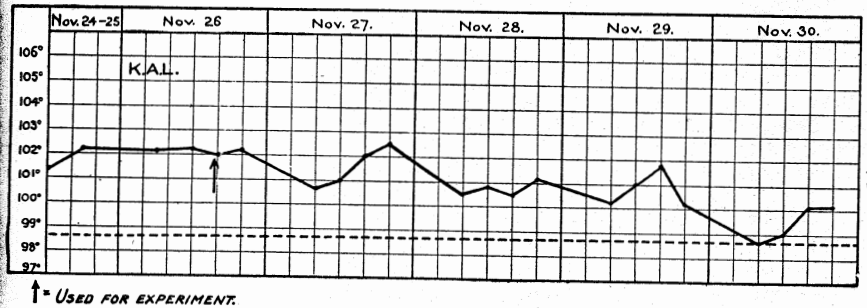
↑ = USED FOR EXPERIMENT.

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



↑ = USED FOR EXPERIMENT.

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



↑ = USED FOR EXPERIMENT.

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

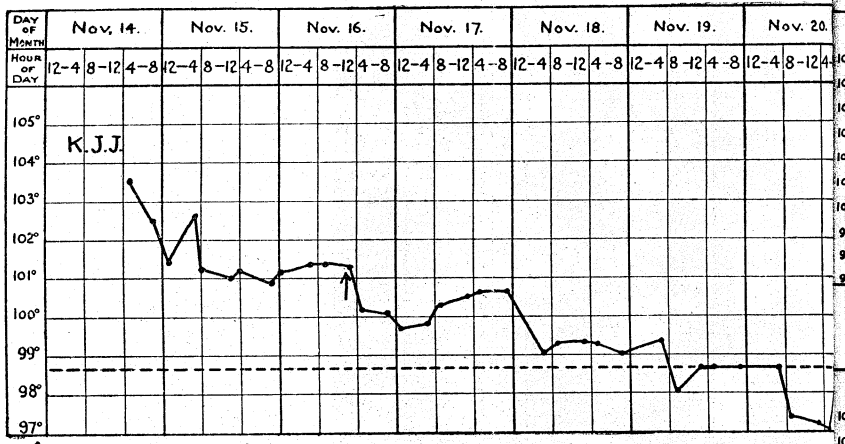


Chart 17.—Temperature curve of donor K. J. J.

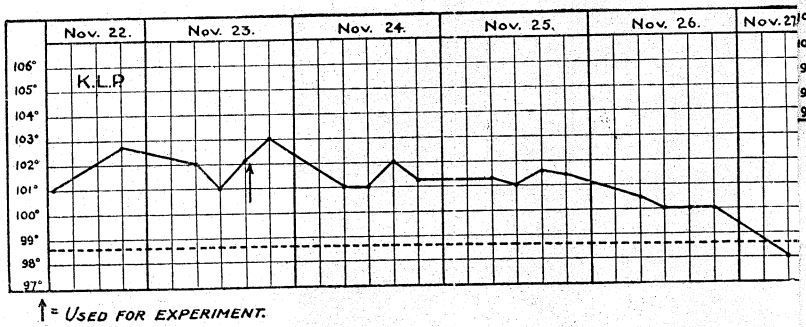


Chart 18.—Temperature curve of donor K. L. P.

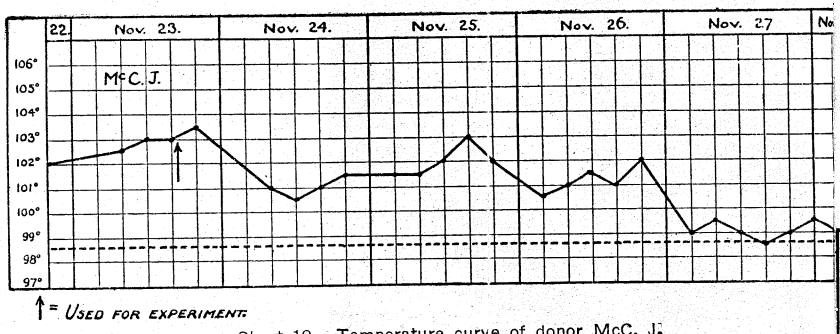


Chart 19.—Temperature curve of donor McC. J.

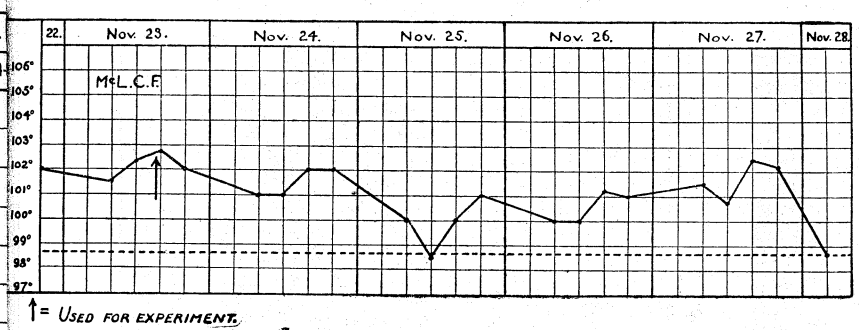


Chart 20.—Temperature curve of donor McL. C. F.

