

Respiratory illness and viral infection in an Edinburgh nursery

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In recent years many outbreaks of respiratory syncytial virus infection have been reported and the associated clinical syndromes are now well documented (Chanock *et al.* 1962; Hilleman *et al.* 1962; Holzel *et al.* 1965). Similarly many accounts of respiratory illness resulting from infection with adenovirus (Bell *et al.* 1955; Chany *et al.* 1958; Hilleman *et al.* 1962), parainfluenza virus (Hilleman *et al.* 1962; Parrott, Vargosko, Kim & Chanock, 1963) and influenza virus (Balducci, Zaiman & Tyrrell, 1956; Holland, Tanner, Pereira & Taylor, 1960) have been published. However, few investigations on these or other respiratory virus infections have been carried out on static populations of normal subjects who were undergoing continuous clinical observation. In this country, Sutton (1962) reported outbreaks of influenza A, parainfluenza 3 and adenovirus infections occurring over a period of 8 months in a nursery containing forty-six children. In the U.S.A., Bell, Rowe & Rosen (1962) described the illnesses occurring in 587 children of the Washington Children's Village over a period of 3 years and described outbreaks of infection with adenoviruses, myxoviruses and coxsackieviruses. Kapikian *et al.* (1961) extensively documented an outbreak of respiratory syncytial virus infection in the Washington Children's Village among ninety children who were under continuous clinical observation.

The present survey was carried out in a community of resident nursery children in Edinburgh during the winter 1963-64 to investigate the prevalence and type of virus infection, as proved by serological means, and to relate this to the respiratory illnesses occurring at the time.

MATERIALS AND METHODS

The population at risk

Forty-nine children were resident in the nursery during the 6 months of the study. Forty-one of these children were included in the final analysis. The remaining eight were excluded either because they were admitted for less than 1 week and during that time remained isolated from the other children in the admission ward, or because they were admitted for less than 1 month and an insufficient number of specimens was obtained from them. The remaining 41 remained in the nursery for at least 11 weeks and 13 were resident in the nursery during the whole 26 weeks of the study. The average duration of the study per child was 18.4 weeks and the total period of observation was 764 child weeks. There were 14 admissions and

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14 discharges during the survey. The sex ratio of children was 25 males to 16 females but their total duration in the nursery was more diverse, i.e. 487 male child weeks to 277 female child weeks.

In the nursery the children were organized into three groups:

(1) *Babies*. Eight babies were included in the study, their ages ranging from 3 to 28 weeks with a mean age of 16.3 weeks. The babies were cared for in one room and had little or no contact with the other children.

(2) *Infants*. Seventeen infants were included in the study, their ages ranging from 7 to 18 months apart from one child aged 28 months who was mentally retarded. They had a mean age of 14.3 months. These children remained separate from the other groups for eating and sleeping but a few of the older children mixed with the toddler group during play.

(3) *Toddlers*. Sixteen toddlers were included. Their ages ranged from 20 to 44 months with a mean age of 32.6 months.

The staff circulated freely among all groups.

Methods of assessment of illness

The nursery was visited twice a week by the same observer (C.A.) and a report taken from one of the three senior members of the staff concerning any ill child. These children were examined. Similarly the progress of any ill child was followed by repeated clinical examinations and reports by the same member of the staff.

The following categories of respiratory illness were recognized:

(1) *Upper respiratory tract infection* (U.R.T.I.). A respiratory illness confined to the nasopharynx showing one or more of the following signs: mucoid nasal discharge, purulent nasal discharge, pharyngitis, cervical lymphadenopathy, middle ear infection, or systemic upset.

(2) *Bronchitis*. A respiratory illness running a benign course, characterized by cough usually associated with signs of lower respiratory tract infection (crepitations, rhonchi, audible wheeze or mild respiratory difficulty) and with systemic upset with or without pyrexia and signs of upper respiratory tract infection.

(3) *Bronchiolitis*. A respiratory illness occurring usually in babies, running a severe course and associated with severe respiratory distress. There were no cases of bronchiolitis during the survey.

(4) *Pneumonia*. A severe lower respiratory tract illness, requiring hospital admission and having at least some radiological evidence of pulmonary consolidation.

(5) *Laryngo-tracheo-bronchitis* (L.T.B.). A respiratory illness, clinically confined to the larynx, trachea and main bronchi characterized by persistent cough, inspiratory stridor, dyspnoea and pyrexia.

