RESPIRATORY VIRUS INFECTION IN
CHRONIC CHEST DISEASE

BY

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Dedicated to my wife Geraldine
Summary

This thesis describes the impact of respiratory virus infections in patients with chronic chest disease and investigates the role of influenza vaccine and the possibility of preventing infection with intranasal interferon.

The thesis begins by defining respiratory virus infection and presenting a brief historical introduction. This is followed by an account of the important respiratory viruses, the major causes of chronic chest disease and the relationship between respiratory virus infections and exacerbations of chest disease. The introduction concludes by describing the nature of interferons and reviews clinical trials of interferon therapy.

The subjects, materials and methods are followed by the results of the clinical and laboratory studies. Respiratory virus infections were significantly more severe in adults with chronic chest disease than in previously healthy individuals. Unfortunately prophylaxis with intranasal interferon was not associated with any benefit. A preliminary study in children with cystic fibrosis showed that rhinoviruses were associated with exacerbations of lung disease. A survey of General Practitioners in the Trent Region revealed that less than 20% of susceptible patients were vaccinated against influenza and identified several factors which were associated with improved vaccination rates. A study in patients with asthma found that only 9% had received influenza vaccine prior to the 1989-90 influenza epidemic. Influenza vaccination was not associated with any significant reduction in the proportion of asthmatic patients who developed influenza-like symptoms during the influenza outbreak and many episodes of illness were thought to have resulted from other respiratory viruses. All studies suffered from difficulties in establishing a diagnosis of respiratory virus infection, with viruses isolated from less than 15% of acute nasopharyngeal swabs and fewer than 25% of paired serum samples showing a significant antibody rise. The implications of the results and future possibilities are discussed.
## Contents

<table>
<thead>
<tr>
<th>Summary</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbreviations used</td>
<td>11</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>13</td>
</tr>
<tr>
<td>Declaration of work done by the author</td>
<td>14</td>
</tr>
<tr>
<td>Publications arising from this work</td>
<td>15</td>
</tr>
</tbody>
</table>

**A. INTRODUCTION**

**Chapter 1. Respiratory viral disease:**  
Definitions and historical background | 18 |

**Chapter 2. Human respiratory viruses** | 21 |
1. Classification, structure and properties | 21 |
2. Epidemiology | 26 |
3. Transmission | 32 |
4. Pathogenesis | 34 |
5. Clinical manifestations | 37 |
6. Pathological features | 40 |
7. Immune response | 41 |
8. Confirmation of diagnosis | 44 |
9. Prevention and control | 48 |

**Chapter 3. Respiratory viruses and chronic airway disease** | 56 |
1. Introduction and classification | 56 |
2. Association between virus infection and pulmonary function | 61 |
3. Mechanisms of airways obstruction induced by respiratory viruses | 70 |

**Chapter 4. Interferon and respiratory virus infections** | 73 |
1. Introduction | 73 |
2. Classification and properties of interferons | 73 |
3. Mechanism of action | 74 |
4. Pharmacokinetic and tolerance studies | 76 |
5. Clinical use of interferons | 78 |
6. Interferon in respiratory virus infections | 80 |
**B. SUBJECTS, MATERIALS AND METHODS**

**Chapter 5. Subjects and materials**

1. Subjects
2. Interferon trial medication
3. Tissue culture materials and buffers
4. Virus transport medium and tissue culture media
5. Cell cultures
6. Materials and buffer for Complement Fixation Tests
7. Materials for haemagglutination inhibition test
8. Materials for coronavirus ELISA test
9. Materials for immunofluorescence tests

**Chapter 6. Methods**

1. Collection of specimens
2. Growth and maintenance of cell cultures
3. Virus isolation and identification in cell culture
4. Haemagglutination inhibition test (HI)
5. Complement fixation test
6. Coronavirus enzyme-linked immunosorbent assay
7. Detection of viruses by immunofluorescence
8. Analysis of interferon trial
9. Statistical analysis of clinical studies
C. CLINICAL AND LABORATORY STUDIES

Chapter 7. Trial of prophylactic intranasal interferon-alpha in patients with chronic respiratory disease

1. Introduction
2. Organisation of the study

RESULTS
3. Recruitment of subjects
4. Characteristics of study population
5. Use of trial medication
6. Number of respiratory tract infections in interferon and placebo recipients
7. Symptomatic days in interferon and placebo patients
8. Symptom scores in interferon and placebo patients
9. Nature and timing of interferon effect in lower respiratory tract infections
10. Peak flow measurements
11. GP consultations, use of antibiotics and hospital admission
12. Estimated cost of respiratory tract infections
13. Effect of respiratory virus infection in patients taking placebo
14. Comparative severity of respiratory illness in symptomatic placebo-using patients and corresponding index cases
15. Results of viral diagnosis
16. Association of viruses with trial outcome
17. Comparison of trial outcome in rhinovirus, coronavirus and influenza episodes
18. Outcome of rhinovirus, coronavirus and influenza infection on index cases
19. Timing of virus infections
20. Bacteriology
21. Adverse effects
22. Determination of interferon activity
23. Discussion of interferon trial

Chapter 8. Preliminary study of the role of respiratory virus infections in cystic fibrosis

1. Introduction
2. Aims of the study
3. Organisation of the study

RESULTS
4. Response rate
5. Outcome of episodes of presumed respiratory virus infection
6. Bacteriology
7. Virology
8. Discussion of cystic fibrosis study
Chapter 9. Influenza vaccination in the elderly. A study of the policies of General Practitioners in the Trent Region

1. Introduction
2. Organisation of the study
RESULTS
3. Response rate
4. Demographic characteristics of responding GPs
5. Vaccination policies
6. Delivery of vaccine
7. Reasons for not accepting vaccine
8. Factors influencing rate of vaccination
9. Discussion of Trent GP study

Chapter 10. Influenza vaccination and influenza-like illness in patients with asthma and previously healthy adults during the 1989-90 influenza epidemic

1. Introduction
2. Organisation of the study of asthmatic patients
RESULTS
3. Response rate
4. Uptake and timing of influenza vaccination
5. Effect of vaccine on incidence of influenza-like symptoms
6. Symptoms of influenza-like illness in vaccinated and unvaccinated groups
7. Exacerbations of asthma
8. GP consultations and use of antibiotics
9. Hospital Admissions
10. Time taken off work or school
11. Influenza haemagglutination inhibition (HI) titres
12. Respiratory illness occurring during and outside the influenza epidemic period in previously healthy adults
13. Discussion of study of influenza-like illness in asthmatic patients and previously healthy adults 1989-90

D. CONCLUSIONS

Chapter 11. Conclusions

E. REFERENCES

Appendix 1. Peak flow recording chart used by interferon trial patients
Appendix 2. Symptom chart used by interferon trial patients
Appendix 3. Symptom chart used by index cases and secondary cases in the interferon trial
## List of figures

| Fig 5.1 | Electron micrograph of coronavirus OC43 | 104 |
| Fig 6.2 | Titration of complement and haemolytic sera | 120 |
| Fig 6.3 | Titration of antiserum and antigen and complement control | 121 |
| Fig 6.4 | Layout of plate for CFT screening of test sera | 123 |
| Fig 6.5 | Coronavirus immunofluorescence. | 128 |
| Fig 7.1 | The interferon spray in action | 137 |
| Fig 7.2 | Mean initial and lowest peak flows associated with episodes of interferon or placebo use | 149 |
| Fig 7.3 | Outcome of exposure to respiratory tract infection in placebo-using patients with asthma, bronchitis and bronchiectasis | 152 |
| Fig 7.4 | Mean symptom scores and symptom days in symptomatic placebo-using patients and corresponding index cases | 154 |
| Fig 7.5 | Outcome of episodes associated with rhinovirus, coronavirus and influenza infection | 160 |
| Fig 7.6 | Rhinovirus, coronavirus and influenza infection in index cases showing comparison of mean age of cases and mean symptom days and symptom scores associated with episodes of infection | 162 |
| Fig 7.7 | Timing of viral infections diagnosed by isolation or serology in the interferon trial | 164 |
| Fig 10.1 | Symptoms of influenza-like illness in vaccinated and unvaccinated asthmatic patients | 194 |
| Fig 10.2 | Complications of influenza-like illness in vaccinated and unvaccinated asthmatic patients | 196 |
| Fig 10.3 | Protective influenza HI titres in vaccinated and symptoms of influenza-like illness | 201 |
| Fig 10.4 | Reciprocal geometric mean influenza HI titres in vaccinated symptoms of influenza-like illness | 202 |
Fig 10.5 Comparative severity of episodes of respiratory illness occurring in previously healthy adults during the 1989/90 influenza epidemic and outside the epidemic period.

Fig 10.6 Comparison of symptoms of respiratory illness occurring in previously healthy adults during the 1989/90 influenza epidemic and outside the epidemic period.

Fig 10.7 Comparative severity of respiratory virus infections in previously healthy subjects, patients with chronic chest disease and children with cystic fibrosis.

Fig 10.8 Respiratory virus diagnostic rate in episodes of acute respiratory illness occurring in previously healthy subjects, patients with chronic chest disease and children with cystic fibrosis.
List of tables

Table 7.1 Characteristics of trial subjects 140
Table 7.2 Other medical problems in trial patients 141
Table 7.3 Drugs on admission to trial 142
Table 7.4 Comparison of medication users and non-users 143
Table 7.5 Comparison of interferon and placebo users 144
Table 7.6 Outcome of courses of medication 145
Table 7.7 Effect of interferon on the timing and nature of symptoms in patients who developed lower respiratory tract symptoms 147
Table 7.8 Summary of viral diagnosis 156
Table 7.9 Positive virus diagnosis in interferon and placebo treatment groups 157
Table 7.10 Adverse effects reported in interferon and placebo groups 165
Table 8.1 Outcome of the 16 documented episodes of respiratory tract infection in children with cystic fibrosis 177
Table 9.1 Demographic characteristics of the 127 centres analysed 182
Table 9.2 Means of initiating influenza vaccine programmes for non-institutionalised patients by 127 practices in Trent Region 184
Table 9.3 Factors associated with an improved rate of immunisation among patients aged 65 and older 187
Table 10.1 Timing of influenza vaccination 193
Table 10.2 General Practitioner consultations of asthmatic patients with influenza-like illness 197
Table 10.3 Protective influenza HI antibody titres and reciprocal geometric mean titres in vaccinated and unvaccinated asthmatics with and without symptoms of influenza-like illness 200
Table 10.4 Convalescent HI titres against influenza A/England/308/89 H3N2 and A/Shanghai/11/87 H3N2 in vaccinated and unvaccinated patients with and without symptoms of influenza-like illness 203
Table 10.5 Results of virus isolation and serology in previously healthy adults with upper respiratory tract infections 206
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS</td>
<td>Acquired immune deficiency syndrome</td>
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<tr>
<td>BME</td>
<td>Eagle's basal media</td>
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<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
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<tr>
<td>CBP</td>
<td>Cap binding protein</td>
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<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
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<tr>
<td>CFT</td>
<td>Complement fixation test</td>
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<tr>
<td>CFTR</td>
<td>Cystic fibrosis transmembrane conductance regulator protein</td>
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<tr>
<td>CMV</td>
<td>Cytomegalovirus</td>
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<tr>
<td>COAD</td>
<td>Chronic obstructive airways disease</td>
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<td>CPE</td>
<td>Cytopathic effect</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>DDW</td>
<td>Double distilled water</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>FCA</td>
<td>Fluorescein conjugated antibody</td>
</tr>
<tr>
<td>FCS</td>
<td>Foetal calf serum</td>
</tr>
<tr>
<td>FEV1</td>
<td>Forced expiratory volume in 1 second</td>
</tr>
<tr>
<td>FVC</td>
<td>Forced vital capacity</td>
</tr>
<tr>
<td>GP</td>
<td>General practitioner</td>
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<tr>
<td>HA</td>
<td>Haemagglutination</td>
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<td>HBV</td>
<td>Hepatitis B virus</td>
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<tr>
<td>HI test</td>
<td>Haemagglutination inhibition test</td>
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<tr>
<td>ICAM-1</td>
<td>Intercellular adhesion molecule 1</td>
</tr>
<tr>
<td>IgA, IgG</td>
<td>Immunoglobulin A, immunoglobulin G, etc.</td>
</tr>
<tr>
<td>IM</td>
<td>Intramuscular</td>
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<tr>
<td>IV</td>
<td>Intravenous</td>
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<tr>
<td>kD</td>
<td>Kilodaltons</td>
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<tr>
<td>LFA-1</td>
<td>Lymphocyte function associated molecule 1</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>MDCK</td>
<td>Madin-Darby canine kidney</td>
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<tr>
<td>MRC</td>
<td>Medical Research Council</td>
</tr>
<tr>
<td>MS</td>
<td>Multiple sclerosis</td>
</tr>
<tr>
<td>MU</td>
<td>Million International Units (of interferon)</td>
</tr>
<tr>
<td>MWt</td>
<td>Molecular weight</td>
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<tr>
<td>OSD</td>
<td>Optimal sensitising dose of haemolytic serum in CFT</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PHLS</td>
<td>Public Health Laboratory Service</td>
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<tr>
<td>RDE</td>
<td>Receptor destroying enzyme</td>
</tr>
<tr>
<td>RF</td>
<td>Replicative forms</td>
</tr>
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<td>RSV</td>
<td>Respiratory syncytial virus</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SSPE</td>
<td>Subacute sclerosing panencephalitis</td>
</tr>
<tr>
<td>TPBS</td>
<td>Tween Phosphate buffered saline</td>
</tr>
<tr>
<td>URTI</td>
<td>Upper respiratory tract infection</td>
</tr>
</tbody>
</table>
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The studies described in this thesis would not have been possible without the guidance and assistance of many people. My initial interest in respiratory virus infections stemmed from discussions with Dr KG Nicholson, following which I accepted the post of Research Registrar at Groby Road Hospital.

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Declaration of work done by the author

The interferon trial protocol was written prior to my arrival in Leicester, however I devised the patients booklets and record cards, recruited and examined all patients, documented all episodes, took all the specimens and analysed the results. I performed virus isolations in cell culture and coronavirus ELISA tests on specimens collected during 1986-87 at the Common Cold Unit with technical assistance from Mrs M Forsythe and Mrs K Brown. Virology on most of the specimens collected during 1987-88 was carried out by Dr Nicholson's technician Mrs J Kent in Leicester, although I did perform a number of the virus isolations and complement fixation tests.

I was responsible for the recruitment of healthy volunteers at Leicester University with assistance from Mrs J Kent. The preliminary study of cystic fibrosis children was organised by myself, Dr Nicholson, Dr Tanner, Mrs Kent and Sister L Wilde who visited children and obtained specimens. Virological analysis, including cell culture, ELISA and immunofluorescence was performed in our laboratories by Mrs J Kent and myself.

The survey of Trent General Practitioners was conceived prior to my arrival in Leicester by Dr Nicholson and Dr A May, however I assisted with questionnaire and telephone surveys of General Practitioners and the analysis and interpretation of the results. I organised the study of asthmatic patients with assistance from Dr Nicholson, Dr Stern and the staff of the Asthma and Allergy Research Unit. I analysed the questionnaire results and took bloods with help from Mrs J Kent and Dr G McHugh. Haemagglutination inhibition titres were determined in our laboratory by Mrs J Kent and myself.
Publications arising from this work


A. INTRODUCTION
Viruses which replicate in the respiratory tract include members of the picornaviridae, coronaviridae, orthomyxoviridae, paramyxoviridae and adenoviridae families. The clinical manifestations depend upon the location of infection. "Upper respiratory tract infections" include rhinitis, tonsillitis, pharyngitis, laryngitis and otitis media and "lower respiratory tract infections" include tracheitis, bronchitis, bronchiolitis, pneumonitis and pneumonia.

The "common cold" is the most frequent manifestation of respiratory virus infection and is characterised by nasal secretion, nasal obstruction, pharyngeal soreness, cough and headache. It was recognised by Hippocrates who noted that bleeding was a worthless treatment (Murphy and Webster, 1985). The infectious nature of the cold was suggested by the fact that people at sea for long periods did not suffer from colds until they re-established contact with other humans (Couch, 1985). Early studies demonstrated that colds could be induced in volunteers inoculated intranasally with secretions from symptomatic patients (Foster, 1916) and transmission occurred if bacteria-free filtrates were used (Dochez et al, 1930) indicating that the causative agent was a virus. Initial difficulties in isolating the infectious agent from nasal secretions in tissue culture were overcome by Price (1956), who successfully isolated members of the picornavirus family in rhesus monkey kidney cells. In 1960 Tyrrell et al observed a cytopathic effect when human or monkey kidney cells were inoculated with nasal washings from volunteers with colds and incubated at 33°C under slightly acid conditions. These studies established the conditions required for successful isolation of rhinoviruses in susceptible tissue culture cell lines and more than 100 distinct serotypes of rhinovirus have subsequently been identified (Couch, 1985).
Human coronaviruses were isolated from patients with symptoms of the common cold by Tyrrell and Bynoe (1965) using organ cultures. Hamre and Procknow isolated an identical virus in 1966 and this strain was designated 229E. The name coronavirus was chosen because of the characteristic crown-like appearance of the virus surface under electron microscopy (Tyrrell et al, 1968). A second human coronavirus which was antigenically distinct from 229E was identified by McIntosh et al (1967) and designated OC43. Subsequent serological surveys have shown that coronaviruses cause 15-34% of common colds (McIntosh, 1985).

Influenza virus, an orthomyxovirus, is responsible for worldwide, periodic, sharply-defined outbreaks of respiratory infection. A characteristic epidemic was recorded by Hippocrates in 412 BC and epidemics were reported at periods throughout the middle ages (Murphy and Webster, 1985). These historical accounts document several features which are still recognised today; namely the occurrence of epidemics at frequent but irregular intervals, the high morbidity and mortality of infection in the elderly and the characteristic westward geographical spread of epidemics from China and Asia to Europe. The pandemic of 1918-19 was particularly severe and is estimated to have killed 20-40 million people worldwide (Murphy and Webster, 1985). In 1931 Shope discovered that swine influenza could be transmitted by filtered mucus and human influenza virus (influenza A) was first isolated in ferrets by Smith et al in 1933. The identification of influenza A was followed by the isolation and characterisation of influenza B (Francis, 1940) and influenza C (Francis, 1950). The use of inactivated influenza virus vaccine was pioneered by Salk and colleagues (1945a, 1945b) and remains the basis of current attempts to control the disease.

Respiratory syncytial virus, a member of the paramyxovirus family, was first isolated from a laboratory chimpanzee with upper respiratory symptoms (Morris et al, 1956). An identical virus was recovered from children with respiratory tract infections, and serological studies showed that the majority of children were infected during the first three years of life (Chanock and Finberg, 1957). The
related parainfluenza viruses were first isolated from infants with respiratory tract infections using human and monkey kidney cell cultures (Chanock et al, 1959; Chanock and McIntosh (1985). Parainfluenzaviruses were subsequently shown to be important causes of respiratory tract infections in infants and young children. Adenoviruses were first reported by Rowe et al (1953) who were attempting to develop tissue culture lines derived from surgically removed human adenoid tissue and found that the epithelioid cells were being disrupted by a transmissible infective agent. Adenoviruses were later recognised as common causes of human respiratory disease (Dingle and Langmuir, 1968).

The common cold is the most frequent infection encountered by humans in the developed world with an estimated frequency of between 2 and 5 colds/person annually (Couch, 1984). Although respiratory virus infections are generally mild in healthy individuals the resulting economic impact is substantial and evidence from the USA suggests that acute respiratory illness is responsible for 36% of time spent absent from work (Couch, 1985). The increased severity of respiratory virus infections in patients with chronic respiratory disease was recognised by Maimonides c1170 AD who wrote, "I conclude that this disorder (asthma) starts with a common cold, especially in the rainy season, and the patient is forced to gasp for breath day and night" (Frick, 1983).

The impact of respiratory virus infections in patients with chronic chest disease forms the basis of this thesis. Strategies which may alleviate the effect of virus infections include the use of antiviral agents for prophylaxis or treatment and influenza vaccination. This thesis compares the severity of respiratory virus infections in previously healthy subjects and patients with chronic chest disease. A series of studies are described which investigate the distribution and efficacy of influenza vaccine and antiviral chemoprophylaxis with intranasal interferon in patients with chronic chest disease.
CHAPTER 2
HUMAN RESPIRATORY VIRUSES

1. Classification, structure and properties

Rhinoviruses and enteroviruses belong to the picornavirus family. The name picornavirus comes from *pico* meaning very small and RNA. The genus *rhinovirus* contains over 100 serologically distinct strains which are associated with symptoms of the common cold. The genus *enterovirus* includes polioviruses types 1-3, coxsackievirus A (23 serotypes), coxsackievirus B (6 serotypes), echovirus (31 serotypes), and enteroviruses (5 serotypes), (Melnick, 1985). Of the enteroviruses only coxsackieviruses and echoviruses are commonly associated with respiratory symptoms (Rueckert, 1985).

All picornaviruses have a similar structure and are symmetrical, approximately spherical viruses of size 25-30nm (Macnaughton, 1982). There is no lipid envelope and the virus consists of an RNA core surrounded by a protein coat (Macnaughton, 1982). Approximately 30% of the weight of the virus consists of RNA (Rossmann et al, 1985). The full genetic sequence of several picornaviruses have been determined over the past decade (Racaniello and Baltimore, 1981; Carroll et al, 1984; Stanway et al, 1984; Hughes et al, 1988; Skern et al, 1985; Hughes et al, 1987). Comparison of the resulting amino acid sequences has revealed considerable diversity between different rhinovirus serotypes with 60% overall conservation of VP1 amino acid sequence between HRV-89 and HRV-2 but only 34% conservation between HRV-2 and HRV-14 (Skern et al, 1987a). Certain domains have been found to be highly conserved in all serotypes and are thought to be responsible for virus function and receptor binding.

All picornaviruses have nucleic acid in the form of single stranded RNA which is polyadenylated at the 3' end. The poly A sequence is believed to be an important factor determining virus infectivity (Spector and Baltimore, 1974). The 5' end of the RNA is attached to a small protein known as VPg which is not required for infectivity but
may anchor RNA to the endoplasmic reticulum during replication (Skern et al., 1987a). Translation of the virus RNA occurs in a rather unusual fashion. The virus RNA genome consists of only one long reading frame which codes for a large polyprotein. This polypeptide is then cleaved to form 11 or 12 smaller proteins which include coat protein subunits, proteases which mediate the polypeptide cleavage and enzymes involved in RNA synthesis and elongation (Rueckert, 1985).

The coat protects the RNA genome of the virus and recognises receptor sites on host cells. The virus RNA is situated in the core of a protein shell formed out of four polypeptide chains VP1-4 (Medappa et al., 1971; Talbot and Brown, 1972). VP4 + VP2 are formed by cleavage of a larger precursor peptide VP0 (Jacobson and Baltimore, 1968). X-ray diffraction studies of poliovirus crystals have shown that the virus coat is formed by 60 protein sub-units with an icosahedral symmetry (Finch and Klug, 1959; Arnold et al., 1984). Dissociation of the coat using acid or urea reveals that the sub-units form pentamers which are assembled in groups of 12 (Dunker and Rueckert, 1971). The RNA is asymmetrical and is inserted randomly into the central cavity of the virus (Rossmann et al., 1985). VP1-3 have epitopes exposed at the surface which may be identified by reacting antibodies (Dernick et al., 1983), whereas VP4 is situated internally, does not occur in empty capsid preparations (Johnston and Martin, 1971) and is bound closely to the RNA core.

Studies with neutralising monoclonal antibodies have shown that rhinovirus 14 has four epitopes involved in neutralisation (Skern et al., 1987b; Colonno et al., 1989). These epitopes correspond to short discrete amino acid sequences situated on a series of exposed peptide loops with a large degree of amino acid variability between serotypes (Strohmaier et al., 1982; Minor et al., 1983; Skern et al., 1987a; 1987b; Greve et al., 1989). Neutralising antibodies interfere with the binding of virus to receptor and lead to virus aggegation (Colonno et al., 1989).

A common feature of picornaviruses appears to be the presence of a deep cleft or canyon on the viral surface approximately 25Å deep and 12-30Å wide, lying between protrusions on 5 VP1 subunits on one side and and VP2 and VP3 on the other
(Rossmann et al, 1985; Palmenburg, 1987). The amino acid residues in this region are highly conserved and it is thought to be the host receptor binding site (Colonno et al, 1988; Greve et al, 1989; Pevear et al, 1989). The dimensions of the canyon are such that a Fab fragment (35Å) would be too large to fit into the cleft and the receptor site is therefore inaccessible to antibodies (Staunton et al, 1989).

Two serotypes of coronavirus commonly cause human respiratory disease; these have been designated OC43 and 229E. Coronavirus size varies between 60-220nm in diameter. The spike protein is formed by trimerisation of monomer subunits (Delmas and Laude, 1990) and has several important properties; it binds to host cell receptors, has the ability to cause cell fusion, is the main antigen recognised by neutralizing antibodies and facilitates the intracellular budding of new virions (Vennema et al, 1990). The inside of the virus consists of a helical ribonuclear protein connected to a transmembrane glycoprotein with a genome consisting of a single linear molecule of positively stranded RNA which is capped, polyadenylated and has a molecular weight of approximately 6 million.

Three types of influenza virus are recognised, influenza A, B and C (WHO, 1980). These are distinguished on the basis of nucleoprotein and matrix antigens. Influenza viruses are named according to their antigenic description, host of origin (for non-human isolates), geographical origin, strain number and year of isolation (eg A/Seal/Mass/1/80). Influenza viruses possess two main antigenic determinants the haemagglutinin (H antigen) and neuraminidase (N antigen). Influenza A viruses are further classified according to the antigenic subtypes (eg H1N1). There are 12 recognised haemagglutinin subtypes and 9 neuraminidase subtypes.

All three types of influenza virus are morphologically similar and appear roughly spherical after passage in culture, although freshly isolated clinical strains may be more variable in shape. The influenza virus consists of eight segments of single-stranded RNA which code for seven structural proteins and at least three non-structural proteins (Klenk and Rott, 1988). Electron microscopy has shown that the virus is surrounded by haemagglutinin and neuraminidase in the
approximate proportion of 5:1 (Wrigley, 1979; Kendal, 1987). X-ray crystallography studies have shown that each haemagglutinin spike is formed from a trimer containing three copies of each subunit with a hydrophobic sequence anchoring the spike to the cell membrane (Klenk and Rott, 1988). The neuraminidase spike is composed of a tetramer formed out of four roughly spherical subunits (Klenk and Rott, 1988) with a central stalk embedded in the membrane (Laver and Valentine, 1969). Neuraminidase has been crystallised allowing the three-dimensional structure to be assessed and the technique of site-directed mutagenesis has been used to define the nature of important epitopes (Air and Laver, 1989). Neuraminidase and haemagglutinin are both attached to a lipid envelope beneath which lies the structural matrix (M1) protein which forms a shell enclosing the virus core. A second protein M2 is a membrane protein which is expressed at the cell surface. A further two non-structural proteins NS1 and NS2 are coded by the genome but the functions of these are uncertain (Klenk and Rott, 1988).

The core of the virus consists of 8 ribonucleoprotein complexes (Compans et al, 1972) each consisting of one molecule of single-stranded RNA together with one or more copies of three polymerase polypeptides and multiple copies of nucleoprotein (Kendal, 1987). The nucleocapsid protein is expressed on the surface of infected cells and is believed to play an important role in cell-mediated immunity (Klenk and Rott, 1988). As each influenza virus contains 8 ribonucleoprotein gene segments, reassortment between two distinct viruses co-infecting the same host allows the possibility of up to 256 possible recombinations to occur. Although the haemagglutinin and neuraminidase components are variable and undergo antigenic change the core proteins of the virus remain stable.

It is thought that antigenic drift occurs by the accumulation of random point mutations in the genetic code of the influenza virus which lead to significant alterations in the amino-acid composition of the haemagglutinin and neuraminidase (Curry et al, 1974; Gerhard et al, 1981; Yewdell et al, 1981; Webster and
Amino acid substitutions found in naturally occurring viruses have been shown to involve T-cell epitopes and antigenic binding sites identified by sequence analysis of influenza haemagglutinin and epitope mapping techniques (Wiley et al, 1981; Barnett et al, 1989). These studies therefore provide molecular evidence for the antigenic diversity generated by antigenic drift (Barnett et al, 1989). The antigenic changes associated with antigenic shift are too great to be accounted for simply by a process of mutation. Various hypotheses have been forward to explain this phenomenon (Lancet editorial, 1988), including reintroduction of latent viruses (Oxford, 1987), or historical strains of virus which have been lying dormant in frozen fresh-water (Nakajima, 1978; Nakajima et al, 1978) and periodic reappearance of virus particles preserved in space (Henderson et al, 1987; Hoyle and Wickramasinghe, 1990a; 1990b). A more plausible explanation is that antigenic shift results from a genetic reassortment of virus between humans and the animal reservoir (Kendal, 1987; Scholtissek and Naylor, 1988). Farming practices which allow close proximity between humans, ducks and pigs are common in South East Asia and may facilitate this process.

The paramyxovirus family is sub-divided into three genera which all infect the respiratory tract; Pneumovirus includes respiratory syncytial virus (RSV), Paramyxovirus includes the human parainfluenza viruses and mumps virus and Morbillivirus includes measles virus. RSV is antigenically heterogeneous, however differences between strains are considered to be of little practical importance as they cannot be distinguished with available serological reagents (Talis and McIntosh, 1991). There are four serotypes of parainfluenza (types 1-4) and type 4 is subdivided into the closely related subtypes 4a and 4b.

All paramyxoviruses possess a lipoprotein envelope and are roughly spherical in shape with a diameter between 100-300nm. Two glycoproteins are found in the envelope. The HN glycoprotein is responsible for both haemagglutin and neuraminidase activity in most strains, however measles virus and RSV do not
possess neuraminidase activity. The F protein or fusion protein is essential for cell penetration and is responsible for the fusion of infected cells which gives rise to the characteristic syncytia (Stott and Taylor, 1985). The F protein is also the major site recognised by neutralising antibodies (Vainionpaa et al, 1989). The lipoprotein envelope is delicate and paramyxoviruses are very sensitive to the effects of heat or dessication. The virus nucleocapsid is a single helix approximately 1μm long. The genome consists of a single molecule of single stranded RNA of negative polarity and the nucleocapsid contains an RNA transcriptase. The nucleic acid sequence has recently been determined for several members of the paramyxovirus family (Kawano et al, 1990; Kondo et al, 1990).

At least 41 human adenoviruses have been described (Horwitz, 1985), the precise classification of these is disputed although they appear to fall into 6 or 7 separate subgenera (Wadell, 1984). Adenoviruses are icosohedral in structure, approximately 80nm in diameter with an outer capsid composed of 252 capsomeres. The capsid is formed out of 12 pentagon shaped "pentons" which form the vertices and 240 "hexons" which make up the 20 triangular faces (Philipson, 1983). Long fibres with terminal knobs project from each of the pentons. The virus does not possess an outer membrane and is therefore ether resistant. There is an inner protein core with helical symmetry containing the genome which consists of a single linear molecule of double stranded DNA of MWt 20-25 million with inverted terminal repetition sequences. A virus-coded terminal protein is attached to each of the 5' termini (Leith et al, 1989).

2. Epidemiology

Several long-term, community-based, epidemiological studies have investigated the frequency of virus isolations in American children and adults with respiratory symptoms (Gwaltney et al, 1966; Hamre et al, 1966; Fawzy et al, 1967; Monto et al, 1971; Fox et al, 1985; Monto et al, 1987). These studies have yielded valuable epidemiological information and have shown that human rhinoviruses are...
responsible for 42-80% of all positive virus isolations with a peak of infection in September and a smaller rise in the Spring (Gwaltney et al, 1966; Monto and Cavallaro, 1971). The rhinovirus isolation rate is highest in infants under the age of one year. In this group up to 1.21 proven infections/person/yr have been reported (Cooney et al, 1972). The isolation rate slowly declines until adult life with a slight increase in the 20-30 year age group, particularly amongst females which is thought to be due to their contact with young children (Cooney et al, 1972; Hamre et al, 1966; Gwaltney et al, 1967; Monto and Ullman, 1974).

Rhinovirus infections are not confined to temperate climates but have a worldwide distribution (Taylor-Robinson, 1965). Respiratory virus infection in the tropics often coincides with the rainy season (Monto and Johnson, 1968). Epidemiological studies have shown that many serological types of rhinovirus may circulate in a given population during the same season (Gwaltney, 1984) and over 48 different serotypes of rhinovirus were identified during a three year study of respiratory virus infections in young adults in Charlottesville, USA (Gwaltney, et al 1967). There is a gradual change in the prevalence of serotypes over time (Calhoun et al, 1974; Fox, 1976), however new types of rhinoviruses are unlikely to be evolving at a rapid rate as Hamparian (1987) recently showed that 90% of current rhinovirus isolates in three locations could be typed with antisera to types 1-89 which were all recognised before 1973. Rhinoviruses are highly species specific and animal reservoirs do not play an important role in the epidemiology of infection (Couch, 1986).

Enterovirus infections are comparatively rare causes of respiratory tract symptoms and were responsible for only 5% of virus isolations from patients with respiratory symptoms in the Tecumseh community study (Monto et al, 1971). Coxsackieviruses A2, A10, A21, A24 and B2-B5 have been isolated from children and adults with respiratory tract infections and Coxsackie A21 has been associated with epidemics of pharyngitis in military recruits (Melnick, 1985). Echoviruses types 1, 11, 19, 20, and 22, and Enteroviruses types 68 and 71 have all been
implicated in episodes of respiratory illness (Melnick, 1985). Enterovirus colds are indistinguishable from those caused by rhinoviruses, however they appear to be more common in the tropics and they have a different seasonal pattern with infections occurring most frequently during the summer and autumn (Monto et al, 1971; White and Fenner, 1986).

Human coronaviruses are responsible for up to 25% of cases of the common cold and have been implicated in the aetiology of viral gastroenteritis and multiple sclerosis (Jouvenne et al, 1990). Seroprevalence studies have shown that coronaviruses have a worldwide distribution and antibodies to 229E and OC43 have been detected by ELISA in 86-100% adults tested in England and Iraq (McIntosh, 1985). Antibodies first appear in early childhood and the prevalence increases rapidly with age (McIntosh et al, 1970). The gradual waning of antibody titres and antigenic heterogeneity of human coronaviruses allows reinfection to occur throughout life (McIntosh, 1985). A study of sequential sera taken from adults in London between 1976-1981 found that most coronavirus infections occurred during two periods, from June-September or December-February (Macnaughton, 1982). Outbreaks generally occur every 2-4 years, these are due to either 229E or OC43 strains as they are seldom present in the community at the same time (Monto, 1984). Coronaviruses are important animal pathogens and infect the respiratory tract, gut and liver of susceptible species including rodents, chickens, swine, dogs, cats and cattle. The species specificity of coronaviruses and the importance of the potential animal reservoir of infection are still unclear (McIntosh, 1985).

Influenza viruses account for up to 15% of positive virus isolations in the population studies based in the USA (Gwaltney et al, 1966; Hamre et al, 1966; Fawzy et al, 1967; Monto et al, 1971; Fox et al, 1985; Monto et al, 1987). Influenza A virus has the unique ability to cause periodic worldwide pandemics of infection. This is believed to be the result of its high infectivity and ability to undergo antigenic change. Antigenic changes in the influenza virus can take place by two mechanisms, known as "antigenic drift" and "antigenic shift". Antigenic drift
results from an accumulation of minor changes in the surface structure of the virus, whereas antigenic shift is due to a sudden major change in the epidemic strain of influenza A. In 1918 the predominant human strain was H1N1 which was replaced by H2N2 in 1957 and H3N2 in 1969. H1N1 reappeared in 1977. With such long intervals between antigenic shifts the new strains encounter a largely non-immune population and cause global pandemics. There is evidence for this process having occurred for many centuries and the new strains of virus all appear to originate in the Far East and spread rapidly westward (Davenport, 1984). Outbreaks of influenza occur annually (Monto et al, 1985) but major epidemics of influenza A are caused by the appearance of a new antigenic strain. Epidemics may be heralded by a few small, isolated, outbreaks in the population as the virus establishes itself (Langmuir, 1961; Glezen, 1982). These are followed by a very steep rise in the number of cases reaching a peak after a period of 3-4 weeks and then gradually declining over the following 3-4 weeks with the whole outbreak lasting approximately 2 months. Smaller epidemics recur at varying intervals, generally every 2-3 years and sporadic cases are also reported. Epidemics of influenza B are less devastating and are seen at intervals of 4-7 years. Sporadic cases of infection may be caused by influenza C. The weekly incidence of influenza in England and Wales is estimated by the number of positive diagnoses of influenza virus infection recorded by Public Health Laboratories (which will only represent a very small fraction of the total number of affected people), and by the system of "spotter" General Practitioners who operate a surveillance scheme for influenza and other designated infections (Campbell et al, 1988; Fleming and Ayres, 1988).

During an influenza epidemic the most susceptible population group are young children, particularly those over 5 years of age (Monto et al, 1985). This group is usually the first to be affected as they have not previously encountered the virus and are readily exposed at school (Davenport, 1984; Cate, 1987). Adult absenteeism, hospital admissions and influenza-related deaths reach a peak later in the epidemic. Mortality is not usually a direct result of influenza infection, but the 1918-1919
epidemic was caused by a particularly virulent strain which gave an increased mortality, even in younger adults. The mortality attributed to influenza infection usually occurs in elderly or compromised individuals with a background of pre-existing disease and may be estimated by the excess mortality observed in a population during an influenza outbreak (Langmuir and Housworth, 1969; Glezen, 1982; Monto, 1987b; Curwen et al, 1990). In this way it has been estimated that influenza in the USA leads to approximately 10,000 excess deaths during non-epidemic years and kills at least 30,000 people during epidemics (Mostow, 1986). Similar calculations indicate that the 1989-90 influenza epidemic in England and Wales was responsible for approximately 26,000 excess deaths (Curwen et al, 1990). Influenza-associated deaths usually result from respiratory complications (Alling et al, 1981) and 80-90% occur in patients over the age of 64 (Lui and Kendal, 1987). The annual cost of influenza in the USA has been estimated at billions of dollars (Kavett, 1977; Schoenbaum, 1987) and influenza is the only illness which has the capacity in modern times to infect a large proportion of the population and lead to the temporary closure of factories or schools.

Influenza viruses have been isolated from many different species including ducks, pigs, horses, mink, whales and seals (Schnurrenberger et al, 1970; Hinshaw et al, 1978; Murphy and Webster, 1985; WHO Memorandum, 1987). Ferrets are susceptible to strains isolated from human patients and have become a useful animal model of disease and immunity (Potter et al, 1973; Smith and Sweet, 1988). Primates may also be experimentally infected and laboratory infection can be induced in the mouse using adapted strains of influenza virus (Raut et al, 1975).

RSV was responsible for 8% of positive virus isolations in the Tecumseh community study (Monto et al, 1971). The incidence of RSV infection is highest in infants between the ages of six weeks and six months (McIntosh and Chanock, 1985). RSV causes bronchiolitis and croup in infants and is the major cause of hospitalisation and infant deaths in the western world (Henderson, 1987; Anderson et al, 1990a; Heilman, 1990). Most children develop antibodies to RSV in the first
two years of life, however reinfections may occur throughout later life (Talis and McIntosh, 1991). Reinfections are generally less severe and usually result in symptoms of the common cold (McIntosh and Chanock, 1985), however, there is increasing evidence implicating RSV as a major pathogen in residential institutions for the elderly (BMJ editorial, 1983a; Morales et al, 1983) where it has been the cause of serious outbreaks of infection with a documented mortality of approximately 20% (Hart, 1984; Sorvillo et al, 1984). The epidemiology of RSV infection shows sharply defined annual winter epidemics. The incidence of infection is higher in industrial and urban areas, probably as a consequence of overcrowding (Stott and Taylor, 1985). RSV and influenza virus may co-exist during the same season and give rise to identical symptoms (Mathur et al, 1980; Morales, 1983; Falsey et al, 1990; Nicholson et al, 1990). Although a wide range of animal species, including primates and rodents can be experimentally infected with RSV only the chimpanzee and owl monkey have been shown to develop symptoms and RSV is therefore thought to have a narrow host-specificity (McIntosh and Chanock, 1985).

