INFLUENZA VIRUS C INFECTION IN ENGLAND

BY

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In 1947 virus was isolated by Taylor (1949) from the throat washings of a man in New York who was suffering from a mild influenzal illness. It resembled the influenza viruses but differed from types A and B antigenically. Francis *et al.* (1950) later reported the recovery of a similar organism from the throat of a patient also suffering from an influenzal illness, and found serological evidence of infection in a number of other cases. They suggested that the new virus be known as influenza virus Type C, and this designation has been generally accepted (Taylor, 1951; W.H.O., 1953).

Isolations of influenza virus C have since been reported from other laboratories in the United States (Gerber *et al.*, 1952; DeMeio *et al.*, 1955) and from Japan (Fukumi *et al.*, 1951) and Czechoslovakia (Styk, 1954). However, few illnesses accompanied by an increase in antibody to this virus have been reported, and Minuse *et al.* (1954), commenting on experience at Ann Arbor, Michigan, stated that "continued search has yielded only a few additional cases." Studies of the distribution of antibody to influenza virus C in American population groups were made by Hilleman *et al.* (1953) and Davenport *et al.* (1953). These surveys showed a sharp rise in the general level of antibody during childhood, particularly in the earlier years. It seems probable that, in the United States, influenza virus C infection occurs widely in young children and may be accompanied by minor respiratory illness. Our own findings and those of Grist (1955) suggest that the same may be true in Britain.

In the course of a number of investigations during the past three years we have had an opportunity to make clinical and laboratory observations on the presence of influenza virus C infection in this country. Histories of illness were studied in persons whose sera showed antibody changes, and sera from these investigations were used to estimate the age distribution of antibody in the population. Cases of influenza virus C infection were found during three investigations. In the winter of 1952-3, 455 factory workers who had volunteered for an influenza vaccine trial were under observation in Luton, Bedfordshire (McDonald and Andrews, 1955). Eighty-two persons with an acute respiratory illness causing absence from work were visited at home. Specimens were taken from 63 of them for laboratory examination, and serological evidence of virus C infection was found in one (Table—Case 1). In the following winter approximately 50 volunteers from each of five factories in widely separated parts of the country were bled at monthly intervals and their respiratory illnesses noted. Evidence of infection was found in one volunteer (Table—Case 2). In a study of 25 families in Wembley, Middlesex, between November, 1953, and April, 1955,

a clinical record of all respiratory illnesses was kept, and blood taken from the adult members at approximately three-monthly intervals. Throat swabs or throat washings for virus isolation were obtained in all febrile illnesses, and in adults additional blood specimens were taken to provide paired sera for any such illness. Laboratory evidence indicated that influenza virus C infection occurred in eight persons during this study (Table—Cases 3 to 10).

Sera for the estimation of age distribution of antibody were obtained from three main sources. Sixty-four out of 75 specimens from children aged less than 5 years had been taken from healthy individuals participating in a whooping-cough vaccine trial at the end of 1952. The remaining 11 sera in this age group and 25 specimens from persons aged 5 to 9 years were received at the Virus Reference Laboratory for various tests in the winter of 1954-5. Six hundred and seventy-seven specimens from 195 persons aged 15 to 64 years were from the 1953-4 factory investigations mentioned above. All specimens of serum were stored at -15°C . or -30°C . until tested. Most specimens for virus isolation were stored at -70°C ., but a few which were tested within seven days of collection were kept at $+4^{\circ}\text{C}$.

Laboratory Methods

The haemagglutination-inhibition (H.I.) test was performed in a cold room at 4°C ., using plastic haemagglutination plates (W.H.O., 1953). The antigen consisted of amniotic fluid from fertile hens' eggs infected with influenza virus C strain 1233 obtained from Dr. A. Isaacs, of the World Influenza Centre. Eight partial haemagglutinating doses were used in the test.

The complement-fixation (C.F.) test was performed in the same plastic plates used for the H.I. test. Serum, complement ($2\frac{1}{2}$ M.H.D.), and antigen were mixed in 0.1 ml. volumes, incubated at 37°C . for 75 minutes, and 0.2 ml. of a 1% suspension of sensitized sheep red cells was added. After 30 minutes at 37°C . the plates were stored overnight at 4°C . and results read next morning. Titres recorded were the highest initial serum dilution showing approximately 75% of red cells unlysed. The antigen, consisting of amniotic fluid from fertile hens' eggs infected with strain 1233, was used without purification, at optimum dilution. A buffered saline diluent containing added calcium and magnesium (Fulton and Dumbell, 1949) was used throughout the C.F. test.

Similar methods were employed for the serological diagnosis of influenza A and B, except that the H.I. tests were performed at room temperature and in the C.F. tests standard preparations of soluble antigen were used.

