

Rhinovirus Infection in Acute Exacerbations of Chronic Bronchitis : A Controlled Prospective Study*

A. C. STENHOUSE,† B.MED.SC., M.B., M.R.C.P., M.R.A.C.P.

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Natural rhinovirus infection in adults is usually associated with an afebrile coryzal syndrome (Hobson and Schild, 1960; Hamre and Procknow, 1961; Reilly *et al.*, 1962; Forsyth *et al.*, 1963), and, depending on the population studied, rhinoviruses as a group are associated with 8 to 25% of minor upper respiratory illnesses in adults and from 5 to 20% of such illnesses in children (Tyrrell and Bynoe, 1961; Johnson *et al.*, 1962; Kendall *et al.*, 1962; Bloom *et al.*, 1963; Hamparian *et al.*, 1964). Forsyth *et al.* (1963) found in Servicemen from whom rhinoviruses were isolated that 50% experienced chest pain and 86% reported a cough. Taylor-Robinson *et al.* (1963) and Hamparian *et al.* (1964) each reported the isolation of a rhinovirus from an adult with bronchitis. In the collaborative study on the aetiology of acute respiratory infections in Great Britain from 1961 to 1964, 179 patients were reported as having bronchitis. No indication was given regarding the age of the subjects, but 23% were associated with infective agents and in four instances rhinoviruses were isolated (Medical Research Council, 1965b). In a direct study of 15 chronic bronchitis patients over a period of two and a half years, Eadie *et al.* (1966) studied 75 acute respiratory illnesses and reported the isolation of 12 rhinoviruses. Eleven rhinoviruses were isolated from 47 illnesses affecting the chest, whereas only one rhinovirus was isolated from 28 illnesses not affecting the chest. The only other study of acute exacerbations of bronchitis which employed techniques for rhinovirus isolation failed to detect these agents (Carilli *et al.*, 1964).

The purpose of this paper is to report the findings of a controlled prospective study designed to determine the significance of rhinovirus infection on the aetiology of acute exacerbations in patients with chronic bronchitis and to describe the clinical picture associated with such infections.

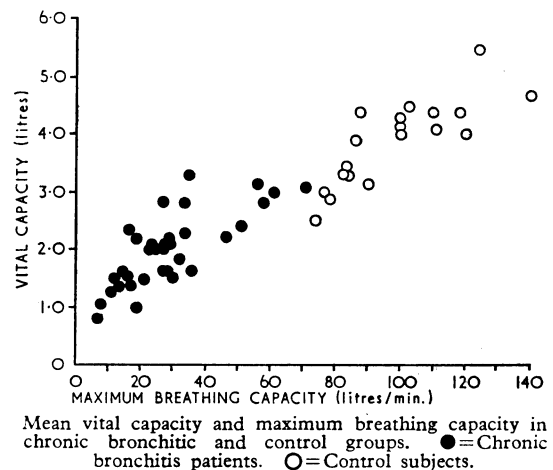
Materials and Methods

Clinical

Thirty-four patients attending the bronchitis clinic, the Royal Hospital, Sheffield, and 19 control subjects enrolled from the staffs of the Sheffield Fire Board and Lodge Moor Hospital, Sheffield, were studied between 18 August 1964 and 1 May 1965. Each patient and subject completed the short questionnaire on respiratory symptoms (*Brit. med. J.*, 1960) approved by the Medical Research Council Committee on the Aetiology of Chronic Bronchitis. The chronic bronchitis patients fulfilled the criteria for chronic bronchitis as defined by the American Thoracic Society (1962) and Medical Research Council (1965a), and they were clearly able to distinguish between quiescent and exacerbation periods. They comprised 31 men and three women, with a mean age of 61 (range 51 to 73) years. On the basis of the subclassification suggested by the Medical Research

Council (1965a) one patient was classified under simple chronic bronchitis, and of the 33 who exhibited airways obstruction, 5 produced mucopurulent sputum and 28 mucoid sputum. The control subjects, comprising 2 women and 17 men with a mean age of 51 (range 32 to 60) years, were shown on the basis of questionnaire findings and ventilatory function studies to be free of chronic bronchitis or other respiratory disease.

