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# Viruses in acute respiratory infection in a general community†

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## INTRODUCTION

The renaissance of tissue-culture techniques a decade ago led to the discovery of many viruses which cause acute respiratory infections. Investigations conducted to assess the role of these viruses mostly involved conveniently accessible groups such as children in hospital and residential institutions, students, and military recruits. These groups consisted of young persons living in socially similar conditions; the turnover in these populations often being rapid. Such conditions favoured the introduction and spread of infection. These studies did not provide information as to the relative importance of different viruses causing acute respiratory infection in normal civilian communities. Comparatively little is known of the aetiology of acute respiratory infections in members of the community living in their own homes. We have, therefore, studied the role of viruses in acute respiratory infections from September 1962 to August 1963 in two general practices in Cambridge.

Parainfluenza infections have been considered in detail in a previous communication (Banatvala, Anderson & Reiss, 1964), and the findings of infection with influenza virus and Eaton agent (*Mycoplasma pneumoniae*) will be presented later. This paper presents the overall results of our survey.

## STUDY POPULATION

The study population was selected to provide a representative sample of the permanent community of Cambridge, i.e. the population apart from students. The population consisted of the patients of two general practitioners (T.B.A., B.B.R.) working in different parts of Cambridge, with a combined total of 5178 patients. A chi squared ($\chi^2$) test was done in order to determine whether the study population was representative of the community of Cambridge. The age and sex structure of the combined practices was compared to that of Cambridge City. The two populations were divided into the following age groups: 0–4, 5–14, 15–39, 40–59, 60 and over. Females in the study population provided a reasonable sample of the

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community ($\chi_4^2 = 7{\cdot}95$, not significant at the 5% level), but there were fewer males aged 0–4 than expected in the study population ($\chi_4^2 = 36{\cdot}1$, significant at the 0·1% level). Apart from the 0–4 age group, the male study population was found to be reasonably representative of the community of Cambridge ($\chi_3^2 = 2{\cdot}82$, not significant at the 5% level).

### *SELECTION AND RECORDING OF CASES*

Details on all patients who needed to consult their general practitioner with an acute febrile respiratory infection either at home or in the consulting room were recorded on standard cards. One of the following clinical diagnoses was made on each patient:

1. Influenza.
2. Common cold.
3. Primary atypical pneumonia (PAP).
4. Acute respiratory disease (ARD).
5. Acute pharyngitis/tonsillitis.

ARD was further subdivided into the following groups:

*a*. Obstructive laryngo-tracheo-bronchitis of infants (croup).
*b*. Acute epidemic bronchiolitis of infants.
*c*. Acute pneumonitis of infants.
*d*. Pharyngo-conjunctival fever.
*e*. Epidemic kerato-conjunctivitis.

This classification is a modification of that devised by Stuart-Harris (1953). Patients seen within 72 hr. from the onset of symptoms and who consented to have specimens taken were immediately referred by the general practitioner for laboratory investigation.

## METHODS

### *Collection of specimens*

On notification of a case by the general practitioner, one of us (J.E.B.) would visit the patient at home and collect epidemiological data together with specimens for virological investigation. These consisted of:

(1) A pharyngeal swab. This was immersed in 4–5 ml. of medium 199 containing penicillin 500 units/ml., streptomycin 500 $\mu$g./ml., and amphotericin B 8$\mu$g./ml. It was transported to the laboratory on crushed ice.

(2) Blood, acute phase sample (10 ml.).

(3) Blood, convalescent phase sample (10 ml.). This was taken 2–3 weeks after the acute phase sample was collected. At this time additional information as to the course of the illness and spread of the infection within the family was obtained. Blood was not taken from infants and young children.

Pharyngeal swab fluid (0·2 ml.) was inoculated into secondary monkey kidney and HEp-2 cell cultures within 2 hr. of collection. Bacterial isolations were only attempted in patients with acute tonsillitis/pharyngitis. Both bacteriological and virological studies were made in a proportion of these cases in order to determine whether viruses had any role in the clinical picture of acute tonsillitis/pharyngitis.

