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An aetiological study of respiratory infection in children, Edinburgh City Hospital, 1961–1963

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The aetiology of acute respiratory infection in young children is still not fully understood and numerous combined clinical and laboratory investigations have been carried out in an attempt to elucidate this problem (Morrison *et al.* 1957; Gardner *et al.* 1960; Holland, Tanner, Pereira & Taylor, 1960; Garrow & Taylor, 1962; Tobin, 1963; Clarke *et al.* 1964). No such study had been carried out in Edinburgh and a fact-finding survey was therefore planned to provide a picture of the bacterial and viral flora occurring in children admitted to hospital with respiratory infection. In this survey, no effort was made to examine a control group of children but it was hoped that by investigating *all* cases of respiratory illness certain aetiological patterns might be found to be associated with certain syndromes. Although the sera were tested for antibodies to respiratory syncytial virus no particular attempt was made to isolate this virus; optimum conditions for isolation of the common cold viruses were likewise not provided. The part played by these viruses may be considerable and must not be overlooked when examining the present results.

MATERIALS AND METHODS

The investigations were carried out during two winter periods; (1) from 1 October 1961 to 30 April 1962; (2) from 1 November 1962 to 31 May 1963. The procedures adopted during the first period are described below; alterations to the protocol made during the second period are listed separately.

Clinical cases and procedures

All children 12 years of age and under admitted to Edinburgh City Hospital with acute respiratory infection were included in the survey. All patients were examined clinically and classified into the following disease categories:

(*a*) upper respiratory tract infection (URTI) which included all coryza-like illness and conjunctivitis, pharyngitis and stomatitis;

(*b*) bronchitis, which included croup;

(*c*) pneumonia which was confirmed radiologically;

(*d*) whooping cough, which was a clinical diagnosis and included complicating bronchitis or pneumonia.

On admission two cough swabs were taken by passing the swab through the

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mouth to the posterior pharyngeal area; one of the swabs was sent to the laboratory for bacteriological examination while the other was immersed immediately in 3 ml. transport medium for virus isolation. A specimen of venous blood and a faecal specimen were also collected for virus studies and in cases of whooping cough a pernasal swab was examined bacteriologically. Ten to fourteen days after onset of the illness a second blood specimen was taken for investigation of virus antibodies.

Bacteriological procedures

The cough swabs were serum-coated according to the method of Rubbo & Benjamin (1951). In all cases the cough swab was cultured on blood agar and on heated blood agar and a direct smear was Gram-stained. The pernasal swab was inoculated on to Bordet–Gengou medium. Sera from cases of whooping cough were examined for antibodies to *Bordetella pertussis* by the tube method (Evans & Maitland, 1939).

Virological procedures

Specimens for virus isolation were transported to the laboratory without delay and stored at $-70°$ C. until cell cultures were available; the transport medium consisted of 2% inactivated horse serum in Hanks's solution and contained penicillin (100 units per ml.) and streptomycin (100 μg./ml.). Serum samples were stored at $-25°$ C. All attempts to isolate virus from throat swabs and faecal specimens were made in secondary cultures of monkey kidney cells and in Hep-2 cells. The maintenance medium for all tissue cultures was medium 199 with 2% calf serum and in all cases penicillin and streptomycin were included in the medium. Tubes were inoculated with 0·2 ml. of the specimen and incubated in roller drums at 35° C.; the cells were examined microscopically for cytopathic effect every 2 days for 18 days. Haemadsorption tests using 0·4% human group 'O' erythrocytes were carried out on all monkey kidney cultures inoculated with throat swabs 10 days after inoculation. Throat swab specimens were also inoculated into the amniotic cavity of 11-day chick embryos; the eggs were incubated for 2 days at 35° C. and the amniotic fluids tested for haemagglutination. A further passage was then carried out.

Complement fixation tests were carried out according to the technique described by Bradstreet & Taylor (1962). The sera were screened at a dilution of 1/16 against the following antigens: influenza A, B and C, Sendai, para-influenza 1, 2, 3, adenovirus, mumps S and V and respiratory syncytial (RS) virus. All complement fixing reagents except RS were obtained from the Standards Laboratory for Serological Reagents, Central Public Health Laboratory, Colindale. The RS antigen was prepared in monkey kidney cells grown on serum-free medium from the Randall strain of virus previously passaged in Hep-2 cells.

Neutralization tests were carried out on patients' serum against any virus isolated from that patient. The virus, diluted to contain 100 TCD 50 per 0·2 ml., was mixed with 0·25 ml. amounts of twofold dilutions of serum. The mixtures were allowed to stand for an hour at room temperature and then 0·2 ml. amounts were inoculated into each of two tubes of the appropriate tissue culture. The neutralizing

titre was taken as the highest dilution showing inhibition of the virus when complete degeneration of the cells was evident in the tubes inoculated with virus alone.

