Papers and Originals

Pilot Study of Factors Associated with Exacerbations in Chronic Bronchitis

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Summary: The factors associated with exacerbations of chronic bronchitis were investigated in a pilot study of 23 male patients over a period of 29 months. There were 63 exacerbations—56 were fully investigated and in seven only serological studies were possible. Evidence of the presence of an infective agent was sought in the early stages of the exacerbations and during relative quiescence. Significantly more potential pathogens, both bacterial (pneumococci and *Haemophilus influenzae*) and viral and mycoplasmal, were found during exacerbations than in quiescent periods. It was not possible to demonstrate that atmospheric pollution or low temperature influenced exacerbations, but the data are insufficient to exclude a small effect.

It is emphasized that the value of future work on these lines would be increased if agreement could be reached on the definition of an exacerbation.

Introduction

Though the place of bacteria, viruses, mycoplasmas, and atmospheric conditions in the aetiology of exacerbations of chronic bronchitis has been investigated with varying degrees of intensity in individual studies, we know of none in which these factors have been assessed in the same patients at the same time. The present pilot study was designed to determine whether this was feasible and to obtain preliminary results.

As a universally accepted definition of an exacerbation has never been proposed, a comparison of the findings of different workers is difficult. Some workers have not defined an exacerbation at all. We have therefore had to devise the definition which has been used in this investigation. Bronchitic patients admitted to hospital are rarely in the early stages of an exacerbation. The patients were therefore seen at home as soon as possible after a change in their respiratory symptoms. In addition they are seen at regular routine clinics. In this way they were considered to act as their own controls. Specimens were taken for the isolation of bacteria, viruses, and mycoplasmas, and for viral and mycoplasmal serology, both at home and at clinic attendances.

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Patients Studied

The study lasted from November 1965 to March 1968. The patients were 23 male chronic bronchitics under the age of 70 years living in Edinburgh. They all had "chronic or recurrent mucopurulent obstructive bronchitis," as defined by the Medical Research Council (1965). In order to facilitate the recognition of exacerbations, patients with a marked and variable wheeze were excluded. Thirteen of the patients had participated in the Medical Research Council (1966) early bronchitis trial, and the remaining 10 were recruited from hospital inpatients or outpatients. The ages of the 23 patients at the beginning of the observation period were: 44-49 years, 2; 50-54 years, 5; 55-59 years, 6; 60-64 years, 6; 65-67 years, 4.

Nine of the patients were at work throughout the observation period, seven did not work, and seven worked for part of the time. At the beginning of the observation period the other members of the household consisted of adults only in 18 instances and of adults plus children in five instances. Fourteen patients were observed for 29 months, four for 12 to 14 months, and five for 1 to 7 months. The total patient-months of observation was 485 (a "month" is defined as a whole or over half a calendar month).

Four patients were withdrawn during the period of study. Two of these died after one and four months of observation respectively, one was withdrawn after six months' observation because he failed to co-operate, and one after seven months' observation because he was too ill.

General Procedure

Specimens Taken at Routine Examinations and at Time of an Exacerbation.—These were (1) sputum for the estimation of purulence and for the isolation of bacteria, mycoplasmas, and viruses; (2) pharyngeal swabs (*Exogen* brand) for the same isolations; (3) nasal washings for viral isolations; and (4) blood for mycoplasmal and viral serology. After an exacerbation a second or "convalescent" specimen of blood was taken two to four weeks from the onset of the illness.

Routine Examination of Patients.—Patients were reviewed by a chest physician (A. J. A.) every two months. Some attended a morning clinic and the remainder attended a Friday evening clinic. At the morning clinic the patients brought an early morning specimen of sputum with them. All specimens were delivered to the laboratory at the end of the clinic. No special storage arrangements were necessary, except that the virology specimens were kept in a cold vacuum flask. After the evening clinic the swabs, nasal washings, and blood were stored overnight. Pharyngeal swabs for bacterial and mycoplasmal isolations were placed respectively in Pike's medium and Difco P.P.L.O. (pleuropneumonia-like organisms) broth and kept at room temperature; those for viral isolations and specimens of blood were kept at 4° C. All specimens were delivered to the laboratory early the next morning. Early morning sputum

specimens were posted to the laboratory by these patients three days after their clinic attendance.

