Papers and Originals

A Collaborative Study of the Aetiology of Acute Respiratory Infections in Britain 1961–4

A Report of the Medical Research Council Working Party on Acute Respiratory Virus Infections*

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In recent years many previously unknown viruses have been isolated from the respiratory tract of patients suffering from respiratory diseases of various sorts. Some of these viruses have been shown to cause respiratory disease of one kind or another in various special groups, such as children admitted to hospital or residential nurseries, military recruits, medical students, or children in boarding schools. Much of the relevant literature has been summarized in conference papers published recently (*Amer. Rev. resp. Dis.*, 1963). However, most acute respiratory diseases occur in persons living at home, and in 1960 practically nothing was known of the cause of such diseases in this country. Therefore in 1961 a collaborative study was organized by the M.R.C. Working Party on Acute Respiratory Virus Diseases in order to investigate a large number of acute respiratory infections occurring in different parts of Britain in a cross-section of the population. Work was carried out in 24 centres, and the laboratory workers and clinicians responsible are listed at the end of this paper.

At first children up to 16 years of age who were living at home were studied. Standard laboratory methods were used for virus isolations, while clinical data were recorded on one or other of two record cards, one of which was a shortened version of the other. Serum was not collected from the patients. An attempt was made to isolate viruses from a matched group of subjects who did not have respiratory disease—contact controls.

In the course of the first two years of the study almost all the viruses for which we were testing had been isolated, but the frequencies were different from those reported in some other studies. In the last year of the study the scope of the investigation was therefore widened by including adults contacted at home or at work. Children admitted to hospital were also studied in order to determine whether, with the techniques used, the viruses recovered from small children with severe respiratory disease were the same as those found in milder cases in general practice.

This report summarizes the methods used and an analysis of the results obtained between June 1961 and June 1964.

Clinical Methods

The methods were defined in some detail for the collaborative workers but are given here in outline. Whenever acute respiratory virus diseases were prevalent an attempt was made to study a constant number of cases each week, in order to help the smooth working of the virus isolation tests; clinicians were asked to choose a representative sample of the illnesses which were occurring—for example, by studying the first two suitable cases to appear at the surgery each week. In fact, however, for local reasons different methods of gaining access to patients were used in different centres, and opinion on what was a suitable case varied somewhat from area to area, so that the proportion of cases studied which fell into each of the clinical categories was not the same in all areas. In some general practices a record was kept of all cases seen, and these and other details will be reported elsewhere (Kendall and Hope-Simpson, in preparation). Patients for study were examined clinically and the history and diagnosis were recorded on a standard form; the "long" form allowed for a day-by-day record of the symptoms and signs, while the "short" form did not. Some clinicians recorded the number of persons in the household and their ages, and also other cases of respiratory disease occurring before and after the index case. Specimens were collected from a healthy member of the household nearest in age to that of the patient (contact controls), but only a proportion of the clinicians did this regularly.

Specimens were collected at first from patients within two days of onset of disease, but in the last two years of the study cases within four days of onset were accepted, since it had been shown that the virus-isolation rate was not affected by this modification (Higgins *et al.*, 1963). A throat swab and a nasal swab were usually taken, but it was sometimes possible to collect more nasal secretion by a nasal "blow-out" or by allowing the swab to soak up secretion in the nose for a few minutes. The specimens were transported in 0.2% bovine plasma albumin in Hanks's saline chilled in ice.

Laboratory Methods

The specimens were inoculated within two hours of collection into cultures of monkey-kidney and HeLa cells and immediately or later into cultures of human-embryo-kidney or human diploid cells (strain WI-38 or HEL-7).

The monkey-kidney cultures were washed and maintained with medium 199 in a roller drum at 33° C. Viruses were detected by examining frequently for cytopathic effect and for haemadsorption with human or guinea-pig red cells at about 5, 10, and 15 days after inoculation.

Enteroviruses were recognized by the cytopathic effect and were typed by neutralization tests with specific immune serum. Haemadsorbing agents were usually identified by haemadsorption-inhibition tests with cholera-filtrate-treated antisera against para-influenza, influenza, and SV5 viruses (Chanock *et al.*, 1958).

The HeLa cells were a strain obtained from Bristol which were maintained in 2% rabbit serum and were known to be

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very sensitive to respiratory syncytial (R.S.) virus (Peacock and Clarke, 1961). Cultures were incubated at 33 or 36°C. and observed for two to three weeks. R.S. virus was identified by its cytopathic effect and a neutralization or complement-fixation test with high-titre rabbit antiserum. Adenoviruses were recognized by their cytopathic effect and by complement-fixation tests on the culture fluid with known positive human serum; they were identified by neutralization tests with specific antisera.

Human-embryo-kidney cultures were maintained in a medium containing 2% calf serum and 0.25% lactalbumin hydrolysate of a pH 7.4 to 6.8 and were rolled at 33° C. (Tyrrell and Parsons, 1960). Human diploid-cell strains were maintained in 2% calf serum with Eagle's medium and were slightly more alkaline. Rhinoviruses were isolated in these cells and were recognized by their cytopathic effect, and identified by their inability to grow in stationary cultures at 37° C. in an alkaline medium, or by being inactivated in acid buffers at *p*H 3 to 4 (Dimmock and Tyrrell, 1962). They were classified as M rhinoviruses if they caused a cytopathic effect in monkey-kidney cells and were further identified by neutralization tests with rabbit antisera.

Herpes simplex virus was identified by its cytopathic effect in several types of cells and by neutralization tests with specific antisera.

Certain laboratories inoculated specimens, mainly from subjects under 10 years of age, by the intracerebral and intraperitoneal route into day-old suckling mice. Coxsackieviruses of group A were recognized by the development of paralysis and identified by complement-fixation tests (Gamble and Kinsley, 1963).

