RESPIRATORY VIRUS INFECTIONS IN HOSPITAL AND IN GENERAL PRACTICE, 1954-6

BY

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In continuation of previous laboratory studies of respiratory illness, tests for a wider range of virus infections were made in a series of patients admitted to hospital and also in cases of milder illness seen in general practice during the winter seasons of 1954-5 and 1955-6. This paper presents evidence of infections with influenza viruses of types A, B, and C, an agent related to the Sendai strain of newborn pneumonitis virus ("influenza D," Jensen *et al.*, 1955), and the adenovirus group (Rowe *et al.*, 1953).

Methods

The population investigated included 167 patients admitted to pneumonia wards of Ruchill Hospital from July, 1954, to April, 1955, 70 admitted to baby pneumonia wards from November, 1954, to May, 1955, and 98 adults and 44 children under 2 years old admitted to these wards from November, 1955, to April, 1956, inclusive. Tests were also conducted in 105 cases of suspected influenza seen in general practice from December, 1954, to April, 1955, and in 92 similar cases seen in the corresponding five months a year later in the course of schemes for the detection of influenza in the community during vaccine trials of the Medical Research Council (M.R.C. Committee, 1955). A number of additional cases were examined in the course of investigations of outbreaks of respiratory illness. Serum samples were taken during the early and late stages of illness. Garglings for attempted virus isolation were collected with autoclaved skimmed milk or saline with added broth. During the 1955-6 season, respiratory secretions of infants were collected from the nose or throat by sterile cotton-tipped swabs which were subsequently extracted each in a few millilitres of broth for one hour at 37° C.

In general, the cultural and serological techniques were those described in previous papers (Grist, 1955; Grist and Landsman, 1955). For attempted virus isolation, embryonated eggs were inoculated by the amniotic route; at least two passages were made before specimens were regarded as negative.

Sera were examined by small-volume complement-fixation (C.F.) tests and by haemagglutination-inhibition (H.I.) tests. Soluble antigens were used in C.F. tests for influenza A and B; in some experiments soluble antigens prepared from locally isolated strains of virus were used. A fixation period of 90 minutes at 37° C. was employed with adult sera, but with sera from infants the cold overnight method was used. Antigens from several of the following strains of virus were used for H.I. tests: PR8 (type A), A/Scotland/1/53, A/Scotland/26/54, A/Scotland/31/56, Lee (type B), B/England/52, B/Scotland/28/55, and the 1233 strain of type C virus (Taylor, 1949). The Sendai strain of newborn pneumonitis virus was received from the World Influenza Centre of the World Health Organization. Allantoic-fluid antigens of this virus were used in C.F. and H.I. tests; positive and negative control sera for C.F. tests were received from the Virus

Laboratory of the School of Public Health, University of Michigan. Type 4 adenovirus (Hilleman and Werner, 1954), together with positive control serum, was received from the Virus Reference Laboratory, Colindale. Adenovirus group antigen was prepared from HeLa cell cultures showing complete degeneration after infection with this virus; the cultures were frozen, thawed, centrifuged at low speed to remove debris, and the supernatant fluid was heated to 56° C. for 30 minutes and stored at 4° C. for use as antigen in the small-volume cold overnight C.F. test. Sera were tested for agglutination of streptococcus M.G. by a standard method similar to that employed at the Virus Reference Laboratory, Colindale. Titres are cited as reciprocals of initial serum dilutions in C.F. and H.I. tests and as reciprocals of final dilutions in streptococcus M.G. agglutination tests.