Investigations of Possible Exacerbations

Directly a patient thought his bronchitis was worse than usual, or if he had a cold in the head or a sore throat, he was requested to post to the laboratory a notification form and a specimen of sputum (if possible the first one coughed up in the morning). The sputum was split into three parts and examined for bacteria, mycoplasmas, and viruses. The bacteriological examination included an estimation of purulence by microscopy.

The patient was visited at home as soon as possible after the receipt of the sputum specimen. Fifty-six exacerbations were fully investigated. Sputum was posted in 39 instances and the home visit was made on the day it arrived in 31, on the next day in four, and on the second or third day in two; in the remaining two instances the patient was admitted to hospital and the history and specimens were taken there. In 10 instances the exacerbation was discovered at a routine attendance and in seven the investigators were informed in a variety of other ways.

At the home visit a history of the illness was taken and the other specimens were collected. These were delivered to the laboratory at once; those for viral isolation were carried in a cold vacuum flask.

The patient was questioned about the following symptoms: coryza, sore throat, hoarseness, increased cough, increased wheeze, increased sputum, and change in colour of sputum to green or yellow. An exacerbation was deemed to be present if the patient reported increased cough, wheeze, or change in colour of sputum, plus at least one other item on the above list, or increased sputum (whether or not this was accompanied by anything else on the list).

A high degree of uniformity in the taking of histories and specimens was achieved. In 51 of the 56 exacerbations which were fully investigated this work was carried out by M. F. and in five by A. J. A. Of the 235 routine examinations the specimens were taken by M. F. in all but one instance.

Laboratory Investigations

Bacteriological Methods

Sputum.—Gram-stained and Leishman-stained films were made from the most purulent part of the sputum allocated for bacteriological study. The remainder was homogenized by shaking with sterile distilled water (Manesty) and a small number of glass beads and inoculated on to two blood agar and two heated blood agar plates. One blood and one heated blood agar plate were incubated in 10% carbon dioxide for 18 hours. The remaining plates were incubated anaerobically for 18 hours. Mice were inoculated with homogenized sputum by the intraperitoneal route. Microscopical purulence of the sputum was graded as follows: M=no pus cells present; PC+=small number of pus cells (1–9 cells per oil immersion ($\times 100$) field); PC++=moderate number of pus cells (10–19 cells/field); and PC+++ = numerous pus cells (20 or more cells/field).

Pharyngeal Swabs.—Blood agar and heated blood agar plates were inoculated and incubated in 10% carbon dioxide for 18 hours. Pneumococci were identified by Optochin (ethylhydrocupreine) sensitivity and inulin fermentation, and *H. influenzae* by morphology, characteristic smell, and satellitism. All strains of pneumococci were serologically typed with sera obtained from the State Serum Institute, Copenhagen.

Sensitivity Tests.—The disc diffusion method (Gould and Bowie, 1952) was used to determine antibiotic sensitivity.

Mycoplasma and Virological Methods

Mycoplasma Culture.—Sputum was homogenized by shaking with about 2 ml. of Difco P.P.L.O. broth base with penicillin and thallium acetate, but no other supplements. Pharyngeal swabs were broken into smaller quantities of broth and squeezed against the side of the bottle before being discarded. The residual broth was used to inoculate plates of Difco P.P.L.O. agar supplemented with

swine serum and low-temperature yeast extract, and also to inoculate 10-ml. quantities of semi-solid agar (for details of cultural methods see Stewart and Chowdray (1968)). The strains isolated were identified by the growth inhibition method of Clyde (1964), typing serum against *Mycoplasma pneumoniae*, *M. salivarium*, *M. orale* type 1, and *M. hominis* (Robbin Laboratories, Chapel Hill, U.S.A.) being used.