In the early part of the study specimens were inoculated amniotically into 13-day-old fertile eggs, during periods when influenza viruses were prevalent, and the viruses were detected by haemagglutination in the usual way.

All the virological methods were demonstrated and discussed at a series of courses run at the Common Cold Research Unit, Salisbury; diagnostic antisera were distributed to all laboratories from the Standards Division of the Central Public Health Laboratory; and monkey-kidney and diploid cells were distributed each week by the M.R.C. Division of Immunological Products Control. In these ways an effort was made to standardize the laboratory methods. The results obtained each month were circulated to all collaborating laboratories, but the final analysis was based on completed clinical cards and laboratory reports which were analysed centrally.

β-haemolytic streptococci were isolated by streaking a freshly taken swab on to blood agar and incubating aerobically. The organisms were usually grouped and often typed, but as this was not required and was not always done no detailed analysis of these data has been made. Of 116 organisms grouped 90% belonged to group A and 6% to group C or G. No attempt was made to isolate other bacteria.

Results

This report is based on results from 24 research groups. Those based on the laboratories at Bristol, Cirencester, Colindale, Epsom, Guildford, and Sheffield produced the bulk of the case records.

Survey of Virus Recovery

The distribution of the patients' ages is given in Table I. It shows that a virus or β-haemolytic streptococcus was isolated from one patient in three below the age of 17 and from one in four aged 17 or over and that viruses were relatively more common in patients below the age of 6. The age distribution of patients studied was similar in all areas, except that relatively few infants and adults were studied in Epsom, while the patients in Chester and Ipswich were predominantly infants admitted to hospital. Altogether 217 cases admitted to hospital were studied, and half of these were in the first year of life.

TABLE I.—*Total Isolation Rates in All Patients by Age Groups*

Age (years):	0–	1–	6–	11–	17+	Total
Patients studied	275	641	362	143	467	1,888
Viruses isolated	80	183	78	29	77	447
β-haemolytic streptococci isolated	1	28	45	17	37	128
% Viruses isolated	29	29	22	20	16	24
% Pathogens isolated	29	33	34	32	24	30

Fig. 1 shows the way in which the study built up over the last two years and how the number of cases studied increased each winter. Influenza A viruses were isolated in the first three months of each year in a pattern which reflected the national prevalence of infection. Para-influenza viruses of all three types

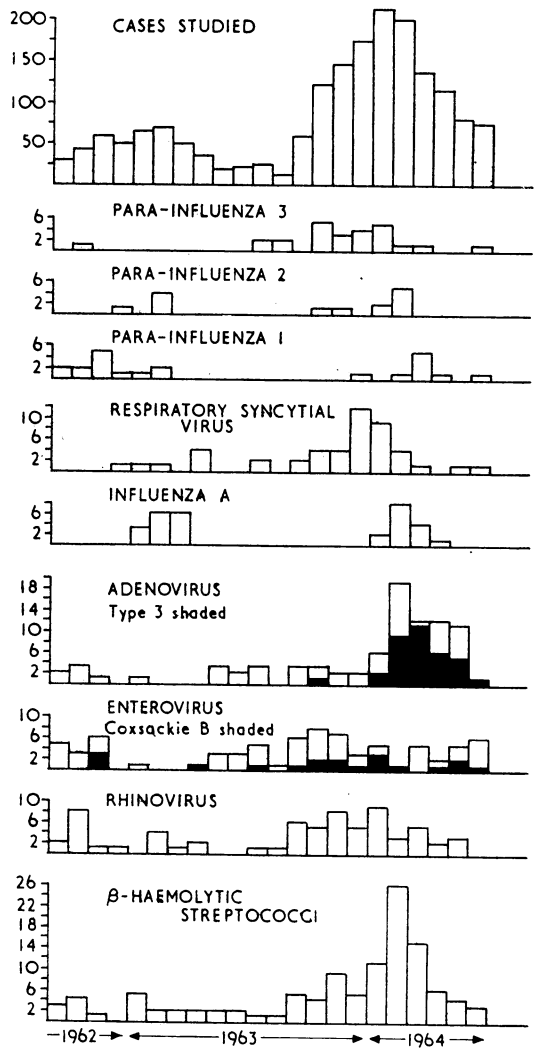

FIG. 1.—Total numbers of patients studied and isolations of viruses and streptococci during the last two years of the study.

appeared each winter in scattered areas, and the returns suggested that some areas had sharp outbreaks lasting a few weeks; one such outbreak of para-influenza 1 in Cambridge has been reported by Banatvala *et al.* (1964). In this study they were noted in Bristol in September and October 1962, in Epsom and Guildford in November 1962, and in Leeds in March 1964. R.S. virus was isolated by most laboratories and the maximum number of isolations was made in December 1963. The peak of isolation of streptococci was later in the same winter.

Enteroviruses were isolated throughout the year, but relatively more often in summer and autumn and less often in the first three months of the year. An unexpected finding was the relative infrequency of adenoviruses in the season 1962–3, although they were relatively common in 1961–2 (not shown in Fig. 1) and again in 1963–4, when a considerable number of adenovirus type 3 infections were recognized, especially in South London (Epsom and Guildford).

Virus Recovery and Age

Table II shows the frequency of isolation of viruses from patients of various ages. R.S. viruses were obtained mainly from children under 6, and over half were from those under 1 year. Para-influenza viruses were isolated mainly from children under 6, but also from under 10's, and adenoviruses and enteroviruses were found roughly equally in all age groups up to 10. Herpes simplex and rhinoviruses were isolated with almost equal frequency from all age groups, and the pattern with influenza was the same, except that this virus group, like β-haemolytic streptococci, seemed to pass by the infants under 1 year of age.

