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Epidemic Influenza B and C in Navy Recruits during Winter of 1951-52. (19965)

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Recognition has recently been given to a strain of virus classified as "Influenza C" (1,2) but multiple isolations of strains of this type from a population during a single winter has not been previously reported.† Fourteen viruses, serologically related to earlier strains of influenza C, were isolated from Navy recruits between January and April of 1952. Evidence that these viruses were epidemic should be of interest to others concerned with the etiology and epidemiology of influenza during the past winter. Epidemic influenza B occurred simultaneously in this population. Eleven influenza B viruses were isolated.

Materials and methods. Observations on influenza have been made among recruits at the Naval Training Center, Great Lakes, during each winter since 1948. Continuing these observations during November, 1951, and June, 1952, throat swabs for virus isolations and acute and convalescent sera were routinely obtained from Navy recruits hospitalized because of acute respiratory infections including suspected cases of influenza and cases of pneumonia. Paired sera also were available from serial bleedings done at various intervals on 13 companies of recruits undergoing training between January and May. Acute and convalescent sera from patients experiencing

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† A few were identified at the University of Michigan during March 1952(5).

TABLE I. Fourfold or Greater Rises in Hemagglutination-Inhibition (H-I) Antibody Titer Against Influenza Viruses PR-8, RM-1, or LEE, and Isolations of Influenza A and B Viruses in Navy Recruits Hospitalized with Acute Respiratory Disease.

Period (1951-52)	Patient's sera: No. pairs sera completed	Patient's sera: No. 4-fold rises in H-I titer to: A or A'	Patient's sera: No. 4-fold rises in H-I titer to: B	Throat swabs or nasal washings: No. of specimens	Throat swabs or nasal washings: No. of viruses isolated: A	Throat swabs or nasal washings: No. of viruses isolated: B	Resp. dis. admissions: No.	Resp. dis. admissions: Rate*
11/11–12/15	181	2	1	116	0	0	236	69
12/16– 1/12	98	1	1	127	0	1	173	52
1/13– 2/9	252	10	30	135	0	3	525	109
2/10– 3/8	238	3	4	102	0	3	464	101
3/9 – 4/5	137	0	6	136	0	3	197	53
4/6 – 5/3	20	1	0	70	0	1	147	48
5/4 – 5/31	22	0	0	18	0	0	159	55
Total	948	17	42	704	0	11	1901	—

* Rate/1000 recruits/period.

influenza-like illnesses during the winter were also supplied from other localities‡ (Table IV).

Nasal washings were collected in 30 ml, and throat swabs were immediately placed in 2 to 3 ml of nutrient broth. Routinely, 1000 units of penicillin and 1 mg of streptomycin were added per ml of filtered nasal washing or throat swab suspension prior to intraamniotic inoculation of 13-day-old embryonated eggs. After incubation for 3 to 4 days at 35°C, the amniotic fluid was harvested and tested for the capacity to agglutinate chicken and guinea pig erythrocytes after being left at 4°C for 60 to 90 min. At least 2 egg passages were made before any specimen was called negative. Salk's modification(3) of the Hirst hemagglutination-inhibition test was employed in the identification of newly isolated viruses, using rooster immune sera§ and 4 agglutinating units of virus. Antibody titrations on sera followed the hemagglutination-inhibition technic of Hirst and Pickels(4).

Observations on influenza among recruits hospitalized with acute respiratory diseases. Data relating to the occurrence of influenza A or B in patients with acute respiratory diseases are summarized in Table I. Admissions include all types of acute respiratory infections hospitalized. A large proportion of the patients in January and February had streptococcal infections.‖ There was little evidence of epidemic influenza as manifested by excessive numbers of admissions for clinically diagnosed influenza at any time during the winter despite the laboratory evidence for the occurrence of this disease. Eight of the 11 influenza B strains were isolated from patients clinically diagnosed as influenza, 2 were from patients diagnosed as acute pharyngitis, and one was from a patient with pneumonia of undetermined etiology. The influenza B strains isolated were immunologically related to the 1210 strain. Fourteen viruses, identified as antigenically similar to the JJ strain of influenza C virus(2), were also isolated from recruits hospitalized because of acute respiratory diseases. Nine of the patients had influenza-like illnesses, 2 were diagnosed as acute pharyngitis, and 2 had pneumonia of undetermined etiology. As in the case of influenza B, there appeared to be nothing distinctive about these illnesses.

The dates of isolation and immunological characterizations of the strains identified as influenza C are given in Table II. Also shown are the antibody titers to the homologous strain isolated from each patient in the patient's acute and convalescent sera. One influenza B strain (GL 704B) and influenza C strain (GL 899C) were selected as prototypes

‡ The Student Health Clinic of the University of Chicago, Fort Ord. Calif., (Dr. E. H. Lennette), Communicable Disease Center, Montgomery, Ala., and the National Institute of Health (Dr. Joseph Bell).