Viral Culture.—Pharyngeal swabs were immersed immediately in transport medium, consisting of medium 199 with added bovine serum albumin (1%), sodium bicarbonate (0.22%), penicillin (100 units/ml.), and streptomycin (100 μ g./ml.). Nystatin (50 units/ml.) was added on arrival at the laboratory. Nasal washings were collected in medium 199, but bovine serum albumin, sodium bicarbonate, and antibiotics were added in the laboratory. Sputum specimens were homogenized in transport medium with glass beads and were then centrifuged. All specimens were kept frozen at -70° C. until tissue cultures were available. Pharyngeal swabs were inoculated into HEP-2, second-pass monkey kidney, and human embryo lung cells. Sputum and nasal washings were inoculated only into human embryo lung cells. The cultures were rolled at 34° C. and were examined for cytopathic effect for 10 days. A further passage was then carried out. Haemadsorption tests, using human group O cells, were performed on all monkey kidney cultures at the end of the second passage. The maintenance media were as follows: (1) 2% calf serum in medium 199, with added sodium bicarbonate (0.18%) for HEP-2 and monkey kidney cells; and (2) 2% calf serum in Eagle's medium for human embryo lung cells. Both contained 100 units of penicillin per ml. and 100 μ g. of streptomycin per ml. Viruses were identified with neutralizing sera from the Standards Laboratory for Serological Reagents, Central Public Health Laboratory, Colindale, London. Rhinoviruses were characterized by means of the acid lability test (Grist *et al.*, 1966).

Serological Methods.—Sera were tested for complement-fixing antibodies according to the technique of Bradstreet and Taylor (1962). The following antigens were used: influenza A, B, and C, Sendai, parainfluenza 1, adenovirus, respiratory syncytial virus (R.S. virus), psittacosis, *Coxiella burnetii*, and *M. pneumoniae*. Sera were titrated only if antibodies were found to be present at a dilution of 1:16. If a significant change in titre was noted the serum was retested in parallel with the previous specimen. All standard antigens and antisera were obtained from the Standards Laboratory, with the exception of parainfluenza 1 and, in the later stages of the investigation, *M. pneumoniae*, which were obtained from Burroughs Wellcome. Both *M. pneumoniae* antigens were prepared from washed and heat-killed organisms without chemical extraction. Cold agglutination tests were carried out on all sera (Cruickshank, 1965). Tests for complement-fixing antibodies to parainfluenza 2 and 3 could not be performed routinely owing to the difficulty of obtaining satisfactory complement. When a virus was isolated and antibody to this virus could not be measured by complement fixation tests, neutralization tests were performed. Routine sera were paired with the previous specimen taken, whether this was a routine or a convalescent specimen.

Data Relating to Atmosphere

Meteorological data and data relating to atmospheric pollution were obtained from the Meteorological Office and the City of Edinburgh Health and Social Services Department respectively.

Investigation of Exacerbations

The 23 patients had a total of 63 exacerbations during the period of observation; the distribution is given in Table I.

TABLE I

No. of Exacerbations per Patient	No. of Patients	Total No. of Exacerbations
0	1	0
1	5	5
2	7	14
3	3	9
4	2	8
5	3	15
6	2	12
	23	63

Fifty-six of the exacerbations came to the notice of the investigators at the time of their occurrence. The remaining seven had to be diagnosed retrospectively when the patient was next seen, and were therefore not fully investigated. These seven incidents are described as "missed" exacerbations, and in these the only source of information about a possible infective agent was in the serological findings.

A record was kept of the interval between the patient's first symptom and the onset of the exacerbation (as defined above under "General Procedure").

Interval in Days between First Symptom and Onset of Exacerbation		No. of Exacerbations
0		45 (including 6 "missed")*
1		11 (including 1 "missed")*
2		3
3		3
4		1
		63 (including 7 "missed")

* The delay in obtaining the history in the "missed" exacerbations makes the time intervals unreliable.