TABLE II.—*Isolations From All Cases by Age Groups*

Virus	Age 0–	1–	6–	11–	17+	Total
Influenza	—	9	5	7	14	35
Para-influenza	15	31	9	1	5	61
R.S. virus	30	19	1	—	—	50
Adenovirus	10	36	26	6	8	86
Herpes simplex	3	11	6	3	6	29
Enterovirus	11	45	20	5	12	93
Rhinovirus	10	25	9	5	29	78
Unidentified	1	7	2	2	3	15
β-haemolytic streptococci	1	28	45	17	37	128
Total studied	275	641	362	143	467	1,888

Isolations of more than one pathogen

	No. of Isolations of Indicated Combination				
	Influenza	Para-influenza	R.S. Virus	Adeno-virus	Rhino-virus
Para-influenza		1	1	1	
Coxsackievirus		1			
Rhinovirus		1		1	
Streptococcus	3	3	1	7	4
Herpes simplex				1	

Virus Serotypes

Two or three pathogens were obtained simultaneously from 25 patients, but for simplicity of tabulation one was chosen, rather arbitrarily, as the probable pathogen. The combinations are shown in the lower half of Table II, which indicates that in 18 instances one organism was the streptococcus; in each of these instances the case was classified as infected by the virus isolated, leaving only seven patients in whom an arbitrary choice between viruses was needed.

Almost all the viruses were typed serologically, and the results are shown in Table III. This shows that para-influenza viruses types 1 and 3 were each about twice as common as type 2. Adenoviruses types 1, 2, and 5, the so-called endemic types, were found just as often as the other "epidemic" types, which were mainly represented by type 3, although other serotypes have often been found both before and since the study. Polioviruses were uncommon, and those isolated were not examined for the characteristics of vaccine strains, although some may have been derived from them. The coxsackieviruses of group A most commonly found—namely, types 2, 4, 5, 6, and 10—are those which have been found to be associated with disease such as herpangina. All serotypes of the coxsackieviruses of group B except type 6 were found. Only echovirus type 6 was found in appreciable numbers, and this type was also being obtained from cases of aseptic meningitis during the period of the study.

TABLE III.—*Serotypes of Viruses Isolated from Cases*

Type No.	Para-influenza	Adeno-viruses	Polio-viruses	Coxsackie-virus A	Coxsackie-virus B	Echo-viruses
1	23	16	3		8	
2	12	13	3	7	4	
3	25	40	—	2	6	
4		2		7	8	
5		11		6	3	
6		1		10		8
7		1		1		
8				1		
9				3		1
10				4		
21		1				
?		1	1	2		3

Survey of Clinical Diagnoses

The case records were next tabulated according to the diagnosis given, and it was found that, although the diagnoses inserted on the record card included common cold, feverish cold, sore throat, bronchitis, and influenza, the largest category of completed cards was that in which some other diagnosis had been specified. Many of these diagnoses were really synonymous with one of those suggested on the card—for example, pharyngitis or tonsillitis instead of sore throat. These diagnoses were therefore revised to correspond with an equivalent diagnosis used by other clinicians. Others were less common diagnoses, such as croup, which had not been mentioned on the card, and these were tabulated separately.

The results of this analysis are shown in Table IV. Obviously most patients were suffering from mild infections of the upper respiratory tract. The cases of bronchiolitis were partly seen in general practice and partly in hospital at Bristol, Chester, and Ipswich. There was a group of cases which ranged from mild laryngitis and simple tracheitis in adults or older children to the full syndrome of acute laryngotracheobronchitis (A.L.T.B.) or croup, which occurred particularly in small children; almost all these patients were treated at home, and all were grouped together for tabulation. The group of pneumonia is also clinically heterogeneous, and includes cases of broncho-pneumonia and a few of primary atypical pneumonia, segmental pneumonia, and collapse. The cases of otitis media include only those in which signs and symptoms of ear disease predominated. Some earache or drum changes were noted in certain cases of tonsillitis and colds, and otitis media or possible otitis media were noted in 27 other patients, in whom the primary diagnosis was common cold, feverish cold, sore throat, or bronchitis. The other diagnoses include cases finally diagnosed as Bornholm disease, measles, pyrexia of unknown origin, stomatitis, glandular fever, and other miscellaneous conditions, although all cases presented as respiratory disease.

TABLE IV.—*Isolations from Patients Suffering from Various Types of Illness*

Diagnosis	Total	Influenza	Para-influenza	R.S. Virus	Adenoviruses	Herpes Simplex	Enteroviruses	Rhinoviruses	Unidentified	β-haemolytic streptococci	Positive No.	Positive %
Common cold	437	1	15	8	8	6	13	28	4	11	94	21
Feverish cold	341	8	13	6	26	1	14	20	2	7	97	28
Influenza	144	23	2	1	3	1	5	4	2	4	45	31
Sore throat	376	1	4	1	31	7	32	8	2	90	176	47
Croup, A.L.T.B., tracheitis, or laryngitis	127	2	11	3	4	1	3	6	1	6	37	29
Bronchitis	179	0	7	8	8	2	8	4	2	3	42	23
Bronchiolitis	46	0	1	10	1	0	4	3	0	0	19	41
Pneumonia, broncho-pneumonia, or primary atypical pneumonia	103	0	4	12	2	5	4	2	0	2	31	30
Otitis media	42	0	1	1	0	1	3	0	1	1	8	19
Other	93	0	3	0	3	5	7	3	1	4	26	28
Total	1,888	35	61	50	86	29	93	78	15	128	575	30

