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A comparative virological study of children in hospital with respiratory and diarrhoeal illnesses

By E. J. STOTT*, E. J. BELL, M. B. EADIE, C. A. C. ROSS and N. R. GRIST

Regional Virus Laboratory and University Department of Infectious Diseases, Ruchill Hospital, Glasgow, N.W.

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INTRODUCTION

Of the many viruses isolated from man in recent years, some show a clear-cut association with specific human disease, others with a range of illnesses, but many show little or no association with any deviation from health. The interpretation of the significance of virus infections diagnosed by laboratory tests can therefore be very difficult, particularly in the case of the increasing number of viruses detected in faecal and respiratory specimens by tissue-culture techniques.

One approach to this problem is the comparison of virus infections in groups of persons suffering from different clinical illnesses but otherwise comparable. This paper describes an investigation of this type. Virological studies of respiratory illness in children admitted to Ruchill Hospital, Glasgow (Ross, Stott, McMichael & Crowther, 1964) were extended to allow comparison with a group of children matched for age and time of admission to the same hospital but suffering from diarrhoeal illness. The results reported here are based on examinations of 113 children in each of the two groups admitted to hospital between October 1963 and April 1965.

MATERIALS AND METHODS

Selection of cases

Cases were selected on certain days of the week from those admitted to hospital within the previous 24 hr. Children suffering from respiratory illness were included only if they were under 10 years of age and could be matched for age (within a month if under 3 months old, within 6 months if aged 3–12 months, within $2\frac{1}{2}$ years if over 1 year) and time of admission (within 1 week) with children with mainly diarrhoeal symptoms.

Collection of specimens

After selection, the children were immediately visited by one of us (M.B.E.) who collected from each a nose swab (a cotton-wool pledget packed into the nose for several minutes) and a throat swab. These were placed in separate bijou bottles each containing 2 ml. of transport medium consisting of Hanks's balanced salt solution (BSS) with 1 % bovine plasma albumen and 0·088 % sodium bicarbonate, 100 units penicillin/ml., 100 μg. streptomycin/ml. and 50 units nystatin/ml. After

* Present address: Common Cold Research Unit, Harvard Hospital, Salisbury, Wilts.

April 1964 nose and throat swabs were placed in the same bottle of transport medium and tested as one specimen. A request was left in the ward for faecal specimens to be collected as soon as possible; acute and convalescent blood sera were requested only from the respiratory cases.

Treatment of specimens

Nose and throat swabs were squeezed with forceps to express excess fluid into the transport medium which was centrifuged at 5000 r.p.m. Of the supernatant fluid 0·2 ml. was inoculated into each of two tubes of each of the following tissue cultures: 'Bristol' line of HeLa cells (BH), primary rhesus monkey kidney cells (RMK) and semi-continuous human embryo kidney fibroblasts (HEKF). The deposit was also inoculated into one tube of each of these cultures and, in alternate pairs of cases, additional antibiotics (100 units penicillin, 100 μg. streptomycin, and 50 units nystatin) were added to each tube containing the deposits. After April 1964 centrifugation was omitted and the additional antibiotics added to all inoculated tissue cultures. Nose and throat swabs were always inoculated within 2 hr. of collection.

Faeces were shaken with phosphate buffered saline (PBS) containing 250 units penicillin/ml. and 250 μg. streptomycin/ml. to form a 20 % suspension which was centrifuged at 3000 r.p.m. Of the supernatant fluid 0·2 ml. was inoculated into two tubes each of primary human amnion (AMN) and RMK. Faecal extracts were inoculated immediately unless tissue was unavailable in which case they were stored at -40° C.

Blood sera were stored at -20° C.

Tissue cultures

For nose and throat swabs

Primary RMK cells (Duncan, 1961) were grown in Eagle's minimum essential medium (MEM) supplemented with 5 % inactivated calf serum, 0·044 % sodium bicarbonate, 100 units/ml. penicillin, and 100 μg./ml. streptomycin. Before inoculation cultures were washed three times in PBS and changed to Parker's 199 medium containing 0·18 % sodium bicarbonate, similar antibiotics but no serum. After inoculation these cultures were rolled at 33° C. and observed for cytopathic effects (CPE) three times weekly and tested for haemadsorption with human group 'O' erythrocytes once weekly for 3 weeks.