An episode of illness had to be present for 24 hr. or more to be included in the results. The last day before the return of the child to his normal health was considered to be the last day of illness. A period of at least 48 hr. free from signs and symptoms of respiratory disease was required to separate any two periods of illness. Some of the children showed a persistent nasal discharge during the whole survey. These children were only considered to have a new respiratory illness if

there was an increase or change in their symptoms. The child's age was recorded on the first day of the illness, to the nearest week in the case of babies and to the nearest month in the case of the infants and toddlers.

Collection of specimens

During the first fortnight of the survey pharyngeal swabs and blood specimens were obtained from each child. Thereafter routine pharyngeal swabs were taken every 4 weeks and routine blood specimens every 8 weeks. Pharyngeal swabs and blood specimens were taken from each child at the beginning of an illness (acute specimens) and again 2 weeks later (convalescent specimens). To minimize the trauma to the children acute and convalescent specimens of blood were not taken if routine blood collection had occurred (in the case of acute specimens) or was to occur (in the case of the convalescent specimens) within 2 weeks. This meant a sacrifice in the accuracy of timing of viral infections but it was felt there would be no loss in the number of antibody rises obtained.

Virological methods

Throat swabs were immediately immersed in 3 ml. of transport medium which consisted of medium 199 containing bovine serum albumin (1%), sodium bicarbonate (5% of a 4.4% solution), penicillin (250 units/ml.) and streptomycin (250 μ g./ml.). These specimens were transported to the laboratory in a cooled thermos flask with the minimum of delay and were kept at 4° C. until inoculation was carried out; in no case was more than 3 hr. allowed to elapse between collection and inoculation of the specimen. The specimens were inoculated into two cultures of HEp-2 cells maintained in 199 and 2% fowl serum and into two cultures of monkey kidney cells maintained in Earle's basic salt solution supplemented with 7.5% liver digest ultrafiltrate (Smith, 1961). The remainder of the specimen was then frozen quickly in alcohol at -70° C. and stored until human embryo lung cells were available. A strain of human embryo lung cells was maintained on Eagle's medium with 2% calf serum and low bicarbonate concentration, i.e. 1% of a 4.4% solution. All cultures were rolled at 34° C. and were examined every 2 days for 10 days; a further blind passage was then carried out on these cultures after freezing and thawing at -70° C. Haemadsorption tests using 0.4% human group 'O' erythrocytes were carried out on all monkey kidney cultures after 5 days on the second passage and again before discarding.

Sera were inactivated at 56° C. for 30 min. and stored at -20° C. They were tested for complement-fixing antibodies according to the technique described by Bradstreet & Taylor (1962) using the following antigens: influenza A, B and C, Sendai, parainfluenza 1, 2 and 3, adenovirus and respiratory syncytial virus. All complement-fixing reagents except the respiratory syncytial antigen were obtained from the Standards Laboratory for Serological Reagents, Central Public Health Laboratory, Colindale. The respiratory syncytial antigen was prepared in monkey kidney cells grown in serum-free medium. The seed virus was the Randall strain of virus previously passaged in HEp-2 cells. Sera were also tested for the presence of cold agglutinins to human group 'O' erythrocytes.

Neutralization tests were carried out on patient's serum against any virus isolated from that patient. The virus, diluted to contain 100 TCD₅₀/0.1 ml., was mixed with 0.25 ml. amounts of twofold dilutions of serum. The mixtures were allowed to stand for 1 hr. 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 cytopathic effect. 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, 14-16, 18-20, 22, 25, 26; adenovirus 1-11, 14-16, herpes simplex. Parainfluenza viruses were identified by haemadsorption inhibition tests.

RESULTS

Those children who showed a fourfold or greater rise in complement-fixing antibody were considered to have had a virus infection, whether the virus was isolated or not.

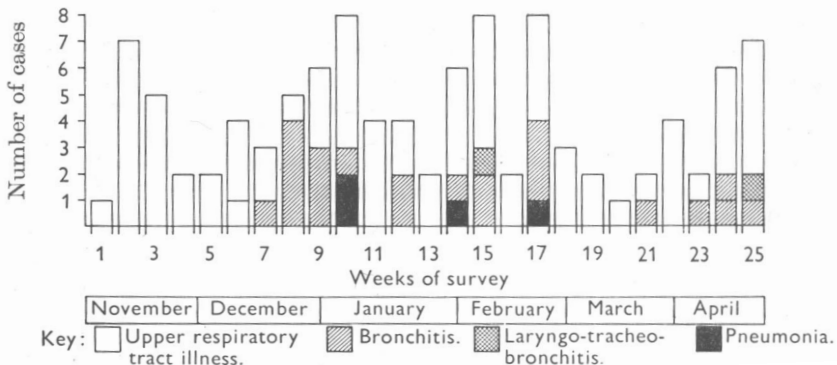


Fig. 1. The incidence of fresh cases of respiratory illness throughout the survey (November 1963 to April 1964).