### *Tissue culture*

*Monkey kidney.* Monolayers were grown in medium 199 containing calf serum 5 %, penicillin 100 units/ml., streptomycin 100 μg./ml., amphotericin B 1·6 μg./ml., SV 5 antiserum 0·02 % and sodium bicarbonate 0·088 %. Maintenance medium was similar to growth medium, except that no calf serum was added. Cultures were thoroughly washed to remove all traces of calf serum before inoculation.

*HEp-2.* Tissue culture monolayers were grown in Eagle's medium containing 10 % calf serum and 5 % human serum. Antibiotics were added as for monkey kidney cultures. Maintenance consisted of Eagle's medium, antibiotics, 1 % calf serum and 0·088 % sodium bicarbonate. Cultures were washed to remove all traces of human serum before inoculation. Both monkey kidney and HEp-2 cultures were incubated in stationary racks at 35° C.

*Fertile hens' eggs.* When influenza was prevalent, specimens were inoculated into the amniotic sac of 11-day-old fertile hens' eggs. The eggs were incubated for 72 hr. at 35° C., after which the aminotic fluid was tested for haemagglutination.

### *Identification of viruses*

Tissue cultures were inspected daily for cytopathic effect (C.P.E.). Fluids from cultures showing C.P.E. were passed into fresh cultures and the virus identified by neutralization tests using specific antisera. Monkey-kidney cultures were tested on the third, fifth and tenth days of incubation for haemadsorption (Vogel & Shelokov, 1957). Fluid from cultures exhibiting haemadsorption together with amniotic fluid exhibiting haemagglutination was passed into fresh monkey-kidney cultures and the virus identified by haemadsorption inhibition tests using rabbit antisera to parainfluenza 1, 2, 3 and SV 5 viruses, and ferret antiserum to influenza A 2 virus according to the method described by Chanock *et al.* (1958).

### *Serology*

#### *Complement-fixation tests (CFT)*

CFT's were carried out using Perspex plates employing overnight fixation at 4° C. as described by Bradstreet & Taylor (1962). Sera were tested against the following antigens: influenza A, B and C, parainfluenza 1, 2, 3 and sendai virus, respiratory syncytial virus (RS virus), adenovirus, psittacosis and R. burneti. CFT's employing Eaton agent antigen were performed on the sera of patients with PAP or on family contacts of such cases who later developed acute respiratory infections. These tests were carried out by Dr B. P. Marmion (Public Health Laboratory, Leeds), using the method described by Goodburn, Marmion and Kendall (1963).

#### *Haemagglutination-inhibition tests (HI)*

Before testing, sera were treated with cholera vibrio extract and then inactivated at 56° C. in order to remove non-specific inhibitors. HI tests for influenza (A 2 Sing. 57) were performed on all patients during the time that influenza was prevalent, according to the method described by the W.H.O. (1953). During the time

that parainfluenza viruses were prevalent, HI tests for parainfluenza 1, 2 and 3 were performed by Dr R. B. Heath (St Bartholomew's Hospital, London) according to the method described by Heath, Tyrrell & Peto (1962).

*Neutralization tests*

Neutralization tests were performed on sera from all patients during the time that influenza was prevalent using fragments of chorioallantoic membrane on egg pieces as described by Fazekas de St Groth, Withell & Lafferty (1958). The same strain of virus was used as in the HI tests. In all these tests a fourfold or greater increase in antibody titre was regarded as significant.

## RESULTS

### *Incidence*

During the investigation there were 592 spells of acute respiratory infection of sufficient severity to require medical attention; this represents an incidence of 11·4 spells per 100 persons. The age and sex specific incidence rates are shown in Table 1. Pre-school children (ages 0–4) had the highest incidence rates (51·6 spells per 100 persons), males having a slightly higher rate than females. The incidence rates decreased in successive age groups.