Parainfluenzaviruses have a worldwide distribution and infection is particularly frequent in infants below the age of 6 months (Henderson, 1987). Reinfection and may occur at any age and outbreaks of infection occur in families and have been recorded in residential homes for the elderly (BMJ editorial, 1983b; Chanock and McIntosh, 1985). Parainfluenzaviruses were responsible for over 20% of virus isolations in the Tecumseh community study (Monto et al, 1971). Parainfluenzaviruses 1 and 2 give rise to well defined autumn outbreaks in alternate years (Anderson et al, 1990a). Epidemics of parainfluenza 3 occur annually in Britain with a peak in the summer months (Easton and Eglin, 1989). A number of rodents are susceptible to RSV infection, including hamsters, guinea pigs, rats and ferrets, however infection in these hosts is usually asymptomatic (McIntosh and Chanock, 1985).
Adenoviruses occur worldwide and cause a wide variety of clinical manifestations of which the most common are upper respiratory tract infections. Approximately 5% of respiratory tract infections in children under the age of five are caused by adenoviruses (Horwitz, 1985) and adenoviruses were responsible for 3% of positive virus isolations in the Tecumseh community study (Monto et al., 1971). Adenovirus infections may be sporadic or epidemic and longitudinal studies have shown a high incidence of asymptomatic infections (Brandt et al., 1969). The most frequent symptoms of adenovirus infection are nasal congestion, coryza and cough, which may be caused by serotypes 1-7, 14, and 21 and are often indistinguishable from other causes of the common cold (Horwitz, 1985). If conjunctivitis occurs in addition to pharyngeal symptoms the illness is known as pharygoconjunctival fever. Adenoviruses are also associated with epidemics of pneumonitis in new military recruits (types 3,4,7), and epidemic conjunctivitis (types 8,11,19,37) (Brandt et al., 1969; Hurwitz, 1985). Seroprevalence studies have shown that 40-60% of children have antibodies to types 1,2 and 5, however the incidence of antibodies to other types is lower in children, hence adenovirus type 3, 4 and 7 infections are more common in adults (Brandt et al., 1969). Persistent shedding of adenoviruses by infected individuals contributes to the spread of infection. Adenoviruses have a high degree of species specificity and animal adenoviruses are not pathogenic to humans, although antibodies to simian, canine and bovine viruses have been detected in human sera (Hurwitz, 1985).

3. Transmission

Transmission of rhinoviruses occurs readily and is related to close personal contact (Holmes et al., 1976). The infectious dose of virus needed to produce infection in susceptible volunteers is small (HID$_{50}$ for rhinovirus =0.032 TCID$_{50}$ in WI-56 human embryonic fibroblast cells) (Couch et al., 1966). The epidemiology of rhinovirus infection suggests droplet spread with infection moving slowly through a population rather than the sudden epidemics of infection caused by viruses spread by
aerosol eg influenza (Douglas, 1970). Several studies have indicated that virus is transmitted via the hands rather than through coughing or sneezing (Hendley et al, 1973; Gwaltney et al, 1978), however Dick et al, (1987) found that aerosol spread was the most important mechanism when they investigated the separate contribution of aerosols, direct contact and fomites. Enteroviruses replicate predominantly in the gastrointestinal tract and are often excreted by asymptomatic individuals (Melnick, 1984). The most important routes of infection are droplet spread and the faecal-oral route (Melnick, 1984).

Coronaviruses are transmitted by the respiratory route and susceptible volunteers have been experimentally infected by intranasal inoculation (Monto, 1984). It is thought that aerosol and droplet spread are both important factors in the spread of coronavirus infection (Monto, 1984).

Transmission of influenza is believed to be primarily due to airborne droplet spread (McLean, 1961; Alford et al, 1966) and close person-to-person contact (Lancet editorial, 1988). Virus may be isolated from both the upper and lower respiratory tract. Peak recovery of virus occurs at 2 days after experimental or naturally occurring infection with recovery rates declining slowly over the following week (Frank et al, 1981).

RSV and parainfluenza viruses are readily transmitted by droplet spread and nosocomial outbreaks of infection have been recorded in paediatric wards and homes for the elderly (McIntosh and Chanock, 1985). Infected infants may continue to shed virus for several weeks (McIntosh and Chanock 1985).

Adenoviruses are highly contagious with droplet, faeco-oral, fomite and venereal transmission causing rapid spread amongst family members and institutional outbreaks (Ruuskanen et al, 1988). Following acute infection adenoviruses may persist for years in adenoid and tonsillar tissue and be excreted in the faeces (Horwitz, 1985).
4. Pathogenesis

The host-specificity and tissue tropism of picornaviruses is related to the expression of specific receptors on susceptible cells (Holland 1961; Macnaughton 1982). All polioviruses appear to share a single receptor. 90% of rhinoviruses share a receptor known as the 'major' receptor with the remaining serotypes recognising a separate 'minor' receptor (Abraham and Colonna, 1984; Staunton et al, 1989). Receptor binding is temperature sensitive and depends on the ionic composition of the medium (Holland and McLaren, 1959; Lonberg-Holm and Whiteley, 1976; Macnaughton, 1982). The major rhinovirus receptor was found to be a 90-Kd glycosylated surface protein (Tomassini et al, 1989) and was subsequently shown to be identical to the intercellular adhesion molecule-1 (ICAM-1) (Greve et al, 1989; Staunton et al, 1989; Lineberger et al, 1990). ICAM-1 is a member of the immunoglobulin supergene family and consists of a 55Kd polypeptide chain with variable complex N-linked carbohydrate groups attached (Staunton et al, 1989). It has been cloned and sequenced and found to contain 5 immunoglobulin-like domains (Staunton et al, 1989). The unpaired immunoglobulin domain is of the correct size to fit into the rhinovirus canyon. The expression of ICAM-1 on host cells is strongly enhanced by cytokines including interferon gamma, tumour necrosis factor and interleukins (Staunton et al, 1989). Less is known about the minor rhinovirus receptor but preliminary studies suggest that it has an apparent MWt of 450 KDa and is therefore likely to consist of a number of subunits (Mischak et al, 1988).

The attachment of the virus particle to the host cell receptor mediates an uncoating reaction with loss of VP4 protein leading to the virus RNA being released into the host cell (De Sena and Mandell, 1977; Smith et al, 1986). The entry of picornaviruses into the cell is achieved by a process of receptor mediated endocytosis and uncoating is thought to take place in the endosomal compartment (Mandel, 1965; Neubauer et al, 1987). Once the virus RNA strand enters the host cell it is translated into a single large precursor which is cleaved into smaller viral proteins.
(Pallansch et al, 1984). The entire polypeptide product has never been observed and it is likely that proteolysis begins before translation has been completed (Skern et al, 1987a) with the initial step involving self-cleavage leading to the production of a virus-coded protease which then continues the process of cleavage (Palmenberg and Rueckert, 1982; Cheah et al, 1988; Sommergruber et al, 1989). The viral protease activity results from the products of areas 2A and 3C of the picornavirus genome which are both sulphydryl proteases, although they have different cleavage sites (Skern et al, 1987a). Protease 2A is responsible for initial cleavage of the polyprotein whereas all other cleavages (except that of VP0) are performed by the product of 3C (Libby et al, 1988; Sommergruber et al, 1989).

The enzymes and energy necessary for the translation reaction are provided by components of the host cell cytoplasm (Crocker et al, 1964). Within an hour of entry of virus into the cell, inhibition of host cell RNA synthesis is observed, followed by synthesis of viral RNA and protein. The mechanism by which virus is able to block synthesis of host protein but allow synthesis of its own viral protein is probably related to the presence of a 'capping' group at the 5' end of host mRNA which is not found on picornavirus RNA (Fernandez-Munoz and Darnell, 1976; Nomoto et al, 1976; Etchison et al, 1984). The virus RNA entering the cell is positively stranded (+) with a poly A tail. Virus replication occurs by the synthesis of negatively-stranded RNA (-) containing a poly U tail. The (-) strand then acts as a template for the synthesis of further (+) strands which are packaged into new virions. In poliovirus this reaction involves a specific viral RNA-dependent RNA polymerase (replicase) (Van Dyke et al, 1982), in addition, other factors including a host protein of MWt 67000 are required for initiation of RNA synthesis (Dasgupta et al, 1980; Morrow et al, 1985).

The assembly of new virus particles is a complex process and involves the cleavage of the large precursor polypeptide VP0 into VP2 and VP4. This reaction is tightly coupled to the insertion of RNA into the capsule shell however the precise mechanism by which this occurs is still uncertain (Putnak and Phillips, 1981).
Crystals of completed virus particles may be found in the cell at this stage and these are released as the cell finally disintegrates.

The nature of the host receptor for human coronaviruses is not known. Once coronavirus has entered the host cell positively stranded RNA is translated into several protein products including an RNA polymerase. The replication of coronaviruses is unusual as viral polymerase directs the synthesis of a full length copy of the viral RNA (-ve strand) which is transcribed into a "nested set" of at least 6 positively stranded overlapping sub-genomic mRNA's each of which extends a variable length from a common 3' terminal with the first being longer than the second etc. These mRNA fragments each direct the synthesis of one protein and are translated from the 5' end; however only the unique sequence that is not shared by the next smallest in the set is translated so that each mRNA translates into a separate protein (Spaan et al, 1988). Virus multiplication takes place in the cytoplasm and a surface envelope is acquired by budding through the membrane of the cisternae of the endoplasmic reticulum and golgi apparatus (Sturman and Holmes, 1983). Mature virus particles are then transported to the cell surface in vesicles.

Influenza haemagglutinin mediates attachment and fusion of the virus to cell surface receptor oligosaccharides containing sialic acid (Krystal et al, 1982; Webster and Rott, 1987d; Rogers and D'Souza, 1989). Haemagglutinin undergoes proteolytic cleavage at the time of binding to the host cell membrane. Cleavage is mediated by cellular proteases which convert the haemagglutinin trimer to mature haemagglutinin consisting of two polypeptide chains (HA1 and HA2) joined by a disulphide bond (Webster and Rott, 1987). Neuraminidase reduces self aggregation of the virus and reduces mucus viscosity allowing the virus to come into close proximity of the host cell. It also has the ability to cleave the haemagglutinin receptor on the host cell allowing elution of progeny virus particles from infected cells (Air and Laver, 1989). Influenza RNA is of negative (non-infectious) polarity and is transcribed by virion associated RNA polymerase into mRNA's which are then translated into virus proteins (Klenk and Rott, 1988).
Attachment of paramyxoviruses to host cells is mediated by the fusion (F) protein which becomes active after extracellular proteolytic cleavage. The F protein mediates fusion of the virus envelope with the host cell membrane allowing the virus to enter the cell directly (Vainionpaa et al, 1989). Virus RNA is transcribed in the cytoplasm and translated into virus proteins. RNA replication takes place and new virus nucleocapsids are synthesised in the cell cytoplasm. Newly formed viruses bud out from the cell surface, however the presence of HN and F protein on the cell surface can lead to fusion with adjacent cells causing syncytia formation. This process allows the virus particles to be passed from cell to cell without being released, thereby evading the action of circulating antibodies.

Adenoviruses penetrate the cell membrane directly and virus uncoating takes place in the cell cytoplasm (Horwitz, 1985). The virus core enters the nucleus where DNA replication takes place (Philipson, 1983). Virus DNA is transcribed to messenger RNA by host cell RNA polymerase and transcription takes place sequentially (Dimmock and Primrose, 1987). mRNA is synthesised from both strands of virus DNA. Genes coding for proteins involved in replication of viral nucleic acid (early genes) are transcribed first followed by genes coding for the structural proteins (late genes) (Moran and Mathews, 1987; Nevins, 1987; Hasson et al, 1989). Core proteins are synthesised late in infection and are believed to attach to viral templates, inhibiting replication and initiating packaging of virus genomes (Leith et al, 1989). New virions are assembled in the nucleus and form crystalline aggregates which are released after cell lysis.

5. Clinical manifestations
The incubation period for rhinovirus is 4-5 days (Douglas, 1986) and infection usually results in symptoms of the common cold with nasal secretion, nasal obstruction, pharyngeal soreness, cough and headache (Couch, 1985; Phillpotts and Tyrrell, 1985). Myalgia and gastrointestinal symptoms may be a feature of infection (Couch, 1985) and cough was found to be more pronounced and prolonged in smokers.
(Gwaltney et al, 1967). Symptoms are generally mild and reach a peak on the second and third days of illness. Recovery occurs after a median period of 7 days, although symptoms last up to two weeks in a quarter of cases (Gwaltney et al, 1967) and occasionally linger for even longer periods (Monto et al, 1987). Complications include otitis media (Arola et al, 1988), sinus infections (Evans et al, 1975), pneumonitis (Craighed et al, 1969; Halperin et al, 1983; Krilov et al, 1986) and secondary bacterial pneumonia (Cherry et al, 1967; George and Mogabgab, 1969; Monto et al, 1987b).

Enterovirus infection is most common in young children and usually gives rise to a non-specific illness with mild fever, malaise and exanthem. In adults enteroviruses may cause upper respiratory tract infections which are indistinguishable from the common cold. Coxsackie virus A21, coxsackie A24, echovirus 11 and echovirus 20 are most consistently associated with respiratory symptoms. Enteroviruses are associated with many other clinical manifestations (Melnick, 1985) including viral meningitis, hand foot and mouth disease, herpangina, acute haemorrhagic conjunctivitis, disseminated neonatal infection (Modlin, 1986; McKinney et al, 1987), myocarditis and cardiomyopathy, (Murray, 1988; Muir et al, 1989; Archard et al, 1987; Easton and Eglin, 1988; Lancet editorial, 1990a) and the chronic fatigue syndrome (Archer, 1987; Calder et al, 1987; Bell et al, 1988; Yousef et al, 1988; Yousef, 1989; Cunningham et al, 1990).

The clinical features of coronavirus infection have been defined by studies of experimental infection in healthy volunteers which have revealed an incubation period of 2-4 days (Monto, 1984). Coronavirus colds generally last about a week with prominent nasal symptoms and mild pyrexia and are indistinguishable clinically from those caused by rhinoviruses (Monto, 1984). Symptomatic subjects excrete virus for a period of 1-4 days (Monto, 1984).

The incubation period of influenza is typically 1-5 days and the illness often begins suddenly with headache, fever, dry cough, anorexia and malaise (Murphy and
Webster, 1985). These symptoms are followed by characteristic muscle pains and nasal stuffiness or rhinorrhea and a sore throat may also be present. Other common complications include otitis-media and conjunctivitis. The median duration of fever is 3 days but cough and malaise often persist for 1-2 weeks. Influenza is usually more severe than the common cold (Monto, 1987), although some individuals suffer only a mild or inapparent illness (Monto et al, 1985; Davies et al, 1986). Recent volunteer studies have shown that clinical and subclinical influenza infection is associated with impaired visual search performance on psychological testing (Smith et al, 1988).

Influenza is most severe in young children, the elderly, patients who are immunocompromised and those who have chronic medical conditions (Stuart-Harris, 1961; Glezen, 1980; Alling et al, 1981). Complications include pneumonitis (Louria et al, 1959; Martin et al, 1959; Wright et al, 1977), febrile convulsions in infants (Kim et al, 1979), Reye's syndrome (Hurwitz et al, 1982), severe myositis (Cunningham et al, 1979), myocarditis, and encephalopathy (Flewett and Holt, 1958). Influenza predisposes patients to bacterial colonisation, particularly with Staphylococcus aureus, Streptococcus pneumoniae and Haemophilus influenzae (Robertson et al, 1958; Murphy and Webster, 1985). Staphylococcus aureus pneumonia is particularly ominous and has a high mortality.

RSV infection occurs predominantly in infants and causes upper respiratory tract infection, bronchiolitis and pneumonia. Infection is particularly serious in infants with underlying cardiac or pulmonary abnormalities (McIntosh and Chanock, 1985). About 1-3% of cases admitted to hospital are fatal, although mortality may be as high as 37% in infants and older children with congenital heart disease (Stott and Taylor, 1985; Groothuis et al, 1990). RSV infection in later life is usually associated with symptoms of the common cold, but may be severe in the elderly (BMJ editorial, 1983a; Morales et al, 1983; Hart, 1984; Sorvillo et al, 1984).
Parainfluenzavirus infection is generally associated with a mild upper respiratory tract infection which is virtually identical to other causes of the common cold (Henderson, 1987). A minority of infections are more severe. In infants under the age of three months parainfluenza 3 may cause a bronchiolitis with pneumonia which is similar to RSV infection (Henderson, 1987). In infants aged 3-12 months parainfluenza types 1 and 2 cause laryngotracheobronchitis (croup).

Adenovirus infection in adults usually results in an illness resembling the common cold although epidemics of acute respiratory disease progressing to a more severe pneumonia have also been noted, particularly amongst new military recruits (Horwitz, 1985). Pharyngitis associated with enlargement of cervical lymph nodes is frequently seen in young children, and may be associated with conjunctivitis (pharyngoconjunctival fever). A more severe form of epidemic keratoconjunctivitis has also been recorded in industrial workers exposed to dusts (Horwitz, 1985). Other manifestations of adenovirus infections include urethritis, cystitis, gastroenteritis, mesenteric adenitis and meningoencephalitis (Horwitz, 1985). Infections may be particularly severe in immunocompromised individuals (Wadell, 1984). Unlike most respiratory viruses adenoviruses can be associated with a high white cell count (>15 x 10⁹/l).

6. Pathological features
Immunofluorescence and scanning electron microscopy studies indicate that the primary site of rhinovirus infection is the epithelial cell layer of the nasal mucosa, including ciliated and non-ciliated cells which are sloughed off during virus infection (Reed and Boyde, 1972; Turner et al., 1982; Winther et al., 1986). Tissue culture cells infected with rhinovirus show characteristic cytopathic changes with vesicles appearing in the cytoplasm approximately 3 hours after infection followed by a degradation of the nuclear membrane, a change in membrane permeability and leakage of cell contents leading to shrinkage of the cell (Cordell-Stewart and Taylor, 1971). Histological examination of nasal biopsies of infected volunteers show
subepithelial oedema with inflammatory cell infiltration and exudation of serous fluid. As peak symptoms are experienced at the time of maximum virus shedding it is believed that the pathological effects are primarily due to the cytopathic effects of rhinovirus.

Influenza infection causes inflammatory changes in the respiratory mucosa with early necrosis of epithelial cells (Hers et al., 1958) and associated exudate, oedema, necrosis and desquamation. Complete resolution of the histological changes takes over one month. Influenza pneumonitis is associated with alveolar thickening due to mononuclear cell infiltration and exudation followed by necrotic changes and loss of the alveolar epithelium (Hers et al., 1958; Martin et al., 1959).

Coronavirus infection results in a patchy destruction of ciliated epithelial cells (McIntosh, 1985). Respiratory syncytial virus infection in infants causes bronchiolitis with destruction of epithelial cells, necrosis of the bronchial epithelium, and oedema of submucosal tissues (McIntosh and Chanock, 1985). Infection may lead to obstruction and collapse of small bronchioles. The histological appearances are of a peribronchial inflammatory infiltrate with thickened interalveolar walls and fluid secretion into the alveolar spaces. Severe parainfluenza infection gives similar pathological changes. Adenovirus infection is associated with characteristic histological features as infected respiratory epithelial cells have swollen nuclei containing basophilic inclusion bodies which are surrounded by a rim of cytoplasm and known as "smudge cells" (Horwitz, 1985).

7. Immune response

Following rhinovirus infection neutralising antibody appears and can be detected in both serum and nasal washes after approximately 14 days (Cate et al., 1966). Studies in human volunteers showed that antibodies could be distinguished in over 90% of subjects who were experimentally infected, although the frequency of antibody response and level of antibody depended on the infecting strain and the infecting dose of virus. Serum antibody levels persist for several years with a slow
fall in titre (Couch, 1985). Under conditions of natural virus exposure pre-existing antibody levels of >8 have been associated with immunity (Hendley et al., 1969) although it is possible to infect volunteers with antibody levels of 32-256 if a very high virus challenge is used (Mufson et al., 1963). Increased nasal secretion is a feature of rhinovirus infection and over 30% of the total secreted protein is IgA (Gwaltney, 1984). Humoral immunity is not thought to play a major role in recovery as neutralising antibodies do not appear until several days after infection (Couch, 1985). Rhinovirus infections activate a systemic cellular immune response (Hsia et al., 1990) and interferon is detected 1-2 days after the peak period of virus shedding. Interferons are therefore believed to mediate recovery from rhinovirus infection (Gwaltney, 1984). Enterovirus immunity is protective against the infecting type only.

The host immune response to influenza involves interferons, humoral immunity and cell-mediated immunity. Interferons are believed to be important in the early stages of recovery as host natural killer cell activity and interferon production reach a peak within the first 2 days after the onset of symptoms (Ennis et al., 1981; Green et al., 1982) and influenza virus is inhibited by interferon in vitro (Merigan et al., 1973).

A rise in serum antibody can usually be detected after acute influenza infection (Fox et al., 1982). The dynamics of the antibody response was investigated using volunteers who were challenged with live-attenuated (H1N1 and H3N2) virus vaccines (Couch and Kasel, 1983). These studies showed that serum IgA, IgG and IgM appeared simultaneously approximately 2 weeks after virus inoculation, however IgM levels declined after 2 weeks whereas IgG levels increased over 4-7 weeks. A gradual fall in serum antibody level is seen over 6 months following natural infection or immunisation, but antibodies may persist at low titre for many years (Couch and Kasel, 1983). Only antibodies directed against haemagglutinin and neuraminidase are associated with protection and the long-term protective effect of the antibody response is limited by the ability of the haemagglutinin molecule to
undergo antigenic change (Couch and Kasel, 1983; Davies et al, 1986). Locally-produced secretory IgA is probably the most important factor mediating initial resistance to infection (Rossen et al, 1970). Cell-mediated immunity is also important in the recovery from influenza and specific cytotoxic T-cells with the ability to lyse virus-infected cells reach a peak approximately 6 days after virus inoculation (Yap et al, 1978; Mitchell et al, 1985). Influenza may be complicated by secondary bacterial infection (Murphy and Webster, 1985). The interaction between respiratory viruses and bacteria is unclear but may involve several mechanisms, including a decreased chemotactic response and phagocytic killing ability of virus infected leucocytes (Larson and Blades, 1976), the enhancement of bacterial adherence and toxin production by the presence of replicating influenza virus (Sanford and Ramsay, 1987; Jakeman et al, 1991) and the activation of haemagglutinin by bacterial proteases (Tashiro et al, 1987a; 1987b).

It has been suggested that some of the pathological features of RSV bronchiolitis may be immunologically mediated and result from an IgE mediated type 1 hypersensitivity reaction (Stott and Taylor, 1985). Previous exposure to infection or persistence of maternal antibody might therefore play a role in the development of infection (Stott and Taylor, 1985). The most important host-defence against RSV infection appears to be local IgA but cellular and humoral immunity also play a role in eliminating virus (Anderson et al, 1990b). Curiously RSV is one of the few respiratory viruses which does not appear to induce host interferon production in man (Isaac, 1989). Immunity against RSV is also incomplete and reinfection may occur (McIntosh and Chanock, 1985).

Immune response to parainfluenza virus infection is type specific (Ray et al, 1990). A high degree of antigenic variation was observed in clinical isolates of parainfluenza 3 suggesting the frequent occurrence of mutations (Coelingh and Tierney, 1989). Mutant viruses might therefore evade the immune system and be the cause of repeated infections. Coronavirus immunity is incomplete and appears to wane so that repeated infections may occur in adult life (Monto, 1984). Immunity
against adenoviruses is usually type-specific. The multitude of serotypes allows repeated infections to occur.

8. Confirmation of diagnosis

Rhinoviruses will grow in a variety of human cell lines including human embryonic kidney, foetal tonsil, human amnion, human embryonic lung diploid fibroblasts, and the HEp-2 and modified HeLa continuous cell lines (Couch, 1985; Geist and Hayden, 1985). Rhinoviruses are acid-labile and are inactivated below pH 6. Many types are thermostable and will survive at temperatures of 50°C (Couch, 1985), although growth is best at a temperature of 33°C and rhinoviruses are probably adapted to the mean nasal temperature (Stott and Killington, 1972). Optimal virus growth occurs if the cell cultures are slowly rotated (12 revs/hr) in the presence of 5% CO2 at pH 7.0-7.2 using standard media eg. Eagles minimal essential medium supplemented with 2-5% foetal calf serum and antibiotics. Cytopathic effect (CPE) due to virus growth in susceptible cell cultures is used to diagnose rhinovirus infection and virus isolation is currently the most sensitive routine diagnostic method. Serological techniques have little to offer for routine diagnosis due to the large number of circulating serotypes.

Recent advances in molecular biology have opened up new diagnostic possibilities for picornavirus infections. Comparison of published nucleotide sequences reveal a high degree of sequence homology in the 5' non-coding region for all picornaviruses (Hughes et al, 1987; Hughes et al, 1988) with blocks of completely conserved nucleotides and intervening areas which are more variable (Skern et al, 1987a). Several studies have investigated the possibility of using gene probes derived from these conserved regions to diagnose picornavirus infection. In initial experiments a single-stranded M13 template consisting of the first 800 nucleotides of the 5' non-coding region of HRV 14 was constructed and used to generate a cDNA probe which was labelled with 32P-dATP (Al-Nakib et al, 1986). Although 54 (96.4%) of 56 rhinoviruses gave positive hybridisation signals the sensitivity of the probe was
very variable. This variability reflected the molecular relationships of the different serotypes but limited the potential use of the probe for clinical diagnosis.

Further studies used radiolabelled 17-base oligonucleotide probes derived from totally conserved sequences of the picornavirus genome (Bruce et al, 1988; 1989). Results showed that the probes hybridised to RNA extracted from all 57 types of human rhinoviruses tested, calf rhinovirus SD1, bovine rhinovirus EC11 and several serotypes of coxsackievirus A and B, echoviruses, and all 3 types of poliovirus. There was no reaction with a range of unrelated viruses including reoviruses, influenza A or B, parainfluenza 3, herpes simplex virus or coronavirus 229E. In clinical studies nasal washes showed positive hybridisation to the probes on at least one day post-inoculation in 54 of 57 volunteers inoculated with HRV 14, compared to only 41 which were positive on tissue culture. Using the probes rhinovirus could be identified for periods of up to 7 days after inoculation.

Oligonucleotide DNA probes have also been used to detect enteroviruses in a blot assay, however the sensitivity was relatively low (Rotbart et al, 1988). Radiolabelled subgenomic RNA probes (riboprobes) are possibly more sensitive and have been used to identify enteroviruses in clinical specimens (Cova et al, 1988; Chatterjee et al, 1988; Zhang et al, 1988).

Conserved oligonucleotides have also been used as primers for the polymerase chain reaction (PCR) which was used to amplify and detect picornavirus nucleic acid in clinical material treated with reverse transcriptase (Gama et al, 1988; Gama et al, 1989). A 380 base amplified fragment was obtained from nasal washes from volunteers inoculated with HRV-2, HRV-3, HRV-9, HRV-14 and HRV-85 and PCR was able to detect coxsackie A viruses and other enteroviruses. The sensitivity was high and the technique was thought to be able to detect as few as 10-20 infectious virus particles (Gama et al, 1989). Modifications of the PCR method have allowed rhinoviruses and enteroviruses to be distinguished by hybridising the amplified product with specific rhinovirus or enterovirus oligonucleotide probes (Hyypia et al, 1989), or using primers designed to yield products of different sizes from...

Although influenza may be diagnosed clinically in patients with typical symptoms during an outbreak, confirmation of the diagnosis is usually provided by virus isolation, immunofluorescence or the demonstration of a rise in antibody titre. Influenza A can be isolated in the amniotic cavity of 12-13 day old chicken embryos and both influenza A and B will grow and produce CPE in several primary kidney cell-lines if trypsin (2μg/ml) is included in the medium. Monkey-kidney and Madin-Darby canine kidney are the most frequently used cell-lines for the laboratory diagnosis of influenza (Frank et al, 1979). Isolation of influenza in tissue culture, even in the absence of CPE, may be demonstrated by the ability to adsorb guinea pig erythrocytes (Davenport, 1984). Serological tests are helpful for diagnosing infection in sporadic cases and for epidemiological surveys (Davenport, 1984). The most common tests used to detect antibody are the complement fixation test and the haemagglutination inhibition test and the tests are most informative if paired sera taken at least 2-3 weeks apart are compared (Davenport, 1984).

The immunofluorescence technique (For discussion of technique see section 7.1) uses cells obtained from nasopharyngeal secretions, pharyngeal aspirates (Westmoreland et al, 1989) or tissue culture and allows rapid diagnosis of infection. However, a recent comparison of immunofluorescence and culture showed that the sensitivity of immunofluorescence was only 43% for influenza A (Ray and Minnich, 1987). Enzyme-linked immunosorbent assay techniques have been developed for detection of influenza antigen in nasopharyngeal specimens using wells coated with guinea pig antiserum. The sensitivity of influenza ELISA is comparable to that of immunofluorescence (Grandien et al, 1985).

Diagnosis of coronavirus infection is not routinely performed as the viruses are difficult to grow in tissue culture although foetal tonsil cells and human rhabdomyosarcoma cells may be susceptible (White and Fenner, 1986). A clone (C16), selected from the MRC-C continuous heteroploid cell line, derived from human embryo lung fibroblast cells, was found to be significantly more susceptible
to coronavirus 229E than the parent cell-line (Phillpotts, 1983), and is used for virus isolation. OC43 grows readily in suckling mouse brain (Monto, 1984). Serological diagnosis of coronavirus infection is possible using an ELISA developed by Kraaijeveld et al (1980). Coronavirus 229E nucleic acid has been cloned and a single stranded RNA transcript labelled with $^{32}$P was used as a gene probe to detect coronavirus in nasal wash specimens from infected volunteers (Myint et al, 1989a, 1989b).

Conventional laboratory diagnosis of RSV infection is by virus isolation, immunofluorescence, or serology; including complement fixation and ELISA techniques (Stott and Taylor, 1985). RSV grows readily in certain cell lines including HeLa cells and HEp-2 cells. The virus is very labile and does not tolerate freezing therefore clinical specimens must be added to cells as soon as possible (McIntosh and Chanock, 1985). Nasopharyngeal aspirates provide a better yield of virus than nasopharyngeal swabs (Ahluwalia et al, 1987) and characteristic CPE with syncytia becomes apparent after 3-10 days. Haemadsorption does not occur and this enables RSV to be distinguished from other paramyxoviruses. Specific monoclonal antibodies have been used for immunofluorescence (Barnes et al, 1989). Radiolabelled probes have recently been developed for the detection of RSV in nasopharyngeal samples using a cDNA probe complementary to the nucleocapsid protein gene; unfortunately the sensitivity was found to be poor in comparison with immunofluorescence and ELISA (Van Dyke and Murphy-Corb, 1989).

Parainfluenza infection may be diagnosed by isolation in tissue culture, ELISA, immunofluorescence and radioimmunoassay (Henderson, 1987). Monkey kidney cell lines are routinely used to grow parainfluenza viruses (Henderson, 1987). CPE is often absent (although type 2 can induce syncytia formation) and diagnosis of parainfluenza virus infection in tissue culture cells is confirmed by detecting adsorption of guinea pig erythrocytes. Parainfluenza is differentiated from other viruses causing similar haemadsorption by performing haemadsorption inhibition or immunofluorescence.
The most susceptible tissue culture cells for adenoviruses are human embryonic kidney cells, HeLa cells and HEp-2 cells. CPE may be difficult to distinguish (Hurwitz, 1985). Serological diagnosis of adenovirus infection includes the use of immunofluorescence, ELISA and complement fixation tests. Adenoviruses have been detected in clinical specimens by DNA hybridization (Hyypia and Pettersson, 1985) and in situ hybridization has been used to investigate the pathology of adenovirus infection in lung tissue (Hogg et al, 1989).

9. Prevention and control
Rhinoviruses are readily inactivated by temperatures above 56°C, UV light and chemical agents including iodine, hydrogen peroxide 2% glutaric acid and sodium lauryl sulphate (Macnaughton, 1982). Treatment with lipid solvents eg. ethanol, hydrocarbons, chloroform and ether has little effect (Macnaughton, 1982). The use of handkerchiefs impregnated with a combination of citric acid, malic acid and sodium lauryl sulphate was found to reduce hand contamination of infected people and block virus transmission (Hayden et al, 1985a; Hayden et al, 1985b) but would be unlikely to alter transmission among infants who are the main source of infection. A number of antiviral agents have activity against rhinoviruses. Some of the first compounds used were benzinidazoles (eg. enviroxime); however oral administration of enviroxime does not lead to high nasal levels and is associated with gastrointestinal side-effects. Placebo-controlled, experimental and field studies of oral or topical enviroxime have shown no significant clinical benefit despite inhibitory drug levels in nasal washes (Phillpotts et al, 1981; Hayden and Gwaltney, 1982; Miller et al, 1985). The reasons for the observed lack of clinical effect may include the irritant and insoluble nature of enviroxime and the possibility of rapid clearance reducing the local drug concentration (Wyde et al, 1988).

Interferons were found to have potent antiviral activity and the clinical use of interferons will be discussed in detail in chapter 4. A number of capsid binding agents have recently been synthesized. These compounds inhibit virus uncoating and
include dichloroflavin, chalcones, R61837 and WIN 5171 (Tisdale and Selway, 1983; Ishitsuka et al, 1982; Ninomiya et al, 1984; Fox et al, 1986; Sperber and Hayden, 1988). Unfortunately clinical trials with oral formulations have failed to demonstrate any significant benefit (Phillpotts et al, 1983a; 1984a; 1984b; Zerial et al, 1985); although intranasal treatment with R61837 was able to suppress colds in volunteer studies provided treatment was begun prior to virus challenge (Alarcon et al, 1986; Al-Nakib et al, 1989). The pyridazinamines and other newer drugs with \textit{in-vitro} antirhinoviral activity await controlled clinical trials (Andries et al, 1988). Combination treatment (e.g. Interferon and enviroxime, dichloroflavin or chalcone) results in a synergistic effect against rhinoviruses \textit{in vitro} (Phillpotts and Tyrrell, 1985; Ahmad and Tyrrell, 1986; Al-Nakib and Tyrrell, 1987) and is worthy of further evaluation.

Newer therapeutic strategies against rhinovirus infections include the use of monoclonal antibodies to block ICAM-1, the major rhinovirus receptor (Colonno et al, 1986; Hayden et al, 1988; Bangham and McMichael, 1990). Human volunteer studies showed that this approach did not reduce overall rates of infection but appeared to modify the course of illness and was associated with a delay in virus shedding and cold symptoms. A soluble derivative of ICAM-1 inhibits binding and infection of rhinovirus in tissue culture and has clinical potential (Marlin et al, 1989).

Vitamin C has been advocated as a treatment for the common cold but an overview of the clinical trials concluded that it was not associated with any benefit (Dykes and Meier, 1975). The therapeutic use of zinc compounds has also attracted great interest. Eby et al (1984) found that zinc lozenges significantly reduced the duration of symptoms in naturally occurring colds but the viral aetiology of the infections was not established in this trial. Further studies in experimentally infected volunteers have not shown convincing prophylactic or therapeutic efficacy (Al-Nakib et al 1987; Douglas et al, 1987; Farr et al, 1987; Geist et al, 1987). The high incidence of the common cold and lack of a specific cure has led to
the marketing of hundreds of symptomatic proprietry medications, mostly of
doubtful clinical value (Graham et al, 1990).

Attempts to produce an effective rhinovirus vaccine have encountered many
problems, including the presence of over 100 distinct serotypes with little natural
cross-immunity and the lack of attenuated strains of rhinovirus (Gwaltney, 1975).
The canyon region in the picornavirus coat is thought to be involved in cellular
binding and provides a possible target for vaccines, however this region does not
elicit host neutralising antibodies (McCray and Werner, 1987; Palmenburg,
1987). Analysis of escape mutant viruses with monoclonal antibodies has resulted in
the identification of four distinct neutralisation sites on the surface of HRV 14
(Sherry et al, 1986) and three neutralisation sites on HRV 2 (Appleyard et al,
1990). Two of the sites correspond in both viruses and provide possible targets for
future vaccines.

There are several approaches to the treatment and control of influenza.
Transmission might be interrupted by appropriate isolation of patients or hygiene
precautions, however the influenza virus is so infectious that these measures are
unlikely to have any real impact except in certain isolated communities (Ravenholt
and Foege, 1982). Amantadine and its analogue rimantadine are the most effective
antiviral agents discovered to date. They have a tricyclic chemical structure with an
amine side-chain and a cage-like configuration and are believed to act through the
inhibition of influenza virus uncoating (Bukrinskaya et al, 1980; Hay et al, 1985,
1986). They inhibit H0N1, H1N1, H2N2, H3N2 and Hsw1N1 strains of influenza A
but have little action against influenza B or C or other respiratory viruses (Grunert
rapidly in the laboratory and the emergence and transmission of resistant strains
has been reported in human trials (Heider et al, 1981; Belshe et al, 1989; Hayden
et al, 1989). The implications of drug resistance are uncertain as no reduction in
efficacy of rimantadine was observed after 20 years of follow up of over 142,000
patients in the USSR (Kubar et al, 1989). Amantadine is the only anti-influenza
drug currently licensed in the UK. It is associated with few serious adverse effects, but headache, light-headedness, dizziness, difficulty concentrating and insomnia have been reported in 5-29% of patients (Bryson et al, 1980), and a teratogenic effect in rats limits its use in women of childbearing age. Amantadine should be prescribed with caution in patients with cardiovascular or cerebral disorders, unfortunately these patients are often at high risk from influenza.

Clinical trials of amantadine and rimantadine have investigated their use both for prophylaxis against influenza and for treatment of established infection. Prophylactic efficacy appears to be high with several studies in children and adults demonstrating at least 50% protection against proven influenza infection and prevention of symptomatic illness in over 70% (Quilligan et al, 1966; O'Donoghue et al, 1973; Monto et al, 1979; Dolin et al, 1982; Sears and Clements, 1987; Tominack, 1987; Brady, 1990). Treatment of established influenza is associated with a reduction in virus shedding and duration of symptoms of approximately one-third, provided amantadine or rimantadine is commenced within 48hrs of onset of symptoms (Wingfield et al, 1969; Togo et al, 1970; Van Voris et al, 1981; Hayden and Monto, 1985; Tominack, 1987).

The Immunization Practices Advisory Committee in the USA currently recommends that amantadine prophylaxis should be given to unimmunised high-risk individuals and associated medical personnel during an outbreak of influenza A infection (CDSC, 1985). It is also recommended that vaccinated individuals should be offered amantadine when the vaccine strains and epidemic strains differ significantly (Betts, 1989). Prophylaxis is advised for the duration of the epidemic, generally a period of 4-8 weeks. If vaccine is given at the start of an outbreak concurrent use of amantadine for approximately 2 weeks will give protection until an antibody response is induced (Mostow, 1987; Douglas, 1990). The Immunisation Practices Advisory Committee proposes that early treatment with amantadine should be considered for high risk patients who develop symptoms of a flu-like illness during periods of influenza A activity. When outbreaks of influenza occur in residential
homes it has been suggested that amantadine should be offered to all residents and staff regardless of vaccination status as it will augment the protection afforded by vaccination (WHO, 1985). Despite the number of well-designed clinical trials showing the efficacy of prophylactic or therapeutic amantadine, its use is very limited in the UK due to a lack of awareness among medical practitioners and concern over efficacy, and possible adverse effects.