Identification of virus strains was carried out by neutralization tests using the following antisera: poliovirus 1, 2, 3, coxsackie B 1–6, A 7, A 9, echo 1–10, 12, 14, 15, 16, 18, 19, 20, 22, 25, 26, adenovirus 1–11, 14, 15, 16, para-influenza 1, 2, 3, herpes simplex. Unidentified viruses were inoculated into litters of unweaned mice to aid identification; the mice were inoculated by the intracerebral and intraperitoneal route and examined for 21 days for signs of paralysis.

Alterations during winter 1962–63

The following changes were made to the above procedures:

(1) the investigations were limited to children of 6 years or under because of the poor isolation rate in those over 6 during the first year;

(2) a nasal swab was examined bacteriologically in an attempt to improve the isolation rate of pneumococci;

(3) the monkey kidney cells were maintained on serum-free medium, i.e. 7·5 % liver digest (Burroughs Wellcome) in Earle's solution (Smith, 1961);

(4) throat swabs were not inoculated into chick embryos as it was considered that most influenza infections would be detected in monkey kidney cells and by the complement fixation test.

RESULTS

During 1961–62, 131 children were included in the survey; of these, 73 % were admitted to hospital during the 3 months October to December. Over the winter of 1962–63, 133 patients were examined; these cases were distributed more evenly throughout the period of study. Approximately a quarter of the patients occurred in each of the four age groups, 0–1 years, 1–2 years, 2–3 years, and over 3 years. Because of the exclusion of children over 6 years of age in the second winter and because 50 % of the children in the oldest age group in the first winter were over 6 it is evident that there were more younger cases in 1962–63.

Distribution of disease categories

The distribution of disease categories throughout the periods surveyed is shown in Fig. 1. During the first winter each disease category was quite evenly distributed throughout the whole winter; 37·4 % of the cases were in the URTI group and 29·8 % in the pneumonia group, while bronchitis and whooping cough were responsible for 16·0 and 16·8 %, respectively. In the second winter pneumonia occurred mainly during the early part of the winter and whooping cough during the latter half. In contrast to the previous year only 29·3 % of cases were in the URTI group; 32·3 % were in the pneumonia group; and bronchitis and whooping cough were again least encountered and were responsible for 18·1 and 20·3 % of the cases. The disease categories were distributed evenly throughout the age groups.

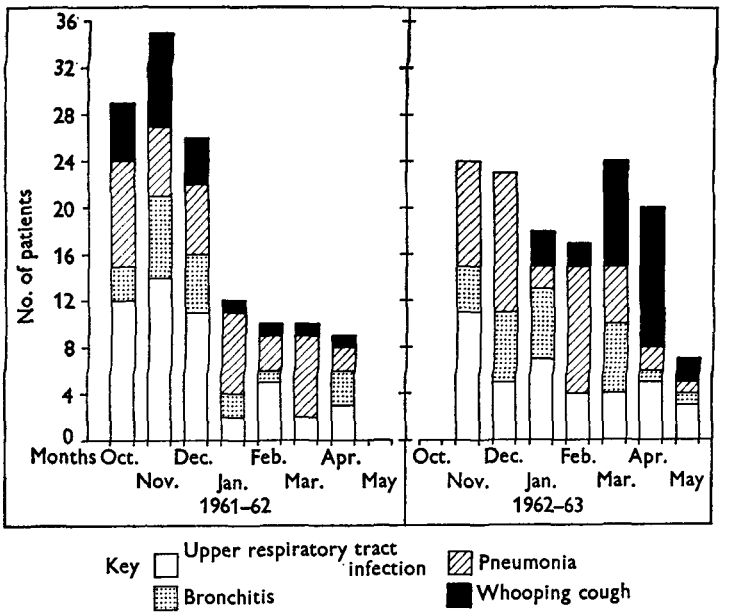

Fig. 1. The distribution of disease categories thoughout the two periods of study.