Table 1. *Age and sex specific incidence rates (spells) per 100 persons*

| Age (years) | No. at risk | | Attack rate (spells) per 100 persons | | |
|---|---|---|---|---|---|
| | M | F | M | F | M and F |
| 0–4 | 145 | 177 | 55·2 | 46·8 | 51·6 |
| 5–14 | 364 | 323 | 25·8 | 23·5 | 24·5 |
| 15–39 | 977 | 872 | 5·6 | 7·7 | 6·6 |
| 40–59 | 693 | 669 | 6·1 | 6·4 | 6·2 |
| 60+ | 420 | 538 | 5·5 | 5·4 | 5·4 |
| All ages | 2599 | 2579 | 11·3 | 11·6 | 11·4 |

### *Seasonal distribution of cases*

The weekly distribution of respiratory infections occurring in the combined practices are compared with the number of new claims to sickness benefit in Cambridge (all causes) in Fig. 1. Laboratory studies confirmed that the sudden increase in cases recorded by the general practitioners in February and March 1963 were due to influenza A (Asian strain). This corresponded to the increase in new claims for National Insurance sickness benefit in Cambridge. The monthly distribution of: (*a*) new claims to sickness benefit, (*b*) cases occurring in the two practices, (*c*) cases referred for laboratory investigation, are shown in Fig. 2. All three curves show similar trends, suggesting a correlation between cases investigated in the laboratory, cases occurring in the combined practices and those occurring in the insured population of Cambridge.

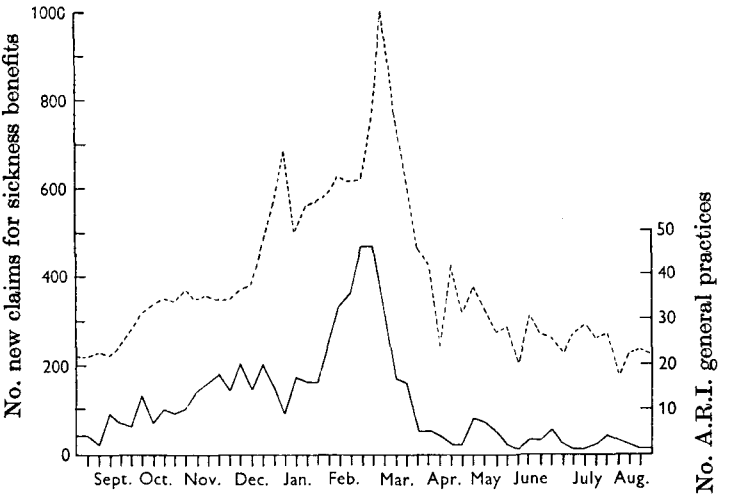


Fig. 1. Weekly distribution of new claims for sickness benefit, and incidence of acute respiratory infection in two general practices. ..., Sickness absence claims; —, general practice records.

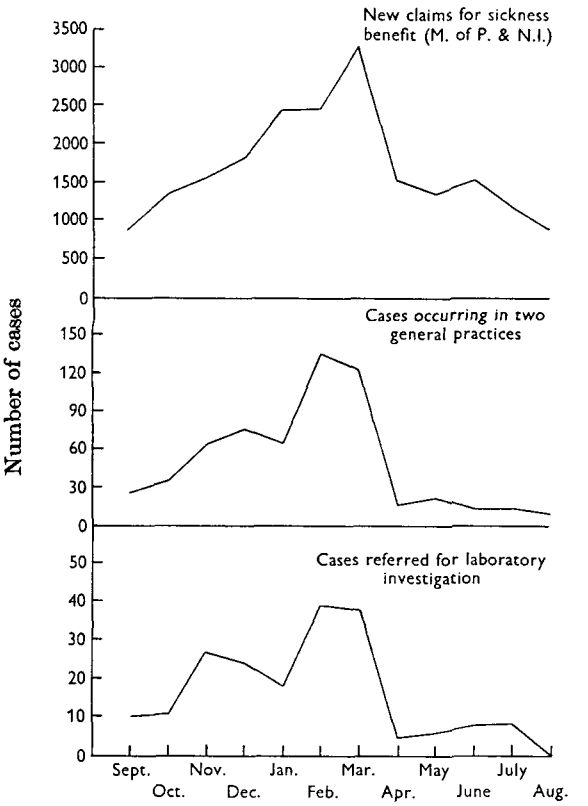


Fig. 2. Monthly distribution of new claims for sickness benefit, acute cases of respiratory infection in two general practices, and cases referred for laboratory investigation.