Other agents with activity against influenza include interferon and ribavirin. The results of clinical studies of interferon in influenza infections have been disappointing. Placebo-controlled trials of intranasal human leucocyte interferon (Merigan et al, 1973), or lymphoblastoid interferon (Phillpotts et al, 1984c) in experimentally-infected volunteers showed only a small clinical benefit even when interferon was commenced before virus challenge. Family studies have confirmed that intranasal interferon has no significant prophylactic effect on viruses other than rhinovirus (Douglas et al, 1986; Hayden et al, 1986).

Ribavirin, a synthetic triazole nucleoside, has a broad spectrum of antiviral activity (Nicholson, 1984). Unfortunately inhibitory levels of ribavirin against influenza viruses have not been achieved after oral administration and hence clinical trials of ribavirin in influenza have generally proved disappointing (Smith et al, 1980; Stein, 1987). Potential toxicity of oral ribavirin includes abnormal serum bilirubin, bone marrow suppression and possible teratogenicity (Galbraith, 1985). Clinical studies of aerosolised ribavirin (Wyde et al, 1986) have shown only a marginal benefit in the treatment of established influenza infection (Bernstein et al, 1988; Wilson et al, 1984).

Current commercial influenza vaccines are multivalent and usually contain an influenza A H1N1 and H3N2 component and influenza B antigens. The antigenic composition of the vaccine is reviewed annually depending upon the prevalent strains. Virus used to make the vaccine is grown up in allantoic fluid, purified and inactivated. The amount of haemagglutinin in each dose of vaccine is standardised, however the titre of neuraminidase is more variable. Two forms of vaccine are
currently available; "split virus" vaccine contains disrupted virus particles which are partially purified using organic solvents and detergents and separated by ultracentrifugation; "surface antigen" vaccine contains highly purified haemagglutinin and neuraminidase antigens. These sub-unit vaccines are well tolerated and evoke good serological responses (Nicholson et al, 1979).

The titre of antibody induced by influenza vaccine depends partly on the dose of vaccine, and whether the subject has been primed by a previous influenza infection. Primed subjects have an enhanced response to vaccination (Parkman et al, 1977; Feery et al, 1979; Nicholson et al, 1979). Studies on the efficacy of influenza vaccine in the elderly have yielded conflicting results with some studies showing a reduced antibody response in elderly subjects and others showing an identical or enhanced response compared to younger subjects (Brandriss et al, 1981). A recent review (Bayer et al, 1989) concluded that the association between age and vaccine response has yet to be established since previous studies had not been controlled for the presence of other diseases, previous exposure to influenza or vaccination. Patients with renal failure or renal transplants have a good antibody response to influenza vaccine (Jordan et al, 1973; Carroll et al, 1974; Pabico et al, 1974; Pabico et al, 1976; Osanloo et al, 1978; Sheth, 1978). The antibody titres induced by influenza vaccine decline over a period of months (Cate et al, 1977).

Annual vaccination is recommended, although there is some evidence that this may ultimately afford little or no protection (Hoskins et al, 1979; Shann, 1990). The efficacy of the vaccine is related to the induced level of antibody to haemagglutinin and the antigenic similarity of vaccine and prevalent strains (Bashe et al, 1964; Stiver et al, 1973; Couch et al, 1979). 70-90% protection against infection can be achieved in young healthy adults when vaccine and epidemic strains are closely matched (Meiklejohn et al, 1978; Feery et al, 1979; Arden et al, 1986), but protection is much lower in institutionalised elderly patients (D'Allesio et al, 1969; Feery et al, 1979). A summary of 17 trials of influenza vaccine in nursing homes (Arden et al, 1986) found that the mean efficacy against influenza A
infection (assessed by comparing attack rates of influenza-like illness in matched vaccinated and unvaccinated groups) was only 27% (range 0-80%). Although vaccine is associated with poor protection against infection in the elderly, studies have shown that vaccination reduces the severity of disease, hospital admissions and mortality by up to 87% (Barker and Mullooly, 1980; Arden et al, 1986; Patriarca et al, 1985; Gross et al, 1988). In addition the herd immunity achieved by vaccinating at least 70% of residents in nursing homes helps to limit the spread of influenza (Patriarca et al, 1986).

The Department of Health (1990) currently recommends that annual influenza vaccination should be offered to persons at special risk, defined as "persons, especially the elderly, suffering from chronic pulmonary disease, chronic heart disease, chronic renal disease, diabetes and other less common endocrine disorders, and conditions involving immunosuppressive therapy". The vaccine should also be considered for elderly persons and children living in residential homes and long-stay hospitals (Dept of Health, 1990). High levels of influenza activity do not normally occur before December so annual vaccination is normally carried out in October and November. Contraindications to vaccination include hypersensitivity to eggs, polymyxin or neomycin. Adverse effects are generally mild and include local tenderness at the site of injection, low-grade fever, myalgia and headache in the first 24 hours after vaccination (Margolis et al, 1990). An increased incidence of Guillain-Barre-syndrome was observed during a vaccination programme against swine influenza in the USA in 1977, but the exact cause remains controversial (Schonberger et al, 1979). Concern over possible adverse effects is often cited by physicians as a reason not to vaccinate but the perceived incidence of severe vaccine adverse-effects was found to be much greater than the actual incidence (McKinney and Barnas, 1989).

Although influenza vaccine is imperfect it reduces mortality and severity of disease and is considered worthwhile (Ruben, 1987) and probably cost effective, particularly in high risk groups (Schoenbaum, 1987). It has been recommended
that vaccination rates should exceed 80% in high risk patients (US Dept of Health, 1987) but it is estimated that the actual vaccination rate is less than 25% in the USA. The reasons for the low uptake are thought to include a poor perception of the potential severity of influenza and concern over vaccine efficacy and adverse effects (Rosenstock 1961; Pachuki et al, 1985; Griffin, 1988; McKinney and Barnas, 1989). Alternative approaches to influenza vaccination include the use of live virus vaccines (Murphy et al, 1973; Feldman et al, 1985), which have been produced by a reassortment using the current haemagglutinin and neuraminidase genes combined with the remaining 6 genes derived from an attenuated cold-adapted strain (Shann, 1990). Live virus vaccines will still suffer from the need to alter the vaccine to cope with the observed antigenic variation of the influenza virus.

Treatment of severe RSV infection includes oxygen, fluids and antibiotics for superadded bacterial infection. The antiviral agent ribavirin inhibits RSV replication and aerosolised ribavirin has been shown to reduce the severity of infection and duration of virus shedding (Breese Hall et al, 1983; Taber et al, 1983). It is licenced for the treatment of severe RSV infection in infants with underlying cardiac or pulmonary abnormalities.

Treatment of patients with coronavirus, parainfluenzavirus and adenovirus infections is merely supportive as symptoms are usually mild and existing antiviral agents are ineffective. Effective vaccines are not currently available against coronavirus, RSV or parainfluenza virus infections.

In conclusion respiratory viruses are among the most important infections to afflict mankind and continue to cause an enormous morbidity and economic cost. Existing methods of prevention and treatment of these infections are inadequate. Our understanding of the molecular pathogenesis of infection has increased dramatically over the past decade and should lead to the development of novel and specific antiviral agents and vaccines.
1. Introduction and definitions

Among the many causes of chronic chest disease asthma, chronic obstructive airways disease, bronchiectasis and cystic fibrosis are of particular interest as viruses have been implicated either in their pathogenesis or in exacerbations. This chapter reviews the clinical and pathological features of these conditions and the role of respiratory viruses.

**Asthma**

Asthma is defined as a disease characterised by airway narrowing which is reversible over short periods of time, either spontaneously or as a result of treatment (Newman-Taylor, 1988a). Asthma is usually diagnosed on the basis of a typical history of episodic wheezing and may be confirmed by showing diurnal and day to day variation in peak flow and response to bronchodilators. Asthma is not one single discrete condition and the characteristics of asthmatic individuals are very variable. The inability to precisely define the disease has led to inevitable difficulties in assessing the true prevalence of the condition (Hill et al., 1989) and interpreting epidemiological and clinical studies (Gregg, 1988). It has been estimated that 5-10% of the population of Britain have experienced asthma at some time (Barnes, 1987; Barnes and Chung, 1989). Asthma can present at any age but approximately 70% of asthmatics develop wheezing in childhood and of these three quarters improve by the age of 15 (Allen, 1988).

Airway-hyperreactivity is the hallmark of asthma (Lopez and Salvaggio, 1987). Bronchoconstriction may be precipitated by a number of factors including extrinsic allergens (eg. cat fur, house-dust mite, grass pollens, *Aspergillus* etc), exercise, upper respiratory infection, stress, gastro-oesophageal reflux and certain drugs or chemicals (Crompton, 1985; Ducolone et al, 1987; Newman-Taylor, 1988a;
1988b; Whyte and Flenley, 1986; Ayres, 1990). Although asthma is essentially a reversible condition persistent airways obstruction may occur in patients with long-standing disease (Connolly et al, 1988; Carpenter et al, 1989).

Asthma is currently viewed as a chronic eosinophilic bronchitis with inflammation leading to a secondary bronchospasm (Barnes, 1987; Stafford, 1988; Gibson et al, 1989; Bousquet et al, 1990; Reed and Hunt, 1990). The physiological basis of inflammation and bronchoconstriction is poorly understood but appears to be the end result of several different mechanisms. Neural, humoral and circulating factors are all believed to be important in the pathogenesis of bronchoconstriction (Tattersfield and McNichol, 1987; Newman-Taylor, 1988a; Holgate, 1990; Stechschulte, 1990). Drugs used in the management of asthma are divided into those which are effective bronchodilators (eg. β-agonists, ipratropium bromide, theophyllines) and those which have a predominantly prophylactic effect (eg. sodium chromoglycate, steroids). The annual mortality of asthma in the UK is approximately 1500 and it has been suggested that inadequate or inappropriate treatment may be contributing to this figure (Flenley, 1981; Benatar, 1986; Tattersfield and McNichol, 1987; Barnes, 1988; Reed and Hunt, 1990; Lancet editorial, 1989a; Lancet editorial, 1990b; Sears et al, 1990; Wong et al, 1990).

Chronic obstructive airways disease

The term chronic obstructive airways disease describes a number of conditions which are related and usually occur together. The definition and classification of chronic bronchitis has been the subject of a Medical Research Council Report (1965).

Chronic simple bronchitis is defined as "chronic cough with production of sputum on most days for at least three months in the year for at least two years". It results from the hypersecretion of mucus. Airways obstruction is not a feature of chronic simple bronchitis. Emphysema is a condition characterised by "dilatation of the terminal air spaces of the lungs distal to the terminal bronchiole with
destruction of their walls". It is a pathological and not a clinical diagnosis. Most patients with chronic obstructive airways disease (COAD) have features of both chronic bronchitis and emphysema (Kutty and Varkey, 1988). COAD is more common in males than females and is responsible for at least 15,000 deaths and an estimated 30 million lost working days/annum in the UK (Brewis, 1985). The development of COAD is very strongly related to cigarette smoking and atmospheric pollution (Brewis, 1985) and the prevalence has decreased over the last 20 years (Wiggins and Lynne, 1988).

Chronic bronchitis is characterised by hyperplasia of the mucus-secreting glands in the large airways and the presence of inflammatory cells in the mucosa. Emphysema is classified into two pathological types; centrilobular or centriacinar emphysema is characterised by distension and destruction of the respiratory bronchioles, whereas panacinar emphysema is associated with distension and destruction of the whole of the acinus. It has been suggested that emphysema is caused by increased alveolar protease activity produced by neutrophils and macrophages which are present in chronic bronchial inflammation (Fujita et al, 1990; Snider 1981; Brewis, 1985).

Bacteria are frequently recovered from the sputum and lungs of patients with chronic obstructive airways disease, most commonly Streptococcus pneumoniae, Haemophilus influenzae and Branhamella catarrhalis (Stuart-Harris, 1968; Tager and Speizer, 1975; Ellis et al, 1978; Brown, 1989). Nicholls et al, (1975) found that 38 of 57 exacerbations of COAD with purulent sputum production were associated with H. influenzae and 10 of 57 were associated with S. pneumoniae.

The long-term effects of chronic obstructive airways disease are respiratory failure, recurrent infections, pulmonary hypertension, right ventricular hypertrophy and congestive cardiac failure ("cor-pulmonale"). Management of COAD includes quitting smoking, prompt treatment of infective exacerbations (Lancet editorial, 1987) and domiciliary oxygen for at least 15 hours/day in hypoxic

The prognosis for established COAD depends on the initial FEV1 and the annual rate of decline (Fletcher and Peto 1977; Peto et al, 1983; Burrows et al, 1987). A prospective study performed by Burrows and Earle (1969) showed that the overall 5 year mortality for patients with symptomatic disease was 47% rising to 67% if the initial FEV1 was <0.75l.

**Bronchiectasis**

Bronchiectasis is defined as "a permanent dilatation of the bronchi" (Crompton, 1982). The pathological features of the condition are divided into *saccular bronchiectasis* where the bronchi are replaced by cysts and the less severe *cylindrical bronchiectasis* where the bronchial wall is infiltrated by neutrophils, usually with destruction of adjacent lung tissue. The major cause of bronchiectasis is repeated bronchial infection. The pathological process often starts with a severe episode of pneumonia in early childhood, which may be caused by whooping cough, measles or tuberculosis. Once bronchial damage has occurred the bronchial wall is weakened and mucociliary clearance is impaired predisposing to further damage by infecting organisms. Less frequent causes of bronchiectasis include congenital anatomical abnormalities, Kartagener's syndrome (dextrocardia and impaired ciliary function), hypogammaglobulinaemia, bronchial obstruction due to a foreign body, tumour or lymphadenopathy compressing the bronchus, allergic aspergillosis and cystic fibrosis (Barker and Bardana, 1988). The prevalence of bronchiectasis has declined in recent years mainly due to an improvement in child health.

The predominant clinical feature is a chronic productive cough (Stockley, 1988). Patients are prone to repeated bacterial infection and may become colonised with *Haemophilus influenzae*, *Staphylococcus aureus*, *Klebsiella spp* or *Pseudomonas aeruginosa* (Stockley, 1988). Complications include recurrent infection, haemoptysis, abscess formation, either local or distant, respiratory
failure, cor-pulmonale and secondary amyloid due to chronic suppuration (Crompton, 1985). Many patients also have evidence of reversible airways obstruction, and respond to bronchodilators or steroids. Aspergillus infection is frequently found in this group of patients (Barker and Bardana, 1988).

The disease is very variable in severity and treatment depends on the clinical features. Management includes physiotherapy and postural drainage, treatment of infective exacerbations and prophylactic antibiotics in patients with frequent infective episodes (Cochrane, 1985; Crompton, 1985; Hill et al, 1988; Stockley, 1988). Surgery may be indicated in patients with localised disease or for repeated haemoptysis (Stockley, 1988).

**Cystic fibrosis**

Cystic fibrosis is the most common lethal genetic disorder. It has a recessive inheritance and affects approximately 1/2000 live births, with an estimated carrier rate of 1 in 20. The primary defect in cystic fibrosis results in an altered cell membrane permeability to chloride ions (McPherson and Goodchild, 1988; Quinton, 1989). The sweat contains an abnormally high level of sodium chloride and clinical manifestations result from abnormally viscid exocrine secretions. Clinical manifestations of cystic fibrosis may occur in the immediate post-natal period with meconium ileus and signs of intestinal obstruction, in infancy as failure to thrive with steatorrhoea, or may become apparent in later childhood after repeated chest infections. Diagnosis is currently made on the basis of the history and an abnormal sweat test (David, 1990).

Recurrent chest infection results in severe bronchiectasis and bacterial colonisation which often follows a sequential pattern with *Haemophilus influenzae* followed by *Staphylococcus aureus, Pseudomonas aeruginosa* (Pier, 1985; Hoiby and Koch, 1990) and *Pseudomonas cepacia* (Isles et al, 1984; Tomashefski et al, 1988). Current management of cystic fibrosis aims to minimise the pulmonary damage and relies on postural drainage and physiotherapy, regular antibiotics and
vigorous treatment of infective exacerbations (Littlewood, 1986). Pancreatic extract and nutritional supplements are usually required. The prognosis of cystic fibrosis has improved greatly over recent years as a result of earlier diagnosis and vigorous treatment of infections with antibiotics and physiotherapy. Approximately 50% of patients now survive until early adult life (Katz et al, 1986; Hodson, 1989). The early results of heart-lung transplantation have been encouraging and offer hope of long-term survival (Hodson, 1989).

The abnormal gene in cystic fibrosis has recently been identified on chromosome 7 (Rommens et al, 1989; Riordan et al, 1989; Kerem et al, 1989; Scambler, 1989). The encoded protein is 1480 amino acids in length and has been named the cystic fibrosis transmembrane conductance regulator (CFTR). Sequence analysis indicates that it probably spans the cell membrane and has a region which binds ATP, suggesting that it is involved in the regulation of ion transport. 68% of cystic fibrosis patients have an identical mutation consisting of a 3 base pair deletion leading to the absence of a single phenylalanine residue. Other patients with CF have a series of different mutations which may give phenotypic variants of the disease (Santis et al, 1990). Possible benefits arising from the identification of the gene include the ability to locate and purify CFTR and therefore elucidate its physiological role (Lancet editorial, 1989b), the development of an animal model (Harris and Bobrow, 1989), carrier detection, reliable prenatal diagnosis (Halley et al, 1989; McIntosh et al, 1989) and ultimately gene replacement therapy (Lancet editorial, 1990c).

2. Association between virus infection and pulmonary function

Previously healthy subjects

Cate et al, (1965) performed one of the earliest studies of lung function after experimental respiratory virus infection and showed that tracheobronchitis occurred after healthy volunteers inhaled a small particle aerosol of rhinovirus, suggesting virus infection of the lower respiratory tract. Studies of airways function
in normal subjects during experimental rhinovirus infection (Blair et al, 1976) and naturally occurring "common colds" demonstrated a change in the frequency dependence of compliance lasting up to 8 weeks (Picken, 1972). When Cate et al, (1973) investigated pulmonary function in healthy medical students with common colds they found that 12 of 14 colds were associated with decreased carbon monoxide diffusing capacity with no consistent effects on spirometry. The abnormal gas transfer was thought to result from an inapparent bronchiolitis caused by virus infection. Empey et al, (1976) demonstrated an increase in the airways resistance induced by histamine in normal subjects with upper respiratory tract infections compared to uninfected controls. This bronchial hyper-reactivity lasted up to 6 weeks and could be prevented with atropine suggesting that bronchoconstriction resulted from smooth muscle constriction.

Bronchial hyper-reactivity was also demonstrated after RSV infection (Hall et al, 1978) and Seidenberg et al (1989) found that RSV infection in infancy was associated with an increase in bronchial airways resistance which lasted up to several months (Seidenberg et al, 1989).

Studies of the pulmonary effects of uncomplicated influenza have shown that infection leads to an increase in bronchial reactivity (Little et al, 1979), abnormalities in small airways function and lung compliance (Hall et al, 1976) and decreased gas transfer (Horner et al, 1973; Johanson et al, 1969). These changes often persist for several weeks but recovery of diffusing capacity was delayed for over 6 months in 20% of patients (Horner et al, 1973). A recent study by Laitinen et al (1991) investigated bronchial reactivity in 14 susceptible healthy male subjects. 6 subjects were inoculated intranasally with live attenuated influenza A virus. All six developed serological evidence of infection but remained asymptomatic, however 5 developed bronchial hyper-reactivity after histamine aerosol which lasted for up to 9 days. Histamine sensitivity was unaltered in the control group of 8 subjects who were inoculated with placebo.
These studies show that upper respiratory viral infection in previously healthy subjects can lead to subtle changes in lung compliance and bronchial reactivity which may last for several weeks. The nature of the abnormalities in lung function suggest that small airways are most affected.

**Children with asthma**

Several studies have investigated the relationship between exacerbations of wheezing in children and viral respiratory infection. McIntosh *et al.*, (1973) followed up 32 asthmatic children aged 1 to 5 during two winters. 139 separate episodes of wheezing were recorded and 58 (42%) were associated with a diagnosed viral infection. Both virus isolation and serology were performed and respiratory syncytial virus (RSV) appeared to be the most virulent agent with 25 infections in total of which 13 were complicated by pneumonia.

In a study of respiratory illness in all children under 13 in a single London suburban general practice (Horn and Gregg, 1973; Horn *et al.*, 1975) a total of 4984 episodes of respiratory illness were recorded in 919 children. Viruses were isolated from nose and throat swabs in 320 (32.6%) of 980 with symptoms of URTI and 18 (64%) of 28 with pneumonia. Rhinoviruses were isolated most frequently and were associated with wheezy bronchitis in children over 3 years of age. RSV infection tended to be most severe in children infected early in life and was an important cause of wheeze in children under the age of 2 years. In a further study (Horn *et al.*, 1979) respiratory viruses were isolated from 49% of episodes of wheezy bronchitis in children aged 5-15 years. Rhinoviruses were isolated most frequently and virus isolation rates were higher in children with more severe symptoms. The recovery of rhinoviruses from sputum samples was higher than from nose or throat swabs suggesting active viral replication in the lower respiratory tract.

Influenza, RSV and rhinoviruses are all capable of precipitating attacks of asthma in children (Minor *et al.*, 1976). RSV infection is strongly associated with
wheezing in the first two years of life and is often the cause of the first documented episode of childhood wheezing (McIntosh, 1976). The relationship between RSV infection and wheezing in infants below the age of 12 months was demonstrated by Sims (1979) who investigated the role of respiratory virus infection diagnosed by culture or immunofluorescence in 776 (74.9%) of 1036 illnesses associated with wheeze (bronchitis, bronchiolitis and asthma). RSV accounted for 83.5% of virus-positive infants with parainfluenza the next most common. In older children (age unspecified) viruses were demonstrated in 207 (39.5%) of 524 wheezy illnesses. RSV accounted for 42% of positive virus diagnoses and appeared to be less important in this older group with a greater proportion caused by parainfluenza, influenza, adenoviruses and rhinoviruses. A further study from Norway (Carlsen et al, 1984) documented respiratory virus infection in 73 (29%) of 256 asthmatic attacks in children over the age of 2 years with rhinoviruses and RSV diagnosed most frequently.

The consequences of respiratory virus infection were investigated by Minor et al, (1974a) who followed a cohort of 16 children with a history of wheezing for a period of eight months. 61 episodes of asthma were recorded of which 42 were associated with symptoms of an upper respiratory tract infection (URTI). Nasal and pharyngeal specimens were taken for virus isolation and bacteriology. Asthmatic attacks were strongly related to rhinovirus and influenza A infection, whereas bacteria were found to be unimportant. Asthmatic children had a significantly greater number of respiratory viral infections (particularly rhinovirus infections) than their non-asthmatic siblings who acted as controls (Minor et al, 1974b). The reason for this increase was unclear but it was suggested that the asthmatic children might have spent more time indoors and thus receive greater exposure to respiratory viruses shed by other members of the family.

Although most studies show a strong correlation between respiratory virus infection and asthmatic attacks in children, Disney et al, (1971) found that seasonal peaks of virus isolation corresponded to peaks of admissions for respiratory
infections, but did not correlate with admissions for acute asthma in a study of paediatric admissions in Birmingham over a 12 month period. A study of children over the age of one year admitted with asthma or wheezy bronchitis found that viruses were isolated from only 39 (14.2%) of 267 specimens (Mitchell et al, 1976).

In summary these studies show that respiratory virus infection is undoubtedly an important precipitating factor in childhood asthma but the results are often difficult to interpret due to differing protocols and techniques for virus diagnosis. In infants RSV and parainfluenza are the most important causes of a wheezy respiratory illness whereas in older children rhinoviruses and influenza appear to be of greater significance.

Adults with asthma

Several studies have investigated the role of respiratory virus infections in adults with asthma. Hudgel et al, (1979) followed a cohort of 19 adults (age range 24-67) with chronic asthma over a 15 month period. A total of 84 wheezing exacerbations were recorded and 76 were evaluated by either viral culture of nasal washes, serology or both (number of each unspecified). Respiratory viruses were implicated in only 8 attacks, however the timing of specimens was not documented and coronavirus serology was not performed. Tarlo et al, (1979) conducted a prospective study of 19 married asthmatics (age range 26-71) over 1 year and found that the asthmatic patients reported 23 upper respiratory infections compared to only 13 in their spouses. Nasal and throat swabs were obtained for viral culture (timing unspecified) and serology was also performed. 14 subjects in this study kept symptom diaries and 35 exacerbations of asthma were recorded. Only 3 (9%) of the 36 exacerbations were associated with cold symptoms and a respiratory virus was identified in only 1 (3%) symptomatic exacerbation. Clarke et al, (1979) followed 51 asthmatic patients (mean age 35.1 years) for up to 18 months. Virus isolation or serology was positive in only 8 (7%) of the 111 exacerbations recorded during the
study period. However swabs for virus isolation were only obtained in 27 episodes (of which 4 were positive), and the authors acknowledged that some specimens were obtained over five days after the onset of symptoms when isolation rates would be expected to be low, thus no valid conclusions on the importance of respiratory virus infections can be drawn from this study. Kava (1986) followed 92 adult asthmatics (age range 15-77) for 6 months and found that 25% of exacerbations were associated with symptoms of upper respiratory tract infection with a mean duration of illness of 11.4 days. Virology was not performed in this study and it was therefore not possible to determine the actual importance of respiratory virus infection. A recent comprehensive study (Beasley et al, 1988) investigated the role of respiratory viruses in 31 patients with atopic asthma (aged 15-56 years) who were followed for 11 months. Nasopharyngeal aspirates were performed each month and additional aspirates were obtained "as soon as possible" after the onset of respiratory tract symptoms or asthmatic attacks. Paired sera were obtained for each symptomatic episode. Viruses were identified in thirty episodes of which 18 (60%) were associated with an exacerbation of asthma. A total of 178 exacerbations were recorded of which 28 were classified as severe (FEV₁<60% or peak flow less than 40% of initial value). Respiratory viruses were identified in 18 (10%) of the 178 exacerbations overall and 10 (36%) of the 28 severe exacerbations.

A different approach was taken by Gregg (1972) who investigated the effect of proven rhinovirus infections in adults with asthma or wheezy bronchitis and found that 20 (87%) of 23 rhinovirus infections were associated with wheezing. These results contrast with those of Halperin et al, (1985) who exposed 21 non-immune asthmatic volunteers to experimental rhinovirus infection. 17 patients developed coryza and 4 volunteers suffered a decrease in FEV₁ of over 10% with increased histamine sensitivity, however the group as a whole showed no significant change in spirometry, but studies of small airway function were not performed. Rhinovirus was isolated from the bronchial washings of only 1 of 4 patients who had bronchoscopy performed. This study clearly showed that rhinovirus infection was
not a major precipitant of asthma in this cohort.

These studies show that respiratory viruses may precipitate attacks of asthma but infection has only been implicated in a minority of exacerbations. As in the childhood studies the interpretation of the results is difficult as optimal specimens for virus culture were not obtained for many of the symptomatic episodes, and coronavirus serology was not performed by any of the investigators. The true importance of respiratory virus infection may therefore have been underestimated.

**Chronic bronchitis**

One of the earliest studies of the association between respiratory viral infections and exacerbations of chronic bronchitis was performed by Carilli *et al.* (1964) who studied 30 subjects with chronic bronchitis and 10 healthy controls over a period of eight months and found that the patients with bronchitis had eight times as many episodes of acute respiratory illness as controls. Although it was not possible to diagnose rhinovirus infections in this study the results showed that 24 (52%) of 46 acute exacerbations were associated with proven viral infection including 8 RSV, 4 influenza A, 4 *Mycoplasma pneumoniae*, 2 parainfluenza 3, and 6 other infections including adenovirus, *Chlamydia psittaci* and *Coxiella burnetii*.

Later studies showed an similar association between rhinovirus infection and exacerbations of chronic bronchitis (Grist 1967). Stenhouse *et al.* (1967) demonstrated rhinovirus infection in 8 (14%) of 56 exacerbations and Stott *et al.* (1968) isolated rhinoviruses from 14 (16%) of 87 exacerbations of chronic bronchitis, including 7 cases where rhinoviruses were isolated from sputum samples suggesting viral replication in the lower respiratory tract. Further studies were performed by Lamy *et al.* (1973) who confirmed respiratory virus infection in 31 (63%) of 49 exacerbations of chronic bronchitis, and Buscho *et al.* (1978) who showed evidence of respiratory viral infection in 25% of 166 exacerbations. Virus isolation was not performed by Busche *et al.* (1978) and virus infection was diagnosed by serology only. Influenza A, parainfluenza and coronavirus
OC43 were most the most frequently diagnosed agents and seroconversion was also documented in 14% of samples taken during periods of remission, showing that virus infection does not necessarily lead to a worsening of bronchitis.

The increased susceptibility of patients with bronchitis to respiratory tract infection was shown by the Tecumseh study which investigated a community in Michigan, USA over one year (Monto et al, 1975). The results showed that patients with chronic bronchitis suffered from an increased total illness rate and a greater number of seroconversions to respiratory viruses than healthy controls. The reasons are unclear but it is possible that long-standing lower respiratory tract disease disrupts normal lung defence mechanisms.

**Cystic fibrosis**

Several studies have investigated the role of respiratory viruses in the progression of pulmonary disease in patients with cystic fibrosis. In 1976 Wright et al, studied 48 episodes of symptomatic respiratory illness in a cohort of 153 CF children. Paired sera were obtained from 36 episodes and respiratory viruses were identified in 7 (39%) of 18 major exacerbations and 5 (28%) of 18 minor exacerbations. RSV and influenza A were the most common agents diagnosed. Petersen et al, (1981) followed 116 CF patients, aged 6 months to 29 years for a period of eight months and recorded 322 acute respiratory exacerbations in 108 subjects (mean 2.9 exacerbations/patient). 76% of the exacerbations were attributed to bacterial infection and 20% were associated with serological evidence of respiratory virus infection, however virus isolation was not performed in this study. The highest rate of virus infection was in those aged 5-9 years and RSV occurred most frequently, followed by parainfluenza, influenza and adenovirus. RSV infections were more common in patients who were colonised with *P. aeruginosa*.

The relationship between respiratory virus infection and clinical state was investigated by Efthimiou et al, (1984) who studied a cohort of 46 adults for a period of 4 months. Nose and throat swabs were taken for virus isolation and
serology was performed. Seroconversion occurred in 29% of patients who had pulmonary deterioration compared to 4.5% of those whose lung condition remained unchanged. Viruses were isolated from only 3 symptomatic episodes (2 adenovirus, 1 varicella zoster) which were each accompanied by a significant rise in specific antibody titre. Unfortunately the study period was very short and it was not possible to assess the longer-term consequences of respiratory virus infection. A more comprehensive prospective study was reported by Wang et al, (1984) who followed 49 CF patients over the age of 6 (mean age 13.7 years) and 19 normal siblings over a two year period. The CF patients reported 3.7 respiratory illnesses/year compared to 1.7/year in the control siblings. A total of 105 infections were identified by virus serology (42 parainfluenza, 26 influenza A or B, 24 RSV and 13 adenovirus) and highly significant correlations were observed between the annual incidence of viral infections and indicators of disease progression (Schwachman score, weight for height ratio, pulmonary function, frequency and duration of hospitalisation). Although nasal washings were taken from the majority of patients during acute exacerbations no viruses were isolated from a total of 1046 specimens. The reason for the inability to isolate viruses was uncertain but the authors postulated that the sputum from these patients might contain an inhibitory substance.

Stroobant (1986) recently studied a group of 30 CF children aged 5-16 years over a period of 12 months. Virus isolation and serology were performed in 63 cases of upper respiratory tract infection and respiratory viruses were diagnosed in 24 (38%) episodes. All 24 proven infections were associated with chest deterioration and a number of agents were responsible (influenza A and B, parainfluenza, rhinovirus, RSV, adenovirus, Mycoplasma pneumoniae and herpes simplex virus). In 1989 Hordvik et al, reported a 2 year prospective study of acute respiratory infections in 10 CF patients aged 5-32 and documented a total of 35 illnesses. Respiratory viruses were isolated in 5 (14%), of which 4 were influenza B and 1 rhinovirus. A further 10 episodes (29%) were associated with serological evidence of virus infection. The 5 subjects with more severe initial abnormalities in
pulmonary function had a significantly greater decrease in peak expiratory flow rate associated with viral infection (mean decrease 42.4%) than the remainder (mean decrease 15.4%) and took longer to recover to baseline measurements (22 days compared to 15 days). 7 of 8 pulmonary exacerbations requiring hospital admission were preceded by viral infections. The study showed that there was a definite relationship between respiratory viral infection and exacerbations of cystic fibrosis and the consequences of infection were most pronounced in patients with severe disease. Unfortunately coronavirus serology was not performed in any of the studies of respiratory virus infection in cystic fibrosis and once again the true importance of these infections may have been underestimated.

3. Mechanisms of airways obstruction induced by respiratory viruses

The relationship between respiratory virus infection and the development of wheeze is strongest for childhood RSV infection (Rooney and Williams, 1971; Frick and Busse, 1988; Sims et al, 1978), but it is still unclear whether RSV infection actually causes asthma or merely precipitates wheezing in an already susceptible child (Busse, 1985). The association of respiratory virus infections with the onset of allergic symptoms was investigated by Frick (1983) who followed up infants with two allergic parents and compared them to a control group of children with no family history of allergy. 21 (87.5%) of 24 susceptible children developed symptoms suggestive of allergy (eczema, rhinitis, asthma, otitis or colic) compared to only 7 (25%) of 28 controls. 62% of the children with allergic symptoms had rising titres to respiratory virus infection coincident with the onset of symptoms and the most common agents were RSV and parainfluenza. Although these studies demonstrate a temporal association between virus infection and development of allergy in these susceptible infants a causative relationship remains unproven.

The mechanisms by which virus infection could affect airways function have recently been reviewed by Busse (1985) and Li and O’Connell (1987). Induced bronchial hyper-reactivity can be blocked by atropine and it has been suggested that
bronchoconstriction may be mediated via a neural mechanism involving sensitisation of the vagus nerve by damaged bronchial epithelium (Hall and Hall, 1979; Frick, 1983; Busse, 1989). Another possibility is that viruses cause bronchoconstriction through the formation of virus-specific IgE with subsequent mediator release. Evidence for this comes from a study performed by Welliver et al. (1980) who found that all RSV-infected infants with bronchiolitis or asthma had cell-bound IgE in nasal secretions compared to only 33% of those who had RSV-infection without wheezing. A further study in 79 infants under 12 months of age revealed anti-RSV IgE in only 1 of 19 infants who did not wheeze but in the majority of the 60 patients with wheezing (Welliver et al., 1981). Parainfluenza virus was found to induce similar effects on IgE release in wheezy children (Welliver, 1983). IgE induced wheezing may be mediated by histamine release as histamine levels were found to be higher in wheezy children (Welliver et al., 1981; Welliver, 1983) and IgE-mediated histamine release from basophils is stimulated if cells are incubated with live or inactivated respiratory viruses or interferon (Lett-Brown et al., 1981; Welliver, 1983; Chonmaitree et al., 1988). An alternative explanation for the association of wheezing and IgE release with RSV infection is that the outcome of RSV infection depends on whether the child is atopic. Laing et al. (1982) found that infants with RSV bronchiolitis were more likely to have previous features of atopy than a matched group of control infants admitted with other acute illnesses.

The effect of sequential virus infections on the lung was recently investigated by Jakab (1990) who induced parainfluenza or influenza A pneumonia in mice. Some of the mice infected with parainfluenza were exposed to influenza A 30 days later. Sacrifice of mice on day 90 showed that those with sequential infections had more severe pneumonia and a higher content of hydroxyproline, indicating more severe fibrosis and collagen deposition. Concomitant infection with influenza and Klebsiella pneumoniae was also associated with a greater rise in hydroxyproline than infection with influenza alone and the results suggest a cumulative effect of viral and bacterial lung infections. The interaction between respiratory virus infection and secondary
bacterial infection was investigated by Clementsen et al, (1989) who showed that influenza virus potentiated the release of histamine induced by Staphylococcus aureus in human leucocyte suspensions and the effect was abolished by inhibition of neuraminidase.

In conclusion, respiratory virus infections are associated with exacerbations of asthma, chronic bronchitis and cystic fibrosis. RSV is the major viral cause of airways obstruction in infants below the age of 2 years with other respiratory viruses becoming more important in older children and adults. It is possible that the early interruption of viral replication might limit the severity and duration of the pulmonary function abnormalities associated with viral exacerbations of chronic chest disease.
CHAPTER 4
INTERFERON AND RESPIRATORY VIRUS INFECTIONS

1. Introduction
One of the important landmarks in virology was the discovery of interferons by Isaacs and Lindenmann in 1957. Their experiments showed that heat-inactivated influenza virus was able to stimulate the production of a factor that interfered with the multiplication of infectious influenza virus in chick chorioallantoic membrane. The recognition of interferons began a vast amount of research which has led to the identification of a family of related molecules with widespread biological activity and therapeutic importance. The identification of interferons was initially hailed with great optimism as they were believed to represent agents which might have a wide spectrum of antiviral and antimitotic activity. The introduction of highly purified interferon, followed by the increased availability of leucocyte and recombinant interferon (Merigan, 1982; Merigan, 1983; Tanaka, 1983) has stimulated many clinical trials of interferon in virus infections and malignant conditions.

2. Classification and properties of interferons
Three major classes of human interferons have been identified, interferon-alpha, beta and gamma. They are antigenically distinct proteins 165-187 amino acids in length with Mwt 17-25Kd (Balkwill, 1989). Interferon-alpha is predominantly produced by leucocytes, usually in response to viral infection, interferon-beta is produced by fibroblasts, also in response to viral infection and interferon-gamma is produced by lymphocytes in response to mitogens or specific antigens. Although interferons are found in all species of vertebrates they have a narrow host specificity and interferons from different species do not usually cross-react.

At least 23 interferon-alpha genes have been identified on chromosome 9 (Burke, 1985; Balkwill, 1989), but the specific functions of the gene products is unclear (Taylor-Papadimitriou et al, 1987). Interferon beta and interferon gamma
are coded by single genes located on chromosomes 9 and 12 respectively (Sehgal et al., 1983; Trent et al., 1982). Interferons are secreted proteins which attach to specific receptors on effector cells (Streuli et al., 1981; Burke, 1985; Balkwill, 1989; Huez et al., 1983; Siemers et al., 1988; Pestka et al., 1987). They resemble local hormones in their mode of action and are extremely potent with as few as 50 molecules having a biological effect at the cellular level. The successful cloning of the interferon genes (Tanaka et al., 1983) has led to the production of virtually limitless amounts of interferon for research and therapeutic use (Merigan, 1982; Miller et al., 1982; Merigan, 1983).

3. Mechanism of action

Interferons inhibit replication of a wide range of viruses (Lai and Joklik, 1973, Kohase and Kohno, 1983) and have wide-ranging effects on cell function and immuno-regulation (Fish et al., 1988). The induction of interferon results from cellular gene transcription (Burke, 1988). Interferon is induced by inactivated viruses, nucleic acid alone and synthetic nucleotides and the final common pathway is believed to be double-stranded RNA (Isaacs and Lindemann, 1957; Dianzani, 1970; Henderson and Joklik, 1978; Winship et al., 1980; Burke, 1985; Joklik, 1985). The induction of gamma-interferon in vitro was shown to be activated by the presence of lymphoblastoid cells and suitable lectins (Reem et al., 1982) and interferon production is also stimulated by a number of cytokines including tumour necrosis factor, interleukins 1 and 2, and colony stimulating factor (Balkwill, 1989). The inter-relationship between interferons, interleukins and other cytokines is complex and poorly understood (O'Garra, 1989a; O'Garra, 1989b).