During the 6 months of the survey there were 105 episodes of respiratory illness (2.6 per child) and of these 44 (42%) were associated with one or more virus infections. Evidence of virus infection was obtained on 67 occasions, 20 children showing no respiratory illness at the time and 3 having evidence of infection with two viruses concurrently. One hundred and seventy specimens of blood were obtained for serological investigation and 255 throat swabs were taken for virus isolation.

Figure 1 represents the number of fresh cases of respiratory illness occurring during each week of the survey and the incidence of upper respiratory tract infection, laryngo-tracheo-bronchitis, bronchitis and pneumonia. It can be seen that at no time was the nursery completely free from respiratory illness and that four major outbreaks were observed. These occurred in November, late December, February and April.

Table 1 tabulates the results of complement-fixation tests carried out on sera obtained from each child at entry into the survey. It will be seen that apart from

Table 1. Results of C.F. tests on sera taken at entry into the survey (number of cases with titre of eight or more)

(No children had titre of eight or more to influenza A, B, parainfluenza 1 or respiratory syncytial virus.)

	Influenza		Parainfluenza		Adeno
	C	Sendai	2	3	
Babies	0	0	0	0	3
Infants	2	1	4	0	15
Toddlers	0	2	2	2	13
Total	2	3	6	2	31

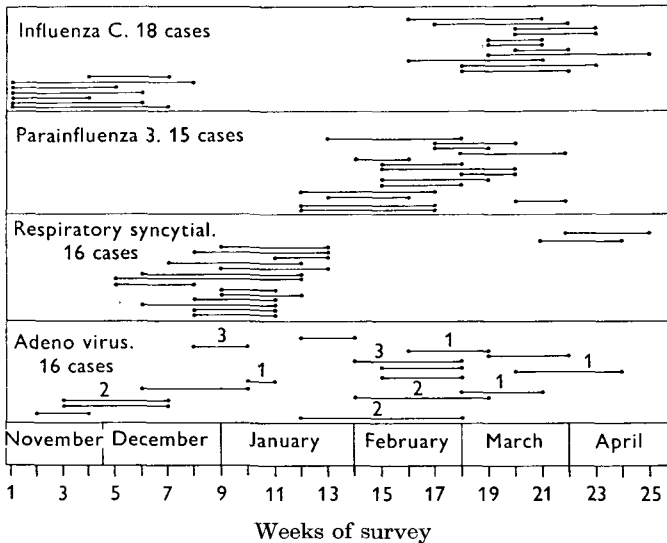


Fig. 2. The seasonal incidence of serologically proven virus infection throughout the survey (November 1963 to April 1964). ●—●, Period over which fourfold rise took place. Figures represent adenovirus types.

Table 2. The incidence of virus infection in each disease category

	No. of cases	No. of cases with virus infection	Cases with virus infection (%)
U.R.T.I.	76	28	37
L.T.B.	2	2	100
Bronchitis	23	13	56
Pneumonia	4	1	25
Total	105	44	42

adenovirus infection there was very little evidence of prior infection with any of the viruses tested.

Figure 2 presents the virus infections observed during the course of the 6 months. It will be seen that there were 16 cases of adenovirus infection, 16 cases of respira-

tory syncytial virus infection, 15 cases of parainfluenza 3 infection and 18 cases of influenza C infection; herpes simplex virus infection was encountered in only 2 cases. The adenovirus infections were distributed evenly throughout the time of the survey whereas the respiratory syncytial, parainfluenza 3 and influenza C infections occurred in well-defined outbreaks. The first three outbreaks of respiratory illness coincided with the outbreaks of infection with influenza C, respiratory syncytial, and parainfluenza 3 virus respectively and these were considered to be the aetiological agents responsible. The last outbreak of respiratory disease was not associated with any particular virus infection and occurred 1 month after the second influenza C outbreak. The total number of virus infections occurring in each disease category is shown in Table 2.