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*Results of laboratory investigations*

From the 592 spells of acute respiratory infection occurring in the combined practices, 195 were investigated in the laboratory (33 %). Diagnosis in adults was established by isolating a virus and demonstrating concomitant significant sero-

Table 2. *Summary of principal laboratory findings*

| Aetiological agent | Isolation* alone | Isolation and serology | Serology alone | Total |
|---|---|---|---|---|
| Influenza A | 11 | 18 | 27 | 56 |
| Parainfluenza 1 | 17 | 3 | 7 | 27 |
| Parainfluenza 2 | 2 | — | — | 2 |
| Parainfluenza 3 | 2 | — | 4 | 6 |
| Adenovirus | 1 | 1 | 2 | 4 |
| Respiratory syncytial virus | — | — | 3 | 3 |
| Eaton agent | — | — | 9 | 9 |
| β-Haemolytic streptococcus | 15 | — | — | 15 |
| All agents | 48 | 22 | 52 | 122 |

* Blood was not taken from young children; diagnosis being by virus isolation alone.

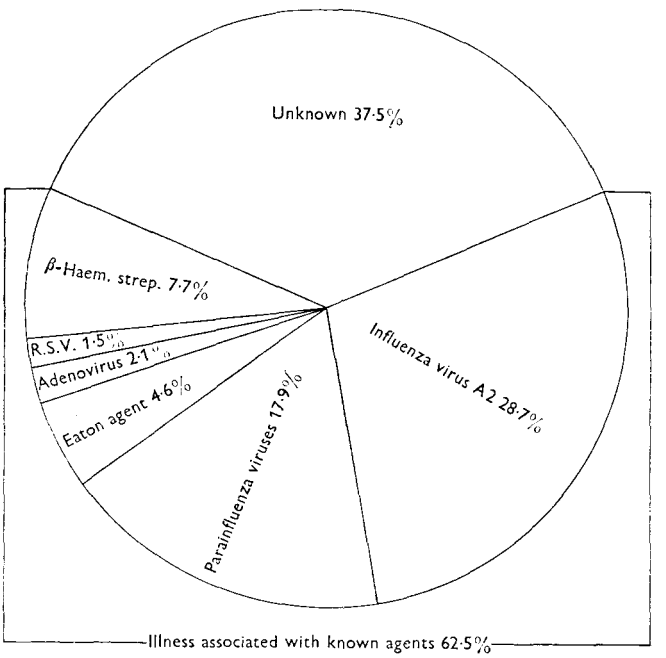


Fig. 3. Distribution of aetiological agents associated with acute respiratory infections in the combined practices.

logical evidence of infection, or by serological evidence alone. Only two adults were reluctant to give blood; diagnosis in these cases was by virus isolation alone. However, particularly in influenza and parainfluenza infection in children, secondary adult cases frequently occurred in the same family. It was often

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possible to isolate from them a similar virus and demonstrate a significant rise in antibody titre to that particular virus antigen.

Table 2 summarizes the laboratory results. An aetiological agent was detected in 122 of the 195 cases investigated (62·5 %). Fig. 3 shows the proportion of infections associated with different aetiological agents. Viruses (i.e. excluding Eaton agent and streptococcal infections) were responsible for 98 of the 195 laboratory investigated cases (50·3 %). Myxovirus infections, i.e. influenza and parainfluenza infections, featured prominently, being responsible between them for 46·6 % of all infections investigated.

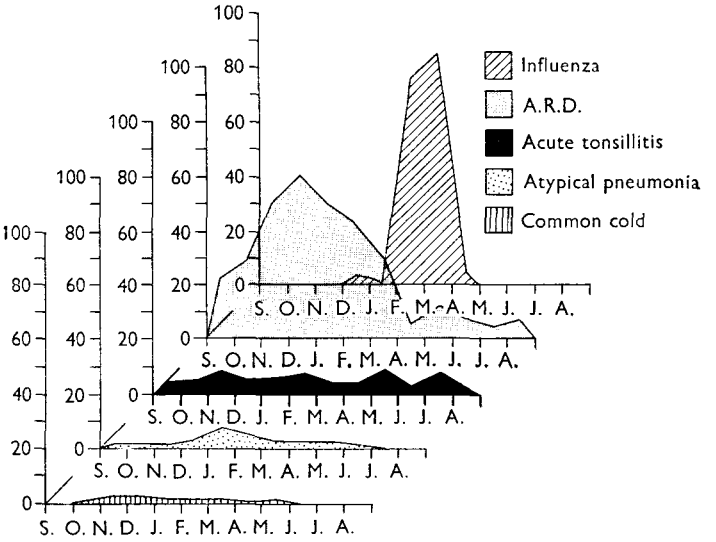


Fig. 4. Monthly distribution of clinical diagnostic categories.