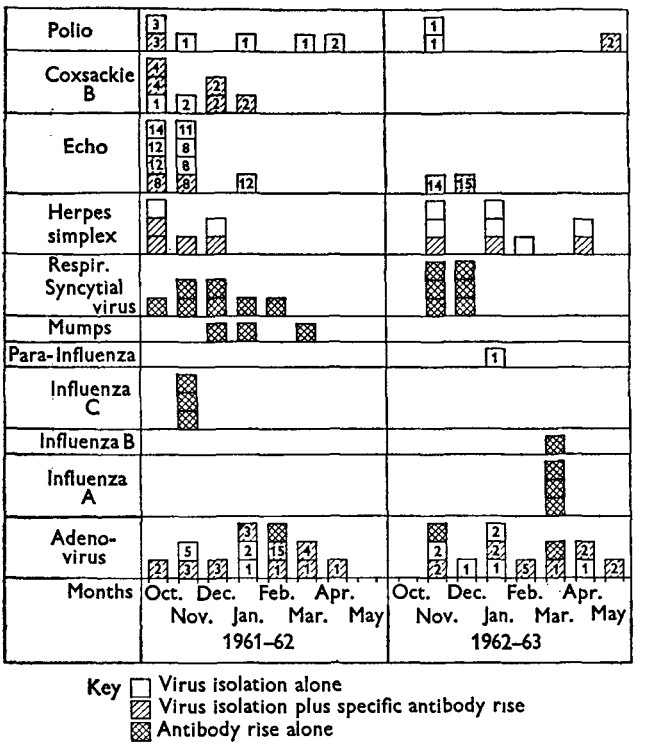

Fig. 2. The seasonal incidence of virus throughout the two periods of study. Numbers indicate type of virus isolated.

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Incidence of virus

The viruses encountered over the two winters are shown in Fig. 2. The percentage incidence of virus during the two periods of study was 41·2 and 28·6 %, respectively. This percentage includes viral isolation with or without antibody rise as well as a fourfold or greater rise in antibody *without* isolation of the virus. Excluded from these figures are twenty-three unidentified viruses isolated over both winters; twenty-two of these were isolated from faecal specimens alone and twenty-one grew only in Hep-2 cells; none was pathogenic for unweaned mice. The general pattern of virus incidence was similar in both periods apart from a difference in the number of enterovirus isolations; these formed 40·7 % of the total viruses in 1961–62, but only 13·2 % in 1962–63; this reduction was due to the complete absence of coxsackie B viruses and a decrease in the number of echo viruses in the second winter. There was no evidence of influenza C or of adenovirus types 3, 4, or 15 in 1962–63. The enteroviruses, apart from poliovirus, were encountered early in the winter in both surveys; influenza A and influenza C infections were also demonstrated within a relatively short period of time as was RS in the second winter. The adenoviruses were more evenly distributed throughout the survey period.

More than one virus was isolated from a patient in eleven cases as follows: adenovirus with mumps, influenza B, influenza C and para-influenza virus respectively in four cases, RS with echo, herpes simplex and adenovirus respectively in three cases, herpes simplex with influenza C and an unidentified virus in two cases; echo 14 and coxsackie B1 occurred together once and two apparently different unidentified viruses were isolated from the same patient in one case.

Relation of virus incidence to disease category

The incidence of virus within the disease categories is shown in Fig. 3. The percentage of patients in each group with evidence of virus can be seen in the first part of Table 1.

Of twenty-three adenovirus isolations ten were from patients in the URTI group, all of whom showed an antibody rise to the virus isolated; one of these cases had associated splenomegaly. Two patients in this group showed a rise in antibody but no virus was isolated. Two cases of pharyngoconjunctival fever were diagnosed and were associated with the isolation of adenovirus type 3; both patients were under 2 years old. The other types encountered in the URTI group were types 1, 2, 4 and 15. Half the patients with URTI had associated diarrhoea. There were three adenovirus isolations (types 1 and 2) in the bronchitis group, which included one patient with croup, but no antibody response occurred with these isolations. Adenovirus types 1, 2, and 5 were isolated from children with pneumonia and from those with whooping cough. The two patients in the whooping cough group who produced antibody rises both had bronchitis; the three whooping cough cases who did not show an antibody rise to the adenoviruses isolated had pneumonia as a complication. Of the twenty-six cases with evidence of adenovirus infection or carriage, 70 % were under 2 years of age.

The three cases of influenza A which occurred in March 1963 were associated in time with eleven adult admissions of influenza and were distributed throughout the age groups. Only one case of para-influenza virus infection was diagnosed virologically; it occurred in a child aged one year with uncomplicated croup.