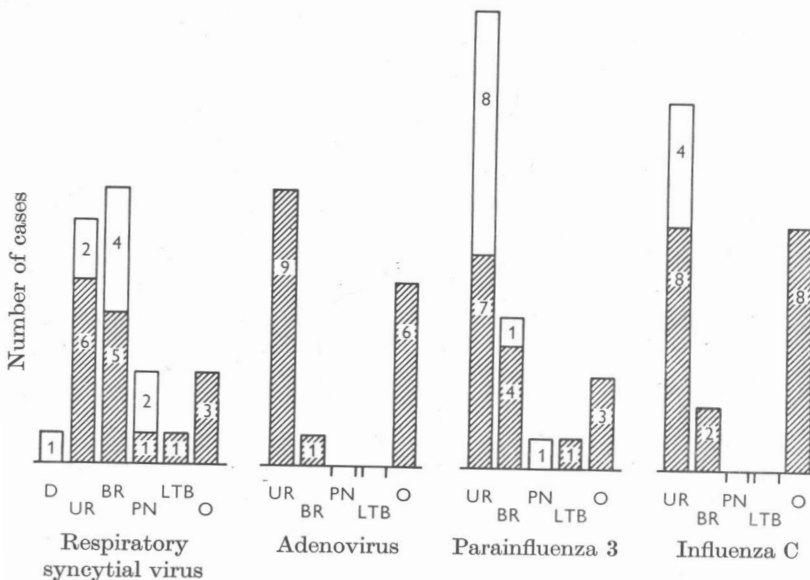


Fig. 3. The incidence and type of respiratory illness occurring in association with virus infection. ▨, Serological evidence of infection; □, clinical association only with virus outbreak. D, Died; UR, upper respiratory tract infection; BR, bronchitis; PN, pneumonia; LTB, laryngo-tracheo-bronchitis; O, no illness.

Influenza C outbreak

During the second week in November there was a sudden outbreak of mild upper respiratory disease amongst the infants and toddlers. Within 5 days 11 (55%) of the 20 children at risk in these two groups developed a profuse mucoid nasal discharge. Of the children with symptoms, 7 developed a fourfold rise in complement-fixing antibody to influenza C antigen. Influenza C infection was encountered again in March when a further 11 cases were recognized—5 toddlers, 4 infants and 2 babies. Of these, 9 were children who had been admitted since the previous outbreak and 2 were children who had shown neither serological nor clinical evidence of infection during the previous outbreak. In the second outbreak only 3 children had clinical evidence of infection: 1 toddler and 1 infant developed

bronchitis and 1 baby had an upper respiratory tract infection. The analysis of the serological response to influenza C infection is shown in Table 3. The frequency with which the different categories of illness occurred is shown in Fig. 3.

Influenza C was not isolated in tissue culture during the course of the survey.

Table 3. *Serological response to influenza C in the different age-groups*

	No. of sera tested	Total with serological evidence of infection	No. with serological and clinical evidence of infection	No. showing clinical illness only
First outbreak				
Babies	7	0	0	0
Infants	10	6	6	1
Toddlers	10	1	1	3
Second outbreak				
Babies	6	2	1	0
Infants	13	4	1	0
Toddlers	11	5	1	0

Respiratory syncytial virus outbreak

During the 3 weeks from 18. xii. 63 to 8. i. 64 twenty (69%) of the 29 children at risk became ill with respiratory tract illnesses and 14 (48%) of the children at risk developed fourfold antibody rises to respiratory syncytial virus antigen. Three of the children who developed antibodies remained free from clinical evidence of infection and eight of the children who were ill had no increase in their respiratory syncytial virus antibody (convalescent serum was not obtained from one child). The type of illness varied with the age of the child. Seven of the toddlers developed upper respiratory tract illnesses only and one developed bronchitis. Five of the infants developed bronchitis, one developed pneumonia (a child who was admitted during the outbreak) and one died. This fatal case was a 17-month-old female child with no previous history of respiratory illness. She developed a mucoid nasal discharge and became irritable on 19. xii. 63. Early on the morning of 20. xii. 63 she suddenly became dyspnoeic and cyanosed and died before the arrival of the nursery doctor. She was afebrile throughout her illness. A specimen of serum and a throat swab had been taken a few days before death but no other specimens were obtained. No evidence of virus infection was found on culture of the pharyngeal swab or from complement-fixation tests. Autopsy was not carried out.