Material for virus isolation was tested by the inoculation of 0.1 ml. into the amniotic cavity of six fertile hens' eggs on the 10th day of incubation. After three days' incubation at 35°C . the amniotic fluids were tested for ability to agglutinate fowl red cells at 4°C . The virus type was confirmed by complement-fixation, using dilutions of egg material against low dilutions of serum from persons convalescent after influenza virus A, B, and C infections. Small quantities of serum from a known case of influenza C infection were kindly supplied by Dr. Elva Minuse, of Ann Arbor, Michigan. All specimens for virus isolation were given at least two egg passages.

Case Reports

Laboratory evidence of recent infection with influenza virus C was found in eight adults and two children; a summary of observations made on them is given in the Table. In the eight adults a fourfold or greater rise in antibody between two bleedings was observed by C.F. or H.I. test, or both. In some of these both titres were low,

but, as a fourfold or greater rise was demonstrated to influenza virus C and not to virus A or B, they are included. In two of the eight adults—Cases 1 and 10—the first blood specimens were taken in the acute stage of an influenza-like illness, mild in one and moderately severe in the other. The second specimens in these two cases were taken 20 to 23 days after onset. Virus isolation was attempted without success on throat washings collected within three days of onset from both these patients.

The two children (Cases 6 and 7) were sisters, and, together with their elder brother aged 13 years, developed a severe running nose on the same day. A throat swab was taken from the youngest child (Case 6), who had a rectal temperature of 99° F. (37.2° C.) and a moderately severe cough when seen on the day after onset, and virus C isolated from it. The specimen was taken with a wooden-shafted swab and the end broken off into a screw-capped bottle containing broth. It is interesting that this specimen was kept at 4° C. until inoculated into eggs four days later and not at -70° C. as was usually done. Blood samples were taken from the three children and from their mother and father 18 days after the onset of illness in the children. The parents, who had been bled three weeks before the episode and again four months later, had no illness of any kind during this period and showed no serological changes. The two sisters had antibody titres of 1/256 and 1/512 to influenza virus C complement-fixing antigen, strongly suggesting recent infection, but that of their brother was less than 1/2.

In the remaining six patients shown in the Table a fourfold or greater antibody rise was noted between two routine bleedings. In Case 2 the gap between the bleedings was five weeks, and it seems possible that the rise was associated with a common cold which occurred seven days before the

second bleeding. It was the only illness during the period. The time interval between specimens in the other cases ranged from three to five months, and though all but one had colds in this period it is impossible to say whether the antibody changes were related to any of these illnesses. Certainly nothing more severe than a common cold resulted from the infection in these five persons.

It is perhaps worth noting that, though the 10 cases shown were spread over three years, the serological changes occurred during the first half of the year in eight of them. It is possible that the same was true of the other two cases, but in these the interval between bleedings was long, the specimens being taken in April and October. The significance of a trend based on so few observations is obviously questionable.

Age Distribution of Antibody

It is not claimed that the 295 subjects from whom blood was available for study of age distribution of antibody were necessarily representative of the population of this country. The great majority were, however, normal healthy persons from widely separated towns. The sera in the 5-14-year age group were least satisfactory, as they were from persons suffering from some febrile illness.

The proportion of sera with a complement-fixing antibody titre of 1/10 or higher in each age group is shown in the accompanying Diagram. A titre of 1/10 was chosen because approximately half the sera from persons over 14 years of age were positive at this level and errors due to non-specific effects were unlikely to be great. The proportion positive rises steadily from a low level (5%) in those under 5 years to a peak (61%) in the 25-34-year age group and remains high thereafter. The fall in the proportion positive

Summary of Findings on 10 Patients from Whom Laboratory Evidence of Infection with Influenza Virus C was Obtained