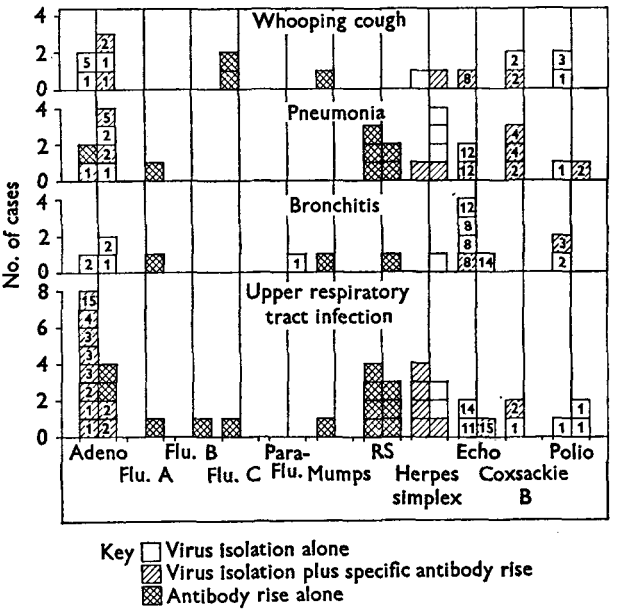

Fig. 3. The incidence of virus within the defined disease categories. 1961–62, left side of column. 1962–63, right side of column. Numbers indicate type of virus isolated. Total patients in each disease category: URTI 89; bronchitis 45; pneumonia 82; whooping cough 49.

Table 1. *The relation between virus isolation and serological response*

	URTI		Bronchitis		Pneumonia		Whooping cough	
	1961–62	1962–63	1961–62	1962–63	1961–62	1962–63	1961–62	1962–63
Percentage patients in each disease category with evidence of virus*	47	39	38	29	30	28	52	14
Percentage patients in each disease category with antibody rise	38·8	18	14·3	8·3	25·7	16·3	22·7	11
Ratio of antibody rise to virus isolation†	13/15	4/6	2/5	0/0	6/7	4/6	2/7	3/4

* Including virus isolation with or without antibody rise as well as a fourfold or greater rise in antibody *without* isolation of virus. Unidentified viruses are excluded.

† Excluding those patients from whom serum was not available and those with antibody rise with no virus isolation.

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Thirteen cases of respiratory syncytial (RS) virus infection were diagnosed serologically. Seven of these had mild URTI, five had pneumonia and one had bronchitis. All the cases except one were over the age of 1 year and half of them were over the age of two.

Herpes simplex was isolated from fifteen patients. Of these, three cases were diagnosed as aphthous stomatitis, one as bilateral conjunctivitis and one as a case of pneumonia which also had facial herpes; these five cases had an associated antibody rise to herpes simplex. The remaining ten cases which were distributed throughout the disease categories showed no clinical evidence of herpes simplex infection although three of them had an associated antibody rise.

Three of the four isolations of echovirus type 8 were made from children admitted from the same day nursery with clinical bacillary dysentery; their ages were 14 months, 2 years and 3 years. All of these children had associated bronchitis and no bacterial pathogens were isolated from their faeces; only one of the children, however, showed an antibody rise. The remaining echo 8 was isolated from a child of 15 months with whooping cough and bronchitis and was accompanied by a rise in antibody. A specific antibody rise was also detected in a case of pneumonia from whom echovirus type 12 was isolated and in a case of URTI with cough from whom echovirus type 15 was isolated. The remaining five echovirus isolations, which included types 11, 12 and 14, were not accompanied by a rise in antibody and occurred throughout the disease categories, showing no special features.

Coxsackie virus type B 2 was isolated from four children, three of whom showed a serological rise. Of these three, one child had a mild aseptic meningitis with URTI, a second had pneumonia with rash and diarrhoea and the third had whooping cough pneumonia. The remaining type 2 virus was isolated from a patient with whooping cough bronchitis. Two B 4 viruses were isolated in the pneumonia group; only one of these produced an antibody rise. One type 1 virus was isolated from a child with uncomplicated URTI; there was no antibody rise.

In only two of the nine cases from whom poliovirus was isolated was an antibody rise detected. One of these occurred in a case of 'croup' from whom poliovirus type 3 was isolated and the other in a pneumonia case with associated diarrhoea. Of the isolations occurring without antibody rise only in one case was a definite history of recent vaccination obtained. This child had bronchitis with diarrhoea. No central nervous system complications occurred in any of the cases with poliovirus isolations.

No special clinical features were associated with the isolation of the unidentified viruses.

Antibody response

Over both winters the percentage of patients with a fourfold or greater antibody response was 28·2 and 17·3 %, respectively, giving an average response of 23 %. In Table 1 the relation between virus isolation and serological response can be seen; the relationship is expressed as a ratio of fourfold antibody rise to virus isolation. From the table it is evident that the antibody response was best in the URTI and pneumonia groups and was variable in the whooping cough and

bronchitis groups. The antibody response in the different age groups was also expressed as a ratio of antibody rise to virus isolation and the response was found to be similar throughout the age groups.