Results

Influenza

Evidence of influenza infection indicated by fourfold or greater rising titres in serological tests was found during the periods shown in Table I. In common with the rest of

TABLE I.—Serological Evidence of Influenza

Period	Category of Case	No. of Cases Tested	No. with Rising Titres to Indicated Type of Influenza
Dec., 1954-Mar., 1955	Adult pneumonia	85	12
	Baby " "	98	2
	" Influenza " "	49	27
April, 1955	Adult pneumonia	28	1
	Baby " "	5	0
	" Influenza " "	7	2
Dec., 1955-Apr., 1956	Adult pneumonia	92	11
	Baby " "	24	2
	" Influenza " "	92	32
Jan., 1956-Apr., 1956	Adult pneumonia	89	5
	Baby " "	19	0
	" Influenza " "	74	1

Britain, Glasgow experienced an outbreak of influenza B during the winter of 1954-5. A few type A infections detected during April, 1955, heralded the following winter's outbreak of influenza A, which was associated with virus C infections as in the outbreak two years before (Grist, 1955). No influenza infections were found at times other than those shown in Table I. As might be expected, a higher proportion of serologically positive cases was found in the general-practice group than among the miscellany of cases admitted to the pneumonia wards, but very few of the infants showed rising titres of antibody. Thus, during the months when type A or B infections were detected, a specific serological diagnosis of influenza A or B was made in 42% of the general-practice cases, and in 12% of adults, but in only 3% of young children in the hospital series. Two type B strains of influenza virus were isolated during the first winter from garglings collected from children involved in an outbreak of illness in a Glasgow school for the deaf in January, 1955. Dr. A. Harboe, of the World Influenza Centre, reported that these strains closely resembled those sent to the Centre from other countries during the previous two years. During the same period outbreaks of influenza were reported from Ayrshire, and serological evidence of type B infection was found in four out of five cases from which paired sera were sent by Dr. C. D. Rigg, general practitioner.

During the second winter outbreak eight strains of virus A were isolated: these viruses were identified by Dr. A. Isaacs, of the World Influenza Centre, as Scandinavian subtype A strains similar to those isolated in Glasgow in 1953 and 1954. Two of these viruses were isolated from garglings from adults suffering from clinical influenza and six from throat swabs of infants or young children admitted to the baby pneumonia ward, of whom two aged 1 year showed fourfold or greater increases in antibody to type A virus. Of the other four children, two aged 3 years gave positive

serological reactions in the earliest serum samples and in the second serum of one; no second serum was available from the other child. The remaining two infants were 3 months old: very weak positive reactions were given by the sera of one and no reaction was found with the sera of the other at a dilution of 1/2 in the cold overnight C.F. test with soluble antigens prepared from PR8 or from current (A/Scotland/26/54) strains of virus.

Table II, constructed from the records of adults in both hospital and general practice series from whom paired sera were examined by both C.F. and H.I. tests, shows that these

TABLE II.—Results of Influenza C.F. and H.I. Tests of Adult Sera

Season and Type of Influenza	Results of C.F. Tests	Results of H.I. Tests	
		Fourfold or Greater Rising Titre	No Rise of Titre
1954-5 B	Fourfold or greater rising titre ..	28	3
	No rise of titre ..	8	176
1955-6 A	Fourfold or greater rising titre ..	24	15
	No rise of titre ..	8	142

tests were of similar efficiency. Five more cases of influenza B were diagnosed by H.I. than by C.F.; of 31 cases tested by H.I. with two type B antigens, all showed significant increases of antibody titre with current virus antigen (B/Scotland/28/55) and 30 did so with antigen of the Lee strain. Only 62% of type A infections gave diagnostic reactions with both tests, and seven more were detected by C.F. than by H.I.; only antigens from recently isolated strains of virus were used in H.I. tests for influenza A.

A specific serological diagnosis of influenza A or B based on rising titres of antibody was made in 34% of the general-practice cases. When sending blood for examination the practitioners were asked to classify the patients as either "typical" or merely "probable" cases of influenza, basing the distinction on their own clinical judgment. Virus A or B infection was confirmed serologically in 41 (31%) of "probable" and 26 (39%) of "typical" cases.

Newborn Pneumonitis Virus

The detailed results of experience with this agent will be reported separately (Sommerville, to be published). Fourfold or greater rising titres were found by one or both of the C.F. and H.I. tests in three adults admitted to pneumonia wards and also in six of the general-practice cases of suspected influenza (Table III). Of these nine cases of antigenic experience, four were found in the 1954-5 season; the other five occurred during the following winter.