Subjects of both groups were seen at monthly intervals and in addition were asked to report as soon as possible after the onset of an acute infection of the upper or lower respiratory tract. On each occasion that a subject was seen a clinical form was completed. The presence of any upper or lower respiratory tract symptoms during the previous month and the state of the subject at the time of completion of the form were noted. In the chronic bronchitis patients the nature and amount of sputum produced, together with the occurrence of wheeze and shortness of breath, were recorded invariably, and at hospital visits the maximum breathing capacity and the vital capacity were measured by a Poulton Barry SP126 (McKerrow *et al.*, 1960). The mean vital capacity and maximum breathing capacity in the chronic bronchitis patients and control subjects are shown in the Chart.



The term "acute exacerbation of chronic bronchitis" embraces a spectrum of clinical disorders and was defined in this study as "an alteration in the quiescent state of the patient with an increase in either the quantity or purulence of the sputum."

Virological

On each occasion that subjects were seen a throat swab was obtained and a nasal washing with phosphate-buffered saline made. The throat swabs and nasal washings were placed in a transport medium containing 10% bovine serum albumin and kept at 4° C. Specimens were either inoculated into tissue-culture tubes within three hours of collection or frozen at -70° C. and stored until tested. Sputum specimens obtained from bronchitic subjects were placed at 4° C. for three hours

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† Wellcome Research Fellow, University Department of Medicine, the Royal Hospital, Sheffield, and the University of Sheffield, Virus Research Laboratory, Lodge Moor Hospital, Sheffield. Present address: Wellcome Virus Research Laboratory, Colonial War Memorial Hospital, Suva, Fiji.

and rendered non-toxic for tissue culture by emulsification in transport medium. They were then centrifuged at 2,000 r.p.m. for 30 minutes and the supernatant fluids stored at -70°C . Blood was obtained by venepuncture, and after separation the serum was stored at -20°C .

Secondary rhesus monkey kidney, human embryo kidney, human diploid (WI-38 and HEL 7 strains), and Bristol HeLa cells were used during this investigation. With the use of standard isolation procedures the HeLa tissue cultures were shown to contain mycoplasma agents. The laboratory procedures for tissue culture and virus isolation studies paralleled the methods described by the Medical Research Council (1965b).

Rhinoviruses isolated at 33°C . were recognized by their inability to grow at 37°C . in an alkaline medium, and lack of haemadsorption with human group O cells. Subsequently an acid stability test at pH 3 was performed (Dimmock and Tyrrell, 1962), and when calculated by the method of Reed and Muench (1938) a hundredfold or greater reduction in titre was taken as evidence of acid lability (Tyrrell and Chanock, 1963). If the rhinovirus was isolated in, or could be propagated in, rhesus monkey kidney cells it was designated as an M strain; otherwise it was classified as an H strain of rhinovirus. Isolates suspected of being herpes simplex virus were identified by neutralization with type-specific antiserum. Homologous rhinovirus neutralization was performed with serial fourfold dilutions of acute and convalescent serum inactivated at 56°C . for 30 minutes, starting at 1 in 8. Serum dilutions were incubated for one hour at room temperature with 50 to 100 50% tissue-culture infective doses (TCID₅₀) of homologous virus. Two WI-38 tubes were inoculated with 0.2 ml. of virus-serum mixture and were rolled at 33°C . Complete suppression of cytopathic effect after 10 days' incubation was used to determine the end-points.

Results

Virus Isolations

A total of 299 specimens from the chronic bronchitis patients and 109 specimens from the controls were obtained during the course of the study. Each specimen consisted of a nasal washing and throat swab; a sputum sample was also examined from the bronchitis patients. From the chronic bronchitis patients 237 specimens were taken during periods of quiescence or at more than 10 days after the onset of the acute respiratory symptoms. From the control subjects 88 specimens were examined when they were well. Of the 62 specimens obtained from the chronic bronchitis patients 10 days or less after the onset of an acute respiratory infection 56 had an acute exacerbation of bronchitis, and 38 of these were associated with upper respiratory symptoms. The 21 specimens from the control subjects were collected within six days of the onset of an acute upper respiratory illness.