During the course of the survey it was noted that when the same virus was isolated from both throat swab and faeces a fourfold or greater rise in antibody to that virus was always associated. In contrast to this only 32 % of viruses isolated from the throat alone and 17 % of the viruses isolated from faeces alone gave a fourfold or greater antibody rise.

Table 2. *The isolation of potential bacterial pathogens within the disease categories*

	URTI		Bronchitis		Pneumonia		Whooping cough		Total
	1961–62	1962–63	1961–62	1962–63	1961–62	1962–63	1961–62	1962–63	
Staphylococcus pyogenes	15	5	5	9	6	13	4	4	61
Pneumococcus	0	5	0	1	0	3	0	4	13
Bordetella pertussis	—	—	—	—	—	—	2	0	2
Haemophilus influenzae	2	0	1	0	1	0	1	0	5
Escherichia coli	5	3	0	3	3	1	3	1	19
Monilia	0	1	0	0	0	1	1	0	3
Pneumococcus plus other bacterial pathogens	0	4	0	2	0	1	0	1	8
Other double bacterial pathogen isolations	0	2	0	0	0	0	0	1	3
Total patients with pathogenic bacteria	22	20	6	15	10	19	11	11	114
Total patients	49	39	21	24	39	43	22	27	264
Percentage with pathogenic bacteria	45	51	29	63	26	44	50	41	43

Bacteriological findings

Potential bacterial pathogens were isolated from 37 % of patients in 1961–62 and from 49 % in 1962–63. These findings are summarized in Table 2, where it may be seen that *Escherichia coli* was included as a potential pathogen. During the first winter, isolations were highest in the whooping cough and URTI groups; isolations in 1962–63 were highest in the URTI and bronchitis groups.

The commonest potential bacterial pathogen isolated was *Staphylococcus pyogenes*. No pneumococci were isolated in 1961–62 but the examination of a nasal swab during the second winter gave isolations of pneumococci in 15 % of cases. *Bordetella pertussis* was isolated twice in 1961–62 but not at all in 1962–63. Agglutinating antibodies to *B. pertussis* were investigated in twenty-one patients during 1961–62 and in nineteen patients during 1962–63. Of the forty patients, eight had

no detectable antibody at 1/30, twenty-five had stable titres of 1/30 or 1/60, four had stable titres of 1/120 or higher, two patients had a fourfold antibody rise and one had a fourfold fall in titre. Seven of the children tested had been immunized; five had stable titres at 1/30, two had stable titres at 1/60. Of the forty-nine cases of whooping cough examined during the survey only nine (18%) had been immunized.

There was no significant difference in the isolation rate of bacterial pathogens between those patients who had received antimicrobial drugs before admission and those who had not. It is worthy of note that half the children from whom *Esch. coli* was isolated had *not* had previous chemotherapy.

Summary of laboratory findings

The combined bacteriological and virological results obtained during the survey are summarized in Table 3. Approximately one third (36%) of the cases had no known aetiology and a further 6% had viruses alone without any serological rise, indicating doubtful aetiology. Serological evidence of virus infection with or without bacteria was shown in 22%, and 29% had bacterial pathogens alone.

Table 3. *Incidence of bacterial and viral infections during the survey*

	B	VI	VS	B+VI	B+VS	Nil	Total
1961–62	32	11	26	7	10	45	131
1962–63	45	6	12	9	11	50	133
Total both winters	77	17	38	16	21	95	264
Percentage both winters	29·2	6·4	14·4	6·0	8·0	36·0	—

B = Bacterial pathogens alone.
VI = Virus isolation alone.
VS = Serological evidence of virus with or without virus isolation.

DISCUSSION

The proportion of respiratory illnesses associated with known viral agents has been found to be high in infancy and childhood (Chanock & Johnson, 1961) and the figures presented in this paper are comparable with those from other surveys (Gardner *et al.* 1960; Hilleman *et al.* 1962; Sterner & Tunevall, 1962). The aetiological structure of respiratory illness in a 'non-epidemic' period is also known to be complex (Sterner & Tunevall, 1962); this is borne out here by the fact that, with the exception of the possible association between upper respiratory tract infection and adenoviruses, no specific virus or virus group was associated with any particular disease category.