§ The JJ strain of influenza C used in preparation of immune rooster sera was kindly given us by Dr. Thomas Francis, Jr.

‖ Oral penicillin prophylaxis for streptococcal disease was given to a majority of recruits during March, 1952.

TABLE II. Hemagglutination-Inhibition (H-I) Titrations for 14 Strains of Influenza C Viruses Using Rooster Immune Sera for A, B, and C (JJ) Strains of Influenza Virus and Patient's Acute and Convalescent Sera with His Homologous Strain of Virus. Titers are expressed as the reciprocal of dilution of sera.

Date of isolation, 1952	Strain	Hemagglutination-inhibition titer of virus to: — Rooster immune sera — FM-1	Lee	1210	JJ	Patient's homologous sera — Acute	Convalescent
Jan. 22	GL 585				1280	64	64
22	596				1280	20	160
Feb. 7	695				640	256	1024
14	756				1280	32	32
18	779				2560	32	32
19	782				640	32	32
26	832	<20	<20	<20	2560	32	1024
26	833				1280	128	1024
Mar. 10	899				1280	40	640
12	919				2560	32	64
12	920				2560	128	128
Apr. 23	1082				1280	32	512
24	1090				1280	128	256
24	1091				2560	64	64

for use in serological studies. The GL 704B strain was isolated from the nasal washings of a recruit during an influenza-like illness on February 8, 1952. The GL 899C strain was isolated from the nasal washings of a recruit ill with pneumonia of unidentified etiology on March 10, 1952.

Relation of influenza B and C to acute respiratory infections in humans. Clinical and bacteriological data collected weekly and sera from 5 bleedings were available for analysis on a company of 82 recruits who entered training on January 15, and departed on April 16, 1952. Hemagglutination-inhibition tests with the PR 8, FM 1, and Lee strains of influenza virus showed that 19% of the men had significant antibody rises to these strains during this period of time. The serial sera of 25 men in this company were picked at random and also analyzed for antibody rise to the GL 899C strain of influenza C virus and to the GL 704B strain of influenza B virus. A 4-fold or greater rise in titer against these viruses was considered to indicate an infection with the virus when all sera from one man were run in a single test. The results are shown in Table III. Twelve individuals showed 4-fold or greater rises in antibody titer to the GL 899C antigen while 7 showed similar rises to the GL 704B strain. Antibody rises to both viruses occurred in 4 men and in only 2 were these rises concurrent. A comparison with previous data (Table I) showed that there was no correlation between rises to the influenza C virus and rises to the PR 8, FM 1 or the Lee antigens. Of the 12 men showing antibody rises to influenza C virus, 6 could be correlated with a minor respiratory illness clinically classified as the common cold (five) or influenza (one), whereas only one of 7 individuals showing rises to influenza B virus had a correlating respiratory tract illness. Minor respiratory infections and influenza-like illness which did not provoke a rise in antibody titer to the influenza antigens also occurred frequently in the 25 men. These data would suggest, however, that the influenza C virus may have been related to a minor respiratory illness, whereas influenza B virus produced symptoms less frequently.

TABLE III. Hemagglutination-Inhibition (H-I) Antibody Increases to the GL 899 Strain of Influenza C and the GL 704 Strain of Influenza B in Serial Sera Obtained from 25 Navy Recruits Between January and April, 1952.

Period of H-I rise	4-fold rises in H-I titer to GL899C	GL704B
1/15–1/31	4	1
1/31–2/14	2	5
2/14–3/31	6	1
3/31–4/16	0	0
Total	12	7

TABLE IV. Hemagglutination-Inhibition Titers to GL 899 Strain of Influenza C Virus in Paired Sera Obtained from Patients with Influenza Like Illness in Other Areas of United States and in Sera of Navy Recruits from Which This Strain Was Isolated.

Source of sera	No. pairs tested	Titers of initial sera							No. 4-fold rises
		32	32	64	128	256	512	1024	
CDC,* Ala.	10				1	2	5	2	0
Ft. Ord., Calif.	20	1	4	4	1	6	4		1
U. Chicago, Ill.	30		1	6	14	8	1		3
Great Lakes, Ill.	14	1	6	3	3	1			6

* Communicable Disease Center.

Other serological studies on influenza A and B among recruits. Sera obtained at the beginning and the end of training from 370 recruits from 5 companies in residence between November and May were tested for antibody rises to influenza A and B virus antigens. Four-fold increases in hemagglutination-inhibition antibody titer for either the PR 8 or FM 1 influenza viruses occurred in 4% and for the Lee influenza virus, in 14% of these men.

Other serological studies of influenza C among recruits. Six pairs of sera were selected at random from each of a total of 12 companies of recruits from which sera were available. Of these 72 pairs of sera, 11 or 15%, showed a 4-fold rise in hemagglutination-inhibition titer to the GL 899 strain of influenza C virus.