Nineteen viruses were isolated during the course of the study, and 18 of these were rhinoviruses and one was a strain of herpes simplex isolated in human embryo kidney cells from the sputum of a patient 14 days after the onset of an acute exacerbation of bronchitis. No agents were isolated by means of the haemadsorption technique.

Table I shows the number of rhinoviruses isolated in relation to the clinical state of the chronic bronchitic and control subjects at the time of isolation. During quiescence five rhinoviruses were isolated from the patients and one from the control subjects in the absence of clinical illness, giving isolation rates of 2.1 and 1.1% for the patients and control subjects respectively. During acute exacerbations eight rhinoviruses were isolated from the patients and four were isolated from the control subjects at the time of an acute respiratory infection, giving an isolation rate of 14.3% for bronchitic subjects and

19% for control subjects. No viruses were isolated from the throat swabs or nasal washings collected more than six days after the onset of an acute respiratory infection except those unrelated to acute symptoms. The two rhinovirus strains isolated from the sputum were not recovered from nasal washings or throat swabs taken at the same time, and one of these was isolated 10 days after the onset of acute respiratory symptoms. Of 18 rhinovirus strains 13 were reisolated after storage for 4 to 12 months at -70°C .

TABLE I.—Number of Rhinoviruses Isolated in Chronic Bronchitic and Control Subjects in Relation to the Clinical State

Subjects	No. of Specimens Tested	No. of Rhinoviruses Isolated	Percentage Isolated
Chronic bronchitic:			
Quiescent	237	5	2.1
Acute exacerbations of bronchitis ..	56	8	14.3
" upper respiratory infections	6	0	—
Control:			
No illness ..	88	1	1.1
Acute respiratory infection ..	21	4	19.0

Neutralizing Antibody Titres

Table II summarizes the results of homologous neutralization tests carried out on six paired sera from subjects whose throat swabs or nasal washings yielded a rhinovirus. One of the patients from whom a rhinovirus was both isolated and reisolated in the quiescent state showed a high level of neutralizing antibody in the acute serum.

TABLE II.—Homologous Neutralization Titres* in Six of the Subjects Yielding a Rhinovirus

Neutralizing Antibody Titres		TCID ₅₀ in Test
Acute	Convalescent	
< 8	32	50
< 8	16	100
< 8	32	50
< 8	8	100
< 8	16	100
64	64	50

* Reciprocal values of titre given.

Clinical Picture

All the eight chronic bronchitic patients from whom a rhinovirus was isolated during an acute respiratory illness developed an acute exacerbation of bronchitis. This was characterized by increased production of sputum—purulent in six of the eight patients—increased wheeziness, shortness of breath, and cough. Five experienced substernal chest pain typical of tracheitis and six had mild rhinorrhoea. The control subjects, on the other hand, presented with an afebrile or febrile rhinorrhoea in association with a rhinovirus infection.

A clinical description of two of the acute illnesses associated with recovery of a rhinovirus from the chronic bronchitis patients is given.

Case 1.—A 62-year-old man experienced increased wheezing and coughing on 28 December 1964. Next day he developed rhinorrhoea, a mild sore throat, and substernal chest pain. His sputum increased in volume without becoming purulent, and he was more short of breath. A diagnosis of a common cold with acute tracheo-bronchitis was made. An H rhinovirus was isolated from his sputum obtained on 30 December. No virus was found in the throat swab or nasal washings taken at the same time. His symptoms gradually improved over the following week.

Case 2.—On 8 November 1964 a 50-year-old man developed malaise; he became more wheezy, and his sputum increased in volume and was purulent. When examined six days later he had improved slightly and there were no symptoms or signs of an upper respiratory infection. A diagnosis of acute exacerbation of bronchitis was made, and an M rhinovirus was isolated from a throat swab taken on 14 November 1964.

Discussion

Before rhinoviruses can be claimed to be a cause of acute exacerbations of bronchitis a modernized version of Koch's postulates must be fulfilled (Tyrrell, 1963).