TABLE III.—Serological Evidence of Infection with Other Respiratory Viruses

Category	Newborn Pneumonitis Virus		Adenovirus		Streptococcus M.G.	
	No. Cases Tested	No. with Rising Titres	No. Cases Tested	No. with Rising Titres	No. Cases Tested	No. with Rising Titres
Adult pneumonia	175	3	103	0	199	1
Baby "Influenza" ..	97	0	32	0	53	0
"Influenza" ..	172	6	70	1	120	0

Adenovirus Infections

C.F. tests with the group antigen were performed with negative results in 103 cases admitted to adult and 32 admitted to baby-pneumonia wards; a rise in titre from <8 to 64 was found in 1 of 70 general-practice cases tested (Table III). One infant pneumonia case admitted in November, 1955, had a C.F. titre of 32 in both serum specimens, suggestive of recent infection. Further evidence of infection with a virus of this group was found during investigation of an outbreak of respiratory illness in a children's residential nursery in Paisley in mid-January, 1956. Eight children aged from 9 months to 4 years became ill on January 14 with

fever followed by the development of pneumonia with marked illness and copious nasal and post-nasal discharge. Serological investigations gave no evidence of infection with the viruses of influenza A, B, C, newborn pneumonitis, psittacosis, or Q fever. No influenza virus was isolated from throat swabs by egg inoculation, but in one instance an agent which produced the cytopathogenic effects and gave the C.F. reaction of a member of the adenovirus group was isolated in tissue culture.

The results of C.F. tests on serum from the children (Table IV) show that antibody was detected in the first two sera of the child from whom virus was isolated and that at least

TABLE IV.—Evidence of Adenovirus Infection in Residential Nursery

Age of Child (Years)	Virus Isolation from Throat (17/1/56)	C.F. Titres of Sera Tested with Adenovirus Group Antigen		
		17/1/56	31/1/56	7/3/56
9/12	—	<5	<5	<8
1	+	40	40	<10
1	—	720 (a.c.)	<10	<10
2	—	N.T.	N.T.	<8
2	—	720 (a.c.)	N.T.	10
2	—	N.T.	N.T.	<8
4	—	<5	<10	<8

a.c. = Anticomplementary. N.T. = Not tested.

one other child had some antigenic experience. These findings do not prove that an adenovirus was responsible for the wave of illness in the nursery, although negative C.F. reactions do not exclude the possibility, since Balducci *et al.* (1956) have reported instances of infected children whose sera contained no demonstrable complement-fixing antibodies but nevertheless neutralized the homologous strain of adenovirus isolated. Serological evidence of infection with an adenovirus was also found during investigation of an epidemic of keratoconjunctivitis on Clydeside in the early months of 1956.

Streptococcus M.G. Agglutination Tests

Of 372 cases tested (Table III), none had antibody titres of 40 or greater or showed significantly rising titres except one man aged 24 years who was admitted to the pneumonia ward in March, 1955. Titres of 10, 160, and 320 were found with sera taken on the 4th, 7th, and 15th days of illness respectively; the serum of the 7th day gave a cold agglutination titre of 256. Clinically, the illness was an atypical pneumonia of gradual onset, with right lower lobar consolidation and without leucocytosis.

Discussion

These investigations showed the continuing seasonal activity of influenza viruses in and around Glasgow and again illustrated the tendency of type A and B viruses to become active in alternate years. Virus C infections were again found only during the epidemic of influenza A. Serological evidence of infection with an agent related to the newborn pneumonitis virus, for which the name "influenza D" has been proposed by Jensen *et al.* (1955), was found mainly during the height of the outbreak of influenza A. Significantly high but unchanging titres in the tests for infections with these four viruses were found mainly during and after the periods when cases with fourfold or greater rising titres were detected.