*Association between laboratory and clinical diagnosis*

Study of the monthly distribution of clinical diagnostic categories (Fig. 4), together with the monthly distribution of aetiological agents (Fig. 5), establishes a link between the clinical and laboratory diagnosis. There were few clinical cases of influenza before February and after April 1963. Cases initially diagnosed as influenza at other times were mostly due to viruses other than influenza, such as the parainfluenza viruses. However, between February and April 1963, a clinical diagnosis of influenza was frequently confirmed by laboratory studies. Thus, from 165 cases of clinical influenza occurring between February and April 1963, sixty-three were referred for laboratory investigation. Evidence of influenza A (Asian strain) was obtained in fifty-six of these patients (89 %). The serological findings are summarized in Table 3. A significant increase in antibody titre by CFT was detected in forty-one of forty-five paired sera tested (91 %), whereas neutralization tests showed a significant rise in twenty-three of forty-two paired sera tested (55 %). HI tests were the least effective, a significant rise occurring in only fifteen of forty-five paired sera tested (33 %). HI and neutralization tests between them revealed only four cases of influenza which were not detected by CFT. During

the influenza epidemic, the convalescent phase sample of blood was usually collected 10–14 days after the acute phase sample. It has been shown (Jensen, 1961) that HI antibodies tend to develop later than CF antibodies. Had the convalescent sample of blood been collected at the end of the third week, then more significant rises by HI and neutralization tests might have been shown.

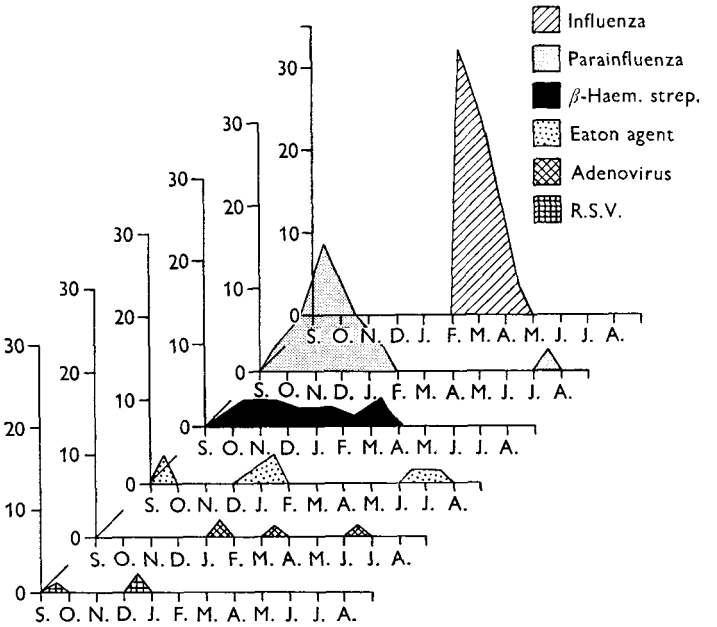


Fig. 5. Monthly distribution of aetiological agents in cases referred for laboratory investigation.

Table 3. *Summary of serological findings in influenza infection*

| Significant rise by... | CFT, HI and neut. | CFT and neut. | CFT and HI | CFT only | Neut. and HI | Neut. only | HI only | Total |
|---|---|---|---|---|---|---|---|---|
| No. of cases | 10 | 9 | 4 | 18 | 1 | 3 | 0 | 45 |

CFT = Complement-fixation test; Neut. = neutralization test; HI = haemagglutinin-inhibition test.