Antibody studies are essential to differentiate between virus carriage and virus infection; infection is defined here as a fourfold or greater rise in antibody whether or not virus has been isolated. We have probably underestimated the proportion of virus isolations which were infections in the true sense. The relatively low figure of 23% may have been due to the fact that insufficient time was allowed between acute and convalescent serum specimens for antibody rise to take place or that convalescent specimens could not be obtained, and in some cases a long period of illness before admission to hospital may have made it impossible to

demonstrate an antibody rise. The latter probably accounts for the apparently poor viral antibody response in the whooping cough group; that most of these cases had had experience with *B. pertussis* is borne out by the agglutination studies, in which 32 out of 40 (80 %) cases were found to possess antibodies, whereas only a very small proportion (18 %) had been artificially immunized with this antigen. The viral infections which did occur in this condition were probably super-infections although we cannot exclude the possible aetiological role of viruses in clinical whooping cough (Goodpasture, Auerbach, Swanson & Cotter, 1939; Chany *et al.* 1958; Farber & Vawter, 1961; Olsen, Miller & Hanshaw, 1964).

The endemic types of adenovirus, types 1, 2 and 5, formed the bulk of our adenovirus isolations, whereas members of the so-called pathogenic group of adeno-viruses, types 3, 4, 7 and 14, occurred in our series only during the first winter. Two of the three cases of adenovirus type 3 infection showed the characteristic syndrome of pharyngo-conjunctival fever, an association which is well documented (Bell *et al.* 1955; Kjellén, Zetterberg & Svedmyr, 1957; Huebner, Rowe & Chanock, 1958). The single isolation of adenovirus type 15 was found in association with upper respiratory tract infection. An antibody rise accompanied 60 % of our adenovirus isolations. These were therefore considered to be infections; of these 67 % occurred with upper respiratory tract syndromes and 20 % in pneumonia. We conclude that although 10 % of our total population had evidence of adeno-virus carriage 7 % actually had virus infection (see Fig. 3); the majority of the viruses were of the endemic type and were probably the cause of respiratory disease predominantly of the upper respiratory tract.

The myxovirus group, which includes the influenza and para-influenza viruses, mumps and possibly respiratory syncytial virus, are all regarded as pathogenic, and evidence of myxovirus infection was found in 9 % of our population, this being the highest infection rate for the groups of virus encountered. Influenza A infection occurred during a localized outbreak in March 1963 when eleven adult cases were diagnosed in our hospital. All the influenza infections encountered occurred throughout the disease categories, two cases of influenza C occurring in the whooping cough group. The single para-influenza infection was associated with croup; this association is now well established (Parrott *et al.* 1962). Two of our three mumps infections were cases of clinical mumps and were included in the survey on account of associated respiratory infection. One of these carried adeno-virus type 2 while the remaining mumps infection was subclinical.

Respiratory syncytial virus has been established as a major cause of respiratory illness, especially of lower respiratory tract infection in infants and children (Chanock & Finberg, 1957; Forbes, Bennett & Gray, 1961; Chanock *et al.* 1962; Hilleman *et al.* 1962). Our apparently low figure of 5 % is probably an under-estimate because of the insensitivity of our serological technique, in which we used only one unit of antigen. Furthermore, no particular attempt was made to isolate the virus. In a recent study of an outbreak of acute bronchiolitis in Sunderland only 50 % gave unequivocal serological evidence of infection with RS virus, pre-sumably because of the inability of the younger children to produce detectable antibodies (Crone, Heycock, Noble & Patton, 1964).

Herpes simplex virus was found in 6 % of our cases and half of these had virus infections. Four of these cases showed a characteristic illness and one other case had facial herpes; it is unlikely that the remaining ten isolations were aetiologically concerned with the associated respiratory illness.

Enteroviruses were found in 10 % of our population and formed the largest viral group encountered. It has been shown by many workers (Walton & Melnick, 1953; Moffet & Cramblett, 1962; Hilleman, 1963) that, during a period of increased prevalence of coxsackie B virus, a variable proportion of minor illness (3–14 %), not infrequently mild respiratory illness, is due to this virus group. Coxsackie B viruses occurred in 7 (3 %) of our cases; five of these represented infection and occurred throughout the disease categories. Certain types of echoviruses, notably types 11, 20 and 28, have been established as causing respiratory illness, usually of a mild type. Of these types only one type 11 was encountered, associated with upper respiratory tract infection: there was no antibody response. The three cases of enteric–respiratory disease from a children's nursery with echo 8 isolations compare with a similar outbreak described by Rosen, Johnson, Huebner & Bell in 1958. The remaining echovirus types in our series, 12, 14 and 15, have not previously been incriminated in respiratory tract disease. Echoviruses were isolated from 11 (4 %) patients, but in only four of these was there a rise in antibody. We conclude that, although coxsackie B virus, when present, may account for a small proportion of respiratory illness, the other enteroviruses did not seem to play an important role in our population. Only one enterovirus, a coxsackie B 2 virus, was associated with clinical involvement of the central nervous system.