The HEKF cultures used in this study were a semi-continuous cell strain derived in this laboratory from a 10-week-old male foetus by methods similar to those described by Hayflick & Moorhead (1961). The cells were grown in Eagle's MEM containing 5 % inactivated precolostral calf serum or 10 % unheated calf serum, 0·088 % sodium bicarbonate, 100 units penicillin/ml., 100 μg. streptomycin/ml. For virus isolation cultures were maintained in Eagle's MEM with 1 % inactivated precolostral serum, 0·044 % sodium bicarbonate, 5 % tryptose phosphate broth and the usual antibiotics. After inoculation these cultures were rolled at 33° C., examined for CPE and the medium changed three times per week for 2 weeks.

The BH cells were obtained from the Common Cold Research Unit, Salisbury, and were grown in Hanks's BSS with 0·5 % lactalbumen hydrolysate, 5 % inactivated rabbit serum, 0·088 % sodium bicarbonate and the usual antibiotics. Before inoculation the cultures were changed to a similar medium containing 2 % inactivated fowl or rabbit serum instead of 5 % rabbit serum and 0·13 % sodium bicarbonate. After inoculation these cultures were held stationary at 36° C. and examined and the medium changed three to four times weekly for 3 weeks, when they were stained with neutral red (1/80,000) and discarded if no syncytia were apparent.

For faecal extracts

Primary RMK cells were grown in Hanks's BSS with 0·25 % lactalbumen hydrolysate, 10 % calf serum, 10 % human serum, 0·044 % sodium bicarbonate and the usual antibiotics. For the growth of AMN cells a similar medium was used, containing 20 % human serum but no calf serum (Duncan & Bell, 1961). Before attempted virus isolation both types of cultures were washed once in PBS and changed to Eagle's MEM containing 1 % calf serum, 0·176 % sodium bicarbonate and antibiotics. After inoculation the tubes were held in a stationary sloped position at 36° C. and observed daily for CPE for 2 weeks.

Serological methods

Complement-fixation tests

All paired acute and convalescent sera were tested for complement-fixing (CF) antibodies against respiratory syncytial (RS) virus, the adenovirus group and parainfluenza type 1 virus by the methods described by Grist, Ross, Bell & Stott (1966); some paired sera were also tested with herpes simplex antigen. The antibody titre of each serum was taken as the reciprocal of the highest dilution of serum showing complete or almost complete fixation of complement. A titre was considered as rising if there was a fourfold or greater increase in titre or an increase from < 8 to 16 between the 'acute' and 'convalescent' serum.

Neutralization tests

Paired sera of children from whom RS virus was isolated were tested for neutralizing antibodies by the methods of Grist *et al.* (1966). The Randall strain of RS virus was diluted in PBS containing 25 % unheated rabbit serum unless otherwise stated. The serum-virus mixtures were inoculated into BH cultures; after 3 days these were changed into fresh maintenance medium containing 1/80,000 neutral red, and read the following day when the virus titration indicated that 32–320 TCD 50 was present.

Paired sera from children from whom rhinoviruses were isolated were titrated for neutralizing antibodies against the homologous rhinovirus. The tests were performed in HEKF and read the day after the virus titration indicated that 10–32 TCD 50 had been used.

The antibody titre was taken as the reciprocal of the highest dilution in the serum-virus mixture which completely neutralized the virus.

Identification of viruses

RS virus, parainfluenza viruses 1–3, herpes simplex virus and adenoviruses 1–7 were initially grouped by their characteristic CPE and were finally identified by neutralization tests with type specific antisera.

Enterovirus strains were identified by neutralization tests using antisera to poliovirus types 1–3, coxsackievirus types A 9 and B 1–6, and echovirus types 1–25. The 'pooled-serum' technique of Lim & Benyesh-Melnick (1960) was used for the preliminary identification of the strains. Final confirmation of their identity was made with type specific antisera.