Three of the babies developed severe bronchitis, two had pneumonia and two remained well. The serological response to infection with respiratory syncytial virus appeared to vary among the children according to age as can be seen from Table 4. It was considered that the illnesses among the babies were due to infection with respiratory syncytial virus despite the lack of serological proof as their illnesses were so closely related in time and clinically to the outbreak of respiratory syncytial virus infection among the older children.

Two children developed fourfold antibody rises to respiratory syncytial virus

during April. One, an infant who had had pneumonia during the previous outbreak but no serological evidence of infection, developed laryngo-tracheo-bronchitis in association with a fourfold rise in titre, and the other, a toddler who was admitted after the outbreak, developed a fourfold antibody rise to respiratory syncytial virus with an upper respiratory tract infection.

Table 4 shows the analysis of serological response to respiratory syncytial virus, and Fig. 3 an analysis of the types of diseases encountered.

Thirty-eight pharyngeal swabs were taken from the children during the outbreak. No respiratory syncytial virus was isolated in tissue culture but adenoviruses were isolated from three swabs (types 1, 3 and 5) and one unidentified cytopathic agent was isolated in human embryo lung cells.

Table 4. *Serological response to respiratory syncytial virus in different age-groups*

(Figures in parentheses represent the two sporadic cases.)

	No. of sera tested	Total with serological evidence of infection	No. with serological and clinical evidence of infection	No. showing clinical illness only
Babies	7	1	1	4
Infants	10 (+1)	5 (+1)	4 (+1)	3
Toddlers	11 (+1)	8 (+1)	6 (+1)	2

Table 5. *Serological response to parainfluenza 3 in different age-groups*

	No. of sera tested	Total with serological evidence of infection	No. with serological and clinical evidence of infection	No. showing clinical illness only
Babies	7	4	4	3
Infants	8	5	5	2
Toddlers	9	6	3	1

Parainfluenza 3 outbreak

Between 6. ii. 64 and 24. ii. 64 twenty-two (71%) of the children at risk became ill with respiratory symptoms. Fifteen children (48%) developed a fourfold rise to parainfluenza 3 over this period and parainfluenza 3 virus was isolated on two occasions. Three of the children who developed antibodies had no respiratory illness, six children who did not develop antibodies had respiratory illnesses at this time and three children had neither respiratory illnesses nor a rise in antibody. Seven children had no convalescent serum tested and of these four had respiratory illnesses. The serological response in the three age-groups was more uniform during this outbreak than in the respiratory syncytial virus outbreak as is shown in Table 5.

The types of illness encountered during this outbreak are shown in Fig. 3. It was

noticed that the severity of illness was greater among the babies. One baby developed bronchopneumonia, 1 laryngo-tracheo-bronchitis which required hospital admission, 3 bronchitis and 3 developed upper respiratory tract illnesses. Of the 23 infants and toddlers, 12 had upper respiratory tract illnesses and 2 had bronchitis.

Adenovirus infection

During the survey adenoviruses were isolated from throat swabs on 21 occasions (adenovirus type 1, 6 cases; type 2, 8 cases; type 3, 6 cases, type 5, 1 case). On 10 occasions the isolation was accompanied by a fourfold rise in neutralizing antibody titre to the virus isolated.

On a further six occasions a fourfold rise in complement-fixing antibody for adenovirus unaccompanied by virus isolation occurred and the type of adenovirus responsible was not ascertained. These sixteen proven infections are shown in Fig. 2 where it will be seen that both the typed and untyped infections occurred sporadically throughout the period of study.

The majority of adenovirus infections were associated with either upper respiratory tract infection (9 cases) or no clinical illness (6 cases). One case of bronchitis in association with adenovirus infection occurred.

A small outbreak of conjunctivitis occurred in the nursery during April, but adenovirus was not isolated from throat or conjunctival swabs from these children.

DISCUSSION

In this Edinburgh nursery two distinct patterns of infection emerged. The adenovirus infections were endemic in the nursery and occurred throughout the period of study. The respiratory syncytial virus, parainfluenza 3 virus and influenza C virus infections were epidemic in character, occurring in well demarcated outbreaks. This type of pattern is similar to that found by Sutton (1962) in his survey in which he found outbreaks of parainfluenza 3 virus infection and influenza A virus infection occurring against an endemic pattern of adenovirus infection. This difference in epidemiological pattern appears to be the result of previously acquired immunity as 31 (76%) of the 41 children showed serological evidence of previous adenovirus infection whereas only 2 children showed evidence of prior parainfluenza 3 infection, 2 showed evidence of influenza C infection and none showed evidence of respiratory syncytial virus infection. It was impossible to obtain sufficient sera from the children for neutralization tests against the prevalent adenovirus types and therefore we were unable to ascertain the type of adenovirus responsible for the previous infections.