Relation Between Virus Isolation and the Presence of Disease

Thirteen groups of workers studied a sufficient number of specimens from contacts of the patients to be worth analysing ; the results obtained are summarized in Table V. If a virus was isolated more often from cases than from controls then this suggested that it caused the illness in the case. Table V shows that para-influenza 1 and 2 viruses and R.S. virus were isolated almost entirely from cases and not from contacts, but there was little difference in the rate of isolation of para-influenza 3 from cases and contacts. Enteroviruses were likewise isolated more often from cases than from controls, but these agents were not isolated in all areas with the same frequency. In order to meet this difficulty estimates were made of the frequency of virus isolations in contacts in those areas in which viruses were also isolated from cases. Coxsackieviruses of group A were isolated from 7.1% of 594 cases and 1.6% of 307 contacts, coxsackieviruses of group B from 2.5% of 950 cases and 1.5% of 333 contacts, and echoviruses from 0.5% of 715 cases and none of 142 contacts. Poliovirus was found in 0.4% of 446 cases and 1.6% of 126 contacts. The adenoviruses of both non-epidemic (1, 2, and 5) and epidemic (3, 4, 7, and 21) types were apparently associated with disease, although the non-epidemic types were isolated from contacts more frequently than epidemic types. Rhinoviruses and streptococci were also found much more often in cases than in controls, although in both instances appreciable numbers of healthy persons yielded the agent.

TABLE V.—*Comparison of Isolation Rates for Cases and Contact Controls from Laboratories Testing More than 10 Contacts*

Type	No. Tested	Para-influenza 1	Para-influenza 2	Para-influenza 3	R.S. Virus	Adenovirus	Enterovirus	Rhinovirus M	Rhinovirus H	β-Haemolytic Streptococci
Cases	1,303	20	12	17	34	85	65	7	47	128
Controls	493	2	0	5	0	8	25	5	5	23

The differences in frequency of isolation between cases and controls would be regarded as significant if conventional statistical tests were applied, but we do not present these because the ratio of cases to controls was not the same in all areas and therefore the population of patients and of contacts studied were not strictly comparable.

There were some further difficulties regarding the virus isolation results from contacts ; it was not always certain whether a contact tested had had a cold recently or developed one within a day or two of being tested, but contacts were often visited again ; sometimes it was not possible to find one whose age was near that of the case ; there was also a danger that specimens from cases were more thoroughly studied than those from contacts, but laboratories were specifically warned about this and almost all specimens were coded until tests were complete. The final results were thought to indicate that in the family setting in which most of these studies took place there was good evidence that most of the viruses and streptococci isolated were responsible for the diseases in which they were found.

Relation Between the Virus Recovered and the Clinical Type of Illness

In Table IV it can be seen that pathogens were recovered most frequently from cases of bronchiolitis and sore throat ; half the pathogens in cases of sore throat were streptococci and half the viruses in bronchiolitis were R.S. The low isolation rate in otitis media and pneumonia may have been due to our ignoring bacteria other than streptococci and not testing ear swabs or sputum ; since all cases were studied soon after onset, viruses should have been isolated if they were there. Isolations from patients with colds may have been unsuccessful because so little virus was shed or because the tests used would not detect the viruses causing the disease ; the latter suggestion is supported by evidence that some of the viruses which cause colds cannot be isolated in any known culture system (Kendall *et al.*, 1962).

The types of disease with which the viruses of each group were associated were analysed in two ways, firstly by tabulating the frequency of the symptoms and signs recorded, mainly at the time that the specimens were collected. This method can be criticized because different clinicians require different degrees of severity before they record a symptom or sign as present, and in this analysis they were recorded as present or absent irrespective of their severity. For this reason a second method was used in which the records were analysed according to the diagnosis given ; this reflects the physician's opinion of which part of the respiratory tract was most severely affected. Since a rather similar picture emerged from both methods of analysis, it is thought that both are likely to be valid. The second also shows the variations in the main area of attack and the severity of illnesses produced by a single infectious agent. Both analyses were based on the clinican's assessment of the general clinical picture at the time the patient was first seen, but a few cases which were thought at first to be mild progressed later to more serious disease, such as pneumonia. There was no satisfactory method of discovering the frequency with which this occurred.

Symptoms and Signs

The analysis of the histories in Table VI and Fig. 2 shows that blockage and running of the nose were each recorded in about half the virus infections, but the nose was apparently drier in adenovirus infections and wetter in enterovirus and rhinovirus infections. Nasal symptoms were less common in streptococcal illnesses. Sore throat was uncommon in para-influenzal infections, and common in adenovirus, enterovirus, and streptococcal infections. Cough was common in infections with the myxoviruses but also occurred in infections with enteroviruses

TABLE VI.—*Occurrence of Symptoms and Signs in Patients Infected With Indicated Agent*

Symptoms	Influenza (30 Type A, 3 Type B)	Para-influenza	R.S. Virus	Adenovirus	Echo and Polio	Coxsackievirus A	Coxsackievirus B	Rhinovirus M or H	β-haemolytic streptococci	?
Nose blocked	13	25	11	37	4	15	6	29	34	40
Nose running	17	28	24	31	11	20	6	48	34	67
Sore throat	14	7	2	37	3	25	10	22	100	45
Cough	24	40	40	45	10	20	11	41	34	75
Sputum	3	2	2	3	1	3	1	1	5	10
Hoarseness	9	9	3	12	2	6	1	9	12	18
Chest pain	1	1	1	3	0	2	6	2	4	3
Wheezing	1	7	8	8	2	5	3	7	4	19
Shivering	11	2	0	14	2	7	3	3	16	15
Sweating	11	5	4	21	3	11	4	8	16	20
Headache	13	8	1	25	6	13	8	16	44	33
Muscle pain	15	4	1	8	1	4	7	2	14	15
Vomiting	7	8	12	17	5	4	5	3	20	21
Diarrhoea	1	2	1	9	0	2	2	1	3	11
Abdominal pain	1	6	1	12	2	6	5	1	4	5
Physical signs in :										
Nose	18	26	22	44	6	17	12	37	34	55
Throat	15	20	10	56	8	31	14	15	103	69
Chest	2	14	36	13	7	5	8	8	6	20
Fever present	28	21	38	65	12	29	18	37	88	61
„ over 101° F. (38·3° C.)	15	9	16	29	5	15	10	10	33	20
Median No. of days in bed	3	2	6	3	3	2	5	< 1	2	3
Median No. of days off work or school	5	—	—	> 8	—	—	> 8	< 1	5	2
Total No. studied*	33	52	48	80	12	41	26	70	124	117