Viruses which produced enterovirus-like CPE most rapidly in RMK or HEKF when the cultures were rolled at 33° C. in a low bicarbonate medium were provisionally classified as rhinoviruses: M types if they produced CPE in both RMK and HEKF, H types if they grew only in HEKF. Such viruses were then tested for chloroform stability (Feldman & Wang, 1961) and acid lability (Tyrrell & Chanock, 1963). Viruses found to be chloroform-stable and acid-labile were considered to be rhinoviruses.

Identification of rhinoviruses was attempted by neutralization tests using antisera against the following 27 types: echovirus 28, Salisbury strains B 632, HGP, FEB, Thomson, Norman, 16/60 (Taylor-Robinson & Tyrrell, 1962) NIH strains 353, 1059, 1734, 11757, 363, 1200, 33342 (Johnson & Rosen, 1963 and Webb, Johnson & Mufson, 1964) Chicago strains 106-F, 140-F, 179-E, 127-1, 137-3, 164 A (Connelly & Hamre, 1964 and Hamre, Connelly & Procknow, 1964) West Point strains 1, 68, 181, 204, 5986, MRH (Ketler, Hamparian & Hilleman, 1962) Baylor type 3 (Phillips, Melnick & Grim, 1965). The antisera were diluted to contain approximately 20 antibody units per 0·1 ml.

RESULTS

Population studied

The 113 children in the respiratory group represented 13 % of the children under 10 years of age admitted to this hospital with respiratory illnesses during the 19-month period. They comprised 64 males and 49 females aged from 3 weeks to 6 years with an average of 17·8 months. This was similar to the diarrhoea group which comprised 61 males and 52 females aged from 1 week to 4½ years with an average of 17·4 months (Table 1).

Three of the children in the respiratory group also had mild enteric symptoms on admission. Eight of the children in the diarrhoea group had some upper respiratory symptoms.

Virus isolation

The viruses isolated from the throat and nose are shown in Table 2. 64 viruses were isolated from 59 (52 %) of 113 children in the respiratory group and 31 viruses from 29 (26 %) of 113 children in the diarrhoea group. Faeces were available from 106 of the respiratory group and 110 of the diarrhoea group (Table 3) and enteroviruses were isolated from similar numbers in both groups (16 % and 19 %

respectively). The same virus was isolated from the throat and nose swabs and faeces in two respiratory cases (coxsackievirus B4 and echovirus 9) and in three diarrhoeal cases (adenovirus 3, echovirus 8 and an untyped enterovirus).

Table 1. *Population studied*

Group		Total cases	Age in years		
			under 1	1–2	2–6
Respiratory:	Male	64	27	15	22
	Female	49	23	11	15
Diarrhoeal:	Male	61	24	16	21
	Female	52	22	14	16

Table 2. *Viruses isolated from throat and nose swabs*

ype of case	No. of cases	RS	PF		HS	RH		AD					Polio	Coxsackie		Echo		UT	Total
			1	3		M	H	1	2	3	5	7	2	B2	B4	8	9		
iratory	113	26	2	7	12	1	9	2	2	1	0	0	0	0	1	0	1	0	64*
:hoeal	113	1	0	2	4	1	9	2	1	1	3	1	1	2	0	1	1	1	31†

Dual isolation in five cases: RS + herpes, Ad 1 + herpes, Rh M + herpes, Pf 3 + Ad 2, RS + Ad 1.
Dual isolation in two cases: Rh H + Ad 5, Pf 3 + RS.
' = parainfluenza, RH = rhinovirus, HS = herpes simplex, AD = adenovirus, UT = untyped ·ovirus.

Table 3. *Viruses isolated from faeces*

Type of case	No. of cases	AD		Polio	Coxsackie			Echo								UT	Total
		1	3	3	A9	B3	B4	3	4	7	8	9	11	12	14		
Respiratory	106*	1	0	0	1	1	2	0	2	2	2	1	0	2	1	2	17
Diarrhoeal	110*	0	1	1	1	0	0	5	1	1	3	0	3	0	2	3	21

* Faeces unavailable from seven children in respiratory group and three in diarrhoea group.