Case No.	Sex	Age (Yrs.)	Dates Bled	Antibody Titres						Egg Inoculation	Date of Onset and Nature of Any Illnesses Associated in Time with the Serological Changes	Other Observations
				C.F. Test			H.I. Test					
				A	B	C	A	B	C			
1	M	47	30/1/53 18/2/53	1/8 1/8	1/4 1/4	* *	1/10 1/20	* *	1/30 1/120	Neg.	29/1/53: Typical influenza of moderate severity—shivering, sweating, aches and pains, irritating dry cough, "tight" chest, slight coryza. Temperature 98° F. (36.7° C.) on 30/1/53	
2	M	38	16/2/54 23/3/54	<1/5 <1/5	1/5 1/5	1/5 1/40	* *	* *	* *	*	16/3/54: Common cold	
3	F	31	30/1/54 25/4/54	<1/5 <1/5	<1/5 <1/5	<1/5 1/40	* *	* *	1/5 1/80	*	31/3/54: Common cold	Husband bled on same dates had no illness and no serological changes. Her 2 children, aged 6 and 4 years, had colds on 29/3/54 and 31/3/54 respectively
4	F	36	3/2/54 22/4/54	1/5 1/5	<1/5 <1/5	1/10 1/20	* *	* *	1/10 1/80	*	3/2/54: " "	Husband bled on same dates had no illness and no serological changes. Her 2 children, aged 6 and 3 years, had colds on 25/1/54 and 26/1/54 respectively. The younger child had a temperature of 99.4° F. (37.4° C.) (P.R.)
5	M	36	6/2/54 6/5/54	<1/5 1/5	<1/5 1/5	<1/5 1/10	1/20 1/20	1/20 1/20	1/10 1/80	*	No illness	Wife bled on same dates had a twofold rise in antibody to virus C but had no illness. The elder of their 2 children, aged 5 and 3 years, had a cold on 2/3/54
6	F	2	31/5/54	<1/5	<1/5	1/256	* *	* *	* *	Virus C isolated	13/5/54: Febrile cold with moderately severe cough	These children were sisters: their brother, aged 13 years, had a cold on 13/5/54 and was also bled on 31/5/54, when his antibody titre to virus C was <1/2. Their mother and father had no illness at the time, and blood taken on 24/4/54, 31/5/54, and 2/10/54 showed no antibody changes
7	F	6	31/5/54	<1/5	<1/5	1/512	* *	* *	* *	*	13/5/54: Common cold	
8	F	31	25/4/54 2/10/54	<1/5 <1/5	<1/5 <1/5	1/5 1/10	* *	* *	1/10 1/40	*	15/5/54: " " 3/7/54: " "	
9	M	17	25/4/54 2/10/54	<1/5 <1/5	<1/5 <1/5	<1/5 1/10†	* *	* *	<1/5 1/10	*	28/4/54: " "	
10	F	40	15/2/55 9/3/55	1/5 1/5	<1/5 <1/5	1/5 1/10	* *	1/40 1/40	1/20 1/80	Neg.	13/2/55: Mild influenzal illness—aches and pains, cough, and "tight" chest. Not febrile	Husband, bled on 5/1/55 and 9/3/55, had no illness and no serological changes. Their children, aged 15, 4, and 3 years, were not ill during the same period

* Not done. † Serum slightly anticomplementary.

between 35 and 44 years is worth noting, though it may well have been due to chance. It seems reasonable to conclude that a high proportion—probably the majority of persons in this country—have encountered the virus at some time during childhood or early adult life. Our results give no indication of the extent to which infection was accompanied by illness nor the part played by reinfection in the maintenance of antibody levels. The fact that 8 of the 10 cases reported here were drawn from 7 out of 25 families observed for only 18 months suggests that infection with influenza virus C is not uncommon.

Diagram showing age distribution of antibody to influenza virus C. (The number of persons tested is shown in each age-group column.)

Summary and Conclusions

Ten cases are reported in which serological evidence of recent infection with influenza virus C was found. Influenza virus C was isolated from a throat swab of one—a girl aged 2 years suffering from a febrile cold and cough. Her sister, aged 6 years, had a cold on the same day and had a high antibody titre to influenza virus C 18 days later. A fourfold rise in antibody was found in paired sera taken from two adults suffering from a mild influenza-like illness. The remaining six cases had a fourfold or greater rise in antibody between two routine bleedings. Five of the six had colds during the period, but it is not known whether influenza virus C infection was responsible.

Blood specimens from 295 persons aged 4 months to 64 years from widely separated parts of the country were tested for complement-fixing antibody to influenza virus C. The proportion with an antibody titre of 1/10 rose from 5% in the 0-4-year age group to 61% in the 25-34-year age group and then remained high.

It is concluded that influenza virus C infection is common in this country and that infection may be responsible for acute respiratory illness.

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INFLUENZA A AND C IN GLASGOW, 1954

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The studies of influenza in pneumonia which began in 1950 and were reported in previous papers from this unit (Grist *et al.*, 1952; Grist and Landsman, 1955) were continued in 1953 and 1954. Because of the particular importance of acute respiratory infections in the very young, and because of the possibility that influenza C virus might be a cause of such infections in the early years of life, the scope of the investigation was extended in two respects. Firstly, infants were included in the study in addition to adults. Secondly, tests for influenza C infection were made in addition to those for type A and B virus infections. The present paper describes the results of these investigations during 1953-4.

Material and Methods

The patients studied were those admitted to adult and baby pneumonia wards in Ruchill Hospital, Glasgow. The adults comprised those admitted to one female ward from October, 1953, to July, 1954, to one male ward from January

TABLE I.—Ages and Diagnoses of Cases Admitted to Adult Pneumonia Wards During February-May, 1954

Diagnosis	Age Groups (Years)				Totals
	7-19	20-39	40-59	60 and Over	
Pneumonia	7	10	30	28	75
Bronchitis	3	3	16	23	45
Other respiratory diseases ..	2	2	7	2	13
Non-respiratory	2	4	3	3	12
Totals	14	19	56	56	145

to July, 1954, and to another male ward from February to May, 1954. Table I shows the ages and diagnoses of 145 cases admitted to these adult wards during the period February-May, 1954, when influenza infections were detected. The infants were an unselected series admitted to wards in which an investigation of respiratory disease