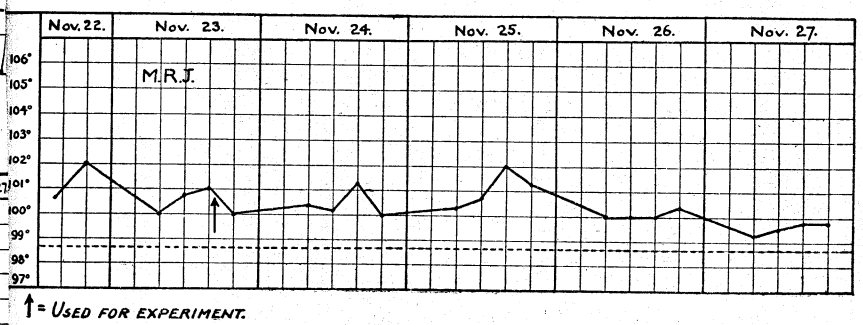


Chart 21.—Temperature curve of donor M. R. J.

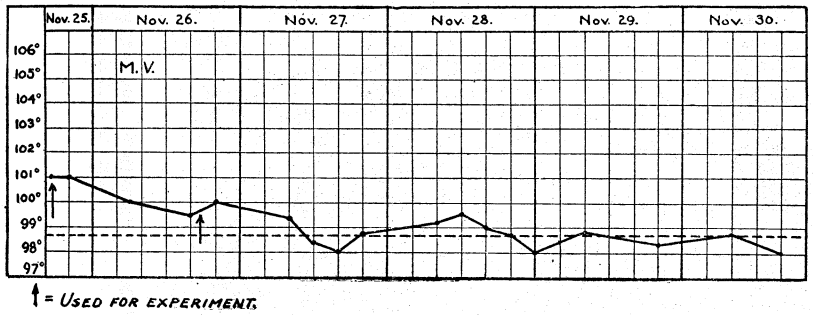


Chart 22.—Temperature curve of donor M. V.

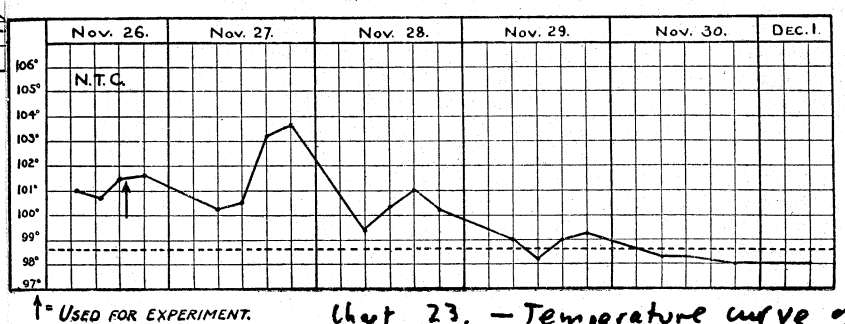
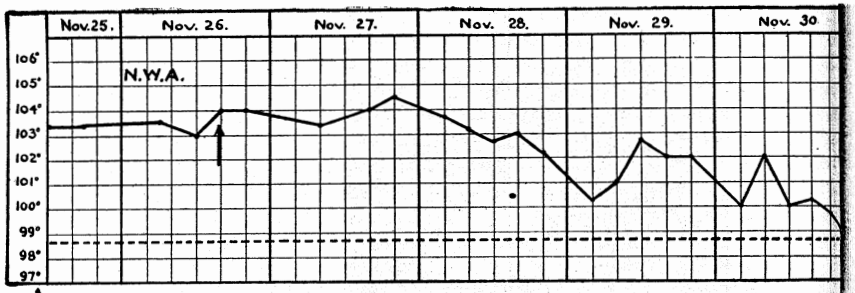
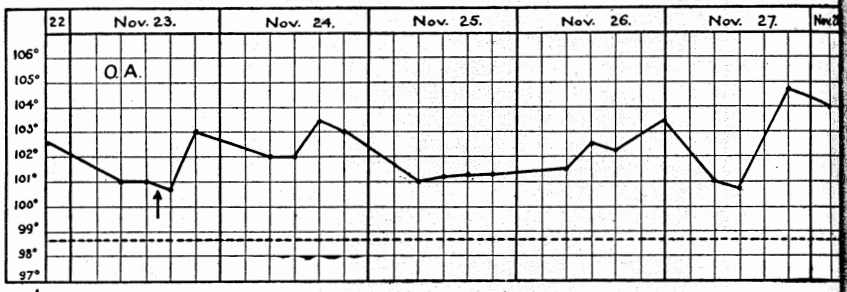


Chart 23. — Temperature curve of donor N.T.C.