Table 4. *Viruses isolated from throat and nose swabs of* 113 *children with respiratory disease according to day of illness*

	Day of illness			
	1–3	4–7	>7	Unknown
Cases tested ...	60	38	10	5
Viruses isolated ...	30 (50%)	27 (71%)	4	3
RS virus	12	11	2	1
Parainfluenza	4	3	1	1
Rhinoviruses	4	5	1	0
Herpes simplex	7	4	0	1
Adenoviruses	2	3	0	0
Enteroviruses	1	1	0	0

Analysis of the virus isolations from the throat and nose of children with respiratory disease in relation to the number of days between the onset of symptoms and the collection of swabs (Table 4) shows that viruses were isolated more

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often from children who had been ill from 4 to 7 days than from those who had only been ill 3 days or less. During the first 7 months of the study different viruses were never isolated from the throat and nose swabs of the same patient. Virus was isolated from the nose alone in three cases, from the throat alone in 16 cases, and from both the throat and nose in 23 cases. We also found between October 1963 and April 1964 that the addition of extra antibiotics (100 units penicillin, 100 μg. streptomycin and 50 units nystatin) to each tissue culture tube inoculated with throat and nose swabs suppressed bacterial and fungal contamination as effectively as centrifugation. The examination of the supernatant and deposit after centrifugation yielded similar results except for five specimens where the supernatant was negative but the deposit yielded RS virus.

Serological tests by complement-fixation

Paired sera were received from 69 of the 113 respiratory cases. A rise in CF antibodies to RS virus was detected in 11 (16%) from eight of which RS virus was isolated; high ($\geqslant 128$) but not rising titres were found in seven of the remaining cases but only one of these yielded RS virus. Rising titres to the adenovirus group were found in four cases (two positive for adenovirus by isolation) and to parainfluenza type 1 in three cases (one yielded parainfluenza type 3); high ($\geqslant 64$) titres to adenovirus group were found in six (one positive for adenovirus by isolation) and to parainfluenza type one in two cases. Thus, infections diagnosed only serologically comprise three RS virus, two adenovirus and two parainfluenza virus.

Table 5. *Virus infections of 226 children according to illness*

Illness	Cases	Infections diagnosed	RS	PF	RH	HS	AD	ENT
Respiratory group								
Pneumonia and bronchopneumonia	47	41 (5)	13	6	4	6	6	6
Bronchiolitis and bronchitis	51	34 (3)*	15	3	3	4	1	8
Croup	3	2 (1)	0	0	0	1	0	1
U.R.T.I.	12	9 (1)	1	2	3	1	1	1
Diarrhoea group								
Bacterial diarrhoea	55	24 (5)	1	1	6	2	3	11
Non-specific diarrhoea	58	25	0	1	4	2	5	13
Total	226	135 (15)*	30	13	20	16	16	40

Figures in brackets are cases with dual infections.
* Includes one case with triple infection.
ENT = enterovirus, other abbreviations as in Table 2.

Type of illness

The relationship between various disease syndromes and total virus infections is shown in Table 5. RS virus infections were mainly found in cases of bronchopneumonia and bronchitis but illnesses associated with parainfluenza viruses were more varied. Rhinoviruses were associated with lower respiratory tract disease in seven cases but were isolated with the same frequency from both respiratory

and diarrhoeal illnesses. Adenovirus and enterovirus infections were found almost as often in children with respiratory illnesses as in those with diarrhoea. Similar numbers and types of virus infections were found in cases with either bacterial or non-bacterial diarrhoea.