No distinctive clinical picture for any of the infections was evident either on clinical examination at the time of infection or on retrospective analysis of the symptoms and clinical signs. It was apparent, however, that infection by respiratory syncytial virus and parainfluenza 3 virus resulted in a much more severe illness, particularly among the infants, than did influenza C virus or adenovirus infection. Respiratory syncytial virus is known to be causally related to epidemic bronchio-

litis in infants (Sandiford & Spencer, 1962) but in the present outbreak no cases of bronchiolitis were recognized.

The most severe cases of respiratory syncytial virus infection showed radiological evidence of bronchopneumonic consolidation and the others developed acute bronchitis, upper respiratory tract illness or showed no evidence of infection. Unlike parainfluenza virus 1 and 2 which are established causes of laryngo-tracheo-bronchitis in young children (Parrott *et al.* 1963), parainfluenza 3 has been found in association with a wide range of respiratory disease from severe pneumonia to mild upper respiratory tract infection (Chanock *et al.* 1963). This pattern was seen in the present outbreak where illness ranged from severe bronchitis in the younger age-groups to asymptomatic infection in the older children and included one case of laryngo-tracheo-bronchitis severe enough to require hospitalization.

It is surprising to find two clear-cut outbreaks of influenza C in our survey as evidence of infection with this virus is unusual in children. The apparent infrequency of influenza C infection in children may be due to the mild illness it produces which results in few cases being investigated virologically.

The most significant factor affecting the severity of the illness appears to be host resistance as reflected by the age of the child. No serious respiratory illness occurred in any child over the age of 9 months whereas seven of the infants, one on two occasions, required hospital admission for severe respiratory illness.

In a compact community such as the nursery where all the occupants were equally at risk it was surprising to find that during the respiratory syncytial virus outbreak only 48% of the children developed antibody. This is much lower than the rate of infection found by Kapikian *et al.* (1961) in the Washington Children's Village outbreak where 78% of the population at risk developed a fourfold rise in antibody titre. In the Washington Children's Village all the children were over 6 months of age and for a direct comparison it is necessary to correct our figures for age by excluding all children less than 6 months old. When this is done 66% of the children are found to have developed a fourfold rise in antibody titre.

Despite the lack of serological evidence among the babies it is highly probable that they were infected by respiratory syncytial virus at this time as five out of seven of them developed severe respiratory illness concurrently with the serologically proven respiratory syncytial virus outbreak among the older children. Thus it is apparent that the antibody response to respiratory syncytial virus infection among the babies was much poorer than in the older children. This difference may be expressed as fourfold rises in antibody titre to respiratory syncytial virus per child: babies (< 7 months), 0.14 fourfold responses per child; older children (7 months and over), 0.63 fourfold responses per child.

This is in contrast with the antibody response during the parainfluenza 3 outbreak where the following fourfold antibody responses to parainfluenza 3 virus occurred: babies (< 7 months), 0.57 fourfold responses per child; older children (7 months and over), 0.64 fourfold responses per child.

These findings suggest that the poor antibody response to respiratory syncytial

virus in the infants was not due to the inability of the infants to produce complement-fixing antibody to virus infection but rather to a failure to produce antibody to respiratory syncytial virus in particular. This apparently inadequate antibody response in infants to infection with respiratory syncytial virus may be of significance in the pathogenesis of the very severe respiratory illnesses characteristic of infection with this virus in infants (Crone, Heycock, Noble & Patton, 1964).

The high incidence of respiratory infections in children's homes is a major problem. The greatest danger is to children of less than 7 months and it is important therefore to take special measures to protect communities of infants from the introduction and spread of viral pathogens.

SUMMARY

Forty-one children in an Edinburgh nursery were observed for evidence of respiratory illness from November 1963 to April 1964. During this period serial specimens of serum from these children were examined for virus antibodies and serial throat swabs were investigated for the presence of virus. Well-defined outbreaks of respiratory illness occurred and could be associated with respiratory syncytial virus, parainfluenza 3 virus and influenza C virus infection. Infection with adenovirus followed a more endemic pattern. The antibody response in infants to respiratory syncytial virus as measured by the standard complement-fixation test was found to be much poorer than the response to parainfluenza 3 virus. It is suggested that this may play a part in the severity of respiratory syncytial virus infection in infants.

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