In calculating the stage of the illness at which the various specimens were obtained the *first symptom* has been taken as the baseline. The intervals between the first symptom and the collection of the specimens (56 exacerbations) are given in Table II.

TABLE II.—Interval in Days Between First Symptom and Specimens

No. of Days	Sputum	Other Specimens (Pharyngeal Swabs, Nasal Washings, and Sera)
0	2	0
1	10	6
2	13	9
3	13	12
4	7	13
5	1	6
6-15	10	9
	56	55 (1 not examined)

The interval between the first symptom of an exacerbation and the first or "acute" serum for viral and mycoplasmal serology (56 exacerbations) was seven days or less in 49 instances. In the remaining seven exacerbations the routine serum preceding the exacerbations was regarded as the "acute" serum; in three instances this serum was collected 6 to 13 days before the first symptom, and in four instances 29 to 41 days.

The interval between the first symptom of an exacerbation and the second or "convalescent" serum for viral and mycoplasmal serology (63 exacerbations) was 12 to 28 days in 48 instances. In 11 of the remaining 15 instances it was obtained before the 37th day.

A "third" serum for mycoplasmal serology was taken at the patient's next routine attendance. The interval between the first symptom and this "third" serum was 31 to 60 days in 42 instances and 61 to 115 days in 19 instances. In two instances no specimen was obtained.

Possible Influence of Long-term Antibiotics on Incidence of Exacerbations.—The 23 patients can be divided into three groups according to long-term antibiotic treatment. (1) Seven received antibiotics throughout the winter; these were patients who had been on this regimen for several years following the cessation of the M.R.C. early bronchitis trial in which they had participated. (2) Eight received treatment throughout the year; these were patients with advanced disease in whom it was hoped by this means to prevent further deterioration. (3) Eight received no long-term treatment. It is impossible to say how much this selection of patients influenced our results, but the incidence of exacerbations (in terms of the number occurring per 100 patient-observation months) was virtually the same whether calculated for each of the groups throughout the year or for groups 1 and 2 combined and group 3 in the winter months.

The seasonal incidence of exacerbations throughout the study period is shown in Table III. Although there is a higher incidence in the first two quarters of the year it is clear that exacerbations are not exclusively a winter phenomenon.

TABLE III.—Seasonal Incidence of Exacerbations (63 Exacerbations. November 1965–March 1968)

	No. of Patient-observation Months	No. of Exacerbations	No. of Exacerbations as Percentage of Patient-observation Months
January (3 years) ..	51	7	
February (3 years) ..	53	10	
March (3 years) ..	52	11	
	156	28	17.9
April (2 years) ..	34	5	
May (2 years) ..	34	5	
June (2 years) ..	33	3	
	101	13	13.0
July (2 years) ..	32	2	
August (2 years) ..	32	3	
September (2 years) ..	32	4	
	96	9	9.4
October (2 years) ..	32	3	
November (3 years) ..	49	6	
December (3 years) ..	51	4	
	132	13	9.8

Results of Laboratory Investigations

Bacteriology

Only the pneumococcus and *Haemophilus influenzae* are included in our results, since these are the organisms generally considered to be of importance in exacerbations of chronic bronchitis. Pneumococci were cultured from 12 exacerbation specimens and eight routine specimens of sputum; four isolations were made from routine specimens by mouse inoculation only, and in two, from exacerbations, the organisms were seen in the smear but not cultured. All 20 strains of *H. influenzae* were isolated on culture. In five instances the growth of pneumococci or *H. influenzae* was scanty and in all other instances moderate or profuse. When these organisms were cultured growth occurred both in carbon dioxide and in anaerobic conditions.