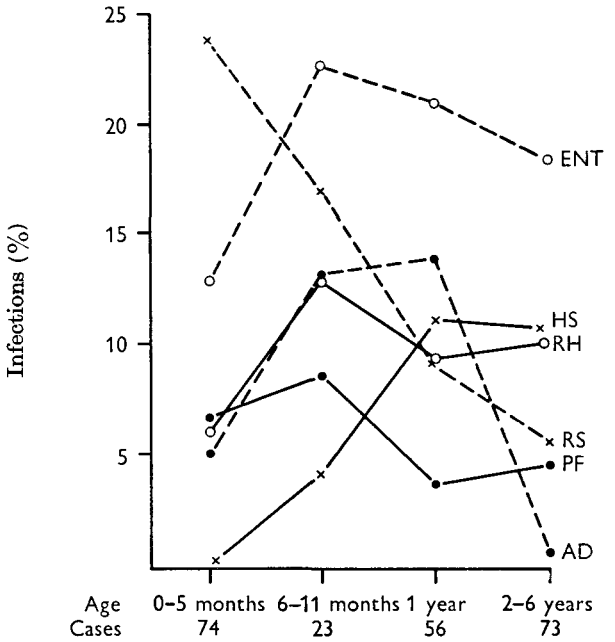

Fig. 1. Distribution of percentage infections with various viruses according to age.

Age of patients

The age distribution of the 135 virus infections in shown in Fig. 1. Most of the RS virus and parainfluenza virus infections were found in children under 1 year of age. Herpes simplex infections were most common after the first year of life and adenovirus infections between 6 months and 2 years. Infections with enteroviruses and rhinoviruses were fairly evenly distributed throughout the age range studied.

Monthly distribution

The RS virus infections, with one exception, occurred during two separate 5-month periods which correlated well with the peak months for admissions of children with respiratory disease (Fig. 2). Infections with all the other viruses studied occurred sporadically throughout the year.

Respiratory syncytial virus infections

RS virus was isolated from 26 respiratory cases but from only one diarrhoeal case (which was also infected with parainfluenza type 3 virus and developed a severe cough with upper and lower respiratory tract signs 3 days later), giving a highly significant difference ($\chi^2 = 25{\cdot}8, P < 0{\cdot}001$); this indicates that RS virus is closely associated with respiratory illness (Table 2). In addition, rising CF antibody

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titres for RS virus were found in three cases from which no virus was isolated. If these are included as RS virus infections 29 (26 %) of the 113 respiratory illnesses were associated with RS virus and 25 (86 %) of them were under two years of age.

Two or more blood sera were received from 14 of the cases from which RS virus was isolated; the titres of CF and neutralizing antibodies are shown in Table 6. The CF test detected a rising titre to RS virus in eight cases and high titres

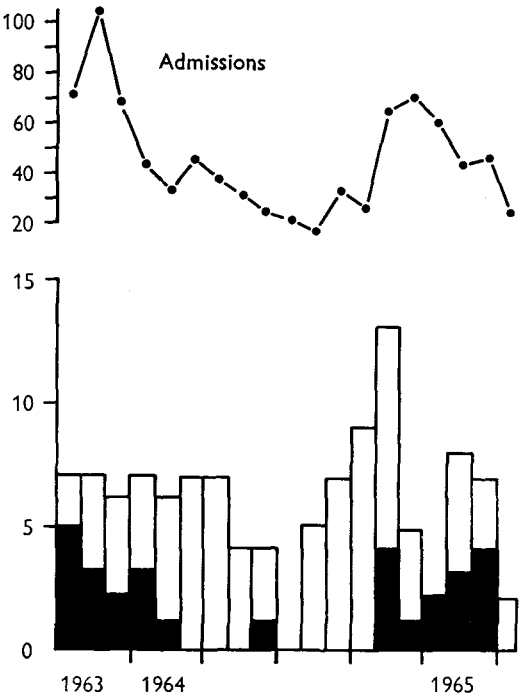

Fig. 2. Monthly distribution of children admitted to hospital with respiratory illness, cases studied and RS virus infections □, Cases studied; ■, RS virus infections.

(128;256) in one case. These children were at least 4 months old, in contrast to the five cases under 4 months in which no CF antibodies were detected. Of eight patients with rising CF titres, seven were also tested for neutralizing antibodies; they all showed titre rises provided unheated rabbit serum was present in the serum-virus mixtures. The absence of unheated serum or its inactivation at 56° C. for 30 min. reduced neutralizing titres two- to eightfold and prevented the detection of rising titres in four of six cases tested. Neutralizing activity without change in titre was obtained with sera from two 2-month-old babies; no antibodies were found by the CF test. The titres of neutralizing antibodies to two strains of RS virus, one isolated during each of the outbreaks, were similar to those obtained with the Randall strain (even in the two cases from whom these strains were isolated).