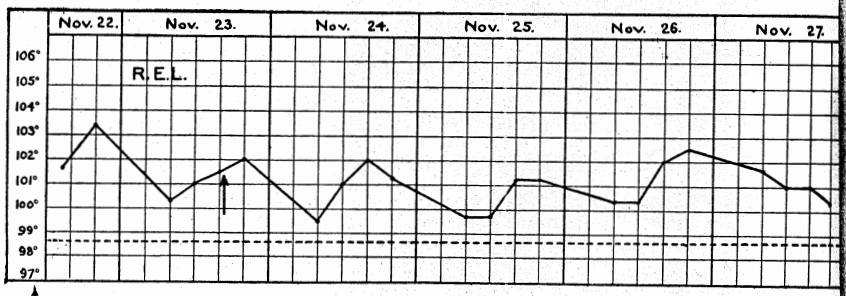
↑ = USED FOR EXPERIMENT.

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



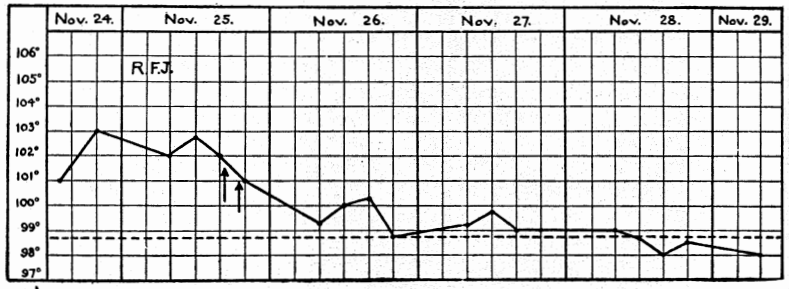
↑ = USED FOR EXPERIMENT.

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



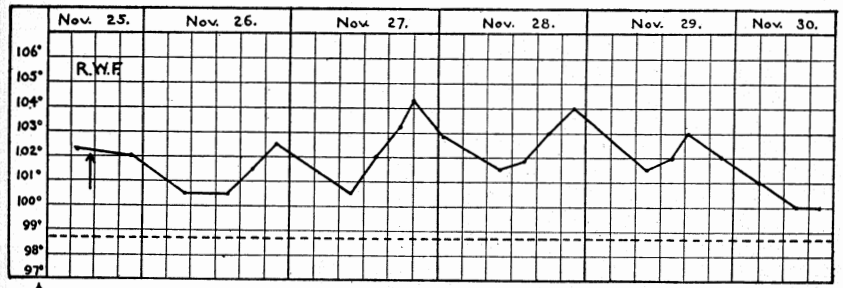
↑ = USED FOR EXPERIMENT.

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



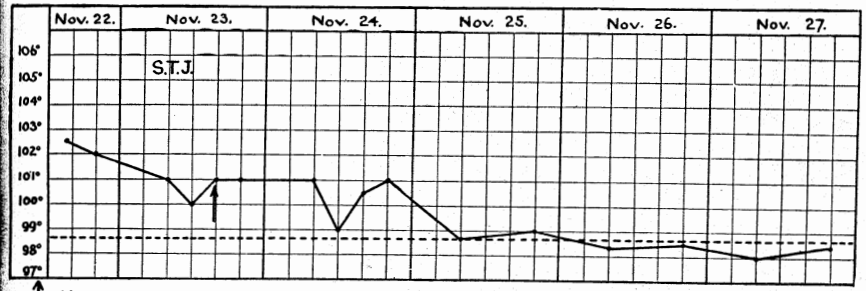
↑ = USED FOR EXPERIMENT.

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



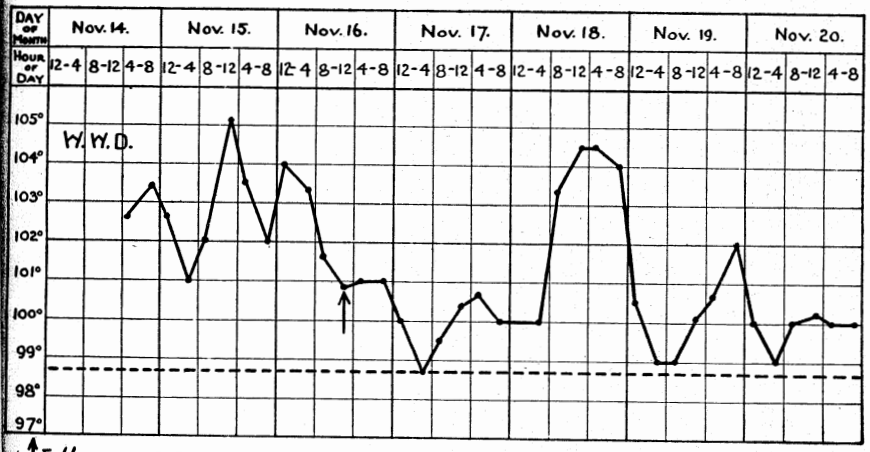
↑ = USED FOR EXPERIMENT.

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



↑ = USED FOR EXPERIMENT.

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



↑ = USED FOR EXPERIMENT.

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