Bacterial pathogens were isolated from half of our total cases but no great emphasis can be put on the presence of pathogens in the throat (Rabe, 1948). It is of interest that, in accordance with the findings of Masters, Brumfitt, Mendez & Likar (1958), the isolation of pneumococci was increased in the second year when nasal swabs were examined. The low isolation rate of *B. pertussis* in whooping cough may be related to the long duration of illness before admission to hospital.

Although bacteria or viruses could be implicated in approximately two-thirds of the cases in this survey, the remaining patients gave negative results. This again emphasizes the need for further investigations in this field, bearing in mind the possible significance of such virus groups as the rhinoviruses. It seems inevitable, however, that the aetiology of a proportion of cases will always be unknown because of the poorer antibody response in the youngest age group; it is therefore important that more should be known of the pathogenesis of the virus groups so that the significance of their presence in disease may be better understood even in the absence of antibody response.

SUMMARY

The findings are described of a combined clinical, bacteriological and virological study which included all children admitted to the City Hospital, Edinburgh, with acute respiratory infection and whooping cough during the winters 1961–62 and 1962–63. During the first winter 131 cases aged 0–12 years and in the second winter 133 aged 0–6 years were examined. The respiratory illnesses were divisible

into upper respiratory tract infection, bronchitis, pneumonia, and whooping cough; many of the cases of whooping cough had respiratory complications with bronchitis or pneumonia.

Paired sera, a throat swab and a faecal specimen were taken from each child and investigated virologically. Over both winters the highest total virus isolation rate was found in the group suffering from upper respiratory disease. Approximately two-thirds of the total number of patients from whom virus was isolated and from whom both acute and convalescent sera were available gave a serological response to the homologous virus; the highest proportion of these patients occurred in the pneumonia and URTI groups. The groups of viruses associated with a fourfold or greater rise in antibodies occurred in the following proportions of the cases: myxovirus 9%; adenovirus 7%; enterovirus 4%; herpes simplex 3%.

Bacterial pathogens were isolated from 37% of patients in 1961–62 and from 49% in 1962–63, *Staph. pyogenes* being the most common pathogen. Isolation of pneumococci was facilitated during the second year by the examination of a nasal swab. Pre-admission chemotherapy did not significantly alter the bacterial isolation rates. Agglutination studies were carried out on forty clinical cases of whooping cough admitted during the two winters and thirty-two showed significant stable titres to *Bordetella pertussis*; only 9 (18%) of these cases gave a history of prophylactic immunization.

A third of the patients had neither bacterial nor viral pathogens.

The findings in this survey illustrate the need for further intensive virological and bacteriological studies of acute respiratory infections in early childhood.

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REFERENCES

BELL, J. A., ROWE, W. P., ENGLER, J. P., PARROTT, R. H. & HUEBNER, R. J. (1955). Pharyngoconjunctival fever. Epidemiological studies of a recently recognized disease entity. *J. Amer. med. Ass.* **157**, 1083.

BRADSTREET, C. M. P. & TAYLOR, C. E. D. (1962). Technique of complement-fixation test applicable to the diagnosis of virus diseases. *Mon. Bull. Minist. Hlth Lab. Serv.* **21**, 96.

CHANOCK, R. & FINBERG, L. (1957). Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (C.C.A.). II. Epidemiologic aspects of infection in infants and young children. *Amer. J. Hyg.* **66**, 291.

CHANOCK, R. M. & JOHNSON, K. M. (1961). Infectious disease: respiratory viruses. *Amer. Rev. Med.* **12**, 1.

CHANOCK, R. M., PARROTT, R. H., VARGOSKO, A. J., KAPIKIAN, A. Z., KNIGHT, V. & JOHNSON, K. M. (1962). Acute respiratory diseases of viral aetiology. IV. Respiratory syncytial virus. *Amer. J. publ. Hlth*, **52**, 918.

CHANY, C., LÉPINE, P., LELONG, M., LE-TAN-VINH., SATGÉ, P. & VIRAT, J. (1958). Severe and fatal pneumonia in infants and young children associated with adenovirus infections. *Amer. J. Hyg.* **67**, 367.

CLARKE, S. K. R., CORNER, B. D., GAMBIER, D. M., MACRAE, J. & PEACOCK, D. B. (1964). Viruses associated with acute respiratory infections. *Brit. med. J.* i, 1536.

CRONE, P. B., HEYCOCK, J. B., NOBLE, T. C. & PATTON, J. B. (1964). Serological evidence of infection by respiratory syncytial virus in outbreak of acute bronchiolitis. *Brit. med. J.* i, 1539.

EVANS, D. G. & MAITLAND, H. B. (1939). Agglutination as a diagnostic test for whooping cough. *J. Path. Bact.* **48**, 468.