Parainfluezna virus infections

Parainfluenza viruses were isolated from nine children in the respiratory group (two type 1 and seven type 3) and two (type 3) in the diarrhoeal group (Table 2)

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showing a significant difference between the two groups ($\chi^2 = 4{\cdot}68$, $P < 0{\cdot}05$). Two additional children in the respiratory group also had a titre rise with parainfluenza type 1 antigen in the CF test, but as there are frequent cross-reactions between the parainfluenza viruses and mumps virus, these antibody responses cannot be considered specific.

Table 6. *Serological results in* 14 *children from whom RS virus was isolated*

			Neutralization titres			
			Randall strain		Epidemic strain*	
Age (months)	Day of illness	CF titres	With rabbit serum	Without rabbit serum	With rabbit serum	Without rabbit serum
1	1	ND†	‡			
	9	<8	—	—	—	—
2	5	ND	32			
	38	<8	32	—	—	—
2	6	ND	32	8	32	<8
	16	<8	32	8	32	<8
3	4	ND				
	11	<8	—	—	—	—
3·5	3	ND				
	18	<8	—	—	—	—
4	3	<8				
	25	16	—	—	—	—
4	3	8	16	8	**16**	**8**
	11	16	64	8	**64**	**16**
	30	128	128	—	**128**	—
5	10	128	<16	<16	<8	<8
	41	256	64	16	128	<16
5	5	<8	<8	—	—	—
	14	16	64			
7	5	<8	8	<8	<8	<8
	12	512	⩾256	128	⩾256	64
7	1	<8	<8	<8	<8	<8
	12	16	32	<16	32	<16
11	3	<8	<8			
	10	128	⩾256	—	—	—
11	4	<8	<8	<8	**<8**	**<8**
	16	16	64	8	**64**	**<8**
13	6	<8	<8	<8	<8	<8
	13	128	64	16	64	16

Figures in bold type are titres of sera from patients from whom strains 121–64 and 143–65 were isolated.

* For 1963–4 winter epidemic strain was 121–64 and for 1964–5 winter, strain 143–65.

† ND = not done.

‡ = insufficient.

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Rhinovirus infections

Rhinoviruses were isolated from 10 cases of respiratory illness and 10 cases of diarrhoeal illness (Table 2). Of the latter, five had no record of respiratory illness before or after entry into hospital, one had a cold 6 days before entering hospital but no symptoms on admission, three had a cough on admission and one developed a cold the day after entering hospital. None of the rhinoviruses isolated from diarrhoeal illnesses was neutralized by the 27 antisera used.

Table 7. *Details of* 10 *respiratory cases with rhinovirus infection*

Age (months)	Sex	Illness	Type and serotype	Day of illness	Neutralization titre to rhinovirus
1·5	M	U.R.I.	H, UK*	—	—
3	M	Pneumonia	H, UK	—	—
4	F	Bronchitis	H, UK	10	16
				24	128
7	M	U.R.I.	H, 181	—	—
11†	F	Bronchitis	M, B632	9	<8
				18	128
12	M	Bronchitis	H, UK	8	
				17	—
21	M	Bronchopneumonia	H, UK	2	<2
				10	2
24	M	Pneumonia	H, UK	—	—
34‡	F	U.R.I.	H, UK	2	<8
				9	<8
36	M	Pneumonia	H, 5986	1	<8
				23	32

* UK = serotype unknown.

† Herpes simplex virus also isolated from throat and nose and echovirus 7 from faeces of this case.

‡ CF antibody titres to RS virus rose from <16 to 64.

Details of the 10 respiratory cases yielding rhinoviruses are shown in Table 7. In seven the lower respiratory tract was involved. Apart from one case of upper respiratory infection which showed a greater than fourfold rise in CF antibodies against RS virus, no evidence of simultaneous infection with RS virus, adenovirus or parainfluenza type 1 virus was found in the six cases with paired sera. Three rhinoviruses were neutralized by specific antisera suggesting that they were serologically identical with strains B632, 181 and 5986 respectively. A fourfold or greater titre rise in antibody to the patient's own virus was detected in three cases; in two cases where the second serum was collected less than 2 weeks after the onset of illness, a low level of antibody was found in one and no antibody in the other.