Acute respiratory disease was prevalent throughout the year, particularly during winter months. Much infection in this category was due to infection by parainfluenza viruses, particularly parainfluenza 1. During the 5 months ending 31 January 1963, parainfluenza viruses were responsible for 40% of all acute respiratory infections investigated. There were few cases of parainfluenza infection after January, influenza then becoming epidemic. Of thirty-five patients with parainfluenza infection, twenty-one were children most of whom had acute laryngo-tracheo-bronchitis (croup) of varying severity. Evidence of parainfluenza infection was obtained from fourteen adults many of whom had an influenza-like illness.

A diagnosis of PAP was made in twenty-five patients, cases occurring throughout the year. Evidence of Eaton agent infection was obtained from seven of ten cases referred for laboratory investigation (70%). A cold agglutinin screen test was employed at the bed-side according to the method described by Garrow (1958) and was positive on four occasions (57%). In addition, evidence of Eaton agent infection was obtained in two patients who were family contacts of patients with PAP, both of whom developed an influenza-like illness without pneumonia. Evidence of adenovirus infection occurred infrequently (four cases). An isolation was made on two occasions (type 2 and type 6). Three patients had ARD (pharyngo-conjunctival fever), whilst the other had an influenza-like illness.

There were three patients with RS virus infections (two adults and one child aged 14 months). One of these adults was the child's aunt, who developed an acute respiratory infection with a sore throat, cough and hoarse voice. The child subsequently developed acute laryngo-tracheo-bronchitis from which he died in hospital.

Since only febrile respiratory infections sufficiently severe to require medical attention were included in this investigaton, few cases of common cold were recorded. We did not attempt to isolate rhinoviruses.

Throat swabs for bacteriologial investigation were taken from fifty patients with acute tonsillitis and group A streptococci were isolated from thirty-two of these specimens (64%). Of seventeen cases submitted for combined virological and bacteriological investigation, group A streptococci were isolated from fifteen, but no viruses were detected.

## DISCUSSION

Surveys in general practice have shown that acute respiratory infections are a leading cause of morbidity in general communities, accounting for between 30 and 35% of all consultations (Pemberton, 1949; Fry, 1957; Davies, 1958; Logan & Cushion, 1958). Recent technical developments have made possible the identification of many viruses responsible for these infections. Nevertheless, few studies incorporating such techniques have been performed on general communities. The regular collection of fresh specimens, the necessity of prompt inoculation into tissue culture and the collection of paired sera present difficulties in surveys on non-institutional communities.

Robinson *et al.* (1962), in a survey on a general population in Atlanta, Georgia, established a diagnosis in 26·4% of cases of acute respiratory infection. As in Cambridge, parainfluenza infection featured prominently, being responsible for 14% of all infections investigated. Higgins, Ellis & Boston (1963), in a general practice survey in Cirencester, identified an aetiological agent in 23·4% of respiratory infections, but performed serological studies in only a limited number of cases. Clark *et al.* (1964), in a survey conducted on children admitted to hospital, together with a few patients with acute respiratory infection who consulted their general practitioner, established a diagnosis in 41% of their cases. As in Cambridge, influenza and parainfluenza viruses featured prominently.

Woodall, Rowson & McDonald (1958), in a general practice survey during the 1957 epidemic of Asian influenza, estimated that 31% of their patients developed

influenza. By contrast, in 1963 in Cambridge, although the volume of the general practitioner's work was considerably increased when influenza was epidemic, only 3·2% of patients developed influenza. Asian influenza, in 1963, was probably performing a 'mopping up' operation, attacking remaining susceptible persons who had escaped infection in 1957–8 and 1958–9, years in which the Asian influenza virus caused major epidemics.

There were few cases of adenovirus infection, for although these viruses are prominent causes of acute respiratory infection in military populations (Woolridge *et al.* 1956; Hilleman *et al.* 1957; van der Veen & Dijkman, 1962), and in children living in closed communities (Kendall *et al.* 1957; Bell *et al.* 1961), studies on civilian communities have revealed that adenovirus infection is responsible for only a small proportion of acute respiratory infections (Jordan *et al.* 1956; Grieble *et al.* 1958; Robinson *et al.* 1962; Higgins *et al.* 1963).