As interferon becomes detectable within hours of viral infection, before the development of the inflammatory response it is thought to be one of the most important factors limiting the early spread of virus (Stiehm et al., 1982). The antiviral actions of interferons depend on the relative susceptibility of effector cells and the concentrations of interferons and infecting virus (Tyrrell, 1987).
Interferons stimulate the production of several proteins in effector cells which induce an antiviral state (Wagner, 1964), and a change in the properties of the cell membrane which inhibits virus replication (Naso et al, 1982; Jay et al, 1983; Whitaker-Dowling et al, 1983). Interferons inhibit translation of viral messenger RNA by causing a failure of viral mRNA and ribosomes to combine and form polyribosomes (Joklik and Merigan, 1966) and have the ability to prevent integration of tumour virus and retrovirus DNA into the host cell (Mozes and Defendi 1979; Avery et al, 1980). In addition, interferons have profound effects on the control of cell function (De Maeyer and De Maeyer-Guignard, 1982) and the immune system (Basham et al, 1982; Rager-Zisman and Bloom, 1985).

Cytotoxic effects are observed in virus-infected cells 3-4 hours after exposure to interferon (Joklik and Merigan, 1966). Interferon-treated cells cannot support virus replication and are destroyed by the immune system, thus helping to terminate the viral infection and increasing the overall chances of cell survival (Sekellick and Marcus, 1980). In some situations there may be a balance between virus cytopathic effect and the cytoprotective effect of interferon resulting in the maintenance of a low level of virus replication and persistent infection (Sekellick and Marcus, 1980).

The molecular basis of the action of interferons is poorly understood, but a number of changes are observed in the function of the host cell following the binding of interferons to their receptors, with alterations in the expression of some 50-100 gene products (Balkwill, 1989). The functions of most of these induced proteins are unknown, however 2 enzymes appear to be particularly important in mediating the effects of interferon, 2-5A synthetase (Kerr and Brown, 1978; Slattery et al, 1979) and a protein kinase (Lebleu et al, 1976; Tovey et al, 1981; Balkwill, 1989).
4. Pharmacokinetic and tolerance studies

Interferons were first obtained from pooled buffy-coat lymphocytes stimulated with parainfluenza virus (Scott and Tyrrell, 1980), however interferon could only be produced in relatively small amounts and was only 0.1% pure. More recently these problems have been overcome by the availability of large amounts of recombinant (Tanaka, 1983) or lymphoblastoid interferons (Pickering et al, 1983).

Interferon preparations may be given subcutaneously, intramuscularly, or topically. A variety of adverse effects have been associated with the use of subcutaneous or intramuscular alpha-interferon including influenza-like symptoms of fever, lethargy, malaise, and myalgia. These symptoms occur frequently but are generally mild and tend to be worse during the initial period of treatment (Ingimarsson et al, 1979). They were originally thought to be due to impurities but similar adverse effects were experienced by subjects using highly purified and recombinant preparations (Scott et al, 1981; Tyrrell, 1987). The tolerance of very high doses of interferon, (up to 198MU) was investigated in cancer patients by Gutterman et al, (1982) who reported mild myelosuppression, and symptoms of peripheral neuropathy in several patients which resolved completely when interferon was discontinued. Smedley et al, (1983) documented other neurological side-effects including somnolence, confusion, upper motor neurone signs and increased EEG slow wave activity. These were related to the dose of interferon and resolved on cessation of treatment.

The clinical use of interferon-beta has also been evaluated. Interferon-beta appears to offer no significant therapeutic advantages over interferon-alpha and blood concentrations after parenteral administration were found to be considerably lower, possibly due to local inactivation (Edy, 1978; Billiau et al, 1979; Eisenberg et al, 1986).

Intranasal interferon has been used for the prophylaxis and treatment of respiratory virus infections and offers potential advantages over parenteral administration—notably maximum concentration can be achieved at the site of virus
replication with relatively little systemic absorption. Pharmacokinetic studies showed that the mean half-life of intranasally applied technitium-labelled albumin was in the order of 20-25 minutes (Aoki and Crawley, 1976; Scott et al, 1982a), however a proportion of interferon activity remained for over 24 hours and not all of the interferon could be recovered by nasal washes (Scott et al, 1982a; Wynne Davies et al, 1983). It was therefore concluded that interferon probably bound to mucosal cells and that single daily spraying might be sufficient. The particle size is another crucial factor which influences the distribution of medication and the intranasal spray devices used in clinical trials against respiratory virus infections were designed to produce relatively large particles which would predominantly collect in the nasopharynx. The use of a small particle aerosol to deliver interferon into the lungs has been shown to prevent pulmonary virus infection in mice (Wyde et al, 1984; Wyde et al, 1985) but has not been used in man.

A large number of clinical studies have shown that short term use (fewer than 8 days) of intranasal interferon in doses between 1.5-22.5MU/day is well tolerated with few adverse effects (Merigan et al, 1973; Greenberg et al, 1982; Scott et al, 1982a; 1982b; Samo et al, 1983). Very high doses of intranasal interferon (42.8MU once daily for 5 days) were associated with transient leucopaenia (WCC<4,000) in 3 of 14 patients (Hayden and Gwaltney, 1983a). Longer-term administration of interferon causes local nasal reactions and blood-stained nasal discharge which resolve rapidly when medication is discontinued (Samo et al, 1983). The long-term tolerance of interferon was investigated by Samo et al, (1984) who gave 1.2MU of intranasal interferon twice daily for 26 days to 20 subjects. 3 subjects discontinued treatment as they developed blood-stained nasal discharge due to superficial mucosal erosions. Hayden et al, (1983b) found that 23% of volunteers given 8.4MU of interferon-alpha daily for 28 days complained of nasal discharge, crusting or bleeding. The nasal abnormalities in symptomatic patients include increased vascularity of the nasal mucosa, ulceration and the presence of occasional fine punctate bleeding points (Hayden et al, 1983b; Douglas
Histology shows pronounced lymphocytic and mononuclear cell infiltration (Hayden et al., 1983b). The changes return to normal 7-14 days after stopping medication (Hayden et al., 1983b; Douglas et al., 1985).

The tolerance of intranasal recombinant beta-interferon was investigated by Sperber et al., (1988) who gave high (12MU/day) or low dose beta-interferon (3MU/day) or placebo spray twice daily for 25 days to healthy volunteers and found similar changes to those reported with interferon-alpha. There was an increase in nasal obstruction or bleeding in patients who received active medication and 54% of the high dose group had lymphocytic infiltration on nasal biopsies compared to 17% of placebo controls.

5. Clinical use of interferons

Interferons have been used clinically in a wide range of viral conditions including herpetic dendritic corneal ulcers (Tommilla, 1963; Emodi et al., 1975; Sundmacher et al., 1976; Jones et al., 1976; Kaufmann et al., 1976; Sundmacher et al., 1978), cutaneous herpes simplex virus infections (Pazin et al., 1979; Overall et al., 1981; Ho et al., 1984; Eron et al., 1987; Egbaria et al., 1990), herpes zoster (Merigan et al., 1978; Arvin et al., 1982), cytomegalovirus (Arvin et al., 1976, Emodi, 1976; Cheeseman et al., 1979; Meyers et al., 1982; Hirsch et al., 1983; Shepp et al., 1984), genital warts (Ikc et al., 1981; Geffen et al., 1984; Schonfeld et al., 1984; Eron et al., 1986; Reichman et al., 1990), juvenile laryngeal papilloma (White et al., 1983), viral encephalitis (Behan, 1981; Prange and Wismann, 1981; Olding-Stenkvist et al., 1982; Mizutani et al., 1985), HIV infection (Hartshorn et al., 1986; De Wit et al., 1988; Lane et al., 1988; Krown et al., 1990), chronic hepatitis B (Greenberg et al., 1976; Merigan et al., 1980; Weimar et al., 1980; Scullard et al., 1981; Levin and Hahn, 1982; Dooley et al., 1986; Eisenberg et al., 1986; Gregory, 1986; Brook et al., 1989; Daniels et al., 1990; Perrillo et al., 1990), NANB hepatitis (Thomson et al., 1987) and hepatitis D (Thomas et al., 1985; Actis et al., 1986; Wiselka and Nicholson, 1987).
The results of these trials have shown that interferon is associated with a small therapeutic benefit in herpes simplex and herpes zoster infections but has been superseded by the use of acyclovir. Interferon is not effective in cytomegalovirus infections and is only associated with a partial response in genital wart infections. Interferon inhibits HIV \textit{in vitro} (Ho et al., 1985; Billiau, 1986) but does not appear to alter the prognosis in patients with AIDS, although skin lesions regress in approximately 30-40\% of patients with Kaposi's sarcoma, and combination therapy (eg. with phosphonoformate) is being evaluated (Johnson, 1990). The only viral infection for which interferon treatment is currently in clinical use is chronic active hepatitis B. Studies have been difficult to evaluate as they have included different groups of patients (Schalm and Heijtink, 1982), however response rates of 30-40\% have been achieved using 10MU alpha-interferon on alternate days for three months. Patients who are most likely to respond to treatment include those with abnormal liver function tests, females, patients with relatively recent infection, and those pretreated with steroids (Omato, 1985). Homosexual men, patients who are HIV positive and those who acquired the infection in the perinatal period are unlikely to respond.

The use of interferon in malignant conditions has generally been disappointing (Dunnick and Galasso, 1979; Dunnick and Galasso, 1980; Scott and Tyrrell, 1980; Goldstein and Laszlo, 1986; Sikora, 1986; Galvani et al., 1988; Balkwill, 1989), however response rates of 70-90\% have been achieved in hairy cell leukaemia (Goldstein and Laszlo, 1986; Balkwill, 1989; Lancet editorial; 1990), chronic myeloid leukaemia (Talpaz et al., 1987) and essential thrombocythaemia (Giles et al., 1988).
6. Interferon in respiratory virus infections

In vitro susceptibility studies

A series of yield reduction and plaque inhibition experiments performed by Merigan et al, (1973) showed that several strains of influenza and rhinovirus were sensitive to human leucocyte interferon. The comparative susceptibility of respiratory viruses to recombinant interferon-alpha and interferon-beta was recently investigated by Sperber and Hayden (1989) using a similar yield reduction assay. Both interferons were found to have high activity against rhinovirus rhinovirus 1A and 39, and coronavirus 229E (90% inhibition at interferon concentrations of $10^{-11}$-$10^{-10}$ gram of protein/ml). Influenza and parainfluenza viruses were inhibited at concentrations of $10^{-9}$ gram of protein/ml but interferons did not inhibit adenovirus or RSV.

Experimental rhinovirus infection

The clinical use of interferon in experimental rhinovirus infection was first investigated by Merigan et al (1973) who gave volunteers a total of 14MU of intranasal leucocyte interferon or placebo in 39 separate doses over a 4 day period and challenged with rhinovirus type 4, 24 hours after commencing medication. Treatment with interferon resulted in a significant decrease in severe symptoms and virus shedding compared to controls and no major adverse effects were seen.

In view of the scarcity and cost of interferon Gaitmaiten et al, (1973) investigated the effect of a locally applied interferon-inducing chemical (N,N-dioctadecyl-N'-bis-[hydroxyethyl]-propanediamine) in healthy volunteers. Although interferon levels increased in some subjects to a degree equivalent to that reached after respiratory virus infection, treatment was not associated with a decreased rate of infection or virus shedding after challenge with rhinovirus 21.

Further placebo-controlled studies have investigated the use of leucocyte (Greenberg et al, 1982; Scott et al, 1982a), lymphoblastoid (Phillpotts et al, 1984c) and recombinant alpha-interferon (Scott et al, 1982b; Hayden and
Gwaltney, 1983a; Samo et al 1983; Samo et al, 1984; Meschievitz and Turner, 1988) in experimental rhinovirus infections. In all studies medication was administered over a period of 4-5 days, and volunteers were challenged with known laboratory strains of rhinovirus 12-48 hours after commencing therapy. The results showed that interferon treatment, in the dose range of 8.1-44.8MU/day, was associated with a significant reduction in the number of colds and recovery of virus. Lower doses of interferon (1.4-3MU/day) were not effective (Samo et al, 1984; Meschievitz and Turner, 1988). A multiple dose regimen was found to be more beneficial than single daily administration (Hayden and Gwaltney, 1983a). Reported adverse effects were generally mild, but included mild nasal stuffiness, sore throat and epistaxis (Scott et al, 1982a; Samo et al, 1983).

The antirhinoviral agent enviroxime has been shown to have marked synergy with interferon in-vitro and the clinical efficacy of combination treatment was investigated by Higgins et al, (1988b). Results showed that interferon alone reduced the daily symptom score and nasal secretion weights in volunteers challenged with rhinovirus type 9. Enviroxime alone had no effect and there was no observed synergy between the two agents. This disappointing result was thought to be due to the rapid clearance of intranasal enviroxime.

A recent clinical trial compared intranasal recombinant interferon-beta (1.5MU or 6MU twice daily for 6 days) with placebo in 36 volunteers who were challenged with rhinovirus after the third dose (Sperber et al, 1988). The higher dose of interferon-beta was associated with a significantly lower total symptom score but interferon-beta did not reduce the rate of infection, seroconversion, or virus shedding. A placebo-controlled study of recombinant gamma-interferon gave even more disappointing results as interferon-gamma did not lead to any reduction in the number of clinical illnesses or seroconversions and interferon recipients experienced higher symptom scores and nasal secretion weights, and a greater incidence of blood-stained nasal discharge than control subjects (Higgins et al, 1988a).
Influenza and coronavirus infections

Several studies have investigated the clinical efficacy of interferon against influenza and coronavirus infections. In 1973 Merigan gave volunteers 0.8MU of leucocyte interferon in 16 doses over 24 hours and challenged with influenza B virus. This relatively low dose of interferon did not prevent illness or reduce severity of symptoms. A more recent trial investigated the use of interferon against experimental influenza A infection in volunteers who were given 2.7MU of interferon or placebo 3 times daily for a total of 13 doses starting 1 day before virus challenge (Phillpotts et al, 1984c). 4 (31%) of 13 interferon-recipients developed a definite illness compared to 10 (59%) of 17 controls, but this result was not statistically significant.

The use of interferon to treat experimental coronavirus infection was investigated by Higgins et al, (1983) who reported a reduction of symptoms in treated volunteers. In a larger study Turner et al, (1986), gave volunteers either intranasal interferon (1MU twice daily for 15 days) or placebo and challenged with coronavirus 229E on day 8. Interferon was associated with a significant benefit as 12 (41%) of 26 subjects who received interferon developed colds, compared to 19 (73%) of 29 controls. In addition, active treatment significantly reduced the intensity of symptoms.

Treatment of established upper respiratory tract infections

The efficacy of intranasal interferon given after experimental virus challenge was investigated by Hayden et al, (1984) who treated susceptible volunteers with interferon-alpha (9MU 3 x daily for 5 days) or placebo, starting 28 hours after inoculation with rhinovirus 39. Although interferon did not reduce the number of rhinovirus infections or colds it was associated with a reduction in symptoms and duration of virus shedding. A further study investigated the use of interferon to treat naturally-occurring colds of less than 48 hours in duration (Hayden et al, 1988). Patients were randomly assigned to receive 5 days of treatment with interferon nasal
spray at 10MU (n=74) or 20MU (n=74) daily in four divided doses or placebo (n=72). Mean duration of symptoms prior to using the trial medication was 27 hours for both the interferon groups and 26 hours for the placebo group. The results showed that active treatment had no effect as the median duration of colds was 10 days in the 20MU group, and 8 days in both the 10MU and placebo groups. Rhinoviruses were isolated from comparable numbers of treated and control patients and both the interferon groups had a higher frequency of nasal bleeding (16-18%) than controls (4%). Overall results of studies using interferon to treat established colds have therefore been disappointing (Sperber and Hayden, 1988).

*Longer term and seasonal prophylaxis*

Several large, placebo-controlled studies have investigated long-term use (22 days-2 months) of intranasal interferon as seasonal prophylaxis against naturally-occurring respiratory virus infections (Farr *et al.*, 1984; Douglas *et al.*, 1985; Monto *et al.*, 1986; 1988; Tannock *et al.*, 1988). These studies have shown that doses of interferon between 1.9MU-10MU/day prevent 76-100% of rhinovirus infections but have no effect on other respiratory viruses (Douglas, 1985) including influenza A, influenza B (Monto, 1988) and parainfluenza (Monto *et al.*, 1986). A lower dose of interferon (1.7MU daily) was found to be ineffective (Monto, 1988) and a study from Australia showed no benefit in volunteers given 1.5MU/3MU/or 6MU daily for 28 days, possibly because the trial period did not coincide with the rhinovirus season. All studies reported adverse effects, particularly blood tinged nasal discharge which occurred during the second or third week of treatment in 20-49% of subjects given interferon compared to 1-22% of controls.
**Interferon in the family setting**

Herzog *et al*, (1983) first reported the use of interferon prophylaxis in volunteer families recruited from the staff of the University of Basle. In this study family members commenced intranasal spraying with alpha-interferon (0.3MU or 1.5MU/day for 5 days) or placebo when one member of the family developed a definite cold. 587 participants were recruited from 147 families and the results showed that 22 (17%) of 126 given 1.5MU interferon/day developed colds compared to 22 (20%) of 110 given 0.3MU/day and 24 (24%) of 101 controls (Herzog *et al*, 1986). Although neither dose of interferon significantly reduced the number of colds the duration of illness and symptom scores were significantly lower in subjects who used 1.5MU/day. Nasal washes were only obtained from half the symptomatic contacts and no significant difference in the rhinovirus isolation rate was recorded in the treatment groups.

The prophylactic efficacy of higher doses of interferon (5MU once daily for 7 days) was investigated by two large family studies which were conducted in Australia (Douglas *et al.*, 1986) and the USA (Hayden *et al.*, 1986). In both studies adult family members commenced spraying with either interferon or placebo as soon as one family member developed a definite cold and the results were remarkably similar. In the Australian study 53 (16%) of 325 interferon courses and 71 (22%) of 319 placebo courses were judged to have been treatment failures. This was only a modest reduction (overall efficacy 27%), but many of those judged to have been failures had only mild symptoms. Definite colds developed in 32 subjects who used interferon compared to 53 control subjects (efficacy 41%). In episodes where rhinovirus was isolated from the index case only 2 of 36 subjects who used interferon developed definite rhinovirus colds compared to 13 of 34 control subjects (efficacy against rhinovirus infections 86%). The mean number of symptomatic days was reduced by 76% in these rhinovirus episodes. There was no evidence of any protective benefit of interferon against any other respiratory viruses. The study concluded that 100 courses of intranasal interferon would prevent 6.8 definite
respiratory illnesses. In the American study respiratory illness developed in 32 (14.2%) of 226 interferon episodes and 52 (23.4%) of 222 placebo episodes (overall efficacy 39%). In episodes where rhinovirus was isolated from the index case only 2 (7.4%) of 27 subjects who used interferon developed proven rhinovirus colds compared to 12 (35.3%) of 34 control subjects (efficacy 79%). Once again there was no evidence of any benefit of interferon against other respiratory viruses. The interferon spray was well tolerated in both studies.

These two studies showed that prophylaxis with 5MU/day of intranasal interferon for 7 days was associated with approximately 40% protection against definite colds and 80% protection against rhinovirus infections. The beneficial effect of interferon spray was thought to be due entirely to its effect against rhinoviruses (Douglas, 1986; Scott, 1986) and the lack of efficacy against other respiratory viruses was demonstrated by a family study which showed no overall benefit of interferon during an outbreak of influenza B (Foy et al, 1986). The dose of interferon is crucial for effective prophylaxis as Monto et al, (1989) found that a lower dose of interferon (5MU day 1 and 2.5MU days 2-5) did not prevent colds in a recent family study which investigated 434 courses of medication and isolated 178 rhinoviruses. At least 5MU daily of alpha-interferon is therefore needed for effective prophylaxis against rhinovirus infections and the potential clinical benefits of interferon must be weighed against its cost (Lancet editorial, 1982).
B. SUBJECTS MATERIALS AND METHODS
# CHAPTER 5

## SUBJECTS AND MATERIALS

### SUBJECTS AND MATERIALS

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Interferon trial</td>
<td>89</td>
</tr>
<tr>
<td>1.2 Children with cystic fibrosis</td>
<td>90</td>
</tr>
<tr>
<td>1.3 General Practitioners in Trent Region</td>
<td>91</td>
</tr>
<tr>
<td>1.4 Patients with asthma</td>
<td>91</td>
</tr>
<tr>
<td>1.5 Healthy volunteers at Leicester University</td>
<td>92</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interferon trial medication</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>92</td>
</tr>
</tbody>
</table>

### 3. Tissue culture materials and buffers

<table>
<thead>
<tr>
<th>Materials</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 Antibiotics</td>
<td>93</td>
</tr>
<tr>
<td>3.2 Phenol red indicator</td>
<td>93</td>
</tr>
<tr>
<td>3.3 Trypsin 2.5%</td>
<td>93</td>
</tr>
<tr>
<td>3.4 EDTA (versene)</td>
<td>93</td>
</tr>
<tr>
<td>3.5 Trypsin/versene solution</td>
<td>93</td>
</tr>
<tr>
<td>3.6 3M Magnesium chloride</td>
<td>94</td>
</tr>
<tr>
<td>3.7 Dimethylsulphoxide</td>
<td>94</td>
</tr>
<tr>
<td>3.8 Tryptose phosphate broth</td>
<td>94</td>
</tr>
<tr>
<td>3.9 Nutrient broth</td>
<td>94</td>
</tr>
<tr>
<td>3.10 Eagles Basal Media (BME diploid)</td>
<td>94</td>
</tr>
<tr>
<td>3.11 Foetal calf serum</td>
<td>94</td>
</tr>
<tr>
<td>3.12 Bicarbonate buffer</td>
<td>95</td>
</tr>
<tr>
<td>3.13 Phosphate buffered saline</td>
<td>95</td>
</tr>
<tr>
<td>3.14 Anti-interferon alpha antibody</td>
<td>95</td>
</tr>
<tr>
<td>3.15 Guinea pig red blood cells</td>
<td>95</td>
</tr>
<tr>
<td>3.16 Tissue culture flasks</td>
<td>96</td>
</tr>
<tr>
<td>3.17 Tissue culture tubes</td>
<td>96</td>
</tr>
</tbody>
</table>

### 4. Cell cultures

<table>
<thead>
<tr>
<th>Cultures</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Ohio HeLa cells</td>
<td>96</td>
</tr>
<tr>
<td>4.2 MRC-5 fibroblast cells</td>
<td>96</td>
</tr>
<tr>
<td>4.3 Madin-Darby canine kidney cells</td>
<td>96</td>
</tr>
<tr>
<td>4.4 C 16 cells</td>
<td>97</td>
</tr>
<tr>
<td>4.5 HEp2 cells</td>
<td>97</td>
</tr>
</tbody>
</table>
5. Virus transport medium and tissue culture media

5.1 Virus transport medium
5.2 Tissue culture growth media
5.3 Tissue culture flask maintenance media
5.4 Ohio HeLa cell tissue culture tube maintenance media
5.5 MDCK cell tissue culture tube maintenance media
5.6 C16, MRC-5 and HEp2 cell tissue culture tube maintenance media

6. Materials and buffer for complement fixation tests

6.1 Virus antigens
6.2 Positive control antisera
6.3 Sodium azide
6.4 Complement
6.5 Haemolysin
6.6 Sheep red cells
6.7 Complement fixation buffer
6.8 "U" bottomed 96-well microtitre plates

7. Materials for haemagglutination inhibition test

7.1 Receptor destroying enzyme (RDE) from cholera filtrate
7.2 Influenza virus strains
7.3 Chicken red cells
7.4 "V" bottomed microtitre well plates

8. Materials for coronavirus ELISA test

8.1 Coronavirus OC43 antigen
8.2 Coronavirus 229E antigen
8.3 Tween phosphate buffered saline solution
8.4 Coating buffer
8.5 Bovine serum albumin (BSA)
8.6 Diluent solution
8.7 Conjugate
8.8 Substrate buffer
8.9 Substrate tablets
8.10 Substrate solution
8.11 NUNC 96 well plastic, flat-bottomed microtitre well plates

9. Materials for immunofluorescence tests

9.1 Freund's complete adjuvent
9.2 Freund's incomplete adjuvent
9.3 Viral antisera
9.4 Preparation of coronavirus 229E antisera
9.5 Fluorescein isothiocyanate (FITC) conjugate
1. Subjects
1.1 Interferon trial

Subjects entering the trial were adults, of either sex aged between 16 and 75 with an established diagnosis of asthma, chronic bronchitis or bronchiectasis. Females of child-bearing age were only included if they were taking adequate contraception. It was our intention to choose patients who lived with children as they were likely to have an increased chance of exposure to upper respiratory tract infections.

Exclusion criteria for the trial were the following:

1. Any subject thought to be unreliable or unwilling to follow the trial protocol
2. Subjects with other active and serious medical conditions (eg diabetes, heart failure, renal failure) as these might complicate the interpretation of the trial.
3. Subjects who were pregnant or felt to be at risk of pregnancy as the safety of the interferon spray in pregnancy has not been fully established.
4. Subjects with nasal polyps or nasal deformity who may not have absorbed interferon adequately or may have been more susceptible to local side effects.
5. Subjects with known hypersensitivity to penicillins, as penicillin was used in the interferon manufacturing process.

Asthma was defined as a history of variable wheezy breathlessness with a documented FEV₁/FVC ratio of less than 60% at least once in their illness and a change in FEV₁ or peak flow rate of at least 15% either spontaneously or as a result of bronchodilator treatment.

Chronic bronchitis was defined as a history of sputum production on most days for at least 3 consecutive months for at least two successive years (MRC, 1965), an FEV₁ of less than 60% of the predicted value and a change of less than 15% in peak flow rate or FEV₁ either spontaneously or as a result of bronchodilator treatment.

Bronchiectasis was diagnosed if the patient had a characteristic long-standing history of chronic purulent cough usually accompanied by typical radiographic changes due to dilated bronchi.
Ethical Committee approval was obtained for the study and potentially suitable subjects were recruited directly from the chest clinic and from patients who had recently been admitted to Glenfield hospital with an acute exacerbation of chronic respiratory disease. Further candidates for the trial were identified from records of lung function tests performed by the Pulmonary Function Laboratory. The patients' case notes were obtained from Medical Records and details of the patient's history, chest X-ray appearance and pulmonary function were recorded. All patients who appeared to meet the criteria for entry to the trial were invited to attend an interferon trial clinic at Groby Road Hospital at a convenient time. At the trial clinic a full history was taken and the purpose and nature of the trial was explained. Written informed consent was obtained including consent for the taking of swabs (and blood if acceptable) from all family members under the age of 16. A full clinical examination was performed and peak flows (best of 3), FEV₁ and FVC were determined. Blood was taken for baseline investigations including full blood count, urea and electrolytes, glucose, calcium and liver function tests. An interferon/theophylline interaction has been identified with an increase in the half-life of theophylline elimination of approximately 15% after administration of intramuscular interferon (Jonkman et al., 1989), baseline drug levels were therefore documented in patients taking theophylline preparations. No clinically apparent interaction was suspected during the course of the trial and subsequent theophylline levels were not recorded. In accordance with MRC policy the volunteers did not receive any financial incentive for their participation in the study, but assistance with expenses for the clinic visit was offered.

1.2 Children with cystic fibrosis.
This study investigated the consequences of respiratory virus infection in children with cystic fibrosis. Parents of the 45 children in Leicestershire known to have cystic fibrosis were informed that the study was taking place and told that it did not entail any extra hospital visits or blood samples. The letter explained the nature of
the study and requested that those who were willing to co-operate should contact the CF Liaison Sister (Sister Wilde) at Leicester Royal Infirmary as soon as their child developed symptoms of an upper respiratory tract infection.

1.3 General Practitioners in Trent Region
During the winter of 1985-86 General Practitioners in the Trent Region were selected at random from the Family Practitioner Committee list and 244 GP's were sent a questionnaire asking details of the size and location of their practice, proportion of patients aged over 65, policies for adult vaccination, implementation of vaccination programme and an assessment of the numbers vaccinated, acceptance rate and reasons for non-acceptance. GP's were questioned about their vaccination policies for patients resident in nursing homes, or other institutions and for ambulant patients, including those with chronic medical conditions. Only one GP in each practice was sent a questionnaire to avoid duplication of information. 48 of the GP's who had not responded were subsequently telephoned and asked to complete the questionnaire by phone.

1.4 Patients with asthma
Subjects were selected from records of patients referred to Dr Stern at the Leicester General Hospital with a diagnosis of asthma. Although patients' dates of birth were not routinely recorded, the majority were aged 20-45 with allergic or atopic asthma, reflecting Dr Stern's interest in this condition.

1099 letters and questionnaires were sent at the end of January 1990. The questionnaire asked if and when influenza vaccination had been given and whether the patient thought they had "flu" during the preceding 2 month period. If they had experienced symptoms of "flu" they were asked to record the impact of the infection ie. symptoms experienced, effect of 'flu on their chest condition, the number of GP consultations, the use of medication, the number of days taken off work or school and need for hospital admission.
A second letter was sent to non-responders 3 weeks later offering an incentive to those who completed the questionnaire with a draw for a £50 dinner voucher in a local restaurant.

1.5 *Healthy volunteers at Leicester University*

This study investigated the impact and diagnosis of respiratory virus infections in healthy volunteers with acute upper respiratory tract symptoms who were recruited from students and staff working in the Medical Sciences Building of Leicester University. Posters advertising the study were prominently displayed on all Departmental notice boards and information about the study was published in the University "Bulletin" received by all members of Staff. In February-March 1990 Mrs Julie Kent, Dr Nicholson's technician, attended the Student Health Centre on Tuesday and Thursday mornings to take specimens from any consenting patients who were thought to have upper respiratory tract infections.

2. *Interferon trial medication*

For each subject the manufacturer (Boehringer Ingelheim) provided a block of four coded nasal sprays with two containing interferon and two placebo. Bottles of medication were stored at 4°C at Groby Road Hospital. Each spray had a code number allocated by the clinical trials department. The randomisation code was not disclosed until the trial had been fully completed and analysed and the patients' records had been returned to the company. It was possible to break individual codes under exceptional circumstances, if severe adverse effects were suspected.

The active spray contained a solution of recombinant alpha2-interferon administered as a metered aerosol delivering 0.5MU per spray. The precise formulation of the spray was not disclosed. A course of medication consisted of three sprays to each nostril twice daily (6MU/day) for five days.
3. Tissue culture materials and buffers

All solutions, media and buffers were autoclaved at 15psi, 121°C for 15 minutes.

3.1 Antibiotics

Penicillin/streptomycin was obtained from GIBCO (Paisley, Scotland) and supplied in 100ml amounts containing 5000 units/ml penicillin and 5000µg/ml streptomycin. Distributed into 1ml aliquots and stored at -20°C.

Gentamicin was obtained from GIBCO (Paisley, Scotland) and supplied in 100ml amounts containing 50mg/ml. Distributed into 1ml aliquots and stored at 4°C.

Amphotericin (fungizone) was obtained from GIBCO (Paisley, Scotland) and supplied in 50ml amounts containing 250µg/ml. Distributed into 1ml aliquots and stored at -20°C.

3.2 Phenol red indicator

Obtained from Flow Laboratories (Rickmansworth, Herts) and supplied in 100ml amounts containing 0.5% wt/vol phenol red indicator.

3.3 Trypsin 2.5%

Obtained from GIBCO (Paisley, Scotland) and supplied as 100ml amounts of 2.5% trypsin solution. Distributed in 5ml aliquots and stored at -20°C.

3.4 Diaminoethanetetra-acetic acid, (EDTA or Versene)

Obtained from GIBCO (Paisley, Scotland) and supplied in 100ml amounts of 1:5000 1x versene solution (0.2g/l). Distributed in 1ml aliquots and stored at 4°C.

3.5 Trypsin/Versene solution

Obtained from GIBCO (Paisley, Scotland) and supplied in 100ml amounts of trypsin/versene solution 1x containing 0.5g trypsin and 0.2g versene/l in modified Puck's saline A. Distributed in 5ml aliquots and stored at -20°C.
3.6 3M Magnesium chloride
Obtained from Fisons (Loughborough, Leics) and supplied as 500g amounts of Magnesium Chloride (MgCl$_2$). To make up 3M solution, 3g MgCl$_2$ was added to 5ml double distilled water (DDW) and stored at 4°C.

3.7 Dimethylsulphoxide
Obtained from Sigma (Poole, Dorset) and supplied in 500ml amounts.

3.8 Tryptose phosphate broth
Obtained from GIBCO (Paisley, Scotland) and supplied in 100ml amounts. Distributed in 20ml aliquots and stored at -20°C.

3.9 Nutrient broth
Obtained from Oxoid (Basingstoke, Hants) supplied as 500g amounts of nutrient broth powder. 13g powder was added to 1000ml DDW. Each litre of the resulting broth contained 1g Lab Lemco powder, 2g yeast extract, 5g peptone and 5g sodium chloride at pH7.4.

3.10 Eagles Basal Media (BME Diploid)
Obtained from GIBCO (Paisley, Scotland) and supplied as a powder containing BME Dipoid with Earle's salts, with L-glutamine without sodium bicarbonate. 1 litre stock made up by adding 9.18g of BME powder and 2.2g sodium bicarbonate to 1000ml DDW. Distributed into 100ml amounts and stored at 4°C.

3.11 Foetal Calf Serum
Obtained from GIBCO (Paisley, Scotland) and supplied in 100ml amounts, stored at -20°C.
3.12 Bicarbonate buffer
Bicarbonate buffer was made up by adding 5.6g sodium bicarbonate and 0.3 ml of 0.5% phenol red indicator to 100ml sterile DDW. Carbon dioxide (CO$_2$) was then bubbled through the buffer solution until an orange colour developed (at least 30 mins). The solution was filtered sterilised through a 0.22µm filter and distributed into 6ml aliquots in sterile bijoux bottles and stored at 4°C. Any discoloured samples were discarded and sterility was routinely checked by adding to nutrient broth and incubating at 37°C.

3.13 Phosphate buffered saline (PBS)
PBS was made up using PBS tablets (modified Dulbecco's formula without Calcium or Magnesium) which were obtained from Flow Laboratories (Rickmansworth, Herts) in batches of 100 tablets. Each tablet was dissolved in 100ml of DDW and distributed in 20ml aliquots.

3.14 Anti-interferon alpha antibody
Monoclonal anti-interferon antibody was kindly supplied by Wellcome Laboratories (Beckenham, Kent) and reconstituted as directed.

3.15 Guinea pig red blood cells
These were used in the haemadsorption test and were obtained fresh from Biomedical Services at Leicester University. Cells were washed x 3 in cold PBS with each wash followed by centrifugation in an MSE Centaur 2 bench centrifuge at 2000 rpm for 10 minutes before resuspending the pelleted cells in fresh PBS. The final cell pellet was diluted in PBS to give a 0.5% suspension (99.5ml PBS added to 0.5ml packed cells).
3.16 **Tissue culture flasks**
NUNC 260 ml flat-bottomed tissue culture flasks were used, obtained from Leicester University bulk store.

3.17 **Tissue culture tubes**
Sterilin Cell Cult 16 x 125mm tissue culture tubes were obtained from Appleton Woods (Selly Oak, Birmingham).

4. **Cell cultures**

4.1 **Ohio HeLa Cells**
This cell line was obtained from MRC Common Cold Unit (Salisbury, Wilts) and Flow Laboratories (Rickmansworth, Hert.). Cells are particularly susceptible to rhinoviruses and enteroviruses which may be distinguished by the acid stability test. Cytopathic effect may also be observed with RSV and adenoviruses.

4.2 **MRC 5 Fibroblast Cells**
Obtained from MRC Common Cold Unit (Salisbury, Wilts) and Flow Laboratories (Rickmansworth, Hert.) at passage 25-35. Cells are particularly susceptible to rhinoviruses, enteroviruses, adenoviruses and herpes viruses.

4.3 **Madin-Darby Canine Kidney Cells (MDCK)**
This cell line was obtained from MRC Common Cold Unit (Salisbury, Wilts) and is particularly susceptible to mumps, influenza and parainfluenza viruses. These viruses do not normally show CPE so presence of virus must be confirmed by demonstrating haemadsorption or by immunofluorescence.
4.4 C 16 Cells
This cell line was obtained from MRC Common Cold Unit (Salisbury, Wilts) and is derived from a clone of MRC-C human embryonic lung fibroblasts which were particularly susceptible to coronavirus 229E (Phillpotts, 1983b).

4.5 HEp-2 Cells
This cell line was obtained from Flow Laboratories (Rickmansworth, Herts) and is particularly susceptible to respiratory syncytial virus.

5. Virus transport medium and tissue culture media
5.1 Virus transport medium
Virus transport medium was made up in batches of 100ml by adding 1.3g of nutrient broth powder to 100ml sterile DDW. 10ml of sterile foetal calf serum (FCS) was added to the nutrient broth solution to make a 10% FCS solution. 1ml stock penicillin/streptomycin (5000u/ml penicillin/5000µg/ml streptomycin), 1ml stock gentamicin (50mg/ml) and 1ml of stock amphotericin (250µg/ml) were added using sterile pipettes.

2.5ml aliquots of virus transport solution were distributed into sterile plastic bijou bottles and stored at -20°C, with a small working stock at 4°C. Sterility was checked by adding samples of transport medium to nutrient broth, incubating at 37°C for 7 days and observing for evidence of bacterial growth. Any cloudy specimens were plated out to identify the contaminants.
5.2 Tissue Culture Growth Medium

This medium was used for growth of all the cell lines used (Ohio HeLa cells, MDCK cells, C16 cells, MRC-5 cells and HEp2 cells) and prepared as shown below and stored at 4°C until required:

- Eagles Basal Media 100 ml
- Foetal Calf Serum 10 ml
- Penicillin/streptomycin stock 1 ml
  (5000u/ml pen / 5000µg/ml strep)
- Amphotericin stock (250µg/ml) 1 ml
- Gentamicin stock (50mg/ml) 1 ml
- Bicarbonate buffer 3 ml

5.3 Tissue culture Flask Maintenance Medium

Medium for maintenance of tissue culture cell-lines in flasks was identical to the composition of the growth medium except for the substitution of 5% FCS. The medium was prepared as shown below and stored at 4°C until required.

- Eagles Basal Media 100 ml
- Foetal Calf Serum 5 ml
- Penicillin/streptomycin stock 1 ml
  (5000u/ml pen / 5000µg/ml strep)
- Amphotericin stock (250µg/ml) 1 ml
- Gentamicin stock (50mg/ml) 1 ml
- Bicarbonate buffer 3 ml
The composition of the medium for maintenance of cell-cultures in tissue culture tubes depended on the cell-lines used and the requirements for virus isolation.

5.4 Ohio HeLa cell Tissue Culture Tube Maintenance Medium
Ohio HeLa cells are particularly susceptible to picornavirus infection. In order to observe characteristic cytopathological effect (CPE) Magnesium Chloride and Tryptose Phosphate Broth must be added to the maintenance medium which was prepared as shown below and stored at 4°C until required:-

- Eagles Basal Media: 100 ml
- Foetal Calf Serum: 2.5 ml
- Penicillin/streptomycin stock: 1 ml
  
  (5000u/ml pen / 5000µg/ml strep)
- Amphotericin stock (250µg/ml): 1 ml
- Gentamicin stock (50mg/ml): 1 ml
- Bicarbonate buffer: 4 ml
- Tryptose Phosphate Broth: 5 ml
- 3M Magnesium Chloride: 1 ml

5.5 MDCK cell Tissue Culture Tube Maintenance Medium
MDCK cells are particularly susceptible to influenza and parainfluenza virus infection. The addition of trypsin to the medium facilitates virus isolation. Foetal calf serum was not required for the maintenance of MDCK cells in Tissue Culture Tubes. The medium was prepared as shown below and stored at 4°C until required:-

- Eagles Basal Media: 100 ml
- Penicillin/streptomycin stock: 1 ml
  
  (5000u/ml pen / 5000µg/ml strep)
- Amphotericin stock (250µg/ml): 1 ml
- Gentamicin stock (50mg/ml): 1 ml
- Bicarbonate buffer: 4 ml
- Trypsin 2.5%: 50 µl
5.6 *C16, MRC-5 and HEp2 cell Tissue Culture Tube Maintenance Medium*

Medium for maintaining these cells in tissue culture tubes was prepared as shown below and stored at 4°C until required:

- Eagles Basal Media 100 ml
- Foetal calf serum 2.5 ml
- Penicillin/streptomycin stock 1 ml
  
  (5000u/ml pen / 5000µg/ml strep)
- Amphotericin stock (250µg/ml) 1 ml
- Gentamicin stock (50mg/ml) 1 ml
- Bicarbonate buffer 4 ml
- Trypsin 2.5% 50 µl

6. Materials and buffer for complement fixation tests

6.1 *Virus antigens*

Virus antigens were supplied by Central PHLS (Colindale) as freeze dried preparations which were reconstituted as directed and stored at 4°C with 0.08% sodium azide added as a preservative. In Leicester University we performed complement fixation tests to determine antibody titres against influenza A, influenza B, respiratory syncytial virus and adenovirus. CFT’s against *Coxiella burnetti*, *Chlamydia psittaci* and *Mycoplasma pneumoniae* were kindly carried out at the Public Health Laboratory in Leicester.