Herpes simplex virus infections

Herpes simplex virus was isolated significantly more often from the respiratory group than from the diarrhoeal group (Table 2). However, paired sera from five of the infected children showed stationary levels of CF antibody against herpes virus.

Adenovirus infections

Adenovirus types 1, 2 or 3 were isolated from the throats of five children and faeces of another in the respiratory group and the throats of four in the diarrhoea group, one of whom had the same virus (type 3) in the faeces (Tables 2 and 3). Three children in the diarrhoeal group had adenovirus type 5 in the throat and one had type 7. There were an additional two children in the respiratory group who had fourfold or greater CF titre rises against adenovirus. Thus, there were 8 adenovirus infections in each of the two groups (Table 5). Paired sera collected from five of the six children in the respiratory group from whom virus was isolated showed a fourfold or greater rise in CF titres for adenovirus in only two.

Table 8. *Multiple infections*

Illness	Throat/nose swab	Faeces	Serological
Tonsillitis	RH H	—	RS
Croup	HS	Echo 4	—
Bronchitis	—	—	AD + PF
	HS + RS	—	—
	HS + RH M	Echo 7	—
Bronchopneumonia	HS + AD 1	—	—
	AD 1 + RS	—	—
	AD 2 + PF 3	—	—
	PF 3	AD 1	—
	RS	Cox. B 4	—
Dysentery	AD 1	UT	—
	AD 5 + RH H	—	—
	RH H	Cox. A 9	—
	RH H	UT	—
Gastroenteritis	RS + PF 3	—	—

Enterovirus infections

There were 16 enterovirus infections in the respiratory group and 24 in the diarrhoeal group (Table 5). In the respiratory group coxsackieviruses were isolated from four cases and echoviruses or untyped viruses from 12; both respiratory and faecal specimens of two cases yielded the same virus (coxsackievirus B4 and echovirus 9 respectively). The 24 enterovirus positive cases in the diarrhoeal group comprised two polioviruses, three coxsackieviruses, 16 echoviruses and three untyped enteroviruses. Throat and nose and faecal specimens both contained virus in one echovirus 8 case and one case with an untyped virus (Tables 2 and 3).

Multiple infections

Multiple infections (Table 8) were found in 10 of the children with respirator illness and five of those with diarrhoeal illness. Herpes simplex was one of th infecting agents in four of the 10 respiratory cases and adenovirus was found wit another viral infection in five respiratory cases and two diarrhoeal cases.

DISCUSSION

The strict criteria for matching children with respiratory illness with thos suffering from diarrhoeal illness for age and time of entry into hospital limited th number of cases available for study. Nevertheless, immediate inoculation withou prior freezing of throat and nose specimens into three types of tissue culture enabled us to isolate a large number of viruses. Collection of these specimen within 24 hr. of admission allowed little opportunity for hospital cross-infectio which is thought to occur with RS virus (Chanock *et al.* 1961). Thus, despite th small numbers studied certain conclusions about the epidemiology of respiratory viruses can be made.

Our findings that of the children with respiratory disease 26 % had evidence of infection with RS virus and 10 % with parainfluenza viruses and that these viruses were isolated more often from children with respiratory disease than from those with diarrhoeal disease agree broadly with the larger studies of others (Holzel *et al.* 1965; Clarke *et al.* 1964; Chanock & Parrott, 1965).