FIG. 1.—Purulence of sputum, determined microscopically, related to incidence of pneumococcus and/or *H. influenzae* in exacerbations and at routine examinations. Note the significant increase in purulence as the isolation rate increases. For exacerbations $\chi^2_0 = 5.26$, 3 D.F., $P < 0.001$. For routine examinations $\chi^2_0 = 7.56$, 2 D.F., $P < 0.05$. M = No pus cells. PC+ = 1–9 cells/oil immersion field. P+ = 10–19 cells/field. P+++ = 20 or more cells/field. In Figs. 1–3 the total exacerbation specimens number 56 and the total routine specimens 233 (there were 235 routine examinations, but sputum was not examined in two instances). In Figs. 1–3 the numbers within the columns represent the number of specimens from which pneumococci and/or *H. influenzae* were isolated.

Fig. 1 shows the purulence of the sputum, determined microscopically, in relation to the incidence of pneumococcus and/or *H. influenzae* in exacerbation and routine specimens. The calculation of significance for routine examinations involved combining the specimens in the two highest grades of sputum purulence because the numbers were small. The more purulent the specimen the higher was the incidence of bacteria. It might be argued that to compare the purulence of sputum in exacerbations with that at routine examinations is unsound, since "change in colour of sputum" was one of the symptoms included in the definition of an exacerbation. Scrutiny of the symptoms in each case, however, showed that in only 32 of the 56 exacerbations did the patient report a change in colour of sputum, and in 30 of these the definition of an exacerbation would have been met on the basis of other symptoms. Eosinophils were present in the sputum of six patients (twice in one); these did not influence the purulence grading, as the latter was based on the microscopical findings with differential staining.

Pneumococci and/or *H. influenzae* were found in 22 of the 56 exacerbation specimens of sputum and in 21 of the 233 routine specimens of sputum. The total frequency of these organisms in exacerbations was significantly higher than in routine examinations (Fig. 2).

FIG. 2.—Incidence of pneumococci and *H. influenzae* in exacerbation and routine specimens of sputum. Note that the total incidence of pneumococci and *H. influenzae* is significantly higher in exacerbations than in routine examinations. $\chi^2_{0.05} = 30.32$, 1 D.F., $P < 0.001$.

Fig. 3 shows the relative frequency of pneumococci and *H. influenzae* in exacerbation and routine specimens of sputum according to antibiotic treatment at the time of the specimen.

FIG. 3.—Incidence of pneumococcus and/or *H. influenzae* in sputum according to whether the patient was receiving antibiotic treatment when specimen was taken. $\chi^2_{0.05} = 6.961$, 1 D.F., $P < 0.01$.

There was a significantly lower incidence of pneumococci in exacerbation specimens of sputum when the patients had been receiving antibiotic therapy.

On seven occasions a positive result was obtained from an exacerbation specimen of sputum while the patient was receiving antibiotic therapy: long-term tetracycline in three instances, long-term ampicillin in one, and tetracycline for two days or less in three. The corresponding figures for the 12 occasions on which a positive result was obtained from a routine specimen of sputum while the patient was receiving antibiotic therapy were nine, two, and one. On all the 19 occasions on which positive results were obtained the organism isolated was sensitive to the antibiotic being given, except for one routine specimen in which *H. influenzae* was isolated and the result of the sensitivity test was doubtful.

Pharyngeal swabs yielded very few positive results. Of 54 specimens taken in 56 exacerbations one produced a growth of pneumococci and one a growth of *H. influenzae*; in both instances the organism was also found in the sputum. Of 234 specimens taken in 235 routine examinations three produced a growth of pneumococci and one a growth of *H. influenzae*; in two of these four instances one or other organism was also found in the sputum.

Ten pneumococcal types (4, 7, 8, 9, 10, 11, 16, 19, 20, and 21) were isolated from the sputum of nine patients. In four patients the same pneumococcal type was present at more than one examination; there were five isolations of type 21 from one patient, and types 4, 8, and 9 were present on two occasions in three patients. The length of time the same type was present ranged from 1 to 30 months.