* A few patients referred to elsewhere were excluded from this analysis because the clinical records were incomplete.

and streptococci and in over half the rhinovirus infections. Hoarseness was noted in infections by all virus groups, but was found most often in influenza and para-influenza virus infections. Chest pain in enterovirus infections may have been due to myalgia, but was generally uncommon. Wheezing was slightly more common, being part of the clinical picture of bronchiolitis caused by R.S. virus, but also being found in about one in every ten patients infected with other viruses, apart from influenza. Constitutional febrile symptoms were common in influenza, adenovirus, and enterovirus infections, and also in those due to streptococci. Their apparent absence in R.S. infections may have been chiefly due to the youth of the patients, but in rhinovirus infections it reflected the fact that many of the illnesses were very mild.

The physical findings were generally consistent with the history. Physical signs in the nose, such as obstruction and clear or purulent discharge, were present in over half the infections except in those due to enteroviruses or streptococci. Chest signs were commonest in infections with R.S. virus but were found in infections in each group, including rhinoviruses. The signs were almost entirely rales and rhonchi. The median number of days in bed and off work or school was calculated where possible, although not all observers provided this information ; the figures given confirmed that rhinovirus infections were short mild illnesses—the patient often continued at work—while R.S. virus, adenovirus, and coxsackievirus B illnesses were the most severe and long-lasting.

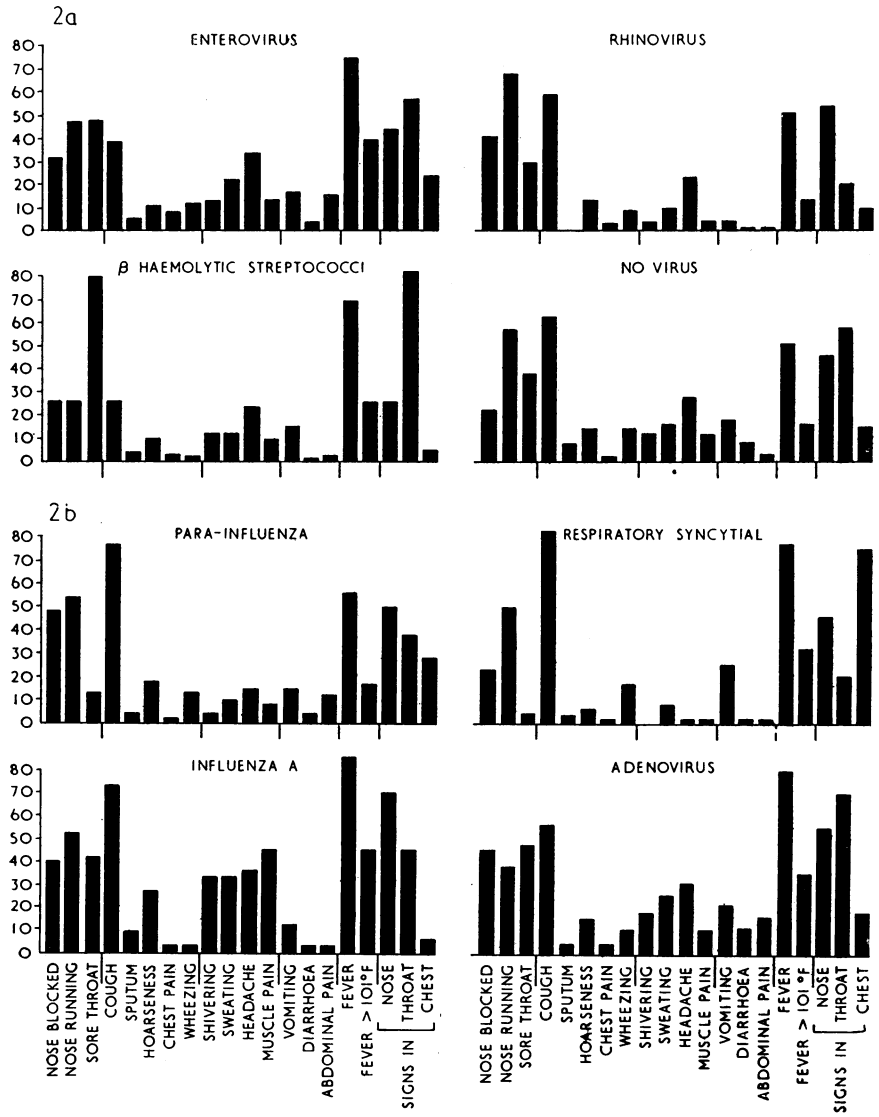

FIG. 2.—Relative frequency of certain symptoms and of clinical involvement at various levels of the respiratory tract in patients shown to be infected with various viruses (data derived from Table VI). Table II shows that a rather high proportion of patients infected with respiratory syncytial virus were infants, and would therefore report no symptoms.