6.2 *Positive control antisera*

"Standard" positive antisera were obtained freeze-dried from the Central PHLS (Colindale) and reconstituted as directed.

6.3 *Sodium Azide*

Obtained from Sigma (Poole, Dorset) supplied as 25g of crystalline sodium azide. A 10% solution was made by adding 1g of sodium azide to 10ml DDW.
6.4 Complement

Obtained from Tissue Culture Services (Slough, Berks) and supplied as lyophilised Guinea Pig serum in Richardson's solution (2ml), which could be stored indefinitely at 4°C. This was reconstituted by adding 2ml of DDW and agitating gently to dissolve the lyophilisate. 0.1% sodium azide solution added for storage. Prior to use the reconstituted complement was diluted with 7 volumes of DDW. The resulting solution was equivalent to a 1/10 dilution of fresh complement in isotonic saline.

6.5 Haemolysin

Obtained from Tissue Culture Services (Slough, Berks) and supplied as 5ml of rabbit haemolytic serum (anti-sheep erythrocyte).

6.6 Sheep red cells

Obtained from Tissue Culture Services (Slough, Berks) and supplied as 100ml amounts of Sheep red cell suspension in Alsever's solution which was used within one week of delivery. Cells were washed x 3 in PBS with each wash followed by centrifugation in an MSE Centaur 2 bench centrifuge at 2000 rpm for 10 mins before resuspending the pelletted cells in fresh PBS. The final cell pellet was diluted in CFT buffer to give a 4% suspension (24 ml buffer added to 1ml packed cells).

6.7 Complement fixation buffer

Obtained from Oxoid (Basingstoke, Hants) and supplied in batches of 100 barbitone-based CFT buffer tablets. 10 tablets were added to 1 litre of sterile distilled water to make up CFT buffer.

6.8 "U" bottomed 96-well microtitre plates

Obtained from Dynatech (Billingshurst, Sussex).
7. Materials for haemagglutination inhibition test

7.1 Receptor destroying enzyme (RDE) from cholera filtrate
Obtained from the Central PHLS (Colindale) and stored at 4°C.

7.2 Influenza virus strains
2 strains were kindly provided by Dr M Chakraverty, Virus Reference Laboratory, Central PHLS (Colindale); influenza A/Shanghai/11/87 H3N2 (titre 160) corresponding to the vaccine strain used in 1989 and influenza A/England/427/88 H3N2 (titre 640), representative of the 1989-90 epidemic strain.

7.3 Chicken red cells
Obtained from Tissue Culture Services (Slough, Berks) and used within 1 week of delivery. Cells were washed x 3 in cold PBS with each wash followed by centrifugation in MSE Centaur 2 bench centrifuge at 2000 rpm for 10 mins before resuspending the pelletted cells in fresh PBS. The final cell pellet was diluted in PBS and stored as a 10% suspension (9ml PBS added to 1ml packed cells). Blood used in the test was further diluted in PBS to 0.5%.

7.4 V-bottomed 96-well microtitre plates
Obtained from Dynatech (Billingshurst, Sussex).

8. Materials for coronavirus ELISA test

8.1 Coronavirus OC43 Antigen
Coronavirus OC43 does not grow in tissue culture and antigen was prepared by inoculation of suckling mice brain. 24 hour old white MHV strain suckling mice (supplied by the animal house facility at Leicester University) were used to prepare coronavirus OC43 antigen.

A 10^-2 dilution of stock OC43 virus in 50% Hanks/50% Nutrient Broth was prepared by adding 0.35ml of virus to 3.15ml of the Hanks/Nutrient broth and
adding 1ml of the resulting 1/10 dilution to a further 9ml of Hanks/Nutrient broth to make up a 10^{-2} dilution. This was distributed into 0.5ml aliquots and stored at -70°C.

Day old suckling mice were each inoculated intracranially with 0.05ml of the 10^{-2} dilution of virus. 4-5 families of mice were infected to obtain sufficient virus antigen and 2 families were used as uninoculated controls.

Mice were checked twice daily and sacrificed by decapitation when showing signs of illness, after 48-72 hours. Negative controls were killed at 72 hours. Heads were stored at -70°C. To harvest the brains the heads were thawed rapidly, the skull cap was removed and the brain withdrawn by suction using a Pasteur pipette. Brain tissue was suspended in small amounts of sterile PBS (approx 5ml/family) and 1ml aliquots were stored at -70°C. Before use the brain suspension was centrifuged in an MSE Centaur 2 bench centrifuge at 3000 rpm for 10 mins. The supernatant contained crude virus antigen which was tested at dilutions of 1/50, 1/100 and 1/200. The supernatant from the uninfected brain suspension was tested at 5% and 10% and subsequently used as control antigen when making diluent for test sera to remove any non-specific inhibition.

Fig 5.1 shows electron microscopy of coronavirus OC43, prepared by this technique. (Micrograph kindly supplied by PHLS, Leicester)
Figure 6.1
Electron micrograph of coronavirus OC43 prepared from suckling mouse brain
8.2 Coronavirus 229E antigen

Coronavirus 229E antigen was prepared by growing the virus in C16 cells (Phillpotts, 1983). Two 260ml tissue culture flasks containing confluent monolayers of C16 cells were each inoculated with 0.75ml of stock coronavirus 229E, after removal of the growth medium. A third flask was inoculated with 0.75ml of growth medium only and provided a negative control antigen.

All flasks were incubated at 33°C + 5% CO₂ for 90 mins. The inoculum was then removed and 20ml of maintenance medium was added to each bottle. Flasks were observed regularly for CPE. When at least 75% CPE was evident flasks were freeze-thawed x3, alternating between -70°C and 37°C. The contents were removed and centrifuged at 2000rpm for 10 mins using an MSE Centaur 2 bench centrifuge. 1ml aliquots of the supernatant were stored at -70°C. To determine the working dilution of the new batch of antigen the original and new stocks of antigen were compared by ELISA.

8.3 Tween Phosphate Buffered Saline solution (TPBS)

Obtained from Sigma (Poole, Dorset). and supplied as 500 ml amounts of Tween 20 (polyoxyethylenesorbitanmonolaurate). 0.5ml Tween 20 was added to 1 litre of PBS to make up TPBS.

8.4 Coating buffer

Obtained from Don Whitley (Shipley, West Yorks) and supplied as 250ml of 10x concentration which was diluted in PBS.

8.5 Bovine Serum Albumin (BSA)

Obtained from Sigma (Poole, Dorset, No A-7030) and supplied in 10g amounts. A 0.5% solution was made up by firstly adding 5g BSA to 100ml PBS to make a 5% solution, and then adding 1ml of 5% BSA to 9ml PBS to make a 0.5% solution.
8.6 Diluent solution
For each plate 2.5ml of 5% (or occasionally 10%) control antigen (229E or OC43) was added to 1ml of 5% stock BSA and 46.5ml of TPBS.

8.7 Conjugate
Obtained from Sigma Laboratories (Poole, Dorset, No A-3150) supplied as 0.5ml Goat anti-human IgG, conjugated with alkaline phosphatase. For each ELISA test plate 10μl of conjugate was added to 10ml of TPBS.

8.8 Substrate buffer
Obtained from Don Whitley (Shipley, West Yorks) and supplied as 2.5 litres of substrate buffer containing diethanonamine buffer pH 9.8 with 0.02% sodium azide and 0.01% MgCl₂.

8.9 Substrate tablets
Obtained from Sigma (Poole, Dorset, No 104) and supplied in batches of 100 phosphatase substrate tablets each containing 5mg p-nitrophenyl/phosphate disodium with filler.

8.10 Substrate solution
Substrate solution was made up by adding 2 substrate tablets to 10ml of substrate buffer.

8.11 NUNC 96 well plastic, flat-bottomed microtitre well plates
Obtained from Dynatech (Billingshurst, Sussex).
9. Materials for immunofluorescence tests

9.1 Freund's Complete Adjuvant (FCA)
Obtained from Sigma (Poole, Dorset) and supplied as 10ml of FCA with each ml containing 1.0mg of *Mycobacterium tuberculosis* heat killed and dried, 0.85ml paraffin oil and 0.15ml mannide mono-oleate. Stored at 4°C.

9.2 Freund's Incomplete Adjuvant (FICA)
Obtained from Sigma (Poole, Dorset) and supplied as 10ml of FICA with each ml containing 0.85ml paraffin oil and 0.15ml mannide mono-oleate. Stored at 4°C.

9.3 Viral antisera
The following viral antisera were supplied by Wellcome as 1ml amounts of lyophilised sera and stored at 4°C.

<table>
<thead>
<tr>
<th>Virus Type</th>
<th>Code</th>
<th>Species</th>
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<tr>
<td>Influenza A</td>
<td>RP 24</td>
<td>chick</td>
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<tr>
<td>Influenza B</td>
<td>RP 25</td>
<td>chick</td>
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<tr>
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<td>RP 26</td>
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<td>Parainfluenza 3</td>
<td>RP 28</td>
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</tr>
<tr>
<td>Adenovirus</td>
<td>RP 27</td>
<td>chick</td>
</tr>
</tbody>
</table>

All samples were reconstituted with 1ml of sterile DDW, dissolved slowly and stored at -20°C in 0.1ml aliquots. For immunofluorescence aliquots were diluted 1 in 10 with PBS.

9.4. Preparation of coronavirus 229E antisera
Coronavirus 229E antisera for use in the immunofluorescence test is not available commercially and was prepared in Leicester by inoculation of one rabbit and three guinea pigs.

Coronavirus 229E was grown in C16 cells and released by freeze-thawing cells x 3. The suspension containing virus and fragmented cells was centrifuged at 3000rpm
for 15 minutes using an MSE Centaur 2 bench centrifuge to precipitate the cell debris. The supernatant, containing virus was emulsified in an equal volume of Freund's complete adjuvant (FCA). Pre-inoculation sera were obtained from all animals. The rabbit was inoculated subcutaneously with a total of approximately 1ml of virus + FCA injected at three separate sites. The guinea pigs were each inoculated subcutaneously with approximately 0.5ml of virus + FCA injected at one site.

6 weeks later repeat inoculations were performed using virus + incomplete Freund's adjuvant. Antibody levels were checked 14 days later and animals with satisfactory antibody levels were bled out.

9.5 *Fluorescein isothiocyanate (FITC) conjugates*

Obtained from ICN Biomedicals (Costa Mesa, California, USA) and supplied as a 1ml solution of conjugate in 0.01M phosphate buffered saline pH7.4, with 1% BSA and 0.1% sodium azide.

<table>
<thead>
<tr>
<th></th>
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</tr>
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<tbody>
<tr>
<td>Anti-chick</td>
<td>65-196</td>
</tr>
<tr>
<td>Anti-bovine</td>
<td>65-164</td>
</tr>
<tr>
<td>Anti-guinea pig</td>
<td>65-166</td>
</tr>
<tr>
<td>Anti-rabbit</td>
<td>65-173</td>
</tr>
</tbody>
</table>

Conjugates were diluted 1 in 32 in PBS by adding 775μl of PBS to 25μl of conjugate. Diluted conjugates were stored at -20°C.
## CHAPTER 6
### METHODS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1. Collection of specimens</strong></td>
<td></td>
</tr>
<tr>
<td>1.1 Nasal and throat swabs</td>
<td>111</td>
</tr>
<tr>
<td>1.2 Acute and convalescent sera</td>
<td>111</td>
</tr>
<tr>
<td>1.3 Sera from asthmatic patients for influenza HI test</td>
<td>111</td>
</tr>
<tr>
<td>1.4 Coding of specimens</td>
<td>112</td>
</tr>
<tr>
<td><strong>2. Growth and maintenance of cell cultures</strong></td>
<td></td>
</tr>
<tr>
<td>2.1 Growth of cells in tissue culture flasks</td>
<td>112</td>
</tr>
<tr>
<td>2.2 Harvesting of cells</td>
<td>113</td>
</tr>
<tr>
<td>2.3 Storage of cells in liquid nitrogen</td>
<td>113</td>
</tr>
<tr>
<td>2.4 Preparation and maintenance of tissue culture tubes</td>
<td>114</td>
</tr>
<tr>
<td><strong>3. Virus isolation and identification in tissue culture</strong></td>
<td></td>
</tr>
<tr>
<td>3.1 Inoculation of clinical specimens into tissue culture tubes</td>
<td>114</td>
</tr>
<tr>
<td>3.2 Passaging of inoculated cells</td>
<td>115</td>
</tr>
<tr>
<td>3.3 Acid stability test</td>
<td>115</td>
</tr>
<tr>
<td>3.4 Haemadsorption test</td>
<td>116</td>
</tr>
<tr>
<td><strong>4. Haemagglutination inhibition test (HI test)</strong></td>
<td></td>
</tr>
<tr>
<td>4.1 Principle of HI test</td>
<td>117</td>
</tr>
<tr>
<td>4.2 Preparation of virus</td>
<td>117</td>
</tr>
<tr>
<td>4.3 Test procedure</td>
<td>118</td>
</tr>
<tr>
<td><strong>5. Complement fixation test (CFT)</strong></td>
<td></td>
</tr>
<tr>
<td>5.1 Principle of CFT</td>
<td>118</td>
</tr>
<tr>
<td>5.2 Standardising of complement and haemolytic sera</td>
<td>119</td>
</tr>
<tr>
<td>5.3 Standardising of antigens and positive control antisera</td>
<td>121</td>
</tr>
<tr>
<td>5.4 Screening of test sera</td>
<td>122</td>
</tr>
<tr>
<td>5.5 Titration of test sera</td>
<td>123</td>
</tr>
<tr>
<td><strong>6. Coronavirus enzyme-linked immunosorbent assay (ELISA)</strong></td>
<td></td>
</tr>
<tr>
<td>6.1 Principle of coronavirus ELISA test</td>
<td>124</td>
</tr>
<tr>
<td>6.2 Test procedure</td>
<td>124</td>
</tr>
<tr>
<td>6.3 Interpretation of results</td>
<td>125</td>
</tr>
<tr>
<td><strong>7. Detection of viruses by immunofluorescence</strong></td>
<td></td>
</tr>
<tr>
<td>7.1 Principle of immunofluorescence test</td>
<td>126</td>
</tr>
<tr>
<td>7.2 Preparation of cells from nasopharyngeal swabs</td>
<td>126</td>
</tr>
<tr>
<td>7.3 Tissue culture preparations</td>
<td>126</td>
</tr>
<tr>
<td>7.4 Staining technique</td>
<td>127</td>
</tr>
</tbody>
</table>
8. Analysis of interferon trial
   8.1 Classification of episodes 129
   8.2 Calculation of symptom score 130
   8.3 Estimated cost of episodes 130

9. Statistical analysis of clinical studies
   9.1 Interferon study 131
   9.2 Survey of influenza vaccination policies of Trent GP's 132
   9.3 Study of asthmatic patients and previously healthy adults during the 1989/90 influenza epidemic 132
1. Collection of specimens

1.1 Nasal and throat swabs

The nasal swab was placed into each nostril and gently rotated to obtain as much secretion as possible. Throat swabs were wiped across the tonsils and pharynx and subjects were asked to cough while the swabs were placed in the pharynx in order to pick up adequate secretions.

Swabs were placed in vials of virus transport medium and inoculated directly into tissue culture or stored at -70°C for later analysis. During the interferon study swabs were taken from patients at home and snap frozen immediately by plunging into liquid nitrogen with subsequent storage at -70°C. Liquid nitrogen was supplied in bulk to the Department of Pharmacology and Therapeutics. Approximately 1 litre was decanted into an insulated bucket which was carried in my car boot during visits.

1.2 Acute and convalescent serum

10ml acute blood samples were taken as close as possible after the onset of symptoms. Convalescent blood samples were obtained approximately three weeks later. In the interferon trial each blood sample was divided with 5ml sent to the Leicester Public Health Laboratory and the remaining 5ml transported back to the University of Leicester where it was centrifuged using an MSE Centaur 2 bench centrifuge at 3000 rpm for 10 minutes to separate serum. Serum samples were then labelled and stored in plastic vials at -20°C for later analysis.

1.3 Sera from asthmatic patients for influenza HI test

Patients with asthma who responded to the questionnaire were invited to attend the Asthma and Allergy Research Unit at Leicester General Hospital to give a 10ml convalescent blood samples. Those patients who were known to be under the age of 16 or who lived over 10 miles from Leicester were not invited. A total of 450 invitations were mailed and patients were asked to attend the Unit at any time during
the hours of 2-8pm on Monday April 9th or Tuesday April 10th. Samples were centrifuged using an MSE Centaur 2 bench centrifuge at 3000rpm for 10 mins. Serum was pipetted into plastic vials and stored at -20°C.

1.4 Coding of specimens

In the interferon study samples were labelled as follows: Each subject was given a family number (F1,F2.. etc) and individuals in the family identified by letters following the number (eg. A for subject, B for spouse, C,D for children, E for colleague at work etc). Each episode of medication use was identified by a number (ie. 1 for the first episode of medication use, 2 for the second episode etc) and samples were labelled either A if they were taken during the acute illness or C for convalescent specimens. For example an acute blood sample from subject number F79A taking his third course of medication would be labelled:- F79A3A. An index case at work might have been identified by the number F79E and a convalescent sample from him from the same episode would therefore be labelled:- F79E3C.

In further trials similar identification systems were used, with the prefix CF for the cystic fibrosis trial. Stored frozen serum samples from each trial were further distinguished by a colour code system-ie, all samples from a particular trial were stored in vials with the same coloured top.

2. Growth and Maintenance of Cell Cultures

2.1 Growth of Cells in Tissue Culture Flasks

All cells were grown in stationary 260ml flat-bottomed plastic flasks containing approximately 25 ml of growth medium (containing 10% FCS, subjects and materials section 4.2) at 37°C + 5% CO₂. The growth medium was changed at around 4-5 days (when 70-80% confluent) to the maintenance medium for cells grown in tissue culture flasks (subjects and materials section 5.3) which contained 5% FCS.
2.2 Harvesting of cells

Flasks containing cell-lines were examined daily under the microscope. Harvesting of cells was performed when a confluent cell layer was present. The procedure was performed under a class II cabinet to ensure sterility. Ohio HeLa cells were dispersed using a solution of phosphate buffered saline (PBS) and EDTA (versene), other cell-lines were dispersed using a trypsin/versene solution.

The Ohio HeLa cell sheet was washed three times in 10ml of PBS before adding 5ml of PBS/versene which was swilled around for approximately 20 seconds. Most of the solution was discarded leaving approximately 1-2ml. The flask containing cells and PBS/versene solution was placed in a 37°C incubator for 2-5 mins to loosen cells. The flask was tapped gently and a milky cell suspension was observed. 5ml of growth medium was added and the entire solution (6-7ml) was transferred to a sterile bijou bottle using a sterile plastic pipette. The solution was gently pipetted up and down to break up clumps of cells. 0.2-0.8ml of cell suspension was transferred into flasks containing growth medium. The split was therefore 1 in 8 or greater and was judged by experience and the observed growth characteristics of the cells. Cell counts were not routinely performed before harvesting.

For all other cell lines 5ml trypsin/versene solution was used instead of PBS/versene, but the method was otherwise identical. MRC-5 cells were relatively slow growing and 2-2.5ml of MRC-5 cell suspension was used to prepare new flasks (ie split of 1 in 2 to 1 in 4).

2.3 Storage of cells in liquid nitrogen

Prior to freezing in liquid nitrogen cells were cultured in growth medium containing 15% FCS and were harvested as previously described above using trypsin/versene or PBS/versene (for HeLa cells). The resulting suspension was transferred to a 10ml bijou bottle and centrifuged using an MSE Centaur 2 bench centrifuge at 1500 rpm for 5 mins. The sediment was removed and added to approximately 5ml of growth medium containing 10% dimethylsulphoxide. The solution was split into
vials, each containing 0.5-1ml which were labelled and frozen slowly (approximately 1°C/min) in liquid nitrogen.

When required for use cells were removed from liquid nitrogen thawed rapidly in a 37°C water bath, added to 5ml of PBS, washed x 3 in PBS, resuspended in growth medium and inoculated into tissue culture flasks containing growth medium. Fresh growth medium was added after 24 hours.

2.4 Preparation and Maintenance of Tissue Culture Tubes

Cells were harvested as described above (methods section 2.2). For the preparation of 20 tubes approximately 1ml of cell suspension was added to 19 ml of growth medium and 1ml of the diluted suspension was pipetted into each tube. Confluent cells were observed after approximately 48 hours when the medium was changed to the tissue culture tube maintenance medium (subjects and materials section 5.4-5.6) the composition of which depended on the cell type and the requirements of the viruses to which the cells were susceptible. Tissue culture tubes were maintained in stationary racks at 37°C + 5% CO₂ prior to inoculation with virus or clinical specimens.

3. Virus isolation and identification in cell culture

3.1 Inoculation of Clinical Specimens into Tissue Culture Tubes

Clinical specimens were inoculated into tissue culture tubes containing an approximately 80% confluent cell monolayer. Clinical specimens were used fresh or fully defrosted if previously stored at -70°C. In the interferon study there was a possibility that residual traces of interferon in the nasopharynx might have inhibited virus growth; therefore anti-interferon antibody, sufficient to neutralise 10,000 units of alpha2-interferon was added to the specimens whilst they were defrosting (based on the method described by Douglas et al, 1986 and Hayden et al, 1986). Specimens were centrifuged using an MSE Centaur 2 bench centrifuge at 3000rpm for 10 mins to deposit debris and 0.2ml amounts of supernatant were
pipetted into tissue culture tubes containing each of the 5 cell-lines. Only one tube from each cell-line was inoculated. Negative control tubes were inoculated with 0.2ml of transport medium only and positive control tubes were inoculated with known virus.

Tubes were placed in rotating racks at 33°C in a 5% CO₂ incubator. The maintenance medium was changed one day after inoculation and after a further 3-4 days. Tubes were observed at daily intervals for characteristic CPE.

3.2 Passaging of inoculated cells
Passaging to increase virus titre was only routinely performed for specimens inoculated into Ohio HeLa cells, however specimens inoculated onto other cell-lines were passaged if they showed an equivocal cytopathic effect or cell-growth was poor.

Cells were passaged after 7 days by freeze-thawing. This was accomplished as follows:- Tissue culture tubes containing inoculated cells were placed in the -20°C freezer for at least one hour and then thawed out in a 37°C water bath. This procedure was repeated for a total of three times. After the third freeze-thaw cycle tubes were centifuged using an MSE Centaur 2 bench centrifuge at 3000rpm for 10 mins to deposit cell debris. 0.1ml of the supernatant from each tube was transferred to a fresh culture tube containing cells which were approximately 80% confluent, and 1ml of tissue culture tube maintenance medium. Passaged cells were propagated as previously described for the initial inoculation (methods section 3.1). The remaining supernatant was stored at -20°C.

3.3 Acid stability test
The acid stability test discriminates between rhinoviruses which are labile under acid conditions and enteroviruses which are acid stable. Specimens showing characteristic picornavirus CPE were exposed to either acid or neutral conditions overnight and subsequent growth in tissue culture was compared. Control enteroviruses and rhinoviruses were included in the test.

Neutral medium was made up by adjusting Eagles Basal Medium (BME) to pH 7 with sodium bicarbonate using a pH meter. Acid medium was made up by adding N/10
hydrochloric acid to BME and adjusting to pH 3. Media were filtered and stored at 4°C. Specimens showing characteristic picornavirus CPE were freeze-thawed three times and centrifuged using an MSE Centaur 2 bench centrifuge at 3000rpm for 10 mins to deposit cell debris. 0.2ml of supernatant was added to 1.8ml of the acid and neutral media to make 1 in 10 dilutions of each which were left at 4°C overnight. The pH was not routinely determined after addition of the supernatant, however it was assumed that there was no significant change in pH as no colour change was detected in the indicator (phenol red). Appropriate rhinovirus and enterovirus controls were included in each test.

On the following day a series of 10-fold dilutions were prepared for each specimen, from the initial 1/10 dilution to 1/100 and 1/1000. Dilutions were carried out in the class II cabinet. Two drops (50μl) from each dilution were transferred to tissue culture tubes containing approximately 80% confluent cells with 1ml of tissue culture maintenance medium, incubated in rotating racks at 33°C + 5% CO₂ in rolling tubes and observed daily for 4-5 days. Characteristic CPE should be observed for rhinoviruses exposed to neutral medium, but should not occur in rhinoviruses exposed to acid media, as they will have been inhibited. In contrast, CPE should be observed for enteroviruses exposed to both neutral and acid media. The tops of rolling tubes were kept loose and incubation was performed in 5% CO₂ to optimise yield of virus under carefully controlled conditions.

3.4 Haemadsorption test

Certain viruses such as influenza and parainfluenza do not always produce a cytopathic effect when grown in susceptible cells. However, virus budding at the cell surface will induce haemadsorption if erythrocytes are added to the cell culture.

The test was performed on MDCK cells 7 and 14 days after inoculation. 0.2ml of 0.5% guinea pig red blood cells in PBS was added to each tube which was tilted slightly and left for 30 mins at 4°C before reading. Clumps of red cells will attach to cells in positive tubes, whereas negative tubes will only show a diffuse suspension only. If tubes were negative the cells were washed in PBS and fresh maintenance
media was added. Positive specimens were identified by immunofluorescence (see methods section 7). The haemadsorption inhibition test can also be used to identify viruses giving positive haemadsorption but was not performed in our laboratory.

4. Haemagglutination Inhibition test (HI test)

4.1 Principle of HI test

The serum antibody titre to specific strains of influenza virus was determined by the HI test. The HI test relies on the property of influenza virus to cause agglutination of red blood cells which is inhibited in the presence of specific antibody. To perform the test the haemagglutination activity of the test virus must first be determined by titration. The patient's sera is then titrated against a standard virus dose (4 HA units). Substances present in serum may interfere with the haemagglutination of red cells and must be removed before performing the test. There are several methods of achieving this (eg. potassium periodate). We used receptor destroying enzyme (RDE) present in cholera filtrate.

The test was performed in 2 working days. On the first day serum was incubated with RDE and the haemagglutination titre of virus was determined. The actual test was then performed on the second day.

4.2 Preparation of virus

The titre of virus was determined before each HI test. Serial twofold dilutions of virus in PBS were prepared, from 1/2 to 1/1280. 0.05ml of each dilution was added to wells in a V-bottomed microtitre plate. An equal volume of 0.5% chicken red blood cells in PBS was added to each well containing diluted virus and the microtitre plate was read after 45-60 mins at room temperature. Positive wells show a discrete pellet of agglutinated red cells whereas negative wells show a diffuse layer of cells. The well showing 50% end-point is conventionally taken as 1 HA unit, hence the titre giving 4 HA units will be at 2 wells back dilution: ie, if 1/320 titre gives 50% end point (1 HA unit) a titre of 1/80 (4 HA units) should be used for the test.
4.3 Test Procedure

Serum samples were diluted five-fold with cholera filtrate (RDE) by adding 0.025ml (1 drop) of serum to 0.1ml of cholera filtrate and incubated overnight at 37°C to destroy non-specific inhibitors of haemagglutination. Samples were then incubated at 56°C for 30 minutes to destroy heat labile inhibitors and to inactivate the neuraminidase in the cholera filtrate.

Serial twofold dilutions of cholera-filtrate treated serum in PBS were prepared, from 1/5 to 1/5120. Successive dilutions of each serum were added to wells 1-11 along each row of a V-bottomed microtitre plate. 0.025ml of virus suspension containing 4 HA units of virus was added to all wells containing serum dilutions, except for the final well which acted as a serum control. Positive and negative control sera were included in each test and in addition a virus control was performed by preparing serial double dilutions of virus in PBS from 4 HA units to 1/4 HA units (to confirm that 1 HA unit would result in a 50% end-point). The microtitre plate was left for 45-60 mins at room temperature, then 0.05ml of 0.5% chicken red blood cells in PBS was added to each well and the microtitre plate was read after a further 45-60 mins at room temperature.

The HI titre of the serum was expressed as the highest dilution which caused 100% reduction of virus haemagglutination.

5. Complement Fixation Test (CFT)

5.1 Principle of CFT

The complement fixation test is a method for determining the titre of specific serum antibodies. The technique has been described by Bradstreet and Taylor (1962) and the test is widely used for the diagnosis of many microbial infections. The principle of the test is that complement and antigen are added to serial dilutions of the patient's serum. If the serum contains specific antibody an antigen-antibody reaction will take place resulting in the fixing of complement. Residual complement is detected by adding sheep red cells sensitised with anti-sheep cell haemolytic serum. If
complement has been fixed by an antigen-antibody reaction there will be no active complement left and sheep cells will not be lysed. This is a positive result. Alternatively if the serum did not fix complement lysis of the sheep cells will take place, a negative result. The test is most informative if antibody titres are compared in acute and convalescent serum samples taken approximately 3 weeks apart.

Complement fixation tests were performed against influenza A, influenza B, respiratory syncytial virus (RSV) and adenovirus in the University of Leicester, CFT's against Coxiella burnetti, Chlamydia psittaci and Mycoplasma pneumoniae were kindly carried out in the Leicester Public Health Laboratory.

To perform the complement fixation test optimal dilutions of each new batch of haemolytic serum, complement, antigen and positive control antisera were determined by checkerboard titrations in U-bottomed microtitre well plates. The patients' convalescent sera were then screened at a dilution of 1/16 followed by titration of any specimens which gave 50% or greater fixation of complement.

5.2 Standardising of Complement and Haemolytic Serum

Complement was reconstituted as directed with double distilled water. Serial dilutions were prepared in CFT buffer, from 1/60 to 1/286 with a 20% difference in concentration between each dilution as shown in Fig 6.2. 0.025mls of each complement dilution were added to wells in the columns of a microtitre well plate as shown in Fig 6.2. The microtitre plate was covered and left overnight at 4°C.

The following day serum containing sheep cell haemolysin was serially diluted twofold in CFT buffer, from 1/25 to 1/800. An equal volume of a 4% suspension of sheep red cells in CFT buffer was added to each haemolytic serum dilution and incubated at 37°C for 20 mins. At the same time the microtitre plate containing complement dilutions was warmed to 37°C. 0.025mls of each haemolytic serum dilution were then added to wells in the rows of the microtitre plate as shown in Fig 6.2. Wells in column 9 and row H were haemolytic serum and complement controls.
respectively. The microtitre plate was shaken, covered and incubated at 37°C for 30 mins with cells resuspended after 15 mins. The microtitre plate was then left at 4°C for 90 mins (or overnight) before reading. The results were scored as follows: 0=no cells remaining, Tr=10%, 1= 25%, 2=50%, 3=75% and 4=100% of cells remaining.

The optimal sensitising dose (OSD) of haemolytic serum is the dilution giving most lysis with the highest dilution of complement (reading of 0) ie. in Fig 6.2 the OSD is 1/100. One unit of complement (HD₅₀) is the dilution which gives 50% lysis (reading of 2) at the OSD of haemolytic serum. In Fig 6.2, 1 HD₅₀ = 1/230. In the test complement was used at 4 HD₅₀ (approximately 1/56). Fig 6.2 shows no lysis in complement or haemolytic serum controls indicating the absence of non-specific lysis.

Fig 6.2 Titration of complement and haemolytic sera.

<table>
<thead>
<tr>
<th>Dilutions of complement-</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<td>Dilutions of haem</td>
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<td>of haem serum</td>
<td>B</td>
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rows
5.3 Standardising of antigen and positive control antisera

A similar checkerboard titration was performed to determine the optimum concentrations of antigens and control antisera. Serial twofold dilutions of antigen and positive control antisera were prepared in CFT buffer. Antigen was diluted from 1/5 to 1/160 and antisera diluted from 1/10 to 1/320. 0.025mls of each antiserum dilution were added to wells in the columns of a microtitre well plate as shown in Fig 6.3. 0.025mls of each antigen dilution were then added to wells in the rows of the microtitre well plate as shown in Fig 6.3. 0.025ml of complement at 4 \( HD_{50} \) was added to each well. A complement control was included in row 12 with wells A-D containing complement at 4, 2, 1 and 1/2 \( HD_{50} \) respectively. Wells in column 7 and row H were antiserum and antigen controls respectively.

Fig 6.3 Titration of antiserum and antigen and complement control.

<table>
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<tr>
<th>Dilutions of antigen-&gt;</th>
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<td>5</td>
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<td>complement control</td>
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The microtitre plate was covered and left overnight. The following day haemolytic serum diluted at OSD was added to an equal volume of 4% sheep red cells in CFT buffer, and incubated at 37°C for 30 mins. At the same time the microtitre plate was warmed to 37°C. One volume of sensitised cells was added to each well, including a cell control. The microtitre plate was shaken, covered and incubated at 37°C for 30 mins with cells resuspended after 15 mins. The microtitre plate was then left at 4°C for 90 mins (or overnight) before reading. Fig 6.3 shows representative results.

The complement control was included to check that the correct concentration of complement was being used and the result should be as shown in Fig 6.3. Antigen and antiserum controls should show total haemolysis (0) indicating the absence of any intrinsic anti-complementary activity. The cell control should show no lysis (4) indicating the absence of spontaneous lysis of sensitised cells. The optimal dilution of antigen is the dilution which gives most fixation with the highest dilution of serum (ie. 1/20 in Fig 6.3). The titre of positive serum is the reciprocal of the serum dilution which gives a reading of 2 with the optimal dilution of antigen (ie. 40 in Fig 6.3).

5.4 Screening of test sera

Sera were routinely screened against a range of antigens at a dilution of 1/16 in CFT buffer. Stored sera including known positive and negative controls were heated to 56°C in a water bath for 30 minutes to inactivate residual complement. 0.025ml of each test serum diluted to 1/16 was added to rows of wells in a microtitre plate, and 0.025ml of each antigen diluted to the optimal concentration was added to the test sera in each column as shown below in Fig 6.4. CFT buffer only was added to the serum control wells. Complement (4 HD₅₀) was added to each well and complement and cell controls (as described in section 5.4) were included in each test. The microtitre plate was covered and left overnight. The following day haemolytic serum diluted at OSD and sensitised cells were added as described in section 5.4. All samples which gave a reading of 4 were titrated out as paired sera.
5.5 Titration of test sera

Stored sera were taken from the -20°C freezer, diluted 1/4 in CFT buffer and heated to 56°C for 30 minutes to inactivate residual complement. Serial twofold dilutions of 0.025ml serum in CFT buffer were prepared, from 1/8 to 1/512 and 0.025ml of antigen diluted to optimal concentration was added to each serum sample. The remainder of the test was performed as described above in section 5.4. Up to 12 tests (6 paired sera) were performed on each microtitre plate.

The titre of serum was recorded as the reciprocal of the highest dilution which gave a reading of 3/4. A four fold rise in paired titre was indicative of recent infection.
6. Coronavirus enzyme-linked immunosorbent assay (ELISA)

6.1 Principle of coronavirus ELISA test

Coronavirus 229E and OC43 IgG antibodies were detected using a technique based on the method described by Kraaijeveld et al, (1980). In the coronavirus ELISA test the antigen (229E or OC43) is adsorbed to flat bottomed microtitre well plates. Acute and convalescent serum samples are then added to separate wells and any specific antibody present in the serum will bind to the adsorbed antigen. Bound antibody is detected using an enzyme-linked antibody (anti-human IgG conjugated with alkaline phosphatase) which reacts with a substrate to give a colour change measured quantitatively by measuring absorbance of light in an ELISA reader. Relative absorbance is then compared in acute and convalescent sera. Positive and negative control sera are included with each test.

6.2 Test Procedure

The optimal concentration of each batch of coronavirus antigen was established prior to performing the test and dilutions of 1 in 100 were found to be most appropriate for both 229E and OC43.

Wells were coated with 100μl amounts of 229E or OC43 antigen diluted 1 in 100 in coating buffer. Wells A1 and A2 were left empty. Microtitre plates were covered with parafilm and incubated at 4°C overnight inside a humidified "tupperware" box containing damp blotting paper.

5μl amounts of test and control sera were placed in labelled glass tubes. 1ml of diluent solution was added to each tube and adsorbed overnight at 4°C. Diluent solution contained control antigen from tissue culture fluid (for 229E) or uninfected suckling mouse brain (OC43) to adsorb any non-specific serum antibodies.

On the following day the microtitre plate was washed x3 in TPBS and shaken dry. 100μl of 0.5% BSA/PBS was added to each well, except A1, and microtitre plates were incubated for 1 hour at 37°C in the humidified box. BSA reduces non-specific binding and increases the sensitivity of the test.
Following incubation the microtitre plate was again washed x3 in TPBS and shaken dry. 100μl of serum+diluent was added to appropriate wells with diluent only added to wells A2-4 (diluent control). Each pair of acute and convalescent samples was tested in duplicate. The microtitre plate was covered, placed in the humidified box and incubated at 37°C for 2 hours.

Following incubation the microtitre plate was washed x4 in TPBS and shaken dry. 100μl of goat anti-human IgG conjugate was added to each well, except A1, and the microtitre plate was incubated for 1 hour at 37°C in the humidified box.

Following incubation the microtitre plate was washed x3 in TPBS and 100μl of substrate solution was added to each well (including A1 and A2). The microtitre plate was incubated at room temperature and absorbance values were read after 15 and 30 minutes at 405nm using an MR600 Microplate Reader (Dynatech Instruments, Billingshurst, Sussex).

6.3 Interpretation of results

The absorbence values of acute and convalescent samples were compared. Several known positive and negative control sera were included in each assay. A consistent ratio of >1.3 between absorbance values of convalescent and acute samples was taken as indicating recent infection. Assays producing borderline rises with convalescent/acute ratios in the range of 1.2-2.0 were repeated.
7. Detection of viruses by immunofluorescence

7.1 Principle of immunofluorescence test

In the immunofluorescence test virus-infected cells expressing virus antigens on the cell surface are bound by a specific antibody (the primary antibody). The virus specific primary antibody may be conjugated with fluorescein or, alternatively, the bound antibody can be detected by a fluorescein-conjugated secondary antibody directed against the primary antibody. Binding of the conjugate is visualised under a fluorescence microscope. The technique can be used to identify infected cells in tissue culture or in clinical specimens eg. nasopharyngeal swabs.

7.2 Preparation of cells from nasopharyngeal swabs

Nasopharyngeal swabs in virus transport medium were vortexed thoroughly and centrifuged using an MSE Centaur 2 bench centrifuge at 1500 rpm for 10 mins. The cell deposit was resuspended in 2ml of sterile PBS, vortexed and centrifuged at 2000 rpm for 10 mins. Following the second centrifugation, the cell deposit was resuspended in 0.15ml PBS.

0.025ml amounts of cell suspension were dropped onto marked wells on glass slides, allowed to air dry then fixed in chilled acetone at 4°C for 10 mins. Slides used the same day were kept at 4°C, unused slides were stored at -70°C.