Viral and Mycoplasmal Results

Table IV shows that significantly more exacerbation than routine examinations were associated with a fourfold or greater rise in viral or *M. pneumoniae* antibodies, with or without isolation of the same agents. In the seven positive viral results occurring in the 63 exacerbations the interval between the first symptom and the "convalescent" serum was 14 to 28 days in five instances and more than 28 days in two. In six of these cases the "acute" serum was collected seven days or less after the first symptom; one was a "missed" exacerbation. Of the eight instances of antibody rise at routine examinations four were associated with symptoms suggestive of a viral infection, but not an exacerbation. All cold agglutinin tests were negative.

TABLE IV.—Exacerbations and Routine Examinations Associated with a Significant (Fourfold or Greater) Rise in Viral and *M. pneumoniae* Antibodies (56 Fully Investigated Exacerbations Plus 7 "Missed" Exacerbations with Only Serological Findings)

Total Examinations	Exacerbation (63)	Routine (189)
Influenza A	3	1
Influenza B	1*	1
Sendai	1†	2‡
Adenovirus	0	1†
R.S. virus	1*	0
Rhinovirus	1†	1
<i>M. pneumoniae</i>	2	2
Total with positive serology	9 (14%)	8 (4%)

$\chi^2 = 6.076$ (1 D.F.), $0.01 < P < 0.02$.

* Associated with isolation of pneumococcus from sputum.

† Associated with isolation of this virus.

‡ One patient in each of these groups also had a significant parainfluenza 1 antibody rise.

(Note: Complications in the collection or examination of specimens for isolation of viruses made it impossible to carry out the full range of tests in all three specimens in eight exacerbations and in seven routine examinations.)

Nine viral isolations occurred without significant antibody response; herpes simplex virus at seven routine examinations and one exacerbation, and one unidentified enterovirus at a routine examination. In no instances was a positive viral finding accompanied by evidence of *M. pneumoniae* infection. *M. pneumoniae* was not isolated during the investigation. Two

exacerbations with significant viral antibody rises were associated with the isolation of pneumococcus from the sputum.

Table V summarizes the positive findings in relation to all the infective agents studied in the 56 fully investigated exacerbations and the seven "missed" exacerbations in which only serological studies were carried out.

TABLE V.—Number of Exacerbations in Which There was Evidence of an Infective Agent (56 Fully Investigated Exacerbations and 7 Exacerbations in Which Only Serological Investigations were Carried Out)

	No. of Exacerbations
Pneumococcus and/or <i>H. influenzae</i> in sputum (no virus)	20 (32%)
Viral infection only	4 (6%)
Viral infection plus pneumococci in sputum	2 (3%)
<i>M. pneumoniae</i> infection	2 (3%)
No evidence of infective agent	35 (56%)
Total	63 (100%)

Atmospheric Pollution and Temperature

There is evidence that both atmospheric pollution and low temperature increase the demand for hospital beds for respiratory illness (Holland *et al.* 1961). There is also evidence that atmospheric pollution has a deleterious effect on chronic bronchitis (Lawther, 1958; Clifton *et al.*, 1960). There have been very few attempts to relate atmospheric conditions specifically to exacerbations, though Eadie *et al.* (1966) state that they were unable to find significant correlation between the incidence of respiratory episodes in their chronic bronchitis and the meteorological data recorded. They did not study atmospheric pollution. We therefore attempted to study atmospheric conditions in relation to exacerbations in our patients.

Records of pollution were not available until June 1966. Thus the data could be studied in relation to only 47 of the 63 exacerbations. Smoke (measured by the method used in the National Survey of Atmospheric Pollution) was considered as an index of the degree of pollution. The readings were taken at one central site in Edinburgh, because it was found that this site usually gave higher readings than the other six sites, and that peaks usually occurred simultaneously at all sites. During each relevant month the mean concentration of smoke was compared on (1) the days on which the first symptoms of exacerbation occurred; (2) the preceding days—that is, day before the first symptom in each instance; and (3) all the remaining days of the months. The means found in both (1) and (2) were slightly higher than those in (3), but the differences were not statistically significant. In none of the nine exacerbations in which no infective factor was found, and in which no antibiotic treatment was being given when the sputum specimen was obtained, was there a peak of pollution on the day of the first symptom or on the preceding day.