Firstly, the respiratory secretions of a comparable group of patients and healthy subjects must be tested under similar conditions for the rhinoviruses. A comparison of the isolation rates of rhinoviruses of the chronic bronchitis patients and the control subjects during acute respiratory infections indicates that both groups are infected to a similar extent. The rhinovirus isolation rate of 2% for the chronic bronchitis patients in the quiescent phase and of 1% for the control subjects in the absence of clinical symptoms is in accord with previously published results from asymptomatic subjects (Hobson and Schild, 1960; Hamre and Procknow, 1961; Hamparian *et al.*, 1964). This indicates, firstly, that in both the chronic bronchitic and control groups the rhinoviruses are probably responsible for the disease produced, and, secondly, that the respiratory secretions of the chronic bronchitic subjects do not harbour rhinoviruses to any greater extent than normal subjects.

The results of the clinical studies show that in the bronchitic subjects rhinovirus infections result in acute exacerbations of bronchitis, while normal persons present a febrile or afebrile rhinorrhoea. These findings are in accord with the observations of Eadie *et al.* (1966), who reported that rhinovirus infections were more often associated with respiratory illnesses which affected the chest than with those which did not. These observations are supported by the findings of Cate *et al.* (1965), who, using a Collison atomizer, which gives a small-particle aerosol, showed experimentally that the lower respiratory tract of adults is quite sensitive to rhinovirus infection by producing, in volunteers, a tracheobronchitis with a low dose (16 to 11 TCID₅₀) of a rhinovirus.

The lower isolation rate of rhinovirus from the chronic bronchitis patients during acute exacerbations as compared with the findings of Eadie *et al.* (1966) is likely to be due to the earlier reporting of illness and the more frequent visitation of their patients. Of the 62 acute respiratory illnesses in the present study only six showed no evidence of involvement of the lower respiratory tract, as compared with 25 out of 75 reported by Eadie *et al.* (1966). This may be a reflection of a more advanced stage of the disease of the patients in the present study, but it is difficult to compare directly the ventilating function studies in the two groups. The report by Eadie *et al.* (1966) of the isolation of five rhinoviruses from the sputum, three of which were not recovered from a throat swab taken on the same occasion, is confirmed by the present study, where two rhinoviruses were isolated from the sputum but not from the throat swab or nasal washing, and indicates that attempts at sputum isolation are worth while.

The second modified Koch's postulate which it is desirable to fulfil is the demonstration of a rise in the titre of the homologous rhinovirus antibody during convalescence, indicating that infection was active at the time at which symptoms occurred and the virus isolation was made. In this study five out of six specimens tested showed a rise in titre of the homologous antibody in the convalescent specimen. The single exception occurred in a patient in the quiescent phase from whom a rhinovirus was isolated and subsequently reisolated from the throat swab. In this instance both the initial serum specimen and one taken four weeks later neutralized the homologous virus to a titre of 1 in 64. The possibilities are that this specimen was contaminated at the time of collection, that a sub-clinical reinfection occurred, or that the patient continued to

excrete the virus for some time after infection—a situation which at present is only speculative.

These results indicate that in normal subjects rhinovirus infection gives rise to an afebrile or febrile coryzal syndrome while in chronic bronchitic patients an acute exacerbation of bronchitis is produced. It is likely that a more complete understanding of the nature of the agents responsible for the initiation of acute exacerbations will parallel the application of advances in techniques for the isolation of viruses from acute respiratory infections as a whole.

Summary

In a controlled prospective study employing virus isolation and homologous neutralizing antibody levels an association between rhinovirus infection and acute exacerbations of bronchitis in chronic bronchitic patients is described. Eight of 56 (14%) of such illnesses yielded rhinoviruses. The isolation of five rhinoviruses from 237 specimens (2.1%) from the same patients in the quiescent phase is in accord with previously published figures on the isolation rate of rhinoviruses from normal asymptomatic subjects.

The chronic bronchitis patients during rhinovirus infection present clinically with an acute exacerbation with or without an associated upper respiratory illness. This pattern of clinical illness is contrasted with a control group who presented a febrile or afebrile rhinorrhoea.

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