7.3 Tissue culture preparations

Negative controls from uninfected cell-monolayers were always compared with the test specimens. Medium from infected and uninfected cell-monolayers was discarded and the cells were briefly washed with 2ml of sterile PBS which was then poured off. 2ml of fresh PBS was added and cells were gently scraped off using a sterile Pasteur pipette. The cell suspension was centrifuged using an MSE Centaur 2 bench centrifuge at 1500 rpm for 10 mins and the supernatant was discarded. The cell deposit was resuspended in 0.15ml of PBS and glass slides were prepared as described above (section 7.2).
Cells deposited on glass slides were covered with one drop (0.025ml) of specific virus antiserum and slides were incubated at 37°C for 30 minutes in a humid container (plastic box with moist tissues). Following incubation slides were washed 3 x 10 minutes in PBS, then drained and air dried.

One drop (0.025ml) of appropriate fluorescein-conjugated anti-globulin (anti-chick, anti-bovine etc, depending on the antisera used) was added to each cell deposit and incubated at 37°C for 30 minutes in humid conditions. Following incubation slides were washed 3 x 10 minutes in PBS then rinsed in double distilled water, drained and allowed to air dry before mounting with PBS/glycerol 10:90 under a coverslip.

When viewed under a fluorescence microscope (Jenamed 2, Carl Zeiss Instruments, Jena, Germany) positive specimens should demonstrate brilliant fluorescence whereas negative samples will show only a very low level of background activity. Fig 6.5 shows the result of coronavirus immunofluorescence and compares C16 cells inoculated with coronavirus 229E with a negative cell control.
Figure 6.5.
Coronavirus immunofluorescence. Slides prepared using antisera to coronavirus 229E raised in guinea pigs and visualised with fluorescein-labelled anti-guinea pig conjugate. a) uninfected C16 cells, b) C16 cells inoculated with coronavirus 229E.
8. Analysis of interferon trial

8.1 Classification of episodes

Subjects, index cases and secondary cases were all issued with symptom diary cards and asked to record the severity of each symptom daily as either 0-nil, 1-mild, 2-moderately severe or 3-severe. When a patient had constant symptoms (e.g., cough) only a change in the severity of that symptom was recorded. The severity of each episode was judged by the number of symptomatic days, total symptom score, need for GP visits, use of antibiotics and need for hospital admission. Diary cards were collected on day 21 and all recorded episodes had ceased by this time. Each course was classified into one of four possible outcomes:

1. "Nil". Subject was asymptomatic.

2. "Doubtful cold". Symptoms scoring no more than 1=mild or involving only one of nose, throat or cough.

3. "Upper respiratory tract infection" (URTI). Symptoms involving two or more of nose, throat, cough or systemic features. At least one symptom scoring 2=moderately severe or worse.

4. "Lower respiratory tract infection". Defined by at least two symptoms of cough, sputum, wheeze or chest tightness lasting at least two days. (If the patient normally complained of chest symptoms only an increasing severity of these symptoms was regarded as an indication of a lower respiratory tract exacerbation). Appearance of cough alone did not lead to classification as an upper or lower respiratory episode.

"Symptomatic day". This was defined as a day in which two or more symptoms were recorded with at least one symptom moderately severe or worse.
8.2 Calculation of symptom scores

The total score for each episode was calculated by simply adding up all the symptom scores for the entire duration of each episode. Separate scores were calculated for all upper respiratory symptoms (ie. sore throat, headache, runny nose, stuffy nose and handkerchiefs used) and all lower respiratory symptoms (ie. cough, wheeze, increased sputum and shortness of breath) to determine whether interferon had a preferential effect on the upper or lower respiratory tract.

8.3 Estimation of cost of episodes

Approximate prices of medication were taken from the British National Formulary (14th edition 1987):

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<th>Drug</th>
<th>Cost (£)</th>
</tr>
</thead>
<tbody>
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<td>Amoxycillin 250mg tds for 7 days</td>
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</tr>
<tr>
<td>Augmentin 1 tablet tds for 7 days</td>
<td>6.00</td>
</tr>
<tr>
<td>Cotrimoxazole 960 mg bd for 7 days</td>
<td>2.00</td>
</tr>
<tr>
<td>Oxytetracycline 250mg qds for 7 days</td>
<td>1.00</td>
</tr>
<tr>
<td>Erythromycin 250mg qds for 7 days</td>
<td>1.00</td>
</tr>
<tr>
<td>Becotide inhaler</td>
<td>10.00</td>
</tr>
<tr>
<td>Becloforte inhaler</td>
<td>23.00</td>
</tr>
<tr>
<td>Salbutamol inhaler</td>
<td>2.50</td>
</tr>
</tbody>
</table>

The cost of hospital admission in 1987 was estimated at £140/day (Dept of Community Health, University of Leicester).
9. Statistical analysis of clinical studies

9.1 Interferon study

Advice on the statistical analysis of the interferon trial was provided by Dr Carol Jagger, Lecturer in Medical Statistics in the Dept of Community Health, Leicester University. The statistical methods used are summarised below:

(i) Unpaired t-tests were used to compare patients' ages, years of illness, years of clinic attendance, peak flow recordings, number of people in households, hours in contact with index cases, and hours between initial contact with infection and commencing medication. Unpaired t-tests were also used to compare symptom days and symptom scores in index cases suffering from different virus infections.

(ii) Paired t-tests were used to compare symptom scores and symptom days in symptomatic placebo-using patients and their corresponding index cases.

(iii) Mann-Whitney U tests were used to compare overall symptom scores, number of symptomatic days and cost of episodes in interferon and placebo recipients. Many episodes included in these comparisons resulted in few or no symptoms and were therefore analysed using a non-parametric test.

(iv) Chi-squared analysis was used to compare incidence of smoking, proportions of patients with asthma, bronchitis and bronchiectasis, proportions of patients using different medications, and number of symptomatic respiratory episodes in patients using interferon and placebo.

(v) Fisher's exact test was used to compare the small numbers of hospital admissions.
9.2. Survey of Influenza vaccination polices of Trent GP's

Comparison of the factors which could influence vaccination policy was made using unpaired t-tests.

9.3 Study of asthmatic patients and previously healthy adults during the 1989-90 influenza epidemic

Chi-squared tests were used to compare the incidence of symptoms, complications, GP consultations and protective antibody titres in vaccinees and non-vaccinees. Reciprocal geometric mean titres were compared by unpaired t-tests after transforming values to $\log_{10}$. The efficacy of the vaccine was calculated by the formula:

$$Efficacy = \frac{\text{Proportion non-vaccinees with flu-like illness} - \text{Proportion vaccinees with flu-like illness}}{\text{Proportion of non-vaccinees with flu-like illness}} \times 100\%$$

In previously healthy volunteers the Chi-squared test was used to compare the incidence of different symptoms associated with episodes occurring during or outside the period of the influenza epidemic. Unpaired t-tests were used to compare symptom days and symptom scores in episodes of respiratory illness occurring during and outside the influenza epidemic season in previously healthy volunteers.

Statistical data was analysed using the "Statview" statistics software on an Apple Macintosh Plus computer and the "SAS" programme on the Leicester University "Vax" computer which was used for Mann-Whitney U tests and Fisher's exact test.
C. CLINICAL AND LABORATORY STUDIES
CHAPTER 7
TRIAL OF PROPHYLACTIC INTRANASAL INTERFERON-ALPHA IN PATIENTS WITH CHRONIC RESPIRATORY DISEASE

1. Introduction
Respiratory virus infections are a frequent cause of exacerbations in patients with chronic chest disease (reviewed in chapter 3), therefore antiviral drugs which prevent or alleviate infection might be of particular benefit in this group of patients. Trials of prophylactic intranasal interferon-alpha (reviewed in chapter 5) demonstrated convincing protection against naturally occurring rhinovirus infection using doses of 3-10MU/day, but little effect against other respiratory viruses. Local side-effects were minimised by giving short courses of treatment lasting less than one week. As short-term interferon prophylaxis appeared effective in previously healthy subjects I co-ordinated a double-blind, placebo-controlled trial of intranasal interferon-alpha in patients with well-documented chronic chest disease. Treatment with interferon at a dose of 6MU/day given for 5 days was initiated after close contact with a presumed upper respiratory tract infection. The end points of the trial were the number and severity of upper and lower respiratory tract infections after interferon or placebo.

2. Organisation of the study
Trial protocol and approval
A protocol was written in conjunction with Dr KG Nicholson and Dr JB Cookson in Leicester, Dr DAJ Tyrrell at the MRG, Common Cold Unit in Salisbury and Dr NJC Snell, Boehringer Ingelheim who kindly provided the interferon. The protocol was approved by the Leicestershire Health Authority Ethics Committee. Boehringer Ingelheim obtained a clinical trials exemption certificate (CTX) from the Department of Health and Social Security in October 1986 and delivery of the trial medication followed shortly afterwards.
Calculation of the power of the study

In order to calculate the power of the study and the number of episodes and subjects needed to show statistical significance at the 5% level we made the following assumptions based on the experience of previous trials:

1. Each subject would be exposed to 5 colds/year
2. Rate of infection after placebo=23%
3. Rate of infection after interferon=15% (ie efficacy=35%)

Using conventional tables for power analysis (Young et al, 1983) it was estimated that approximately 350 exposures (ie 70 patients) would be needed to give a significant result at the 95% level.

Subject participation

Study participants were given a coded bottle of trial medication and instructions on its use. They were also given a peak flow meter and coloured symptom diary cards for themselves and symptomatic contacts. Subjects were requested to perform peak flow readings twice weekly throughout the study and record them on a green sheet provided (Appendix 1). This gave information on the individual variation of peak flow between episodes and was thought to encourage compliance by providing a constant reminder of the study.

Use of medication

Subjects were given a coded bottle of medication on enrollment into the study and shown how to administer it using a dummy bottle containing water. They were advised to store the bottles at 4°C and to start treatment following close contact for at least six hours with a family member or colleague with symptoms of a viral respiratory illness (defined as the presence of at least two of rhinorrhea, nasal congestion, sneezing, sore throat, cough, hoarseness, fever, chills muscle aches, for
at least 24 hours). Subjects were asked to commence spraying as soon as they had confirmed the presence of a cold in the contact and to inform a member of the trial upon starting medication (24 hour answerphone service was available).

The medication spray was designed to give a fine mist of particles which would distribute predominantly in the nasopharynx (Fig 7.1).

Recording of symptoms and peak flows

Subjects were issued with pink cards (Appendix 2) to record symptoms throughout the 5 days that they were using medication and for 5 days thereafter. Symptoms were therefore recorded for at least 10 days whether or not the subject developed a respiratory tract infection. If symptoms carried on for longer than 10 days additional symptom cards were completed until the subject became symptom free. The cards specified symptoms of both upper and lower respiratory tract disease and subjects were asked to score each symptom daily as: 0=Nil, 1=mild, 2=moderately severe and 3=severe.

During this period of close observation the subject was asked to increase the frequency of peak flow readings to twice daily. The index case and any other secondary cases were similarly requested to record symptoms for a period of ten days. These were documented on blue forms (Appendix 3) which were similar in design to the pink forms but did not include lower respiratory tract symptoms.
Figure 7.1
The interferon trial medication in action.
Supervision of subjects and collection of specimens

After being informed that one of the trial members had been in contact with a cold I visited the subject and index case at home at a convenient time, within 24 hours whenever possible. During this visit I confirmed that the index case had symptoms of an upper respiratory tract infection and checked that medication was being used correctly. I recorded the time spent in contact with the index case and the interval between first contact with the index case and nasal spraying. Nose and throat swabs and acute blood samples were taken from both the subject and index case.

Subjects were asked to contact me by telephone if they developed respiratory symptoms or if any secondary cases occurred. Additional home visits were made to review symptoms and collect swabs if the subject developed symptoms of an upper or lower respiratory tract infection. Any secondary cases were asked to record their symptoms on the blue diary cards and provide a sample for serology and nose and throat swabs.

Subjects were reviewed 7 and 21 days after commencing medication. Nasal speculum examination was performed and recorded at each visit, and on day 21 convalescent blood samples were taken from the subject, index case and any secondary cases, symptom diary cards were collected, and a new bottle of trial medication was issued. The trial was designed so that at least 16 days elapsed between courses of medication to minimise the occurrence of local side-effects.

Treatment courses were considered evaluable if the entire course of medication was taken and the index case had a definite upper respiratory tract infection documented on the symptom chart. The residual medication left in the bottle following the course of treatment was checked to estimate compliance. Courses were excluded from analysis if the medication was commenced more than 72 hours after contact with the index case. Regular phone calls (at least monthly) were made to all trial participants and newsletters informed them of the progress of the trial and reminded them to take the medication. Subjects who had not used the spray after a period of several months were also visited by myself to encourage co-operation.
RESULTS

3. Recruitment of subjects

Patients were recruited between June 1986 and August 1987. The majority were seen before October 1986. Letters explaining the nature of the trial were sent to a total of 332 adults. 214 replies were received (64%) and 185 stated that they would be willing to take part. 52 patients were thought to be unsuitable as they had little regular contact with other people. The remaining 133 patients were seen at Groby Road Hospital and 123 of these were enrolled into the study. Ten subjects were excluded from the trial during the consultation; 6 were excluded as they did not fulfil the trial criteria (two had other significant coexisting medical conditions and a further subject was excluded after liver function tests were found to be grossly deranged, one was trying to become pregnant, one had a history of nasal polyps and one had a history of hypersensitivity to penicillin) and 4 declined to participate in the trial at this stage. The remaining 123 patients were enrolled in the study.

4. Characteristics of study population

The characteristics of the trial subjects are summarised in Table 7.1. The mean age of the patients with asthma (41.5 years) was significantly less than the mean age of those with chronic bronchitis (62.4 years), (unpaired t-test, p<0.0005). 32 (86%) of the 37 patients with chronic bronchitis were male compared to 29 (37%) of 79 asthmatics ($X^2$, p<0.001) and 1 (14%) of 7 with bronchiectasis. 34 (92%) of the 37 patients with chronic bronchitis had a history of smoking compared to 35 (44%) of the 79 patients with asthma ($X^2$, p<0.001), however only 17% of trial patients admitted to current smoking.

Although we attempted to select subjects living with children this was not always possible and many, particularly the more elderly with chronic bronchitis, lived in small family units. Patients with asthma lived in households containing a mean of 3.46 people (including the patient), compared to a mean household of 2.70 for those with chronic bronchitis (unpaired t-test, p<0.005) and those with asthma lived
with a mean of 0.77 children under the age of 16 compared to 0.35 children for those with chronic bronchitis (unpaired t-test, p<0.025).

The overall mean respiratory history was 19.5 years and patients had been attending a chest clinic for a mean period of 10.7 years, indicating the chronic nature of their disease. Those with bronchiectasis had a particularly long history with a mean of 37.3 years.

Table 7.1 Characteristics of trial subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Asthma</th>
<th>Bronchitis</th>
<th>Bronchiectasis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (%)</td>
<td>79 (64)</td>
<td>37 (30)</td>
<td>7 (6)</td>
<td>123</td>
</tr>
<tr>
<td>Number of males (%)</td>
<td>29 (37)</td>
<td>32 (86)</td>
<td>1 (14)</td>
<td>62 (50)</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>41.5 (17-74)</td>
<td>62.4 (45-73)</td>
<td>48.1 (37-56)</td>
<td>48.1 (17-74)</td>
</tr>
<tr>
<td>Mean respiratory history (yrs)</td>
<td>16.6</td>
<td>22.3</td>
<td>37.3</td>
<td>19.5</td>
</tr>
<tr>
<td>Mean No of years attending clinic</td>
<td>9.6</td>
<td>9.4</td>
<td>30.2</td>
<td>10.7</td>
</tr>
<tr>
<td>History of smoking (%)</td>
<td>35 (44)</td>
<td>34 (92)</td>
<td>2 (29)</td>
<td>71 (58)</td>
</tr>
<tr>
<td>Present smoker (%)</td>
<td>14 (18)</td>
<td>7 (19)</td>
<td>0</td>
<td>21 (17)</td>
</tr>
<tr>
<td>Mean No in household (inc pt)</td>
<td>3.46</td>
<td>2.70</td>
<td>3.71</td>
<td>3.24</td>
</tr>
<tr>
<td>Mean No under 16</td>
<td>0.77</td>
<td>0.35</td>
<td>0.43</td>
<td>0.62</td>
</tr>
</tbody>
</table>
Although patients with other serious co-existing medical conditions were excluded from the trial several patients did have a history of other medical complaints in addition to their respiratory disease eg, well-controlled angina, mild heart failure, osteoarthritis. These are summarised in Table 7.2. None of these medical conditions were felt to be severe enough to influence the outcome of the interferon trial.

Table 7.2 Other medical problems in trial patients

<table>
<thead>
<tr>
<th>Medical condition</th>
<th>Asthma</th>
<th>Bronchitis</th>
<th>Bronchiectasis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischaemic heart disease</td>
<td>1</td>
<td>4</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2</td>
<td>2</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Arthritis</td>
<td>2</td>
<td>1</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Previous TB</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Bronchopulm aspergillus</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Previous PE</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Eczema</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Coeliac disease</td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>
Table 7.3 shows the medication taken by patients on admission to the trial. There were no significant differences in the use of medication between the different groups except for ipratropium bromide ("atrovent") inhalers which were used by 16 (20%) of 79 patients with asthma compared to 17 (46%) of 37 patients with chronic bronchitis ($x^2$, $p<0.01$).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Asthma (n=79)</th>
<th>Bronchitis (n=37)</th>
<th>Bronchiectasis (n=7)</th>
<th>Total (n=123)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Beta 2 agonist</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhaled</td>
<td>74 (94)</td>
<td>35 (95)</td>
<td>6 (86)</td>
<td>115 (94)</td>
</tr>
<tr>
<td>Volumetric device</td>
<td>58 (78)</td>
<td>30 (86)</td>
<td>5 (83)</td>
<td>93 (81)</td>
</tr>
<tr>
<td>Nebulised</td>
<td>14 (19)</td>
<td>4 (11)</td>
<td>1 (17)</td>
<td>19 (17)</td>
</tr>
<tr>
<td><strong>Ipratropium Bromide</strong></td>
<td>16 (20)</td>
<td>17 (46)</td>
<td>3 (43)</td>
<td>36 (29)</td>
</tr>
<tr>
<td><strong>Inhaled steroids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Becotide/Pulmicort</td>
<td>54 (69)</td>
<td>26 (70)</td>
<td>3 (43)</td>
<td>83 (68)</td>
</tr>
<tr>
<td>Becloforte</td>
<td>29 (37)</td>
<td>16 (43)</td>
<td>0</td>
<td>45 (37)</td>
</tr>
<tr>
<td><strong>Cromoglycate inh.</strong></td>
<td>3 (4)</td>
<td>0</td>
<td>0</td>
<td>3 (2)</td>
</tr>
<tr>
<td><strong>Oral B1 agonist</strong></td>
<td>8 (10)</td>
<td>4 (11)</td>
<td>0</td>
<td>12 (10)</td>
</tr>
<tr>
<td><strong>Oral theophylline</strong></td>
<td>26 (33)</td>
<td>17 (46)</td>
<td>1 (14)</td>
<td>44 (36)</td>
</tr>
<tr>
<td><strong>Oral steroids</strong></td>
<td>11 (14)</td>
<td>8 (22)</td>
<td>0</td>
<td>19 (15)</td>
</tr>
<tr>
<td><strong>Regular antibiotics</strong></td>
<td>1 (1)</td>
<td>0</td>
<td>2 (29)</td>
<td>3 (2)</td>
</tr>
<tr>
<td><strong>Domiciliary oxygen</strong></td>
<td>1 (1)</td>
<td>1 (1)</td>
<td>0</td>
<td>2 (2)</td>
</tr>
<tr>
<td><strong>Diuretics</strong></td>
<td>6 (8)</td>
<td>5 (14)</td>
<td>0</td>
<td>11 (9)</td>
</tr>
</tbody>
</table>
5. Use of trial medication

117 courses of medication were taken by 69 separate patients. Unfortunately 54 patients (44%) did not use the trial spray despite reminders, phone calls and visits. 41 patients (33%) used 1 course of medication, 15 (12%) used 2 courses, 7 (6%) used 3 courses, 5 (4%) used 4 courses and 1 (1%) used a total of 5 courses.

Table 7.4 compares patients who took medication with those who did not. The mean number in each household for those who did not use medication was 3.01 compared to 3.42 for those who did use medication (unpaired t-test, p<0.05). There were also fewer children aged under 16 years in the households of those who did not use medication (mean 0.51 vs 0.70), although this difference was not significant. Non-users were slightly older than users (mean 48.8 years compared to 46.9 years). These results suggest that exposure to fewer household contacts was the main reason for not using medication.

<table>
<thead>
<tr>
<th>Table 7.4</th>
<th>Comparison of medication users and non-users</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Users No (%)</td>
</tr>
<tr>
<td>------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Males</td>
<td>33 (47)</td>
</tr>
<tr>
<td>Mean age</td>
<td>46.9</td>
</tr>
<tr>
<td>Asthmatic</td>
<td>43 (56)</td>
</tr>
<tr>
<td>Chronic bronchitis</td>
<td>21 (57)</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td>5 (71)</td>
</tr>
<tr>
<td>Mean number in house</td>
<td>3.42</td>
</tr>
<tr>
<td>(Number under 16)</td>
<td>0.70</td>
</tr>
<tr>
<td>Total</td>
<td>69 (56)</td>
</tr>
</tbody>
</table>

NS=No significant difference at 5% level between users and non-users
11 of the 117 episodes (9%) were excluded - five as patients took medication when they developed symptoms with no index case, 4 as data sheets were not filled in correctly and 2 patients lost their record cards. Although the exclusions were not included in the analysis of outcome they provided information on adverse effects. A total of 106 episodes of medication use were fully analysed (48 interferon, 58 placebo). Comparison of subjects who used interferon with those who used placebo is shown in Table 7.5. There were no significant differences in the characteristics of the two groups which might have confounded the analysis of the results.

Table 7.5 Comparison of interferon and placebo users.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Interferon</th>
<th>Placebo</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age</td>
<td>47.4</td>
<td>48.4</td>
<td>NS</td>
</tr>
<tr>
<td>Male (%)</td>
<td>19 (40)</td>
<td>26 (45)</td>
<td>NS</td>
</tr>
<tr>
<td>Asthma (%)</td>
<td>33 (69)</td>
<td>35 (60)</td>
<td>NS</td>
</tr>
<tr>
<td>Chronic bronchitis (%)</td>
<td>13 (27)</td>
<td>15 (26)</td>
<td>NS</td>
</tr>
<tr>
<td>Bronchiectasis (%)</td>
<td>2 (4)</td>
<td>8 (14)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean years of illness</td>
<td>20.25</td>
<td>20.7</td>
<td>NS</td>
</tr>
<tr>
<td>Hx of smoking (%)</td>
<td>31 (65)</td>
<td>36 (62)</td>
<td>NS</td>
</tr>
<tr>
<td>Present smoker (%)</td>
<td>5 (10)</td>
<td>4 (7)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean peak flow</td>
<td>30.3</td>
<td>28.8</td>
<td>NS</td>
</tr>
<tr>
<td>Mean no in household</td>
<td>3.9</td>
<td>3.6</td>
<td>NS</td>
</tr>
<tr>
<td>Mean no under 16</td>
<td>0.85</td>
<td>0.84</td>
<td>NS</td>
</tr>
<tr>
<td>Mean hours in contact with index case</td>
<td>26.4</td>
<td>30.6</td>
<td>NS</td>
</tr>
<tr>
<td>Mean hours between contact and starting Rx.</td>
<td>34.7</td>
<td>36.1</td>
<td>NS</td>
</tr>
<tr>
<td>Index case:-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean symptomatic days</td>
<td>5.8</td>
<td>5.4</td>
<td>NS</td>
</tr>
<tr>
<td>Mean symptom score</td>
<td>71.3</td>
<td>60.2</td>
<td>NS</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>58</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS=No significant difference between interferon and placebo groupos at the 5% level.
6. Number of respiratory tract infections in interferon and placebo recipients

The outcome of patients taking interferon or placebo is summarised in Table 7.6. There were no significant differences between the groups. 7 (15%) of the 48 patients who used interferon developed an upper respiratory tract infection compared to 9 (15.5%) of 58 who used placebo and 11 (23%) of the 48 patients who used interferon developed a lower respiratory tract infection compared to 16 (28%) of 58 who used placebo ($X^2$, $p=0.58$).

Table 7.6 Outcome of courses of medication

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Interferon</th>
<th>Placebo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no (%)</td>
<td>no (%)</td>
<td>no (%)</td>
</tr>
<tr>
<td>Nil</td>
<td>19 (40)</td>
<td>21 (36)</td>
<td>40 (38)</td>
</tr>
<tr>
<td>Doubtful cold</td>
<td>11 (23)</td>
<td>12 (20)</td>
<td>23 (22)</td>
</tr>
<tr>
<td>URTI</td>
<td>7 (14)</td>
<td>9 (16)</td>
<td>16 (15)</td>
</tr>
<tr>
<td>Lower RTI</td>
<td>11 (23)</td>
<td>16 (28)</td>
<td>27 (25)</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>58</td>
<td>106</td>
</tr>
</tbody>
</table>

7. Symptomatic days in interferon and placebo patients

Comparison of symptomatic days in interferon and placebo groups showed that interferon was associated with non-significant reduction in the mean length of illness from 5.0 days (median 2 days, range 0-33 days) in placebo users to 3.3 days (median 1 day, range 0-12 days) in interferon users (Mann-Whitney U test, $p=0.63$). Interferon was associated with a borderline significant reduction in duration of lower respiratory infections from a mean of 13.9 days (median 10 days, range 4-33 days) in placebo users to 6.9 days (median 5 days, range 5-12 days) in interferon users; Mann-Whitney U test, $p=0.05$, 95% confidence intervals -12 to 0 days).
8. Symptom scores in interferon and placebo patients

Comparison of symptom scores in interferon and placebo groups showed that the mean symptom score for each interferon episode was 51.4 (median 37, range 0-207) compared to 51.6 (median 26, range 0-316) for those who took placebo. Patients who developed lower respiratory tract infections had a mean symptom score of 104.6 (median 79, range 48-207) for each interferon-treated episode compared to 113.8 (median 86, range 33-316) following placebo (Mann-Whitney U test, p=0.9). This modest reduction in the symptom score contrasts with the marked reduction in the number of days of illness.

9. Nature and timing of interferon effect in lower tract respiratory infections

Use of interferon resulted in a significant reduction in the mean number of symptom days and a small reduction in the mean symptom score in patients who developed lower respiratory complications in addition to an URTI. In order to define the action of interferon in these episodes more clearly I investigated the effect of medication on the timing of symptoms and on the upper and lower respiratory tract components of the overall illness. The results are shown in Table 7.7.

Comparison of upper and lower respiratory tract symptoms showed that interferon had no effect on the upper respiratory component (mean score of 70.9 vs 70.3) but did reduce the lower respiratory component (score 33.7 vs 43.4) although this reduction was not significant.

Interferon reduced early symptoms (13.4 interferon vs 22.7 placebo for the first 3 days and 34.9 interferon vs 43.8 placebo for the first 5 days) but these figures were not significantly different. The symptom scores after the first 3 days of illness were virtually identical.
Table 7.7  Effect of interferon on the timing and nature of symptoms in patients who developed lower respiratory tract symptoms.

<table>
<thead>
<tr>
<th>Symptom score</th>
<th>Mean symptom score</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interferon (n=11)</td>
<td>Placebo (n=16)</td>
<td></td>
</tr>
<tr>
<td>Symptom score first 3 days after starting Rx</td>
<td>13.4</td>
<td>22.7</td>
</tr>
<tr>
<td>remaining days of illness</td>
<td>91.2</td>
<td>91.0</td>
</tr>
<tr>
<td>Symptom score first 5 days after starting Rx</td>
<td>34.9</td>
<td>43.8</td>
</tr>
<tr>
<td>remaining days of illness</td>
<td>69.7</td>
<td>69.9</td>
</tr>
<tr>
<td>Total upper respiratory symptom score</td>
<td>70.9</td>
<td>70.3</td>
</tr>
<tr>
<td>Total lower respiratory symptom score</td>
<td>33.7</td>
<td>43.4</td>
</tr>
<tr>
<td>Combined total score</td>
<td>104.6</td>
<td>113.7</td>
</tr>
</tbody>
</table>

NS=No significant difference between interferon and placebo groups at the 5% level (Mann-Whitney U test)
10. Peak flow measurements

The mean of the peak flow readings taken on the first day of medication was compared to the mean of the peak flow measurements recorded on the worst day for each episode. The results are shown in Fig 7.2.

Initial peak flow values in the interferon (mean 303 l/min, sd 102, range 70-530) and placebo groups (mean 288 l/min, sd 99, range 80-505) were not significantly different. The mean lowest peak flow recorded in episodes of interferon use was 241 l/min (sd 96, range 70-480). This was a mean reduction of 62 l/min (20%) compared to the initial peak flow (paired t-test comparing initial and lowest peak flows in interferon recipients, p<0.0005). The mean lowest peak flow recorded in episodes of placebo use was 244 l/min (sd 98, range 65-500). This represented a reduction of 44 l/min (15%) compared to the initial peak flow (paired t-test, comparing initial and lowest peak flows in all placebo recipients, p<0.0005). There was no significant difference in the overall reduction of peak flow associated with episodes of interferon or placebo use (unpaired t-test comparing interferon and placebo groups, p>0.1).

A similar comparison of initial and lowest peak flow recordings in episodes resulting in lower respiratory tract complications showed that interferon episodes were associated with a 28% reduction in peak flow (mean initial peak flow 295 l/min, sd 106, range 160-445; mean lowest peak flow 213 l/min, sd 74, range 140-390; paired t-test comparing initial and lowest peak flows, p<0.005) and placebo episodes were associated with a 27% reduction in peak flow (mean initial peak flow 246 l/min, sd 86, range 80-400; mean lowest peak flow 179 l/min, sd 76, range 65-340; paired t-test comparing initial and lowest peak flows, p<0.005). The effect of virus infection on peak flow was therefore virtually identical in interferon and placebo recipients.
Figure 7.2

Mean initial and lowest peak flows (and standard deviation) associated with episodes of interferon or placebo use.
11. GP consultations, use of antibiotics and hospital admission
There were no significant differences in the GP consultation rate or use of antibiotics in the two groups. The patient's GP was consulted after 12 (25%) of 48 episodes of interferon use compared to 16 (28%) of 58 placebo episodes (p=0.76). Antibiotics were prescribed following 9 (19%) of 48 interferon episodes compared to 14 (24%) of 58 placebo episodes (p=0.50).

1 (9%) of 11 lower respiratory episodes which followed the use of interferon required admission to hospital compared to 4 (25%) of 16 episodes following placebo (Fisher's exact test, p=0.29). The four patients who took placebo and required hospitalisation were admitted for periods of 1, 4, 10 and 14 days respectively giving a mean of 7.25 days in hospital. The single patient who took interferon and required hospital admission spent 4 days in hospital.

The total estimated cost for the 48 patients who received interferon was £753 (£15.70/episode, median £0, range £0-560) compared to a total estimated cost of £4155 (£71.64/episode, median £0, range £0-1962) in the group of 58 patients who received placebo (Mann-Whitney U test comparing costs, p=0.78). If the lower respiratory tract episodes alone were considered the mean cost per episode was £64.50 (median £7, range £0-560) in those who used interferon and £259.25 (median £6, range £0-1962) in those who took placebo (Mann-Whitney, p=0.65).

Although there appears to be a large difference between the mean costs in the two groups the cost of most episodes was either nil or very small and the major part of the costs associated with the exacerbations in chest disease were due to the expense of the small number of hospital admission. Nevertheless, the overall costs are likely to be underestimates as the additional cost of the General Practitioner's services and absences from work were difficult to assess and were not included.
13. Effect of respiratory virus infection in patients taking placebo

As placebo had no therapeutic effect the outcome of exposure to infection in placebo patients should have been an accurate reflection of the overall impact of respiratory virus infection in patients with chronic chest disease.

There were a total of 58 episodes in the placebo group. Overall 25 (43%) of the 58 placebo recipients developed symptoms of a definite respiratory infection, 16 (27%) had a lower respiratory tract infection and 9 (16%) had an URTI. Therefore of the 25 symptomatic individuals the majority (16 of 25 = 64%) had a lower respiratory infection. 4 placebo patients were admitted to hospital; this represented 7% (4/58) of the total of 58 patients exposed to infection and 25% of the 16 patients who developed lower respiratory tract complications.

The outcome of exposure to respiratory tract infection was compared in placebo patients with asthma, chronic bronchitis and bronchiectasis (Fig 7.3). 18 (51%) of the 35 patients with asthma remained asymptomatic after exposure to infection compared to only 1 (7%) of the 15 patients with chronic bronchitis (Chi-squared test, p<0.05), and 2 (25%) of the 8 patients with bronchiectasis. Lower respiratory complications occurred in 6 (17%) of the 35 asthmatics, 7 (47%) of the 15 bronchitics and 3 (37.5%) of the 8 patients with bronchiectasis. (Chi-squared test, p>0.1 comparing proportion of asthmatics and bronchitics who developed lower respiratory tract complications).
Figure 7.3
Outcome of exposure to respiratory tract infection in placebo-using patients with asthma, bronchitis and bronchiectasis.
14. Comparative severity of respiratory illness in symptomatic placebo-using patients and corresponding index cases

The severity of illness in the 25 placebo recipients who developed upper or lower respiratory tract illness was compared with the severity of illness in their corresponding index cases, recorded during the same episode (Fig 7.4). This analysis therefore provided a direct comparison of the severity of illness in patients with chronic chest disease and previously healthy index cases.

The mean number of symptomatic days in patients was 10.6 days (sd 8.0, range 2-33) compared to 5.7 days (sd 2.7 days, range 0-10) in index cases (paired t-test, p<0.005). The mean symptom score in patients was 100.6 (sd 68.0, range 33-316) compared to 62.2 (sd 35.2, range 5-15) for index cases (paired t-test, p<0.01). The mean age of the patients was 50 years (sd 14, range 17-71 years) compared to 33 years (sd 19.8, range 3-62) for the index cases (paired t-test p<0.05).
Figure 7.4
Mean symptom scores and symptom days (and standard deviations) in symptomatic placebo-using patients and corresponding index cases

**Mean symptom days**

- **Index cases**
  - Mean symptom days: 8

- **Patients**
  - Mean symptom days: 18

* p<0.005

**Mean symptom score**

- **Index cases**
  - Mean symptom score: 10

- **Patients**
  - Mean symptom score: 18

x p<0.01
15. Results of viral diagnosis

Viral isolations were performed at the MRC Common Cold Unit and University of Leicester. Nose and throat swabs and paired serum samples from the 1986-87 season were transported to Salisbury in dry ice. A total of 120 swabs were inoculated onto tissue culture in Salisbury and the remaining 140 swabs from the 1987-88 season were analysed in Leicester. Coronavirus 229E ELISA tests were performed in Salisbury, with repeat confirmatory tests and coronavirus OC43 ELISA later carried out in Leicester. All CFT's were performed in Leicester together with virus isolations and coronavirus ELISA tests on the 1987-88 samples.

The results of virological diagnosis on the complete 240 swab samples and 194 paired serum samples are summarised in Table 7.8 which shows that the isolation rate in tissue culture was 6.9% with all except one positive specimen identified as rhinovirus. The other specimen showed a very marked cytopathic effect characteristic of that produced by herpes simplex virus. The isolation rate in Leicester was comparable to that at the Common Cold Unit. Serology was more helpful with 23% of paired sera giving a positive result, the most common viruses implicated were coronaviruses with a total of 31 positives (16%) and influenza viruses (11 positives, 6%), although 1986-87 and 1987-88 were not major epidemic years for influenza. There was one positive result for each of respiratory syncytial virus, adenovirus and Mycoplasma pneumoniae.
Table 7.8 Summary of viral diagnosis

<table>
<thead>
<tr>
<th></th>
<th>No specimens</th>
<th>No +ve</th>
<th>Virus isolated</th>
<th>% positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Viral isolations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salisbury</td>
<td>120</td>
<td>8</td>
<td>rhinovirus</td>
<td>6.7%</td>
</tr>
<tr>
<td>Leicester</td>
<td>140</td>
<td>9</td>
<td>rhinovirus</td>
<td>6.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>?HSV</td>
<td>0.7%</td>
</tr>
<tr>
<td>Total</td>
<td>260</td>
<td>18</td>
<td></td>
<td>6.9%</td>
</tr>
<tr>
<td>b) Serology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronavirus OC 43</td>
<td>194</td>
<td>18</td>
<td></td>
<td>9.3%</td>
</tr>
<tr>
<td>Coronavirus 229E</td>
<td>194</td>
<td>13</td>
<td></td>
<td>6.7%</td>
</tr>
<tr>
<td>Influenza A</td>
<td>194</td>
<td>5</td>
<td></td>
<td>3%</td>
</tr>
<tr>
<td>Influenza B</td>
<td>194</td>
<td>6</td>
<td></td>
<td>3%</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>194</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV</td>
<td>194</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. pneumoniae</td>
<td>194</td>
<td>45</td>
<td></td>
<td>23%</td>
</tr>
</tbody>
</table>

16. Association of viruses with trial outcome

The association between viral infection and subsequent outcome in interferon and placebo groups is shown in Table 7.9. The table does not include viruses which were implicated in episodes which were subsequently excluded from outcome analysis (i.e., two rhinoviruses, one adenovirus and one HSV).

In the 106 episodes that were fully analysed respiratory viruses were diagnosed in the index case(s) in 32 (30%) episodes and in the trial subject in 22 (21%) episodes.
Table 7.9 Positive virus diagnosis in interferon and placebo treatment groups.
(Episodes where no virus was diagnosed are not shown)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Interferon</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient</td>
<td>Index case or secondary case(s)</td>
</tr>
<tr>
<td>Nil</td>
<td>-</td>
<td>OC43</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>Inf B + OC 43</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>RV</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>RV</td>
</tr>
<tr>
<td></td>
<td>RV</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>RV</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>Inf A</td>
</tr>
<tr>
<td></td>
<td>Inf A</td>
<td>Inf A</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>RV</td>
</tr>
<tr>
<td>Total with +ve virus diagnosis</td>
<td>2/19</td>
<td>8/19</td>
</tr>
</tbody>
</table>

Doubtful

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Interferon</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>229E</td>
<td>RV</td>
</tr>
<tr>
<td>Cold</td>
<td>RV</td>
<td>229E</td>
</tr>
<tr>
<td></td>
<td>OC43</td>
<td>RV</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>RV</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total positive</td>
<td>3/11</td>
<td>4/11</td>
</tr>
</tbody>
</table>

URTI

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Interferon</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>OC 43 (x2)</td>
</tr>
<tr>
<td></td>
<td>OC 43</td>
<td>OC 43</td>
</tr>
<tr>
<td></td>
<td>Inf B</td>
<td>Inf B</td>
</tr>
<tr>
<td>Total positive</td>
<td>2/7</td>
<td>3/7</td>
</tr>
</tbody>
</table>

Lower RTI

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Interferon</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>229 E</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>229 E</td>
<td>RV</td>
</tr>
<tr>
<td></td>
<td>229 E</td>
<td>229 E</td>
</tr>
<tr>
<td></td>
<td>Inf A</td>
<td>Inf A + OC 43</td>
</tr>
<tr>
<td></td>
<td>OC 43</td>
<td>229 E</td>
</tr>
<tr>
<td>Total positive</td>
<td>6/11</td>
<td>5/11</td>
</tr>
</tbody>
</table>

Overall positive

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Interferon</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13/48</td>
<td>20/48</td>
</tr>
<tr>
<td></td>
<td>(27%)</td>
<td>(42%)</td>
</tr>
</tbody>
</table>
Virus infections were diagnosed in 13 (27%) of 48 subjects who used interferon and 9 (16%) of 58 placebo episodes ($X^2$, $0.1 < p < 0.2$). Overall, respiratory viruses were implicated by positive isolation or serology in the subject and/or index case or secondary case(s) in 41 (39%) of the 106 episodes.