FARBER, S. & VAWTER, G. F. (1961). Clinico-pathological conference. The Children's Hospital Medical Center, Boston, Mass. *J. Pediat.* **58**, 876.

FORBES, J. A., BENNETT, N. MCK. & GRAY, N. J. (1961). Epidemic bronchiolitis caused by a respiratory syncytial virus: clinical aspects. *Med. J. Aust.* ii, 933.

GARDNER, P. S., STANFIELD, J. P., WRIGHT, A. E., COURT, S. D. M. & GREEN, C. A. (1960). Viruses, bacteria, and respiratory disease in children. *Brit. med. J.* i, 1077.

GARROW, D. H. & TAYLOR, C. E. D. (1962). An investigation of acute respiratory disease in children admitted to hospital in the South-West Metropolitan region. *Arch. Dis. Childh.* **37**, 392.

GOODPASTURE, E. D., AUERBACH, S. H., SWANSON, H. S. & COTTER, E. F. (1939). Virus pneumonia of infants secondary to epidemic infections. *Amer. J. Dis. Child.* **57**, 997.

HILLEMAN, M. R. (1963). Respiratory viruses and respiratory virus vaccines. *Amer. Rev. Resp. Dis.* **87**, 165.

HILLEMAN, M. R., HAMPARIAN, V. V., KETLER, A., REILLY, C. M., MCLELLAND, L., CORNFIELD, D. & STOKES, J. (1962). Acute respiratory illnesses among children and adults. Field study of contemporary importance of several viruses and appraisal of the literature. *J. Amer. med. Ass.* **180**, 445.

HOLLAND, W. W., TANNER, E. I., PEREIRA, M. S. & TAYLOR, C. E. D. (1960). A study of the aetiology of respiratory disease in a general hospital. *Brit. med. J.* i, 1917.

HUEBNER, R. J., ROWE, W. P. & CHANOCK, R. M. (1958). Newly recognised respiratory tract viruses. *Annu. Rev. Microbiol.* **12**, 49.

KJELLÉN, L., ZETTERBERG, B. & SVEDMYR, A. (1957). An epidemic among Swedish children caused by adenovirus type 3. *Acta paediat., Stockh.*, **46**, 561.

MASTERS, P. L., BRUMFITT, W., MENDEZ, R. L. & LIKAR, M. (1958). Bacterial flora of the upper respiratory tract in Paddington families, 1952–54. *Brit. med. J.* i, 1200.

MOFFET, H. L. & CRAMBLETT, H. G. (1962). Virus isolations and illnesses in young infants attending a well baby clinic. *New Engl. J. Med.* **267**, 1213.

MORRISON, B., BASS, D., DAVIS, J. A., HOBSON, D., MADSEN, T. I. & MASTERS, P. L. (1957). Acute lower respiratory infections in childhood. *Lancet*, ii, 1077.

OLSON, L. C., MILLER, G. & HANSHAW, J. B. (1964). Acute infectious lymphocytosis presenting as a pertussis-like illness: its association with adenovirus type 12. *Lancet*, i, 200.

PARROTT, R. H., VARGOSKO, A. J., KIM, H. W., BELL, J. A. & CHANOCK, R. M. (1962). Acute respiratory diseases of viral aetiology. III. Myxoviruses: Parainfluenza. *Amer. J. publ. Hlth*, **52**, 907.

RABE, E. F. (1948). Infectious croup: I. Aetiology. *Pediatrics, Springfield*, **2**, 255.

ROSEN, L., JOHNSON, J. H., HUEBNER, R. J. & BELL, J. (1958). Observation of a newly recovered echovirus and a description of an outbreak in a nursery. *Amer. J. Hyg.* **67**, 300.

RUBBO, S. D. & BENJAMIN, M. (1951). Some observations on survival of pathogenic bacteria on cotton wool swabs. Development of a new type of swab. *Brit. med. J.* i, 983.

SMITH, S. E. (1961). A maintenance medium for tissue culture virus studies. *Brit. J. exp. Path.* **42**, 232.

STERNER, G. & TUNEVALL, G. (1962). Acute respiratory illnesses in children. A combined bacteriological and virological study. *Acta paediat., Stockh.*, **51**, 349.

TOBIN, J. O'H. (1963). The isolation of respiratory viruses from children in hospital in south-east Lancashire. *Proc. R. Soc. Med.* **56**, 991.

WALTON, M. & MELNICK, J. L. (1953). Coxsackie virus antibody and incidence of minor illness during the summer. *Publ. Hlth Rep., Wash.*, **68**, 1167.