Little evidence of specific viral aetiology for respiratory infections of infants admitted to hospital was found in the present study, but our experience again indicated the greater difficulty in demonstrating serum antibodies in this age group. Not only is the antibody response of infants highly specific for the infecting virus, as compared with the broader response of the antigenically more-experienced adult, but also their antibodies react more weakly than those of older subjects in the C.F. test with influenza antigens (Anderson *et al.*, 1953). In Glasgow, Russell (1956) has found that the period of maximum notifications of pneumonia in children under 1 year old often precedes by some weeks the

seasonal outbreak of influenza which coincides with peak notifications of pneumonia in adults. Similar observations that maximal respiratory illness in young children may not coincide with outbreaks of influenza were reported by Stuart-Harris *et al.* (1949) and by Garrow and Fawcett (1953). The epidemics of acute bronchiolitis in infants in 1951, 1953, and 1955, observed by Heycock and Noble (1956), were all in the pre-Christmas season (Heycock, personal communication), whereas influenza epidemics usually occur later in the winter. It seems likely that influenza viruses are not the main agents responsible for the winter seasonal increase in serious respiratory illness of infants. Nevertheless, influenza infection was detected in several infants in the present study; the clinical aspects of their illnesses will be described in a separate report (Thomas, to be published).

McDonald and Andrews (1955) investigated diagnostic methods in an influenza vaccine trial and concluded that assessments based on purely clinical grounds were apt to underestimate while those based on purely serological data were apt to exaggerate the protective power of the vaccine. They recommended that the assessment should be based on the numbers of cases diagnosed as influenza on clinical grounds during the limited period when laboratory tests showed that influenza was present in the community. The general-practice study described above was organized primarily in connexion with vaccine trials. Both hospital and general-practice investigations indicated the activity of influenza viruses in Glasgow during similar periods. A higher proportion of serologically positive cases was found among those seen by the practitioners, and during the second winter influenza A was detected earlier and for a longer period in this group than in hospital. On the other hand, laboratory investigation of patients admitted to hospital with acute respiratory illness is more easily organized, and earlier studies at this laboratory have confirmed that the results of such tests reflect the influenzal experience of the community as judged by other criteria (Grist and Landsman, 1955).

The standard C.F. test with soluble antigen was again found to be a convenient and satisfactory method of diagnosis in adults, though an additional 23% of infections were detected when this test was supplemented by H.I. tests. The difficulty of clinical diagnosis of influenza is illustrated by the similarity in the proportions of confirmed cases in the groups considered "typical" and "probable" influenza by the general practitioners. It should be understood that each practitioner was encouraged to submit specimens from two patients a week whose illnesses might conceivably be influenzal in origin. This was done in order to secure a representative sample of minor febrile and respiratory disease throughout the study, and doubtless resulted in some dilution of influenzal with other cases. Nevertheless, the "typical" group did not contain a significantly higher proportion of cases in which laboratory tests indicated specific infection with influenza virus A or B.

Evidence of infection with members of the adenovirus group has been reported in Britain by Zaiman *et al.* (1955), Andrews *et al.* (1956), and Balducci *et al.* (1956). In the present study these infections were infrequently found except during investigation of an epidemic of keratoconjunctivitis. Rising titres of C.F. antibody were found by Balducci *et al.* (1956) in 3 out of 67 adults with pneumonia or bronchitis in a Sheffield hospital, whereas none of 103 similar cases in the Glasgow hospital study showed a rising titre. The pathogenicity of these viruses for man is incompletely defined, but in this country they have now been associated with the syndromes of febrile catarrh, pharyngo-conjunctival fever (Bell *et al.*, 1955), and epidemic keratoconjunctivitis.

Atypical pneumonia accompanied by the development of cold agglutinins and agglutinins for streptococcus M.G. has continued to be uncommon in Glasgow since these investigations of pneumonia cases began in 1950. Glasgow did not appear to share the epidemic experience of this disease in 1954 reported from East Anglia and London by Wormald *et al.* (1956), Wood (1956), and Garrow (1956).