There were four cases where two separate virus infections were diagnosed in the same person (one influenza A with coronavirus OC43, one influenza A with coronavirus 229E, one influenza B and coronavirus OC43 and one rhinovirus with influenza B, see Table 7.9). In five episodes the virus diagnosed in the index case was different to that diagnosed in the trial subject (two rhinovirus infections in the index case and coronavirus 229E in the patient, one rhinovirus in the index case and coronavirus OC43 in the patient, one coronavirus 229E in the index case and rhinovirus in the patient and one coronavirus 229E in the index case and coronavirus OC43 in the patient, see Table 7.9). These episodes of mixed infection are likely to have resulted from more than one circulating virus causing infections at the same time, although the possibility of false positive diagnostic tests cannot be excluded (for discussion see Section 23).

17. Comparison of trial outcome in rhinovirus, coronavirus and influenza episodes

The trial outcome in episodes associated with rhinovirus, coronavirus and influenza infection is shown in Fig 7.5. Rhinoviruses were implicated, by diagnosis in the patient and/or index case, in 15 of the episodes which were fully analysed (includes the episodes where other viruses were diagnosed in the same episode in addition to rhinovirus). 10 rhinovirus episodes were associated with the use of placebo and 5 with the use of interferon. 7 (47%) of the 15 rhinovirus episodes resulted in no symptoms in the trial subject, 6 (40%) resulted in a doubtful cold, none were associated with URTI and only two (13%) were associated with lower respiratory tract infection. It was therefore not possible to determine the efficacy of the interferon spray against rhinovirus infections alone.
Coronavirus infection was implicated in 24 episodes (13 interferon, 11 placebo, including 6 where coronavirus infection was diagnosed in both the subject and the index case, 8 where coronavirus was diagnosed in the trial subject only and 10 where coronavirus was diagnosed in the index case only. 8 of the 24 coronavirus episodes (33%) resulted in no symptoms in the trial subject, 5 (21%) resulted in a doubtful cold, 4 (17%) resulted in an URTI and 7 (29%) were associated with a lower respiratory tract infection (6 interferon, 1 placebo). The proportion of lower respiratory episodes after coronavirus infections was greater than that after rhinovirus infections, but this difference did not reach statistical significance (7/24 coronavirus vs 2/15 rhinovirus, Chi-squared, 0.1<p<0.2). Coronavirus infection was implicated in 6 of 11 episodes where patients developed lower respiratory tract complications after interferon compared to only 1 of 16 episodes where patients who developed lower respiratory complications after placebo (Chi-squared, p<0.05).

Influenza A or B infection was associated with 8 episodes (5 interferon, 3 placebo), including 3 where influenza was diagnosed in both the trial subject and index case, and 5 where influenza was diagnosed in the index case only. 4 of the influenza episodes (50%) resulted in no symptoms in the trial subject, 2 (25%) resulted in a doubtful cold, 1 (12.5%) resulted in an URTI and only 1 (12.5%) was associated with a lower respiratory tract infection.
Figure 7.5
Outcome of episodes associated with rhinovirus, coronavirus and influenza infection.
Outcome of rhinovirus, coronavirus and influenza infection on index cases

Index cases were generally fit, healthy individuals who did not use the trial medication. The relative effect of different respiratory virus infections was compared in this group (Fig 7.6). Cases of combined infection were not included in this analysis. The mean age of the 11 cases of rhinovirus infection was 22.3 years (sd 11.2, range 12-50), compared to 26.5 years (sd 11.2, range 14-37) for the 4 cases of influenza and 38.6 years (sd 18.6, range 14-64) for the 14 cases of coronavirus infection. Index cases with coronavirus colds were significantly older than those with rhinovirus colds (unpaired t-test comparing ages of index cases with rhinovirus and coronavirus colds, p<0.05). Mean symptom days and symptom scores associated with each virus were not significantly different.
Figure 7.6
Rhinovirus, coronavirus and influenza infection in index cases showing comparison of mean age of cases and mean symptom days and symptom scores (with standard deviations) associated with episodes of infection.

Mean age

\[ \text{Mean age} \]

\[ * \text{ p} < 0.01 \]

Mean symptom days

\[ \text{Mean symptom days} \]

Mean symptom score

\[ \text{Mean symptom score} \]
19. **Timing of virus infections**

Figure 7.7 shows the number and aetiology of episodes occurring weekly during the study period. The number of reported episodes increased towards the end of 1986 with rhinovirus and coronavirus both being implicated. Low numbers of rhinovirus infections continued during the first three months of 1987 but in addition coronavirus infections were still implicated and influenza A appeared on week 4 of 1987. The number of episodes reached a peak of 8 in week 11 of 1987 with several viruses occurring simultaneously. In the autumn of 1987 the number of episodes again increased with rhinoviruses and coronaviruses being implicated. 2 cases of influenza B infection occurred in week 9 of 1988.

20. **Bacteriology**

Although purulent sputum was routinely sent from patients with symptoms of a lower respiratory tract infection only one specimen gave a positive result with the isolation of ampicillin sensitive *Haemophilus influenzae* from a patient with asthma who used interferon spray and developed a lower respiratory tract exacerbation after influenza virus infection.

21. **Adverse effects**

114 courses of medication use (52 interferon, 62 placebo) were evaluated. This included 12 courses which were subsequently excluded from the outcome analysis. The incidence of adverse effects was similar in those taking interferon and those taking placebo (Table 7.10). No adverse effects were consistently associated with the use of the active interferon spray. One patient collapsed while on the final day of treatment with interferon and was admitted to hospital for investigation of a suspected cerebrovascular accident. Full investigation including CT-scan, EEG, serial ECGs and carotid ultrasound proved negative and she subsequently made a full recovery. It was felt extremely unlikely that this event was associated with the use
Figure 7.7
Timing of viral infections diagnosed by isolation or serology in patients or index cases during the interferon trial

No. of episodes

HIV  RSV  Influenza B  Influenza A  229E  OC43  M Pneumonia  Rhinovirus  Adenovirus  Nil Isolated

1986  1987  1988

Weeks

1  2  3  4  5  6  7  8  9  10
of interferon spray but the Committee of Safety of Medicines was notified. The patient's personal symptom records were lost for this episode and it was therefore excluded from the outcome analysis.

Nasal speculum examinations showed abnormalities of the nasal mucosa on days 1 and/or day 7 in 8 (15%) of 52 patients who used interferon and 15 (24%) of 62 patients who used placebo (Chi-squared, 0.3<p<0.5). Active mucosal bleeding was seen in three subjects who had all used interferon, other patients with nasal abnormalities had mucosal hyperaemia only. In four patients (3 interferon and 1 placebo) nasal hyperaemia was still present on day 21.

Table 7.10 Adverse effects reported in interferon and placebo groups.

<table>
<thead>
<tr>
<th>Adverse effect</th>
<th>Interferon</th>
<th>Placebo</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal bleeding</td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Stuffy nose</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Dry nose</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Runny nose</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Sore nose</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Itchy nose</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Sneezing</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dry mouth</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Funny taste</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Gritty eyes</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Headache</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Migraine</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Urticarial rash</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Muscle aches</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Indigestion</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Possible CVA</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>13</strong></td>
<td><strong>15</strong></td>
<td><strong>28</strong></td>
</tr>
</tbody>
</table>
22. Determination of interferon activity
We were unsure if the active trial medication would retain 100% activity after storage at 4°C for 12 months and the suppliers, Boehringer Ingelheim were unable to guarantee continuing potency over this period. In order to ensure interferon activity fourteen randomly selected bottles of spray medication which had been stored for 12 months were sent to Dr A Meager at the National Institute for Biological Standards and Control for independent analysis by antiviral assay and immunoassay. Newly manufactured standards were provided by Boehringer Ingelheim and comparison of the activity of the stored specimens and standards showed that seven bottles contained active medication with no detectable loss of potency. The remaining seven bottles had no detectable activity and contained placebo.

23. Discussion of Interferon Trial
This study documented the impact of respiratory virus infections in patients with chronic chest disease and the effect of prophylactic intranasal interferon. Previous volunteer studies and field trials in normal individuals have shown that prophylactic intranasal alpha2-interferon can prevent rhinovirus infections (Merigan et al, 1973; Greenberg et al, 1982; Scott et al, 1982a; Scott et al, 1982b; Samo et al, 1983; Hayden et al, 1983; Phillpotts et al, 1984; Farr et al, 1984; Herzog et al, 1986; Monto et al, 1986; Douglas et al, 1986; Hayden et al, 1986). Patients with chronic chest disease would be expected to gain particular benefit from prophylactic antiviral medication and a study was organised to investigate the use of prophylactic interferon in the family setting where the subject of the trial suffered from chronic chest disease.

Trial subjects were selected from patients with well-documented chronic asthma, bronchitis or bronchiectasis who had relatively severe disease and had recently been admitted or were regularly attending the chest clinic. Males and smokers predominated in the group with chronic bronchitis and many of the men with bronchitis had worked in local coal mines with occupational dust exposure. The
overall mean history of respiratory disease in the trial patients was nearly 20 years, emphasising the chronic nature of their condition. Those with bronchiectasis had a particularly long history with a mean of 37.3 years. As bronchiectasis commonly results from severe chest infections in childhood such a long history is characteristic. Nearly all trial patients were using regular medication, 94% were using a β2-agonist and 68% were on inhaled steroid preparations, reflecting the comparative severity of their condition and the local management of obstructive airways disease.

123 patients were recruited and followed over an 18 month period but only 117 courses of medication were taken during the entire study period. It has been estimated that most people suffer from 2-5 common colds/year (Couch, 1984) and the number of episodes was much lower than we had anticipated. The probable reasons include the large proportion of elderly subjects (particularly those with chronic bronchitis) who did not have regular contact with children, and the observation that some patients had learnt to avoid relatives or colleagues with colds. In addition a proportion undoubtedly found themselves unable to comply with the trial despite regular phone calls and visits to encourage co-operation.

The results showed that patients with chronic chest disease may develop severe exacerbations if they are in close contact with someone who has a cold. 27 (25%) of 106 patients in this study developed lower respiratory complications after contact with a cold and 5 (19%) required admission to hospital for treatment of complications. Patients with chronic bronchitis were significantly more susceptible to developing symptoms after exposure to respiratory tract infection than patients with asthma. The mean length of illness and symptom scores in symptomatic placebo-using patients was almost double that recorded in their corresponding index cases during the same episodes. Index cases were slightly younger than the patients and Monto et al, (1987b) found that patients over the age of 40 years tended to have more prolonged symptoms after respiratory virus infections. However, age alone is unlikely to account for the observed difference in severity between index cases and
patients. The results therefore emphasize the importance of respiratory viruses in chronic chest disease and their undoubted morbidity, mortality, and economic significance.

Unfortunately intranasal alpha\textsubscript{2}-interferon was not associated with any significant reduction in the number or severity of upper or lower respiratory tract episodes. The mean number of symptomatic days and mean symptom scores were similar for both the interferon and placebo recipients. Interferon did result in a reduction in the duration of episodes of lower respiratory illness of borderline significance but this was not matched by a similar reduction in the recorded symptom scores. This apparent discrepancy may have resulted from missing data from some of the patients who had been admitted to hospital and did not record their symptoms during admission. As four placebo episodes resulted in hospitalisation compared to only one interferon episode this would tend to underestimate the overall symptom scores associated with the use of placebo.

A respiratory virus was implicated in 39\% of episodes which is comparable to the diagnostic rate in other similar studies (Farr et al, 1984; Monto et al, 1986; Douglas et al, 1986; Hayden et al, 1986; Monto et al, 1988). The range of cell-lines used were designed to allow the possible isolation of all commonly occurring respiratory viruses, however only rhinoviruses were diagnosed by isolation and all other infections were identified by serology. The yield of viruses might have been greater if nasal washes or aspirates had been obtained although this was considered impractical. In addition, the isolation of fragile enveloped viruses (particularly RSV) will have been reduced by freezing specimens prior to culture, but it was not logistically possible to inoculate fresh specimens directly into tissue culture. There is no reason to suspect that these difficulties would result in any differences between interferon and placebo groups.

The timing of infections showed a predominance of rhinovirus isolations in the late autumn although sporadic rhinovirus infections occurred during the winter and spring of 1987. Coronavirus infection occurred throughout the study period. In
1986 and early 1987 both 229E and OC43 occurred simultaneously, however all infections after week 42 of 1987 were due to OC43. A similar 2-3 year cyclical pattern of coronavirus 229E and OC43 infections has previously been recognised (Macnaughton, 1982). Influenza A infection first appeared in week 4 of 1987 and continued until week 11. Influenza infections occurring after this time were due to influenza B. The study showed that several respiratory viruses may be circulating in the community at the same time giving rise to very similar symptoms. In week 11 of 1987 symptomatic episodes resulted from rhinovirus, influenza A, herpes simplex virus, coronavirus 229E and coronavirus OC43. This simultaneous circulation of multiple respiratory viruses is being increasingly recognised (Mathur et al, 1980; Morales, 1983; Nicholson et al, 1990) and underscores the need for effective and accurate diagnosis in clinical and epidemiological studies.

Coronaviruses were associated with a particularly severe illness in this group of patients. Although two small trials showed that intranasal interferon was associated with a reduction in symptoms after volunteers were inoculated with coronavirus (Higgins et al, 1983; Turner et al, 1986) we found a significantly greater number of coronavirus infections in those patients who had lower respiratory symptoms after interferon compared to placebo. This finding may have been purely coincidental as the numbers of episodes were small but needs to be investigated by further field studies.

Different viruses were diagnosed in the index case and subject in five episodes. This was not surprising as it has been shown that several respiratory viruses may circulate in the community at the same time (Nicholson et al, 1990). Coronaviruses were implicated in each of these episodes and an alternative explanation might be a lack of specificity of the coronavirus ELISA test. This was less likely as all but one of these coronavirus infections was associated with an antibody ratio of greater than two between acute and convalescent sera. There was evidence of two distinct virus infections in the same index case in four episodes. These cases presumably had simultaneous or sequential virus infections.
There are several possible explanations for the overall lack of benefit associated with intranasal interferon in comparison to previous studies performed in healthy family members (Douglas et al, 1986; Hayden et al, 1986). Rhinoviruses were only implicated in 15 episodes and this number was too small to determine the efficacy of interferon against rhinovirus infection alone. Other field studies involving healthy subjects have consistently shown no effect of interferon on illness caused by viruses other than rhinoviruses (Douglas et al, 1986; Hayden et al, 1986). The efficacy of interferon might have appeared greater if the study had been conducted in a greater number of individuals over a shorter time period coinciding with rhinovirus activity. The timing of treatment is also likely to be of crucial importance as prophylactic interferon spray was found to be ineffective against established naturally occurring colds (Hayden et al, 1988). The mean period between initial contact with the index case and commencing interferon medication in our study was 34.7 hours. This may have been too late to prevent infection in many cases. The mean period between contact and starting medication was not documented in the family studies in Australia (Douglas et al, 1986) and the USA (Hayden et al, 1986), but may have been lower than in our trial.

The efficacy of interferon depends upon the distribution and duration of action of the intranasal spray. Aoki and Crawley, (1976) found that the mean half-life of intranasally applied labelled albumin was only 24.3 mins, which would necessitate frequent dosing, however similar studies using a labelled intranasally applied interferon spray showed persistence of interferon for over 24 hours, and concluded that interferon probably bound to nasal mucosal cells with twice daily spraying likely to give adequate protection (Wynne Davies et al, 1983). The particle size is a crucial factor determining the penetration of the medication (Wyde et al, 1984). The interferon nasal spray used in our study was designed to give a mist of sufficiently large particle size to distribute in the nasal passages with comparatively little deposition in large airways or alveoli (Fig 7.1). Interferon had no obvious effect on bronchial reactivity as peak flow recordings were similar in the interferon
and placebo groups. The development of lower respiratory disease after respiratory virus challenge is thought to depend primarily on the inhalation of infective virus particles (Cate et al, 1964), however many factors contribute to the associated deterioration in lung function, including the release of histamine and other inflammatory mediators (Busse, 1987; Busse, 1989). The possibility that interferon delivered into the lungs might be more effective at preventing the pulmonary complications of respiratory virus infection has been suggested by Wyde et al, (1985) who showed that aerosolized interferon was able to protect mice against challenge with vesicular stomatitis virus. Nebulised antiviral therapy could have a role to play in the prevention of lower respiratory complications in susceptible patients and warrants further investigation.

Interferon spray was very well tolerated. A number of minor adverse effects were reported but these were not clearly associated with active interferon medication and many of the mild nasal and upper respiratory symptoms may have been due to virus infection. Other studies have similarly found that short-term use of intranasal interferon is well tolerated (Greenberg et al, 1982; Herzog et al, 1986; Douglas et al, 1986; Hayden et al, 1986; Samo et al, 1984), but unfortunately use of intranasal interferon for periods of over two weeks is associated with an unacceptable incidence of nasal irritation and bleeding which limits its potential for seasonal prophylaxis (Samo et al, 1983; Farr et al, 1984; Monto et al, 1986; Hayden et al, 1986; Samo et al, 1984).

In conclusion this study showed that patients with chronic chest disease are at definite risk of developing severe exacerbations of their condition if they are in close contact with someone who has a cold. Prophylactic intranasal interferon spray did not prevent or ameliorate these exacerbations. Further studies might be considered using combinations of antiviral medication and comparing intranasal and nebulised delivery.
1. Introduction

Respiratory virus infections are thought to be one of the most important causes of acute exacerbations occurring in patients with cystic fibrosis (reviewed in chapter 3). Viruses may cause deterioration through a direct cytopathic effect on the bronchial mucosa or as a result of secondary bacterial colonisation. Our understanding of the epidemiology and consequences of viral infection in this group of patients is poor and some studies have reported difficulties isolating viruses from CF patients (Wang et al., 1984). It is important to increase our knowledge of the role played by these viruses as new antiviral drugs are being developed which may benefit these patients. As we had been looking at the impact of respiratory virus infection in adult patients with chronic chest disease we decided to organise a study to assess the role of respiratory virus infections in children with cystic fibrosis. In view of the potential difficulties of working with chronically ill infants and children a preliminary study was undertaken to investigate the feasibility of the project.

2. Aims of study

The aims of a definitive study of respiratory virus infections in cystic fibrosis were planned as follows:-

1) To document the number and nature of respiratory virus infections in children of all ages with cystic fibrosis.

2) To investigate the subsequent effect of virus infections on clinical state and lung function.
3) To compare conventional methods of virus diagnosis using tissue culture and serology with newer techniques including the use of immunofluorescence, enzyme-linked immunosorbent assay (ELISA), radiolabelled oligonucleotide probes, polymerase chain reaction and the use of saliva or urinary specimens for viral diagnosis. The possibility of developing alternatives to blood samples was thought to be particularly important as repeated venepuncture is not desirable in these children.

The preliminary study was organised at the beginning of 1990 with the assistance of Dr S Tanner, Senior Lecturer in the Department of Paediatrics, University of Leicester and Sister Lucy Wilde, the cystic fibrosis liaison nurse. Approximately 45 children (age 0-16) with cystic fibrosis reside in the catchment area of the Leicester Royal Infirmary (LRI) which includes Leicester city and most of Leicestershire. We decided that they could all be potentially recruited into the preliminary study which took place over a relatively short period (late Jan-Easter 1990). As no extra therapeutic measures or interventions were involved in the preliminary-study beyond the routine care received by these patients, Ethics Committee approval was deemed unnecessary.

3. Organisation of the study
Families of children with cystic fibrosis in Leicester were requested to contact Sister Wilde when their child developed symptoms of an upper respiratory tract infection. Sister Wilde visited the family as soon as possible after the call, usually within 24 hours.

At the first visit brief details of the patient's medical history were noted and the history of the symptomatic episode was recorded, including the time of onset of respiratory symptoms. Nasal and throat swabs were taken from the child and other symptomatic family members. Sputum samples were sent for bacteriological culture at the Public Health Laboratory in Leicester. All samples for virology were placed
immediately in cold virus transport medium (kept on ice) and were brought to our laboratory within several hours of collection.

The family of each symptomatic CF child was asked to complete a questionnaire documenting the child's symptoms for a period of approximately 20 days (or until symptoms ceased). Other symptomatic family members were also asked to complete a questionnaire documenting their own symptoms. Families were told to note only a deterioration of symptoms above the usual severity. The following information was recorded:

i) total number of symptomatic days
ii) total symptom score
iii) any extra visits by the CF sister or GP
iv) additional medication used
v) results of sputum bacteriology
vi) need for hospital admission.

Regular lung function tests were not included in the preliminary study. Lower respiratory symptoms were defined as an increase in at least two of cough, wheeze, shortness of breath or phlegm. A symptomatic day was defined as any day in which respiratory symptoms were recorded which were more severe than those usually experienced by the patient.

Upon receipt of the swabs in virus transport medium in the laboratory, nasopharyngeal cells were separated by centrifugation for immunofluorescence and the supernatent was divided. 0.4mls was added to 0.1ml of human placental ribonuclease inhibitor (HPRI) in transport medium and stored at -70°C for later analysis using picornavirus oligonucleotide probes and PCR. 0.2ml of the remainder was inoculated directly into each cell culture. 5 cell lines were used (Ohio HeLa, MRC-5, MDCK, C16 and HEp-2 cells). Any residual sample was stored at -70°C.
RESULTS

4. Response rate

Letters inviting children and their parents to participate in the study were sent to 45 families. 17 children (38%), age range 1-16, developed symptoms of an upper respiratory tract infection during the study. Sixteen children had only one cold, but one girl had four symptomatic episodes. A total of 20 episodes were therefore recorded.

Symptomatic children were aged between 1 and 16 years. The family size was documented for 16 of the 17 children with symptoms. Ten (62.5%) had sibs and two of these had sibs who also suffered from cystic fibrosis.

5. Outcome of episodes of presumed respiratory virus infection

Symptoms were fully documented for 16 of the 20 episodes, occurring in 13 separate children. Table 8.1 shows the outcome in these 16 episodes. 3 had mild symptoms only and were classified as doubtful colds, 10 children had symptoms consistent with upper respiratory infection and three had lower respiratory symptoms. Two of the 3 episodes of lower respiratory tract infection were associated with rhinovirus infection. The mean number of symptomatic days for each episode was 10.8 days (sd 9.5 days, range 2-20 days). The mean symptom score was 45.9 (sd 29.9, range 3-103).

Only one episode led to a consultation with the child's General Practitioner, however, the CF Sister was asked to visit 5 children and she made a total of 9 calls to these five patients. Antibiotics were given in 7 (45%) episodes.

6. Bacteriology

Bacteria were cultured from samples taken during 8 (50%) episodes. S. aureus and B. catarrhalis were each cultured alone in 1 episode; P. aeruginosa was found in four episodes and mixed infection was documented in 2 episodes (Table 8.1). The requirement for antibiotics was not evidently related to the isolation of bacteria as 3
of 8 (37.5%) children from whom bacteria were isolated were prescribed antibiotics, and antimicrobials were also prescribed for 4 of 8 (50%) episodes where no bacteria were isolated.

7. Virology

34 nasopharyngeal specimens were received from CF children or symptomatic contacts. Viruses were isolated from only 5 (14.7%) specimens overall (Table 8.1). Specimens were received from the symptomatic CF child in all 20 episodes (including the 4 episodes which were not fully documented) and viruses were isolated from 3 (15%), including 2 (10%) rhinoviruses and one (5%) enterovirus, identified as a coxsackie B virus. In addition, specimens were received from 14 cases who were symptomatic contacts of the CF children. Viruses were isolated in 2 (14%) of these 14 samples. One of the 2 viruses isolated from symptomatic contacts was identified as a rhinovirus and one was coxsackie B, which appeared identical to the virus isolated from the CF patient during the same episode.

One of the CF patients from whom a rhinovirus was isolated had a lower respiratory tract infection and the other had an upper respiratory tract infection. The third episode in which a rhinovirus infection was implicated (by isolation in the contact) resulted in a lower respiratory tract infection in the CF child. Coxsackie B virus resulted in an upper respiratory tract infection.

Immunofluorescence tests were all negative, but it was noted that the specimens taken from CF children were often very viscous and purulent resulting in difficulties in making suitable cell smears for immunofluorescence. Bacterial contamination of tissue culture also occurred following inoculation of specimens from CF patients. This was overcome by adding gentamicin to the tissue culture medium.
Table 8.1 Outcome of the 16 documented episodes of respiratory tract infection in children with cystic fibrosis

<table>
<thead>
<tr>
<th>Pt</th>
<th>Result</th>
<th>Symptom Days</th>
<th>Symptom Score</th>
<th>GP calls</th>
<th>Calls to CF sister</th>
<th>Bacteriology</th>
<th>Virology</th>
<th>Virology Patient</th>
<th>Virology Contact</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>URTI</td>
<td>6</td>
<td>34</td>
<td>0</td>
<td>1</td>
<td>S. aureus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Flucloxacillin</td>
</tr>
<tr>
<td>2</td>
<td>Lower RTI</td>
<td>5</td>
<td>81</td>
<td>0</td>
<td>2</td>
<td>P. aeruginosa</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>Doubtful cold</td>
<td>18</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Salbutamol</td>
</tr>
<tr>
<td>4</td>
<td>Lower RTI</td>
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<td>77</td>
<td>0</td>
<td>3</td>
<td>P. aeruginosa</td>
<td>Rhinovirus</td>
<td>-</td>
<td>-</td>
<td>Ciprofloxacin</td>
</tr>
<tr>
<td>5</td>
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<td>32</td>
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<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>6</td>
<td>URTI</td>
<td>15</td>
<td>55</td>
<td>0</td>
<td>0</td>
<td>B. catarrhalis</td>
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<td>-</td>
<td>-</td>
<td>Erythromycin</td>
</tr>
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<td>7</td>
<td>URTI</td>
<td>20</td>
<td>66</td>
<td>0</td>
<td>0</td>
<td>P. aeruginosa</td>
<td>Enterovirus(? Cox B)</td>
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<tr>
<td>8</td>
<td>URTI</td>
<td>6</td>
<td>23</td>
<td>0</td>
<td>0</td>
<td>P. aeruginosa</td>
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<td>-</td>
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</tr>
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<td>9</td>
<td>URTI</td>
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<td>25</td>
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<td>P. aeruginosa/ H. influenzae</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>10</td>
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<td>7</td>
<td>13</td>
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<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>11</td>
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<td>103</td>
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<td>-</td>
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<td>3</td>
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<td>H. influenzae/ B. catarrhalis</td>
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</tr>
<tr>
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<td>URTI</td>
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<td>91</td>
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<td>-</td>
<td>Rhinovirus</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
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<td></td>
<td>4</td>
<td>20</td>
<td>0</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
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<td>49</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Magnapen</td>
</tr>
<tr>
<td>URTI</td>
<td></td>
<td>8</td>
<td>39</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Magnapen</td>
</tr>
</tbody>
</table>
8. Discussion of Cystic Fibrosis Study

This preliminary study provided some information on the impact of respiratory virus infection in children with cystic fibrosis and showed the feasibility of a study in this group of patients. A large degree of co-operation was achieved and over the 10 week study period a total of 20 suspected colds were reported. Although children with CF often suffer from chronic ill-health and persistent respiratory symptoms the majority of episodes were discrete with a well defined period of respiratory symptoms and only 3 (19%) episodes were thought to be doubtful colds. Rather surprisingly the results showed that symptoms were confined to the upper respiratory tract in 10 (62.5%) of the 16 episodes and were equivalent in length and severity to colds suffered by normal healthy individuals (eg. the index cases in the interferon study). Evidence of an exacerbation affecting the lower respiratory tract was found in only 3 (19%) episodes, of which 2 were associated with rhinovirus infection. Fortunately none of the episodes resulted in hospital admission. The study was performed during the spring, if it had taken place during the period of maximal rhinovirus activity (Sept-Oct) the proportion of lower respiratory episodes may have been greater. The relatively severe outcome after rhinovirus infection indicates a potential therapeutic use of interferon in these patients.

Viruses were isolated from 3 (15%) of 20 specimens obtained from the children with CF. This was comparable to the isolation rate achieved from specimens taken from healthy subjects and in the interferon trial and showed that there was no particular problem with isolating viruses from specimens obtained from CF patients provided that fresh specimens were inoculated directly onto tissue culture and bacterial contamination was avoided.

The results of microbiological analysis showed that half of the children were colonised with bacteria including S. aureus, H. influenzae, B. catarrhalis and P. aeruginosa. The significance of bacterial isolation during periods of symptoms is uncertain as most patients are chronically infected; however there is no doubt that virus infection can lead to bacterial overgrowth and invasion and hence antibiotic
therapy is logical for all except the mildest episodes (Fick and Stillwell, 1989; David, 1990). The precise nature of the interaction between respiratory viruses and colonising bacteria is still poorly understood.

The most important study of virus infections in cystic fibrosis was performed in the USA by Wang et al, (1984) who showed that children with cystic fibrosis had more virus infections than their healthy siblings and demonstrated highly significant correlations between the annual incidence of viral infections and indicators of disease progression. There were several major drawbacks in this study, as it only looked at children over the age of 6 and virus diagnosis was only achieved in a minority of episodes with no viruses isolated from nasal washings. In comparison our study demonstrated that it is possible to isolate respiratory viruses from specimens obtained from CF patients. Newer techniques including the use of gene-probes, PCR, detection of secretory IgA in saliva or nasopharyngeal aspirates and detection of salivary or urinary antigen should lead to further improvements in diagnostic accuracy.

The preliminary study was invaluable and has led to the funding of a comprehensive long-term study which will start in summer 1991 and continue for 12-24 months. This aims to be a definitive study of virus infections in CF patients and will follow-up all consenting local CF children. The study also aims to evaluate novel techniques for diagnosing respiratory virus infections. Cystic fibrosis is frequently diagnosed shortly after birth and affected babies will be included in the study from the time of diagnosis so that the role of respiratory viruses can be assessed during this crucial period when the lungs are particularly susceptible to microbial infection.
CHAPTER 9

INFLUENZA VACCINATION IN THE ELDERLY. A STUDY OF THE POLICIES OF GENERAL PRACTITIONERS IN THE TRENT REGION

1. Introduction

In the United Kingdom annual influenza vaccination is recommended for the elderly and for patients with chronic medical conditions (DHSS, 1984; Department of Health, 1990), as influenza A infection has been associated with excess mortality in these groups (reviewed in chapter 1). The elderly living in residential homes or long stay hospitals are particularly susceptible to influenza, with attack rates of up to 60% during an outbreak of influenza A and case-fatality rates of over 30% (DHSS, 1984; MMWR, 1987). Influenza vaccination has been shown to reduce the morbidity and mortality of infection in high risk adults (Barker and Mullooly, 1980; Patriarca et al., 1985; Barker and Mullooly, 1986) and the Immunization Practices Advisory Committee in the USA therefore advises that at least 80% of adults with chronic medical conditions and those resident in nursing homes or other chronic care institutions should receive annual vaccination (MMWR, 1984). In spite of the official recommendations there is very little information about the uptake of influenza vaccine in the United Kingdom and the vaccination policies and practices of General Practitioners. This study aimed to investigate the use of vaccine by General Practitioners in the Trent Region, and identify the strategies and practice policies which resulted in an improved vaccination rate.
2. Organisation of the study

Questionnaires were sent to 244 randomly selected General Practitioners in the Trent Region during the winter of 1985-86 asking about the size and location of their practice, the number of patients over 65 years of age, policies for vaccination, implementation of vaccination, reasons for non-acceptance of vaccine and overall immunization rate. A further questionnaire was sent to those GPs who did not respond. Those who did not reply to the second questionnaire were contacted by telephone.

RESULTS

3. Response rate

141 questionnaires were returned (58% response). Of these 12 were not completed. The reasons stated for non-completion included; retirement or death of the doctor concerned, change of address, no fee offered, student health centre with no elderly patients and questionnaire considered too complex. Of the 129 that were completed, 2 were discarded - one where two partners in the same Health Centre had replied and one that was answered inconsistently. 127 were therefore evaluable (52%).

To assess whether those who replied had different policies compared to those who did not reply, 48 of the 103 non-responders were selected at random and telephoned. 13 of the non-responding GPs were unavailable or had moved away, 5 refused to answer, but 30 completed the questionnaire by telephone. The responses from the GP's who were telephoned were not included in the subsequent analysis, but were found to be similar to the responses of the GP's who had returned the questionnaire.
4. Demographic characteristics of responding GP's

The demographic characteristics of these practices is shown in Table 9.1. Practices covered the whole of the Trent Region with all counties represented and a spread of practices from wholly urban, urban and rural to wholly rural. The majority of practices had between 2 and 5 partners, with a mean of 3.8 partners (sd 1.6, range 1-8) and an overall mean list size of 8194 (sd 4265, range 995-20,000) of whom 16% were estimated to be over 65.

Table 9.1  Demographic characteristics of the 127 centres analysed

<table>
<thead>
<tr>
<th>Demographic characteristic</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
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</tr>
<tr>
<td>Derbyshire</td>
<td>15</td>
</tr>
<tr>
<td>Leicestershire</td>
<td>16</td>
</tr>
<tr>
<td>Lincolnshire</td>
<td>9</td>
</tr>
<tr>
<td>Nottinghamshire</td>
<td>16</td>
</tr>
<tr>
<td>South Yorkshire</td>
<td>44</td>
</tr>
<tr>
<td>Wholly rural</td>
<td>15</td>
</tr>
<tr>
<td>Wholly urban</td>
<td>36</td>
</tr>
<tr>
<td>Mixed urban and rural</td>
<td>48</td>
</tr>
<tr>
<td>Not stated</td>
<td>1</td>
</tr>
<tr>
<td>No of partners</td>
<td></td>
</tr>
<tr>
<td>1 - 2</td>
<td>20.5</td>
</tr>
<tr>
<td>2.5 - 5</td>
<td>63</td>
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<tr>
<td>&gt;5</td>
<td>15.7</td>
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<tr>
<td>Not stated</td>
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<tr>
<td>Dispensing practices</td>
<td>21</td>
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<tr>
<td>Practices looking after homes for the elderly</td>
<td>49</td>
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</table>
5. Vaccination policies

The questionnaire investigated the vaccination policies of GP's, and asked about the policy for patients resident in nursing homes or other institutions and for ambulant patients, including those with chronic medical conditions.

88 of 127 (69%) practices had a policy for vaccination. 62 practices were responsible for homes for the elderly and 47 (76%) of these practices had a policy which covering nursing home residents. 113 (89%) practices offered influenza vaccine to non-institutionalised patients. These patients were identified during consultations or by sending letters (Table 9.2). 8.7% of practices had a policy of sending letters to all mentally alert patients over 65 and 43.3% were willing to vaccinate all patients aged 65 or older who attended for a consultation. 20% of practices sent postal reminders to all patients with chronic diseases. If patients with chronic disease attended for consultations between 65.4-81.1% were offered vaccination.

90 practices provided details of their vaccination strategy for institutionalised patients; 52% offered the vaccine to all elderly residents, 81% vaccinated all mentally alert residents and 84% ensured that all mentally alert residents with chronic medical conditions were vaccinated. Only 30% considered vaccinating psychogeriatric patients. 47% of practices offered vaccination to staff working in residential institutions but only 44% of the staff who were offered the vaccine actually accepted it.
<table>
<thead>
<tr>
<th>Means of initiating vaccination</th>
<th>No of practices</th>
<th>% of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sending letters offering vaccination to <strong>all</strong> mentally alert people aged 65 or over living at home</td>
<td>11</td>
<td>8.7</td>
</tr>
<tr>
<td>Sending letters offering vaccination to <strong>all</strong> mentally alert people aged 65 or over living at home with: Chronic heart disease</td>
<td>25</td>
<td>19.7</td>
</tr>
<tr>
<td>Chronic chest disease</td>
<td>26</td>
<td>20.5</td>
</tr>
<tr>
<td>Diabetes</td>
<td>26</td>
<td>20.5</td>
</tr>
<tr>
<td>Chronic renal disease</td>
<td>25</td>
<td>19.7</td>
</tr>
<tr>
<td>Consultation in the practice with <strong>all</strong> mentally alert people aged 65 or over living at home</td>
<td>55</td>
<td>43.3</td>
</tr>
<tr>
<td>Consultation in the practice with <strong>all</strong> mentally alert people aged 65 or over living at home with: Chronic heart disease</td>
<td>93</td>
<td>73.2</td>
</tr>
<tr>
<td>Chronic chest disease</td>
<td>103</td>
<td>81.1</td>
</tr>
<tr>
<td>Diabetes</td>
<td>84</td>
<td>65.4</td>
</tr>
<tr>
<td>Chronic renal disease</td>
<td>83</td>
<td>65.4</td>
</tr>
<tr>
<td>Consultation in the practice with selected patients considered at risk</td>
<td>10</td>
<td>7.9</td>
</tr>
</tbody>
</table>

N.B. Figures for patients with chronic disease includes practices where offer was extended to all patients
6. Delivery of vaccine

Of the 112 practices providing details, 64 (57%) vaccinated ambulant patients during medical consultations only and 18% would vaccinate only at specially arranged vaccination session. The remainder vaccinated both during consultations and in special clinics, or on an ad hoc basis, usually by the practice nurse. In 39% of practices vaccine was only given by a doctor, in 36% both doctors and nurses gave vaccines, in 22% only nurses gave the vaccine and 3% involved other staff eg. health visitors. 91% of practices were willing to vaccinate non-ambulant patients in their homes. Vaccination was given to these patients by the GP alone in 42%, by the nurse alone in 20% and by either in the remaining 38%.

Practitioners were asked to estimate the number of vaccinations given by their practice. The 127 respondents and their partners gave a mean of 67 vaccinations (95% confidence limits 55.5-78.4, range 0-500) which covered 19.5% of the estimated number of patients aged over 65. The overall vaccine acceptance rate was estimated to be 72% (95% confidence limits 67.3-76.8%), hence 27% (95% confidence limits 22.3-34.5%) of elderly people were considered to have been offered the vaccine.

In practices responsible for homes for the elderly the overall vaccination rate for residents was estimated to be 54%, with 48% of practices achieving a vaccination rate of over 80%.

7. Reasons for not accepting vaccine

14 practices (11%) did not give the vaccine and 9 of these gave reasons for this policy which included being uncertain of the efficacy (8/9), concern over possible side effects (4/9) and insufficient staff (3/9).

GP's were also asked to give reasons why patients did not accept vaccine. Concern about the vaccine's efficacy was the most common reason followed by concern over local and systemic reactions, dislike of needles, failure to arrange or keep appointments and difficulty in getting to the practice.
8. Factors influencing rate of vaccination

The factors found to be associated with an improved rate of vaccination are summarised in Table 9.3. When practices combined several of these factors the rate of vaccination was particularly high. For example, practices which had an agreed policy for vaccination, who sent letters to all mentally alert patients aged 65 or over, and held regular immunisation sessions, achieved a mean vaccination rate of 29.8%; over four times greater than the practices which did not have these policies (mean vaccination rate 7.6%, p<0.0005).