Clinical Diagnoses

Table IV and Fig. 3 show the absolute and relative frequency with which virus infections of each type fell into various clinical categories. The spectrum of illness (Tyrrell, 1963) plotted in Fig. 3, and the analysis of signs and symptoms just considered, have many parallel features. Influenza virus infections were apparently recognized as such in most instances, probably because of the frequency of constitutional symptoms and general mild involvement of the respiratory tract (Fig. 2) and the presence in some instances of other associated cases. Para-influenza virus infections showed a wider clinical spectrum, the nose was involved rather than the throat, so colds were diagnosed rather than sore throat, and in the lower respiratory tract laryngitis or croup was a more common diagnosis than bronchitis. R.S. virus infections usually took the form of either colds or bronchiolitis. The physical signs or the extension of the disease mean that some of the latter diagnoses merged with those of bronchitis or bronchopneumonia, though many of these cases had prominent nasal symptoms.

Adenovirus infections were usually recorded as sore throats. The symptoms and signs were more numerous in those infected with type 3 than in those infected with non-epidemic types but otherwise the diseases were similar ; chest signs were commoner in those infected with non-epidemic types, possibly because of their youth, and conjunctivitis was more common in cases infected with epidemic types (25%) than in the other cases (5%). Sore throat was also the usual diagnosis in enterovirus infections, and the symptom analysis suggests that the clinical picture was very similar to that in adenovirus infections ; although exudate was occasionally seen in the throat in adenovirus infections and almost never in enterovirus infections, while conjunctivitis was noted in a number of cases of coxsackievirus A infections. Rhinovirus infections were almost always called colds, but in a few the cough and lower respiratory symptoms were apparently sufficiently prominent to change the diagnosis to tracheitis, bronchitis, or even bronchiolitis. It is very striking that almost all cases infected with β-haemolytic streptococci were diagnosed as having sore throats. The cases from which no virus was isolated presented in the aggregate a rather intermediate type of clinical picture.

Spread of Illness in Families

Where full family records were available these were analysed in order to try to obtain a picture of the setting in which the diagnosed cases had occurred. They were first arranged to show the age structure of the households. Table VII shows that there was a preponderance of small children in households infected with para-influenza viruses, R.S. virus, and adenovirus types 1, 2, and 5, suggesting that these are among the first viruses to invade the growing family. The other viruses were found in households which, as a whole, had the expected proportion of younger and older children, and this is consistent with the data in Table II,

which indicate that these viruses were isolated relatively often from older children as well as from younger children.

All cases of respiratory disease occurring within a period of two weeks, including the date of onset of the case which was diagnosed by laboratory tests, were then tabulated. Secondary attack rates were calculated, and most fell between 0.2 and 0.3, as had been reported by others in acute respiratory infections in which no laboratory diagnosis was made—for example, Lidwell and Sommerville (1951), Badger *et al.* (1953), and Buck (1956). However, the attack rates for influenza and para-influenza were much higher, although the number of cases was not large ; the high rate in influenza has been noted before, but that with para-influenza has not, although it has been shown that the latter viruses may spread very rapidly in a residential nursery (Chanock *et al.*, 1961 ; Sutton 1962).

The successive cases of each family outbreak of respiratory disease were also tabulated chronologically, taking the day of onset of the first case as day zero. The data thus obtained are shown in Fig. 4. This shows that the cases which were apparently secondary to a case of influenza occurred usually three to five days later, whereas those following a rhinovirus infection occurred most often two to three days after the first. Since the incubation period of an experimental rhinovirus infection is two to three days, this suggests that transmission usually takes place on the day of onset of a case.

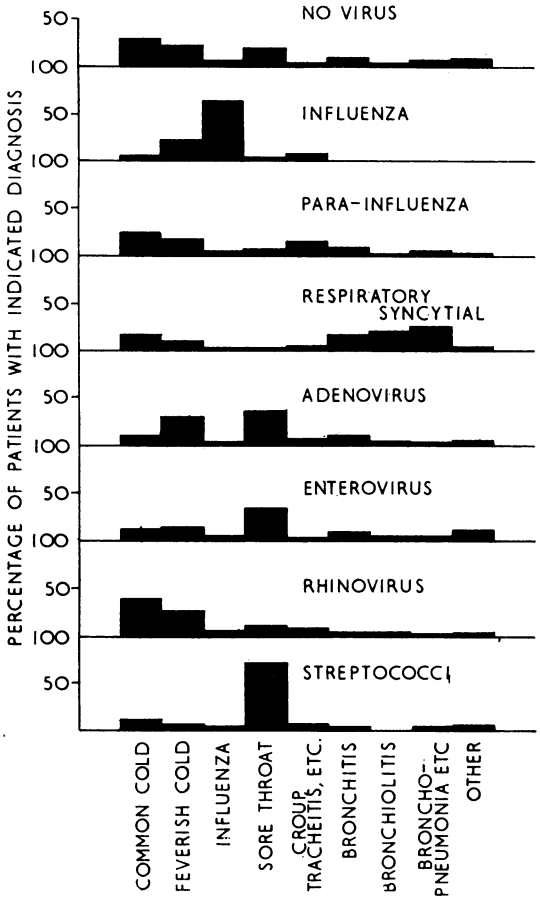

FIG. 3.—Relative frequency of various clinical diagnoses among patients infected with viruses and streptococci and a 10% random sample of patients from whom no pathogens were recovered (data derived from Table IV).