Influenza C elsewhere. Sera obtained elsewhere were also tested with the GL 899C strain and the results are shown in Table IV. Three pairs from the National Institute of Health were also run and showed no rise. For comparison, the titers in the sera of Navy recruits from whom the virus was isolated are included in Table IV. Four-fold rises occurred in 3 of 30 pairs of sera from the University of Chicago and in one of 20 from Fort Ord, California, indicating that the virus may be widespread in the population.

Biological characteristics of influenza C viruses. The presence of the virus was always detected in the first or second egg passage by the capacity of the amniotic fluid to agglutinate chicken erythrocytes at 4°C. Serial amniotic passages of the GL 899C strain have been successful through 21 passages. The hemagglutination titers range from 512 to 2048 and the egg infectivity was 10^{-8} at the 21st passage. Several efforts have been made to adapt the virus to the allantoic membrane. Varying the age of the chick embryo, the length of incubation, and the concentration of the inoculum has seemed to have little effect on the virus growth obtained. The results have been inconsistent and only occasional titers as high as 1:128 have been obtained. Attempts at serial allantoic passage have failed. The virus agglutinated chicken erythrocytes at 4°C only. Neither guinea pig nor human type 0 erythrocytes were agglutinated. The hemagglutination titer of infected amniotic fluid decreased rapidly upon storage at 4°C, making it impossible to store the virus for serological tests at this temperature. The titer was maintained when stored in sealed ampules at —70°C. Two attempts were made to adapt the virus to Swiss mice weighing 12 g by intranasal inoculation. No lesions were observed in the lungs during 6 blind passages nor was there any virus detectable in mouse-egg-mouse passages.

Discussion. The experience with influenza B at Great Lakes is similar to that reported from many other parts of the United States during the winter of 1951-52. Patients from whom viruses were recovered had relatively mild clinical illnesses and developed antibody increases to the epidemic B strain and less regularly to the Lee strain. Of special interest also was the isolation of several strains of influenza virus type C from individuals showing similar illnesses. The routine performance of the hemagglutination test at 4°C in isolation and typing procedures was essential for the recovery of the influenza C viruses.

The recovery of 14 influenza C viruses and the immunological evidence of their prevalence during a period when influenza B was also prevalent in the same population is of interest since the isolation of both the JJ and the 1233 strains occurred during A and A′ epi-

demics. This serves further to set influenza C apart from influenza A or B as a distinct entity. Studies on the biological and immunological characteristics of the GL 899C strain of influenza C indicate its close resemblance to the JJ strain of Francis(2). Its relationship to the 1233 strain of Taylor(1) is under study.

Neither influenza B nor C viruses isolated at Great Lakes during 1952 seemed to possess marked virulence as only a small fraction of men showing diagnostic antibody rises had clinical illness of any degree of severity. However, in view of the past experience with influenza A and B, it is conceivable that the pathogenicity of the influenza C virus may change in subsequent years or in different localities. Thus, it would appear justifiable to look for this influenza virus strain with other etiological agents when investigating epidemic respiratory disease.

Summary. Data have been presented on the occurrence of influenza in Navy recruits at Great Lakes Naval Training Center during the winter of 1951-52. Serological evidence of occurrence of epidemic influenza B was confirmed by the isolation of 11 viruses which appear to resemble the 1210 strain of influenza B. The isolation of 14 strains characterized as influenza C and the serological evidence that influenza C was also epidemic are presented. One of these viruses, the GL 899C strain, was studied in more detail. Its biological and immunological properties and its relation to previously isolated strains were studied. Data were also presented indicating that influenza C infections occurred in other localities throughout the United States. The importance of performing routinely the CRC agglutination and CRC agglutination-inhibition tests at 4°C for the detection of influenza C viruses and antibody titer is stressed.

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Electrophoretic Studies on Cerebrospinal Fluid.* (19966)

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In a previous publication(1), a component (X-protein), migrating faster than albumin, was found to be a normal constituent of cerebrospinal fluid. This component has also been observed in a few cases by earlier workers(2,3). Labhart, Schweizer and Staub(4) analyzed 178 cerebrospinal fluids of healthy and diseased individuals with the aid of the Jamin interferometric optical system and failed to describe the presence of the rapidly migrating component. Recently Bücher, Matzelt and Pette(5), using paper strip electrophoresis, noted a rapidly migrating component in each of 10 cerebrospinal fluids which were classified as "normal". In the present paper, cerebrospinal fluids of patients with neurological disturbances were analyzed in the Tiselius apparatus in order to study the distribution of the components, particularly those migrating faster than albumin.

Method. Cerebrospinal fluids (50-220 ml) were obtained from patients undergoing pneumoencephalograms for diagnosis. Each fluid was centrifuged to remove cells and concentrated by pressure dialysis to final volumes varying from 3 to 5 ml. The sample was dialyzed against phosphate buffer of pH 7.85

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