The isolation of rhinoviruses from 7 % of children with lower respiratory illness is a slightly higher rate than the 3·1 % found by Bloom, Forsyth, Johnson & Chanock (1963) and the 4·2 % found by Portnoy, Eckert & Salvatore (1965), in similar groups. This may be partly due to the use of HEKF which revealed 30 % more rhinoviruses when used in parallel with WI 38 cells (unpublished data). The rhinovirus isolation rate of 8·8 % in the diarrhoeal group is also higher than that found in the control group of other studies, but this may be partly due to the inclusion in our diarrhoeal group of some cases with mild respiratory symptoms. Hamparian, Leagus, Hilleman & Stokes (1964) reported the isolation of rhinoviruses from 14 children with lower respiratory disease but did not show a causal relationship although an earlier report from this group (Reilly *et al.* 1962) stated that cases selected for rhinovirus study were among those whose paired sera failed to give evidence of infection with influenza, parainfluenza, reovirus (group), adenovirus (group), and RS virus. Portnoy *et al.* (1965) found serological evidence of simultaneous infection with RS virus or parainfluenza type 3 virus or both in five of 13 (38 %) children with lower respiratory disease associated with rhinovirus infection. Serological tests on six of our cases with rhinovirus infection revealed only one with evidence of infection with another (RS) virus. Thus, we did not obtain convincing evidence that rhinoviruses caused lower respiratory disease in this group of children although the ability of these viruses to infect the lower respiratory tract was shown in adult volunteers by Cate *et al.* (1965) and also suggested by our own study of adults with chronic bronchitis (Eadie, Stott & Grist, 1966).

Although herpes simplex virus was isolated significantly more often from the children with respiratory illness than from those with diarrhoeal illness, the stationary antibody levels in the five respiratory cases tested suggest that these were not primary infections with herpes virus, but reactivated latent infections secondary to, not a cause of, the respiratory illness.

Neither adenovirus nor enterovirus infections showed any significant difference between the two groups. This may be due either to low pathogenicity of these viruses or to their ability to cause both respiratory and diarrhoeal symptoms. The infection rates (Table 5) for enteroviruses of 21% for children with diarrhoeal illness and 14% for children with respiratory illness are closely similar to those found by Sommerville (1958) in a previous study in this hospital and do not suggest that enteroviruses were significant causes of diarrhoeal illness during our study.

In a previous study (Ross *et al.* 1964) development of CF antibodies to RS virus was found to be slow in some children, the highest titre not being attained until between the fourth and sixth week of illness. In the present study many of the convalescent sera were collected in the second to third week of illness before the children left hospital; this may explain our failure to detect a rise in CF antibody to RS virus in five children from whom RS virus was isolated. However, as all these five were under 4 months of age this might support the finding of previous workers (Beem *et al.* 1960; Chanock *et al.* 1961; Gardner, Elderkin & Wall, 1964) that a CF antibody response to RS virus infection is rarely detected during the first few months of life. In our earlier study differences in neutralizing antibody titres had been detected by the use of a strain of RS virus isolated locally but we could not repeat this finding with strains isolated during the present study. This discrepancy could be explained by differences of 'avidity' or antigenic variation between the three local strains used; work is in progress to elucidate this point for although antigenic differences have been found between RS virus strains these have not been detectable in neutralization tests with human sera (Coates, Kendrick & Chanock, 1963; Doggett & Taylor-Robinson, 1965). The value of unheated rabbit serum in the measurement of neutralizing antibodies against rubella virus has been reported by several workers (Neva & Weller, 1964; Plotkin, 1964; Parkman, Mundon, McCown & Buescher, 1964), but our finding that RS neutralizing antibody titres could be increased two- to eightfold by the addition of unheated serum does not appear to be widely known.

SUMMARY

Between October 1963 and April 1965, 113 children with respiratory disease and 113 children with diarrhoeal disease were matched for age and time of entry into hospital and studied by virus isolation and serological techniques.

Infections with respiratory syncytial (RS) virus, parainfluenza virus and herpes simplex virus respectively were found in 29, 11 and 12 children in the respiratory illness group but in only 1, 2 and 4 children in the diarrhoeal group. Rhinoviruses were isolated from 10 children in each group and in seven cases were associated with lower respiratory disease. Adenovirus infections were found in nine children

with respiratory disease and eight with diarrhoea. Of the 40 enteroviruses isolated 16 were associated with respiratory disease and 24 with diarrhoea.

A poor or delayed serological response in children under 4 months with RS virus infection was observed. Addition of unheated rabbit serum increased the sensitivity of the neutralization test with RS virus.

These findings indicate that respiratory syncytial and parainfluenza virus infections were clearly associated with respiratory illness but the pathogenic role of the other viruses was not clear.

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