TABLE VII.—*Family Age Structure and Secondary Attack Rates by Aetiological Groups*

Infection Identified	No. of Families	No. of Members	Percentage Aged (Years)		Secondary Attack Rate
			< 6	6–16	
Influenza	17	68	18	38	0·55
Para-influenza	10	39	41	10	0·65
R.S. virus	11	57	32	26	0·22
Adenovirus 1, 2, and 5	19	76	35	18	0·26
Adenovirus 3, 4, and 21	26	112	13	38	0·38
Enterovirus	46	212	20	27	0·30
Rhinovirus	23	114	20	34	0·30
No virus	41	171	22	25	0·26
β-haemolytic streptococci	58	280	15	41	0·27

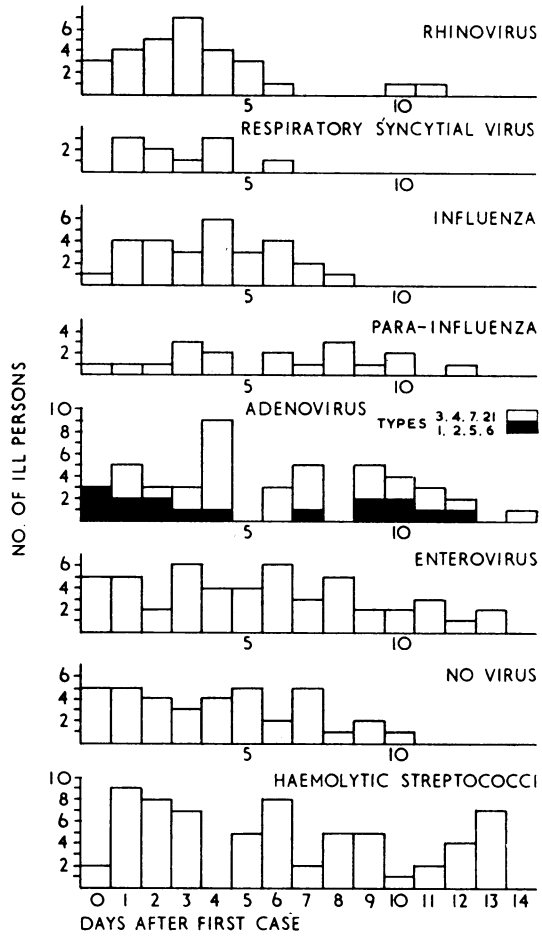

FIG. 4.—Interval between day of occurrence of the first and apparent secondary cases in families in which a virus or streptococcus was isolated from at least one case (further data in Table VII).

The few cases of R.S. secondary infection appear to follow in a pattern similar to influenza, but the para-influenza infections seemed to spread much more slowly through the family. Type 3 adenovirus infections seemed to give rise to a peak of secondary cases about four days after the first, but the other adenovirus serotypes were followed by cases at irregular intervals for the whole period of observation. A similar straggling distribution was seen after enterovirus and streptococcal infections, and this might be related to the rather longer incubation period and duration of carriage of these organisms.

Patients Seen in Hospital

Most of the observations are drawn from general practice, but about one-tenth of the patients were seen in hospital. They were almost all under the age of 5 (Table VIII), but they did not all have severe respiratory infections ; some, for example, were admitted because a convulsion occurred in the course of an otherwise mild cold. The results are of interest because they show that, although exactly the same methods were used, the types of virus isolated from this group of patients were rather different from those in the main study. The pattern corresponded to large studies in children elsewhere (Gardner *et al.*, 1960 ; Clarke *et al.*, 1964 ; Holzel *et al.*, 1965), particularly in

emphasizing the importance of R.S. virus in bringing small children into hospital and the relative unimportance of β-haemolytic streptococci.

TABLE VIII.—*Recovery of Viruses from Patients Seen in Hospital*

Age Group	Total	Influenza	Para-influenza	R.S. Virus	Adenovirus	Herpes Simplex	Enteroviruses	Rhinoviruses	Unidentified	β-haemolytic streptococci	Positive	
											No.	%
0–12 months ..	106		5	15	2	2	6	2	1		33	31
1–5 years	86	1	3	5	4	3	5	4	1		26	30
6–16 years.. ..	25		1			1		1		2	5	20
Total ..	217	1	9	20	6	6	11	7	2	2	64	29

Discussion

The results of this investigation have answered some of the questions which were in mind at the time the studies were initiated. They show that viruses of all the groups sought, except for the reoviruses, were found infecting patients with mild acute respiratory disease living at home in Britain. Although the study was not designed as a full epidemiological survey, we have obtained useful data on the distribution of serotypes in different years and in different age groups of the population. It is very probable that the viruses isolated were in many cases causing the disease observed, for the isolation rates from patients were substantially higher than those from their contacts. The results are not entirely satisfactory, for contacts were not tested in all sections of the study and at all times of the year, so the pooling of results may have introduced some errors. However, comparison with a group of case contacts is a more stringent test of the aetiological importance of a virus than is comparison with cases of non-respiratory diseases admitted or seen at a clinic at the same time ; for spread within the family is likely to occur and some viruses may be excreted for a long time. In this respect the present figures are a better test of aetiological association than others published previously.

It is difficult to describe respiratory diseases very exactly, and our information on clinical syndromes and signs and symptoms may be criticized because of the combined effect of observer error and differences in diagnostic labelling between clinicians. However, the fact that the figures are compiled from the findings of a number of clinicians observing a mixture of cases, and that most of them recorded all their findings before they were aware of the laboratory results, means that the final tabulations are relatively free of diagnostic bias. In support of their validity it should be noted that the association between certain symptom complexes and certain virus infections were evident in the figures from individual centres analysed separately, and that the clinical pictures of influenza and streptococcal disease built up from our "blindly" recorded case histories are strikingly similar to those recognized in intensive outbreaks of infections with these agents. It is therefore reasonable to conclude that the clinical spectrum of infection shown in the cases infected with other viruses is equally valid.

Some of the research groups contributing to this study carried out more extensive investigations of their own in the same area, and reports on some of these have now appeared (Higgins *et al.*, 1963, 1964 ; Clarke *et al.*, 1964), and include the results of laboratory tests on some of the patients whose records they contributed to this study. There has been another study of para-influenza virus infections in Cambridge (Banatvala *et al.*, 1964). So far as they are comparable with the collaborative study all these results are in good agreement with those presented here. However, it has now been decided to undertake larger and more thorough collaborative studies of patients seen both in general practice and in children's hospitals, and the results of these should amplify considerably those we have reported here.