There were few cases of RS virus infection in the combined practices during 1962–63. RS virus is frequently associated with lower respiratory infection in infants and young children, particularly with bronchiolitis and pneumonia (Beem *et al.* 1960; Lewis, Rae, Lehmann & Ferris, 1961; Andrew & Gardner, 1963). Infection tends to occur in well-defined outbreaks, and it is probable that 1962–63 represented a year in which these viruses were not prevalent in Cambridge.

Since bacteriological investigations were only performed on patients with acute tonsillitis/pharyngitis, it is not possible to assess the role of pathogenic bacteria as a cause of other types of respiratory infection seen in this survey. However, other investigations have revealed that, apart from acute tonsillitis, pathogenic bacteria play a relatively small role in the aetiology of acute respiratory infections. Dingle *et al.* (1953) concluded from a study of 4428 acute respiratory infections over a two-year period in Cleveland, Ohio, that only 2·5% of infections were of bacterial origin. Higgins *et al.* (1963), in their general practice investigation, found $\beta$-haemolytic streptococci associated with 3·5% of acute respiratory infections, whilst Hilleman *et al.* (1962), in a study of respiratory infections in 677 children in hospital and outpatient clinics, together with a group of 155 industrial workers, found that there was no evidence of primary bacteriological cause in any of their cases.

In Cambridge an aetiological agent was not detected in 37·5% of cases investigated. Had blood been taken from young children and techniques for the identification of rhinoviruses adopted, it is probable that more laboratory diagnoses would have been made. Rhinoviruses typically cause common colds (Hobson & Schild, 1960; Hamre & Procknow, 1961), and therefore would be unlikely to be encountered frequently in our survey since only febrile infections requiring medical attention were studied. However, there is some evidence that rhinoviruses may cause more severe illness in young children (Reilly *et al.* 1962). In addition, evidence of Eaton agent infection was sought only in patients with PAP or their contacts in this survey. Chanock *et al.* (1961), in an outbreak of respiratory infection in a recruit training camp, demonstrated that Eaton agent caused upper respiratory and inapparent infections more commonly than pneumonia. The role of Eaton agent infections in upper respiratory infections in civilian communities

has yet to be established. One may, finally, speculate as to whether some of the infections in which no aetiological agent was detected were due to agents which remain to be discovered.

This survey was conducted over a limited period and only a relatively small number of cases were studied. One cannot be certain that at other times and in other places similar findings will occur. There is a clear indication for more prolonged observation employing the newer techniques for the identification of such agents as the rhinoviruses and Eaton agent so that the role of these agents in mild as well as severe infections can be assessed.

## SUMMARY

The aetiology of acute respiratory infections between September 1962 and August 1963 was studied in two general practices in Cambridge. These practices were reasonably representative of the permanent community of Cambridge.

There were 592 spells of acute respiratory infection in the combined practices, representing an incidence of 11·4 spells per 100 persons. Children aged 0–4 had the highest rates (51·6 spells per 100 persons).

It was possible to establish a diagnosis in 62·5% of cases investigated. Influenza and parainfluenza infections featured prominently, being responsible between them for 46·6% of all respiratory infections investigated. From September 1962 to January 1963, parainfluenza viruses were prevalent, causing acute laryngo-tracheo-bronchitis in children (croup), and an influenza-like illness in adults. From February to April 1963, influenza A (Asian) was epidemic, a clinical diagnosis of influenza being frequently confirmed by laboratory studies at this time. There were nine cases of Eaton agent infection, seven of which had PAP, the other two being family contacts who later developed influenza-like illnesses.

Adenovirus (four cases), and RS virus (three cases) were not prevalent to any large extent in Cambridge during the survey.

We are indebted to many for their contributions to this study: Dr A. P. Waterson for his help and encouragement throughout this investigation; Mrs I. Nitkin, for her technical assistance; Miss E. R. Dalton, Department of Human Ecology, Cambridge, for statistical advice, Dr C. M. P. Bradstreet, Central Public Health Laboratory, Colindale, for supplying many diagnostic reagents; Dr G. Swan and Dr R. Bangham for clinical assistance; Dr C. G. Eastwood, M.O.H., City of Cambridge, for providing National Insurance sickness absence claims. The National Polio Research Fund gave generous support and enabled this investigation to be made.

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