There were no significant differences in the immunisation rates of small (up to 2 partners), medium (2.5-5) or large practices, between urban and rural practices, or between those looking after homes for the elderly and those not responsible for old peoples' homes.
Table 9.3. Factors associated with an improved rate of immunisation among patients aged 65 and older

<table>
<thead>
<tr>
<th>Factors</th>
<th>Versus</th>
<th>Vaccination rates (%)</th>
<th>Increase (%)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Policy versus no policy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agreed practice policy for adult vaccination</td>
<td>None</td>
<td>23.2 vs 11.1</td>
<td>110</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Demented and others with poor quality of life</td>
<td>Not offered</td>
<td>27.2 vs 16.2</td>
<td>68</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Nursing home staff offered vaccine</td>
<td>Not offered</td>
<td>24.8 vs 16.5</td>
<td>50</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Means of initiating policy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Letters to all mentally alert people aged 65 or over</td>
<td>Not sent</td>
<td>38 vs 18.4</td>
<td>107</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Letters to all mentally alert people aged 65 or over</td>
<td>Vaccine offered to all mentally alert people during consultation</td>
<td>38 vs 28.6</td>
<td>43</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Vaccine offered to all mentally alert people aged 65 or over</td>
<td>Vaccine offered to mentally alert people with chronic disease during consultation</td>
<td>26.6 vs 13.2</td>
<td>63</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Vaccine offered to all mentally alert people aged 65 or over</td>
<td>No offer</td>
<td>26.6 vs 13.2</td>
<td>102</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Implementation of the policy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A regular or special vaccination session</td>
<td>None</td>
<td>25.6 vs 18.5</td>
<td>38</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Vaccine given by nurse and GP</td>
<td>Given by GP only</td>
<td>24.4 vs 17.1</td>
<td>43</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Immobile patients vaccinated at home</td>
<td>Home vaccinations not given</td>
<td>20.4 vs 10.2</td>
<td>100</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Follow up activities</td>
<td>None</td>
<td>30.4 vs 17.7</td>
<td>77</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>
9. Discussion of Trent GP Study

This study evaluated the policies for influenza vaccination in the elderly and in patients with chronic medical conditions in a large cross-section of General Practitioners in the Trent Region. The practices sampled in this study were responsible for the care of approximately one fifth of all the patients in the Trent Region and included urban, rural and mixed practices. The majority of GP's were in practices with 2.5-5 partners, and 49% had responsibilities for residential homes for the elderly. In 1984 the population of Trent Region was 4.61 million of whom 668,000 (14.5%) were aged 65 years or over. This proportion is very similar to the estimate of 16% provided by the GP's in this study. Information from vaccine suppliers suggested that approximately 225,000 doses of vaccine were distributed to the Trent Region during the winter of 1985-86. Assuming that half to two thirds was given to the elderly an estimated 17-22% of those aged 65 or over would have received vaccine, a proportion which agrees closely with the overall figure of 19.5% for the practices in the study. These practices were therefore thought to be entirely representative of the Trent Region as a whole.

Vaccination rates and policies differed markedly between practices. The most significant factor associated with a higher vaccination rate against influenza was the presence of an agreed practice policy which resulted in a vaccination rate 110% higher than practices which had no policy. Practices which had policies offering vaccine to demented patients and those with a poor quality of life, and practices which offered vaccine to nursing home staff had significantly higher vaccination rates than practices which did not include these groups. The organisation and implementation of the vaccination programme also influenced the vaccination rate. Practices which sent letters to all mentally alert patients over 65 had significantly higher vaccination rates than practices which did not send letters, and practices which offered vaccine to all mentally alert patients had significantly higher vaccination rates than those who only offered vaccine to patients with chronic illness. Significantly higher vaccination rates were also found in practices which organised regular vaccination
sessions or special immunisation clinics, practices which involving doctors and nurses in the vaccination programme, and practices which vaccinated immobile patients at home. Each of these factors was individually associated with a 38-110% increase in vaccination rate, however when several factors were combined the vaccination rates increased more than four times. The results show that vaccination rates might be improved substantially if practices reviewed their vaccination policies and programme.

It was estimated that 54% of resident patients in this study were vaccinated and 48% of practices achieved a level of vaccination of 80%. This is the target recommended by the Immunisation Practices Advisory Committee of the US Public Health Service (MMWR, 1987) as serious outbreaks are more likely to occur if the overall vaccination rate amongst staff and residents is not maintained at this high level (Kendal et al, 1986). Only 30% of the practices who responded to our questionnaire vaccinated psychogeriatric patients and only 44% of nursing staff in residential homes were vaccinated. These figures explain why serious outbreaks of influenza still occur in residential institutions. Institutionalised psychogeriatric patients are amongst those at the very highest risk of mortality or significant morbidity after influenza. Vaccination of a high proportion of residents will confer a beneficial herd effect and provide a degree of protection for residents and staff. However, there may be problems obtaining consent for vaccination from these demented individuals and the decision whether to vaccinate may be very difficult.

Concern over vaccine efficacy and possible adverse effects were thought to be the major reasons why some patients did not accept the offer of vaccination. Older vaccines were associated with painful local reactions and transient malaise and myalgia but newer highly purified split-product vaccines are more acceptable with few adverse effects (Nicholson et al, 1979). A study amongst institutionalised elderly patients in the USA (Patriarca et al, 1985) found that the overall efficacy of vaccination in preventing infection among institutionalised elderly patients was only 35%, however vaccination was associated with a 40-50% reduction in
pneumonia or hospitalisation and an 80% reduction in mortality. In a non-institutionalised elderly population influenza vaccination was associated with a 72% reduction in hospitalisation and an 87% reduction in mortality (Barker and Mulooly, 1980). The proportion of unvaccinated residents was found to be the most important factor associated with occurrence of influenza outbreaks in residential homes, with the lowest frequency of outbreaks in homes with high vaccination rates (Kendal et al, 1986). These studies show that influenza vaccine reduces the severity of disease as well as the incidence of infection and high vaccination rates are associated with protection against outbreaks in residential institutions. The general public and health-care workers clearly need to be made more aware of the benefits of vaccination.
CHAPTER 10
INFLUENZA VACCINATION AND INFLUENZA-LIKE ILLNESS IN PATIENTS WITH ASTHMA AND PREVIOUSLY HEALTHY ADULTS DURING THE 1989-90 INFLUENZA EPIDEMIC

1. Introduction
Although annual influenza vaccination has been recommended to groups considered at high risk, including those with chest disease (Dept of Health, 1990), the survey of Trent GP's and other studies in Leicester (Kurinczuk and Nicholson, 1989, Nicholson et al, 1990) had indicated that the use of influenza vaccine in these patients was patchy and depended to a large extent on the views and policies of the patients' General Practitioners. The public and many medical practitioners still remain largely unconvinced of the benefits of influenza vaccination. At the end of 1989 there was a relatively large outbreak of influenza A infection (PHLS, 1990) which provided an opportunity to study the impact of influenza infection and the efficacy of influenza vaccine in a group of high risk patients with asthma. In addition a study of respiratory virus infections in previously healthy adults was initiated during the autumn of 1989 and coincided with the influenza outbreak. This study allowed us to compare episodes of respiratory illness occurring during and outside the period of the influenza epidemic.

2. Organisation of the study of asthmatic patients
Patients with asthma who were under the care of Dr M Stern, Consultant Physician at Leicester General Hospital, were sent an explanatory letter and questionnaire in early February 1990, shortly after the influenza epidemic. The questionnaire asked patients whether they thought they had influenza and if they had received vaccine. Patients who had been vaccinated were asked to give the approximate date of vaccination. Patients who had experienced an influenza-like illness during the epidemic period were asked to record the number of days taken off work or school,
the number of visits to their GP, the use of additional medication and whether they had been admitted to hospital as a result of their illness. These factors documented the impact of influenza-like illness and were compared for vaccinated and unvaccinated groups. To investigate the accuracy of diagnosis haemagglutination inhibition (HI) antibody titres to vaccine and epidemic strains of influenza were determined in a representative sample of patients to see whether the presence of convalescent antibodies correlated with the patients’ perception of illness.

RESULTS

3. Response rate

1099 questionnaires were sent. 477 (43%) replies were received after the first mailing and a further 165 (15%) replies were received after the second mailing. Altogether 642 replies were received (response rate=58.4%). Of these 642 replies 110 were returned by the Post Office because the patients had moved elsewhere and 8 questionnaires were returned incomplete and could not be analysed. A total of 524 questionnaires were fully analysed.

4. Uptake and timing of influenza vaccination

Only 78 (14.9%) of the 524 respondents had received the influenza vaccine. Table 11.1 shows the timing of vaccination. 47 (60%) of the 78 vaccinees received vaccine in September, October or November, but 23 (29%) were given vaccine in December or January, at the height of the epidemic, when the vaccine may have been given too late to have any preventative effect. There was no significant difference in the timing of vaccination in those patients who had influenza and those who did not; 20 (65%) of 31 vaccinees with influenza-like symptoms were vaccinated prior to December compared to 28 (60%) of 47 who did not have symptoms (Chi-squared, 0.8<p<0.9).
Table 10.1 Timing of influenza vaccination

<table>
<thead>
<tr>
<th>When vaccine given</th>
<th>Pts with flu</th>
<th>Pts without flu</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Sept 1989</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>September</td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>October</td>
<td>9</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>November</td>
<td>7</td>
<td>14</td>
<td>21</td>
</tr>
<tr>
<td>December</td>
<td>7</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>January 1990</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Not specified</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>47</td>
<td>78</td>
</tr>
</tbody>
</table>

5. Effect of vaccine on incidence of influenza-like symptoms

232 of 524 (44.3%) patients had an influenza-like illness during the epidemic period. 31 (39.7%) of the 78 vaccinees suffered an influenza-like illness compared to 201 (45.1%) of the 446 asthmatics who did not receive vaccine (Apparent efficacy of vaccine =11.8%). Vaccination did not lead to a significant reduction in the incidence of influenza-like illness (Chi-squared test comparing incidence of influenza-like illness in vaccinees and non vaccinees, 0.5<p <0.7).

6. Symptoms of influenza-like illness in vaccinated and unvaccinated groups

The symptoms of influenza-like illness in vaccinated and unvaccinated patients are shown in Fig 10.1. The majority experienced headache, muscle pains, sweats, sore throat, cough and phlegm. There were no significant differences (by Chi-squared comparison) in the frequency of symptoms experienced by the patients who were vaccinated and those who were not vaccinated.
Figure 10.1
Symptoms of influenza-like illness in vaccinated and unvaccinated patients
7. Exacerbations of asthma

The impact of influenza-like illness on the patients' underlying asthma is shown in Fig 10.2 which compares the complications experienced by vaccinated and unvaccinated patients. 167 (72%) of the 232 vaccinated and unvaccinated subjects with influenza-like illness became more wheezy during the attack of influenza and most of these patients increased their use of bronchodilators. 124 (53%) consulted their General Practitioner; of these 105 (85%) were prescribed antibiotics and 89 (72%) were told to increase their use of steroids. 68 (76%) of the 89 patients who were treated with steroids used inhaled preparations and 21 (24%) were given oral steroids. There were no significant differences (by Chi-squared comparison) in the incidence of each complication between vaccinees and non-vaccinees.
Figure 10.2
Complications of influenza-like illness in vaccinated and unvaccinated asthmatic patients

- Vaccinated n=31
- Unvaccinated n=201

- Worsening wheeze
- Increased bronchodilator use
- GP consulted
- Antibiotics prescribed
- Increased steroids required
8. GP Consultations and use of antibiotics

The number of General Practitioner consultations of asthmatic patients with influenza-like illness is shown in Table 10.2. 124 (53%) of the 232 symptomatic patients were seen by their GP, including 20 (65%) of the 31 symptomatic vaccinees and 104 (52%) of the 201 symptomatic non-vaccinees (Chi-squared, 0.3 < p < 0.5). Altogether 202 consultations were made by the 232 patients who had influenza-like illness (mean=0.87 consultations/symptomatic episode).

A total of 54 GP consultations were recorded in the group of 31 symptomatic vaccinees (mean=1.74 consultations generated by each symptomatic episode in vaccinees), compared to a total of 148 consultations recorded in the group of 201 symptomatic non-vaccinees (mean=0.87 consultations generated by each symptomatic episode in non-vaccinees). The difference in consultation rates between symptomatic vaccinees and non-vaccinees was highly significant (Chi-squared, 54/31 vs 148/201, p < 0.001). Both surgery appointments and home visits were significantly increased in vaccinees (Table 10.2).

Table 10.2.

General Practitioner consultations of asthmatic patients with influenza-like illness.

*Mean visits = (Total number of visits/Total number of episodes of influenza) for each group of patients.*

<table>
<thead>
<tr>
<th></th>
<th>Vaccinated (31)</th>
<th>Unvaccinated (201)</th>
<th>Total (232)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>Mean</td>
<td>No</td>
</tr>
<tr>
<td>Number consulting GP</td>
<td>20</td>
<td>104</td>
<td>124</td>
</tr>
<tr>
<td>Total number of visits</td>
<td>54  (1.74)</td>
<td>148 (0.73)</td>
<td>202 (0.87)</td>
</tr>
<tr>
<td>Surgery visits</td>
<td>43  (1.39)</td>
<td>121 (0.6)</td>
<td>164 (0.71)</td>
</tr>
<tr>
<td>Home visits</td>
<td>11  (0.35)</td>
<td>27 (0.13)</td>
<td>38 (0.16)</td>
</tr>
</tbody>
</table>
105 (45%) of 232 patients who developed influenza-like illness received antibiotics. There was no significant difference in the use of antibiotics in the vaccinated and unvaccinated groups with 15 (48%) of 31 symptomatic vaccinees receiving antibiotics compared to 90 (45%) of 201 symptomatic non-vaccinees. 98 patients used one course of antibiotics, 4 patients used 2 courses and there were single patients prescribed 3, 4 and 5 courses respectively. A total of 118 courses of antibiotics were therefore taken. Antibiotics prescribed included amoxycillin, cotrimoxazole, trimethoprim, erythromycin, oxytetracycline and augmentin.

9. Hospital admissions
Altogether 9 (4%) of 232 symptomatic patients were admitted to hospital. This included 2 (6.5%) of the 31 vaccinees and 7 (3.5%) of the 201 non-vaccinees (Chi-squared, 0.3<p<0.5). The reasons for hospital admission were uncontrolled asthma in 7 patients, influenza in 1 patient and pneumonia in a further patient.

10. Time taken off work or school
139 (60%) of 232 patients with influenza-like illness took time off work. Interestingly only 12 (39%) of 31 symptomatic vaccinees took time off work compared to 127 (63%) of 201 symptomatic non-vaccinees (Chi-squared, p<0.05). A total of 890 days off work or school were recorded for the 232 symptomatic episodes (mean absence=3.84 days/symptomatic episode); the 139 patients who took time off were therefore each absent for a mean period of 6.4 days.

93 days of absence occurred in the group of 31 symptomatic vaccinees (mean absence=3 days/episode). As only 12 vaccinees took time off they were each absent for a mean period of 7.75 days. 797 days of absence occurred in the group of 201 symptomatic non-vaccinees (mean absence=3.97 days/episode). The 127 non-vaccinees who took time off were each absent for a mean period of 6.3 days. Chi-squared test comparing days of absence in vaccinees and non-vaccinees, 93/31 vs 797/201, 0.2<p<0.3).
11. Influenza Haemagglutination inhibition (HI) titres

HI titres were determined against two strains of influenza virus:-

i) Influenza A/Shanghai/11/87 H3N2, corresponding to the virus contained in the 1989 vaccine and

ii) Influenza A/England/427/88 H3N2, which was representative of the influenza viruses causing the UK epidemic in 1989-90.

Both strains kindly provided by Dr M Chakraverty at the Central PHLS, Colindale.

A total of 124 serum samples were obtained, 28 from vaccinated patients (12 symptomatic and 16 asymptomatic) and 96 from unvaccinated patients (42 symptomatic and 54 asymptomatic). An antibody titre of 40 or greater was considered protective (Hobson et al, 1971; Nicholson et al, 1979; Stuart-Harris et al, 1985; Zuckerman et al, 1990). Protective influenza HI titres and reciprocal geometric mean HI antibody titres were compared for each group. The results are shown below in Table 10.3 and illustrated graphically in Figs 10.4 and 10.5. The actual titres for each patient are recorded in Table 10.4.

Comparison of titres in asymptomatic patients showed that all had reciprocal HI antibody titres $\geq 40$ against A/Shanghai/11/87 regardless of vaccination status, but the reciprocal geometric mean titres were higher in vaccinees than non-vaccinees (207 vs 132 respectively, $p<0.05$). A significantly higher proportion of asymptomatic vaccinees had reciprocal HI antibody titres $\geq 40$ against A/England/308/89 than asymptomatic non-vaccinees (15/16 vs 19/54 respectively, Chi-squared, $p<0.05$), and the reciprocal geometric mean titres were also significantly different (247 vs 17 respectively, $p<0.05$).

Among non-vaccinees 20 (47%) of 42 with influenza-like symptoms had reciprocal HI antibody titres $\geq 40$ to A/England/308/89 compared to 19 (35%) of 54 who remained asymptomatic (Chi-squared, $0.3<p<0.5$), and reciprocal geometric mean titres were similar in both groups (27 vs 17 respectively, $p>0.3$).
Table 10.3 Protective influenza HI antibody titres and reciprocal geometric mean titres in vaccinated and unvaccinated asthmatics with and without symptoms of influenza-like illness.

<table>
<thead>
<tr>
<th></th>
<th>Vaccinated patients (n=28)</th>
<th>Non-vaccinated patients (n=96)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asymptomatic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A/Eng/308/89</strong></td>
<td>Hl titre ≥ 40</td>
<td>15/16 (94%)</td>
</tr>
<tr>
<td></td>
<td>Reciprocal GMT</td>
<td>247</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19/54 (35%)</td>
</tr>
<tr>
<td><strong>A/Shang/11/87</strong></td>
<td>Hl titre ≥ 40</td>
<td>16/16 (100%)</td>
</tr>
<tr>
<td></td>
<td>Reciprocal GMT</td>
<td>207</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54/54 (100%)</td>
</tr>
<tr>
<td><strong>Influenza-like illness</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>A/Eng/308/89</strong></td>
<td>Hl titre ≥ 40</td>
<td>11/12 (92%)</td>
</tr>
<tr>
<td></td>
<td>Reciprocal GMT</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20/42 (48%)</td>
</tr>
<tr>
<td><strong>A/Shang/11/87</strong></td>
<td>Hl titre ≥ 40</td>
<td>12/12 (100%)</td>
</tr>
<tr>
<td></td>
<td>Reciprocal GMT</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td></td>
<td>41/42 (98%)</td>
</tr>
</tbody>
</table>
Figure 10.3
Protective influenza HI titres in vaccinated and unvaccinated asthmatic patients with and without symptoms of influenza-like illness.

% with HI titres ≥40

Influenza A/Shanghai/11/07 H3N2
Influenza A/England/427/98 H3N2

Asymptomatic

Influenza - like symptoms

Vaccinated (n=16)
Non-vaccinated (n=34)

Vaccinated (n=12)
Non-vaccinated (n=42)
Figure 10.4
Reciprocal geometric mean influenza HI titres in vaccinated and unvaccinated asthmatic patients with and without symptoms of influenza-like illness.
Table 10.4

Convalescent HI titres against influenza A/England/308/89 H3N2 (Eng) and A/Shanghai/11/87 H3N2 (Shang) in vaccinated and unvaccinated asthmatic patients with and without symptoms of influenza-like illness.

<table>
<thead>
<tr>
<th>Symptom Status</th>
<th>Eng</th>
<th>Shang</th>
<th>Eng</th>
<th>Shang</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated Asymptomatic</td>
<td>640 320</td>
<td>320 160</td>
<td>160 160</td>
<td>160 160</td>
</tr>
<tr>
<td>Not vaccinated</td>
<td>640 160</td>
<td>10 160</td>
<td>160 160</td>
<td>160 160</td>
</tr>
<tr>
<td>Vaccinated IL</td>
<td>320 320</td>
<td>- 160</td>
<td>320 320</td>
<td>5120 1280</td>
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12. Respiratory illness occurring during and outside the influenza epidemic period in previously healthy adults

In the autumn of 1989 a study was set up to investigate respiratory virus infections in previously healthy adult volunteers. The study had two principal aims:

1) To document the impact of respiratory virus infections in this group of previously healthy subjects.

2) To provide nasopharyngeal swabs and paired sera for comparison of conventional diagnosis techniques and later analysis using oligonucleotide probes and the polymerase chain reaction (PCR).

The study period lasted from September 1989-April 1990 and spanned the period of the national outbreak of influenza A infection. This allowed us to compare episodes of respiratory tract infection which occurred during or outside the period of the 1989-90 influenza epidemic.

Local Ethics Committee approval was granted for the study and specimens were obtained from students and staff at Leicester University who had symptoms of intercurrent respiratory virus infection but were otherwise healthy. Symptomatic volunteers were asked to report to our laboratory as soon as they developed symptoms of a cold. On their first visit personal details were recorded ie. sex, date of birth, other significant medical conditions, days since onset of symptoms, index case (if known) and point of contact.

Nasal and throat swabs were taken and placed in virus transport medium. Each specimen was then divided, part was stored at -70°C in medium containing human placental ribonuclease inhibitor, the remainder was usually inoculated directly onto tissue culture, although a few specimens were temporarily stored at -70°C during holiday periods when cells were not available. An acute blood sample was also taken from willing subjects. Volunteers were asked to complete a 10-day questionnaire identical to that used by index cases in the interferon study, which documented symptoms of upper respiratory infection and handkerchief usage. When symptoms
persisted beyond 10 days further questionnaires were completed until the subject recovered fully. After approximately 21 days the subject returned the completed questionnaire and a convalescent blood was collected. A small compensation in the way of a chocolate bar was offered to subjects who completed the study satisfactorily, however there was no financial inducement.

During the 1989-90 respiratory virus season (Sept 1989-April 1990) a total of 72 episodes of upper respiratory tract infections were reported to our laboratory. Twelve subjects did not return their forms and the outcome of these episodes could not be assessed. Completed forms were obtained from a total of 60 episodes of upper respiratory tract infection occurring in 57 separate subjects (3 subjects recorded 2 episodes of respiratory infection). One episode was excluded as it was considered to be a very doubtful cold; the remaining 59 episodes were fully analysed.

34 (58%) of the 59 episodes occurred in men and the mean age of the subjects was 28.3 years (sd 10.6, range 8-57 years). The mean time interval between onset of symptoms and collection of diagnostic specimens was 2.9 days (sd 2.2 days, range 0-10 days). The mean number of symptomatic days was 6.5 (sd 3.4, range 1-23 days) and the mean symptom score was 82.6 (sd 54.9, range 15-361).

Nasopharyngeal swabs and paired sera were taken for virology. The results of virus isolation and serology are summarised in Table 10.5. Results of PCR and oligonucleotide probe analysis are not yet available.
Table 10.5 Results of virus isolation and serology in previously healthy adults with upper respiratory tract infections (includes results from nasopharyngeal swabs taken from the 13 episodes which were subsequently excluded from outcome analysis).

<table>
<thead>
<tr>
<th>Agent</th>
<th>No of positive episodes</th>
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<tr>
<td>Rhinovirus</td>
<td>2</td>
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<tr>
<td>Echovirus</td>
<td>1</td>
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<tr>
<td>Adenovirus</td>
<td>2</td>
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<tr>
<td>Influenza A</td>
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<td>Influenza B</td>
<td>1</td>
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<td>RSV</td>
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<tr>
<td>Coronavirus 229E</td>
<td>1</td>
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<tr>
<td>Total (n=72)</td>
<td>9 (12.5%)</td>
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</table>

Swabs were taken from subjects during the acute phase of all 72 episodes (including the thirteen which were excluded from final analysis) and inoculated into tissue culture. There were five positives (6.9%), 2 adenoviruses, 2 rhinovirus and 1 echovirus. Paired acute and convalescent serum samples were obtained from 32 episodes (54.2%). Sera were titrated for complement fixing antibodies to influenza A, influenza B, adenovirus, RSV and Mycoplasma pneumoniae and coronavirus 229E and OC43 ELISA tests were performed. The results showed a significant antibody rise in 4 subjects (12.5%), one with influenza A, one with influenza B, one with RSV and one with coronavirus 229E. One episode of rhinovirus infection occurred in a subject who did not return the symptom forms. A diagnosis of respiratory virus infection was therefore confirmed in 8 (13.6%) of the 59 episodes which were fully analysed.
The symptoms and severity of the 20 (40%) episodes beginning during the epidemic period (defined as the period between November 19th 1989 and February 9th 1990; Curwen et al, 1990; PHLS, 1990) were compared with those documented in the 39 (60%) episodes occurring either before or after this period (Fig 10.5). The mean number of symptom days for episodes occurring during the influenza epidemic was 7.5 (sd 1.8, range 4-10) compared to 6.1 (sd 3.9, range 1-23) for other episodes (unpaired t-test, 0.05<p<0.1). There was no significant difference between the mean symptom score associated with episodes occurring during the influenza epidemic (mean score 85.3, sd 31.6, range 37-174) and episodes occurring outside the epidemic period (mean score 81.3, sd 64.0, range 15-361), (unpaired t-test, 0.375<p<0.4). There were similarly no significant differences in the symptoms associated with episodes occurring during or outside the epidemic period (Fig 10.6). The low frequency (25%) of muscle aches in episodes during the influenza season was particularly surprising as myalgia is often considered to be a characteristic feature of influenza.

Figure 10.7 compares the severity of respiratory virus infections in these 59 previously healthy subjects with the severity of infections occurring in the 16 children with cystic fibrosis (chapter 8) and the 25 symptomatic placebo-using patients with chronic chest disease who participated in the interferon trial (chapter 7). Symptomatic episodes occurring in the placebo-recipients with chronic chest disease (mean symptom days=10.6; mean symptom score=100.6) were significantly more severe than episodes occurring in the previously healthy volunteers (mean symptom days=6.5, unpaired t-test p<0.01; mean symptom score=82.6, unpaired t-test, p<0.005). Episodes recorded in children with cystic fibrosis lasted a mean period of 10.9 days (unpaired t-test compared with previously healthy volunteers, p<0.05), but the mean symptom score was only 45.9 showing that respiratory virus infections in these children gives rise to a prolonged but relatively mild illness.
Figure 10.8 compares the virus diagnostic rate in these three studies. Viruses were isolated from just 6.9% of nasopharyngeal swabs taken in the interferon trial and were isolated from 6.9% of samples taken from the previously healthy volunteers. Viruses were isolated from 14.7% of nasopharyngeal swabs taken from symptomatic children with cystic fibrosis (Chi-squared test, comparing virus isolation rates in cystic fibrosis children, previously healthy volunteers and patients with chest disease $0.3<p<0.5$). Serology was rather more helpful and was positive in 23% of paired sera taken in the interferon trial and 12.5% of paired samples from previously healthy subjects. Serology was not performed in the cystic fibrosis study. Overall, respiratory viruses were implicated in 39% of episodes in the interferon trial, 13.6% of episodes of upper respiratory tract infection in previously healthy subjects and 14.7% of symptomatic episodes in children with cystic fibrosis. The diagnostic rate in the interferon trial was higher because specimens were taken from the index case and secondary cases in addition to the trial subject.
Figure 10.5
Comparative severity of episodes of respiratory illness occurring in previously healthy adults during the 1989/90 influenza epidemic (n=20) and outside the epidemic period (n=39)
Figure 10.6
Comparison of symptoms of respiratory illness occurring in previously healthy adults during the 1989/90 influenza epidemic (n=20) and outside the epidemic period (n=39)
Figure 10.7
Comparative severity of respiratory virus infections in previously healthy subjects (n=59), patients with chronic chest disease (n=25) and children with cystic fibrosis (n=16)
Figure 10.8
Respiratory virus diagnostic rate in episodes of acute respiratory illness occurring in previously healthy subjects (n=59), patients with chronic chest disease (n=25) and children with cystic fibrosis (n=16).

(serology was not performed in the cystic fibrosis study)
13. Discussion of study of influenza-like illness in asthmatic patients and previously healthy adults 1989-90

The asthma study documented the use and apparent efficacy of influenza vaccine in a high-risk group of patients with asthma and the overall impact of influenza-like illness during the period of the national influenza A outbreak. The 1989/90 epidemic was the largest recorded since 1976 and was responsible for an estimated 20,000-30,000 excess deaths, mainly in those aged over 65 (Curwen et al, 1990; PHLS, 1990). 2798 cases of confirmed influenza A virus infection were reported to the Public Health Service Communicable Disease Surveillance Centre between weeks 44 of 1989 and 25 of 1990 (PHLS, 1990). Reporting began to rise at the end of November 1989, reached a peak in the second week of December and declined rapidly thereafter to reach normal seasonal levels by the beginning of February. All 796 isolates of influenza A received by the PHLS Virus Reference Laboratory were subtype H3N2, and most were similar to the A/England/427/88 variant seen in the 1988/89 influenza season (PHLS, 1990).

Studies conducted in young healthy adults showed a good match between the A/Shanghai/11/87 subtype represented in the 1989 vaccine and a representative epidemic virus, A/England/308/89; with 99% of vaccinees acquiring protective antibodies to the epidemic strain (Zuckerman et al, 1990). Vaccination should therefore have conferred a high degree of protection. In the asthmatic patients all 16 asymptomatic vaccinees who provided convalescent serum samples had protective antibody titres against A/Shanghai/11/87 and 15/16 also had protective titres against A/England/308/89. In addition, asymptomatic vaccinees had significantly higher geometric mean antibody titres against both strains of influenza, compared to asymptomatic non-vaccinees, confirming a close match between the vaccine and epidemic strains.

Although influenza vaccination has been recommended for patients with chronic chest disease the results of this retrospective study showed that less than 15% of asthmatic patients in Leicester had received influenza vaccine and only 47 (9%) of
the 524 respondents had been vaccinated prior to the onset of the epidemic. The vaccination rate was low but was comparable to that found in other local studies of high risk groups, including patients with serious heart disease (Kurinczuk and Nicholson, 1989) and the institutionalised elderly (Nicholson et al, 1990). The vaccination rate was also comparable to the results of vaccination in the elderly as revealed in the survey of GP's in Trent Region.

Altogether 45% of the respondents reported symptoms of influenza-like illness, but it is acknowledged that this high infection rate may reflect a bias since patients who had been symptomatic were more likely to have replied than those who remained well. Nevertheless, population-based studies in Houston, USA, showed that the annual infection rate for influenza ranges from around 20% to nearly 50%, with an average of nearly 33 infections per 100-person years (Glezen et al, 1986), and comparable results were noted in a 5-year study of influenza in families in the UK (Mann et al, 1981; Glezen et al, 1982). As local perceptions were of a severe epidemic resulting in closure of some schools and cancellation of routine hospital admissions at the peak of the outbreak, it did not seem surprising that the attack rate in these asthmatic patients was so high.

Vaccination did not reduce the overall incidence or severity of the symptoms of influenza-like illness and without serological data it could have been concluded that vaccination was ineffective. Other investigators have estimated the efficacy of influenza vaccine solely by comparing the attack rates of influenza-like illness in vaccinated and non-vaccinated groups, and have assumed that virtually all influenza-like illness during epidemics is actually due to influenza (Barker and Mullooly, 1980; Hayden et al, 1980b; Van Voris et al, 1981; Dolin et al, 1982; Patriarca et al, 1985; Barker and Mullooly, 1986; Fleming and Ayres, 1988; Paul et al, 1988). Similar assumptions were also made by the Committee on Issues and Priorities for New Vaccine Development in the United States (Institute of Medicine, 1985) when they evaluated the economic importance of influenza.
The study in asthmatics had several limitations as we did not perform virus isolations during the period of symptoms and we were not able to obtain both acute and convalescent sera. However, the failure to demonstrate any significant differences between the antibody profiles of non-vaccinees with and without symptoms of influenza-like illness suggests that symptomatic illness is a poor guide to attack rates. An alternative explanation is that wild-type virus generates a poor or short lasting antibody response, but this is most unlikely as antibody responses to both live and inactivated virus vaccines have been shown to persist at high titre for months (Ikic et al., 1977; Noble et al., 1977; Foy et al., 1981; Couch and Kasel, 1983). Therefore the most plausible reason for the apparent vaccine failure was that many of the symptomatic episodes were due to viruses other than influenza A. These findings are important because they question the validity of the studies which have investigated the apparent efficacy of influenza vaccine or treatment without virological confirmation of infection.

Although the incidence of complications was similar in vaccinated and unvaccinated groups there were differences in the rate of GP consultations and absence from work. The mean number of GP consultations for episodes of influenza-like illness occurring in vaccinees was significantly greater than the mean number of consultations for episodes of illness occurring in non-vaccinees, however the severity of illness and the use of antibiotics and steroids was similar in both groups. Paradoxically, significantly fewer vaccinees took time off than non-vaccinees. Possibly this reflects the motivation of patients who receive regular vaccination and the attitude of GP's who recommend its use.

This study again highlights the importance of acute respiratory viral disease in patients with chronic respiratory disease. Overall 72% of symptomatic patients became more wheezy, 53% consulted their GP, 45% required antibiotics and 4% were hospitalised. A better understanding of the role of viruses in asthma is clearly required as this should ultimately provide improved methods of prevention and treatment.
The study of normal healthy volunteers with acute upper respiratory tract infections during 1989/90 provided a comparison of the symptoms and severity of episodes which occurred during the influenza epidemic with those that occurred before or after the epidemic period. Virtually all the subjects had nasal symptoms, usually accompanied by headache, cough and sore throat and the subjects who presented during the period of the influenza epidemic had an illness indistinguishable from the subjects who presented at other times. Only one of the subjects had a demonstrable rise in complement fixing antibody titre to influenza A virus and both adenovirus and coronavirus infections were diagnosed during the period of the national influenza outbreak. Although the CFT test is relatively insensitive and may have missed some cases of influenza, a possible explanation is that many of the symptomatic subjects did not actually have influenza, thus reinforcing the conclusions of the study in asthmatics and suggesting that other respiratory viruses were prevalent in the community at the same time as influenza A. The presence of several circulating viruses during an influenza epidemic has been documented previously (Mathur et al, 1980; Morales, 1983; Falsey et al, 1990; Nicholson et al, 1990). The findings of our studies in asthmatics and healthy volunteers might explain why many doctors and patients are sceptical of the efficacy of vaccination and question the assumptions made in past estimates of the economic impact of influenza and the value of influenza vaccines.
D. CONCLUSIONS
CHAPTER 11
CONCLUSIONS

The studies described in this thesis documented the impact of respiratory virus infection in patients with chronic chest disease and investigated the possibilities for preventing or ameliorating these infections by the use of prophylactic intranasal interferon or influenza vaccine.

The interferon study showed that patients with chronic chest disease may suffer serious complications after exposure to respiratory viruses. In this study 16 (64%) of the 25 symptomatic patients who took placebo reported a worsening of their chest condition and 25% of those with chest symptoms required hospital admission. A clear association between respiratory virus infection and exacerbations of chest disease was demonstrated in the study of asthmatic patients during the influenza epidemic of 1989/90, with 70% of those who developed influenza-like symptoms reporting a deterioration of their asthma.

We found that episodes of respiratory illness occurring in patients with chronic chest disease were significantly more severe than episodes of illness occurring in their corresponding index cases, or in previously healthy adults attending the University. This confirmed the relatively severe impact of respiratory virus infection in adults with chronic chest disease. In contrast, respiratory virus infection in children with cystic fibrosis appeared to give a prolonged but relatively mild illness.

Unfortunately prophylactic intranasal interferon-alpha did not reduce the number or severity of symptomatic episodes in patients with chronic chest disease. Prophylactic interferon has only been shown to be effective against naturally occurring rhinovirus infections and must be given as soon as possible after exposure. The expense, lack of efficacy against other viruses and frequency of side-effects with prolonged use, means that intranasal interferon is unlikely to find a role in the prophylaxis or treatment of respiratory virus infections.
Although effective chemotherapy against respiratory viruses is not currently available influenza vaccines have been in use for over 30 years. Current vaccines have few adverse effects and annual vaccination is strongly recommended for high-risk patients including the elderly and those with chronic medical conditions. However, many patients and medical personnel are sceptical of the efficacy of influenza vaccination. Our studies showed that fewer than 10% of asthmatic patients had been vaccinated prior to the 1989/90 influenza epidemic and General Practitioners in Trent Region were vaccinating less than a fifth of susceptible elderly patients. The Trent GP study found that the vaccination rates and policies varied considerably between different practices. Several factors were associated with an improved rate of vaccination including having an agreed practice policy, identifying high-risk patients, sending out reminders and organising special vaccination clinics. These observations have helped to identify strategies for GP's to increase their vaccination rates, but suggest that patients and GP's need to be made more aware of the potential benefits of vaccination.

The study of asthmatics during the 1989/90 influenza epidemic showed that vaccination had little effect on the incidence or severity of influenza-like symptoms, however, comparison of convalescent HI titres suggested that many of the patients did not have influenza and were probably infected by other respiratory viruses. The results from previously healthy adults who developed symptoms of upper respiratory tract infection during 1989/90, similarly suggested that many of the symptomatic episodes which occurred during the period of the national influenza epidemic were probably caused by other respiratory viruses. These studies demonstrate the crucial need to establish a diagnosis of respiratory virus infection in clinical studies of vaccine or antiviral agents.

The diagnosis of respiratory tract infections represented a formidable problem in these studies with virus isolation rates for nasopharyngeal swabs varying between 6.9% and 14.7%, despite the use of 5 susceptible cell lines. Between 12.5% and 23% of paired serum samples showed a significant antibody rise indicating recent
respiratory virus infection. The relatively low diagnostic rates probably resulted from a combination of factors, including late presentation of some patients, inadequate collection or storage of specimens and insensitive diagnostic techniques. The use of nasopharyngeal washes rather than swabs might have increased the yield of viruses and freezing of specimens will have inactivated fragile enveloped viruses (eg. respiratory syncytial virus). Ideally specimens should have been inoculated directly onto tissue culture immediately after collection, however this was not always possible as many specimens were obtained away from the laboratory. Recent advances in molecular biology have led to the development of newer techniques for diagnosis of respiratory virus infections, including the use of gene probes and the polymerase chain reaction (PCR). These techniques offer several potential benefits over existing diagnostic methods with increased speed and sensitivity and the ability to process many fresh or stored specimens simultaneously. We have begun to evaluate these techniques in our laboratory. Future improvements in diagnosis will undoubtedly lead to a greater understanding of the impact and pathogenesis of these important infections.
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Appendix 1.

Peak flow recording chart used by interferon trial patients

(Actual chart was printed on green card)

Patient Name ........................................... Patient Identification Number[ / / / / ]

Peak Flow Results Throughout Study Period : Date of start  [ ] [ ] [ ] [ ]

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Appendix 2

Symptom chart used by interferon trial patients

(Actual chart was printed on pink card)

**TREATMENT EPISODE** [ 1 ]

**PEAK FLOW MEASUREMENTS**

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**DIARY OF SYMPTOMS**

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<tr>
<td>Temperature (at night, oral)</td>
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<td>37.3-37.5 = 1</td>
<td>37.6-38.0 = 2</td>
<td>38.1-38.5 = 3</td>
<td>over 38.6 = 4</td>
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<tr>
<td>Handkerchiefs</td>
<td>0-3 = 0</td>
<td>4-8 = 1</td>
<td>9-16 = 2</td>
<td>over 16 = 3</td>
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<td>Red or watery eyes*</td>
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* = severity scale: 0=None; 1=slight; 2=moderate; 3=severe

**CHEST SYMPTOMS**

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<td>Normal volume/purulent = 1</td>
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<td>Over 4xnormal volume/purulent = 3</td>
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<td>GP called 1=yes; 2=no</td>
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Appendix 3

Symptom chart used by index cases and secondary cases in the interferon trial

(Actual chart was printed on blue card)

**TREATMENT EPISODE**

**INDEX CASE / SECONDARY CASES**

**Trial identification number:**

**Date and time of onset of symptoms**

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<td>37.6-38.0 = 2</td>
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<td>&gt; 38.6 = 4</td>
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<tr>
<td>Handkerchiefs</td>
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<td>4-8 = 1</td>
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<td>&gt; 16 = 3</td>
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<td>Malaise*</td>
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<td>Muscle aches*</td>
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<td>Chills*</td>
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<td>Cough*</td>
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<td>Painful swollen neck glands*</td>
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<td>Other symptoms (specify)*</td>
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Nasopharyngeal swab taken?  
No. .................  
[ ] [ ] [ ] [ ] : Time ..........  
D M Y

Acute phase blood taken?  
No. .................  
[ ] [ ] [ ] [ ] : Time ..........  
D M Y