Summary

Specimens taken from 1,888 patients suffering from acute respiratory infections and mostly encountered in general practice have been tested for the presence of respiratory viruses and 575 specimens were positive ; β-haemolytic streptococci, influenza, para-influenza, and respiratory syncytial viruses were isolated as well as adenoviruses, coxsackieviruses of group A and B, echoviruses, and rhinoviruses. Relatively few of these agents were isolated from 493 contacts in the same households.

The symptoms and signs of the illnesses observed were analysed, and it was shown that different viruses appeared to produce on the average different diseases. It was also shown that the temporal distribution of secondary cases within the household varied according to the virus isolated.

Within the large group there was a small group of patients who were admitted to children's hospitals, and in this group the pattern of illness and causative organisms was significantly different from that in the group as a whole.

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* The numbers in parentheses indicate the number of patients studied by each group.

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Malabsorption During and After Recovery from Acute Intestinal Infection

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In recent years increasing interest has been shown in small-intestinal malabsorption associated with a variety of chronic diarrhoeal diseases. The effect on absorptive capacity of acute intestinal infection, the most common form of gastro-intestinal disease the world over, has received little attention. Many cases of acute enteritis are either too mild to require hospital admission or are discharged from hospital soon after symptomatic recovery. Occasional patients have been reported, however, in whom a sprue-like syndrome associated with malabsorption appeared to follow acute intestinal infection (Achor and Smith, 1955 ; King and Joske, 1960). In the past year we have also encountered two cases in which diarrhoea and loss of weight with malabsorption appeared to follow episodes of acute intestinal infection in previously healthy individuals (Lindenbaum, 1965). Interest in these cases, as well as in the possible relationship of previous bouts of acute bowel infection to the asymptomatic malabsorptive state common in tropical countries (Baker, Ignatius, Mathan, Vaish, and Chacko, 1962 ; Sprinz, Sribhibhadh, Gangarosa, Benyajati, Kundel, and Halstead, 1962 ; Aziz, 1965 ; Lindenbaum, 1965), prompted a study of absorption during and after recovery from acute intestinal infections in East Pakistan. Preliminary results are presented here.

Methods and Materials

The 95 patients included in this study were Pakistanis admitted to the Pakistan-SEATO Cholera Research Laboratory hospital ward for acute diarrhoeal illness of less than two weeks' duration. Nearly all patients were admitted after only one to two days of acute illness. While most were young adults of either sex, the entire group ranged in age from 7 to 65 years.

In most cases a bacteriological diagnosis was established by culture of rectal swabs. *Vibrio cholerae* was obtained in 47 ; shigellae (types A, B, or D) in 6 ; and salmonellae (one type D, one type B) in 2. In four patients who had the classical clinical picture of staphylococcal food-poisoning *Staphylococcus aureus* was isolated on stool culture and from food recently ingested by all of them. Nine patients, from whom no known pathogens were isolated, had an acute severely dehydrating illness resulting in profound circulatory collapse unassociated with rises in antibody titre against *Vibrio cholerae* ("non-vibrio cholera"). Clinical features of these cases will be reported elsewhere (Lindenbaum, Greenough, Benenson, Oseasohn, Rizvi, and Saad, 1965). In the remaining 27 patients, all of whom had an acute gastro-intestinal illness of one to four days' duration not associated with circulatory collapse, no pathogens were isolated despite stool cultures on SS, MacConkey's, gelatin, and tellurite-taurocholate-gelatin agars ("acute gastro-enteritis").

The five-hour urinary excretion of D-xylose after a 25-g. oral dose, as well as plasma-xylose levels at two hours, were measured by the method of Roe and Rice (1948). Normal values for this laboratory for five-hour urinary xylose excretion in asymptomatic Europeans and Americans range from 5 to 10 g. Folic-acid absorption was studied by microbiological assay of serum with *Streptococcus faecalis* after an oral dose of 40 μg. of folic acid per kg. Values of >40 mμg./ml. at one or two hours are considered normal (Chanarin, Anderson, and Mollin, 1958). The 24- and 48-hour urinary excretion of vitamin B_{12} was measured after an oral dose of 1 μg. of ^{58}Co-labelled cyanocobalamin (Amersham) given simultaneously with 50 mg. of intrinsic factor. "Flushing" doses of 1 mg. of non-radioactive vitamin B^{12} were given at 0 and 24 hours (Schilling, 1953 ; Ellenbogen, Williams, Rabiner, and Lichtman, 1955). Normal values in this laboratory are 9–25%/24 hours and 13–36%/48 hours.

Since the tests of xylose and vitamin-B_{12} absorption depend on urinary excretion of the absorbed substances, care was taken to exclude patients with impaired renal function. Oliguric patients were not included in the study. Those who had subnormal urinary xylose excretion associated with two-hour plasma-xylose levels greater than 36 mg./100 ml. were considered to have renal impairment and were excluded from the study. Cases whose urinary excretion of ^{58}Co-cyanocobalamin during the second 24 hours after the oral dose exceeded that of the first 24 hours were also excluded. Patients who vomited within several hours of the administration of the test substances also were not included.

Stool was collected for gross description and volume measurement over eight-hourly periods. The end of the last eight-hour period before the passage of formed stools of normal appearance was defined as "end of diarrhoea." In some patients studies of xylose, folic-acid, and/or vitamin-B_{12} absorption were performed soon after admission (after dehydration and circulatory collapse had been corrected with intravenous fluids) in the presence of continuing diarrhoea. Others were first studied during the first week after the end of diarrhoea. In 62 patients serial studies of one or more parameters of absorption were performed.

* From the Pakistan-SEATO Cholera Research Laboratory, Dacca 5, East Pakistan. This report is based on a presentation delivered to the Cholera Research Symposium at Honolulu on 28 January 1965.