Summary

Examinations for virus infection were made in 197 cases of suspected influenza seen in general practice and in 265 adults and 114 children admitted to pneumonia wards during the winters of 1954-5 and 1955-6.

Cases of influenza B were detected in all groups during the first winter.

In the second season an outbreak of influenza A occurred; during this period were found several influenza C infections and also instances of antigenic experience with an agent related to the Sendai strain of newborn pneumonitis virus.

Few adenovirus infections were detected except in cases of epidemic keratoconjunctivitis, but a virus of this group was isolated during investigation of an outbreak of respiratory illness in a children's nursery.

One case of atypical pneumonia with agglutinins to streptococcus M.G. was found.

The investigation showed the practicability of detecting the time and type of influenza outbreak by serological tests of cases selected in general practice or admitted to hospital with the diagnosis of pneumonia.

Combined complement-fixation and haemagglutination-inhibition testing revealed more influenza cases than either test alone. The weak immune response of infants made serological diagnosis difficult.

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REFERENCES

- Anderson, S. G., Donnelley, M., French, E. L., Kalra, S. L., and White, J. (1953). *Med. J. Aust.*, **2**, 44.
 Andrews, B. E., McDonald, J. C., Thorburn, W. B., and Wilson, J. S. (1956). *British Medical Journal*, **1**, 1203.
 Balducci, D., Zaiman, E., and Tyrrell, D. A. J. (1956). *Brit. J. exp. Path.*, **37**, 205.
 Bell, J. A., Rowe, W. P., Engler, J. I., Parrott, R. H., and Huebner, R. J. (1955). *J. Amer. med. Ass.*, **157**, 1083.
 Garrow, D. H. (1956). *British Medical Journal*, **1**, 1105.
 — and Fawcett, J. W. (1953). *Lancet*, **2**, 795.
 Grist, N. R. (1955). *British Medical Journal*, **2**, 994.
 — and Landsman, J. B. (1955). *Glasg. med. J.*, **36**, 69.
 Heycock, J. B., and Noble, T. C. (1956). *British Medical Journal*, **1**, 438.
 Hilleman, M. R., and Werner, J. H. (1954). *Proc. Soc. exp. Biol. (N.Y.)*, **85**, 183.
 Jensen, K. E., Minuse, E., and Ackermann, W. W. (1955). *J. Immunol.*, **75**, 71.
 McDonald, J. C., and Andrews, B. E. (1955). *British Medical Journal*, **2**, 1232.
 M.R.C. Committee (1955). *Ibid.*, **2**, 1139.
 Rowe, W. P., Huebner, R. J., Gilmore, L. K., Parrott, R. H., and Ward, T. C. (1953). *Proc. Soc. exp. Biol. (N.Y.)*, **84**, 570.
 Russell, S. J. (1957). *Scot. med. J.* In press.
 Stuart-Harris, C. H., Laird, J., Tyrrell, D. A. J., Kelsall, M. H., Franks, Z. C., and Pownall, M. (1949). *J. Hyg. (Camb.)*, **47**, 434.
 Taylor, R. M. (1949). *Amer. J. publ. Hlth*, **39**, 171.
 Wood, P. (1956). *British Medical Journal*, **1**, 711.
 Wormald, P. J., Dowsett, L. M., and Walker, J. H. C. (1956). *Ibid.*, **1**, 709.
 Zaiman, E., Balducci, D., and Tyrrell, D. A. J. (1955). *Lancet*, **2**, 595.

A film entitled "Hearing Tests in Cerebral Palsied Children" has been made at the Centre for Spastic Children, Chelsea, in co-operation with the audiology unit of the Royal National Throat, Nose, and Ear Hospital. It shows some of the methods and difficulties in testing these children. The film is silent, 16 mm., and 650 ft. (198 m.) in length. A script to accompany the film is available or a lecturer can be supplied. The film is available from the Centre for Spastic Children, 61, Cheyne Walk, Chelsea, London, S.W.3.