The same procedure was followed in relation to the mean daily temperature recorded over the whole observation period of 29 months at another site in Edinburgh. Though a fall in temperature was sometimes observed at the time of an exacerbation there was no suggestion of a sufficiently frequent association to justify statistical analysis. This was true both for all exacerbations and for those in which no infective factor was found and in which no antibiotic treatment was being given at the time of the sputum specimen.

Discussion

Factors associated with exacerbations of chronic bronchitis have been investigated by a number of workers, but no attempt seems to have been made to isolate bacteria, viruses, and mycoplasma at the same time. In a very few studies—for example, Eadie *et al.* (1966)—patients have been seen at home during exacerbations, but we know of no previous investigation in which patients have been used as their own controls by being seen both during exacerbations in their home and at regular routine reviews, with the same standard investigations on all occasions.

In chronic bronchitis the symptoms fluctuate from day to day, so it is difficult for a patient always to recognize the onset of an exacerbation. The difficulty is increased by the absence of an agreed definition of an exacerbation. Only a few investigators in this field have suggested definitions, and these vary from worker to worker. This makes a comparison of results very difficult. The definition used in our investigation gave rise to no problems of interpretation. On only two occasions, when a notification was sent, was a patient found not to have an exacerbation as defined by us. On one of these the patient complained of a "tight sore chest" and on the other of shortness of breath. The notification of exacerbations depended on the reliability and intelligence of the patient or his wife. Though the patients were constantly reminded that we wanted to know about "colds" as well as exacerbations, on no occasion on which a notification was sent did the patient have only an upper respiratory tract infection. Of a total of 63 respiratory illnesses that fitted our definition 56 were notified in time to be fully investigated.

The possible role of bacteria in producing exacerbations has been investigated by many workers. There is much evidence that *H. influenzae* and pneumococci are often isolated from the sputum of chronic bronchitis, but the exact part they play in producing exacerbations remains undecided. The problem is discussed by Stuart-Harris (1968). He says that "all observers agree that there are few differences between sputa collected during acute exacerbations and in the relatively chronic phases of the disease," but points out that "quantitative data to establish contrasts are mostly unavailable." In our study the frequency of isolations of *H. influenzae* and pneumococci was significantly greater during exacerbations than at routine examinations ($P < 0.001$) (Fig. 2). This difference does not seem to have been convincingly documented before. We have also shown that there was an increase in the isolation rate of these organisms with increasing sputum purulence (Fig. 1), a finding in agreement with May (1953). The association is more striking for exacerbations ($P < 0.001$) than for routine examinations ($P < 0.05$). Our results suggest that *H. influenzae* and pneumococci are frequently aetiological factors in exacerbations. Support for this view can be found in some reports of the efficacy of certain antibiotics in reducing both the purulence of the sputum and the isolation of these organisms in acute exacerbations—for example, Citron *et al.* (1968)—particularly when treatment is started early in the exacerbation (Malone *et al.*, 1968). Antibiotic treatment, however, has not been found consistently effective (Stuart-Harris, 1968).

Increases of viral or mycoplasmal antibody, with or without isolation, were significantly more frequent in exacerbations than in routine examinations in our patients (Table IV). The four positive serological results for influenza viruses in exacerbations all occurred when infections due to these viruses were known to be present in the general population. In the 56 fully investigated exacerbations viruses were isolated three times. These were influenza B, rhinovirus, and herpes simplex. The influenza B and rhinovirus had confirmatory antibody rises in the blood. *M. pneumoniae* was not isolated from our patients but significant antibody rises occurred in association with two exacerbations and at two routine examinations. The failure to isolate this organism might have been partly due to inefficient cultural techniques. *M. salivarium* was isolated in exacerbations and at routine examinations, but there is no evidence that this organism plays any part in exacerbations of chronic bronchitis (Stewart and Chowdray, 1968).

The association of viruses with exacerbations of chronic bronchitis has been investigated by several workers, with widely differing results. Using techniques available at the time, Jack and Gandevia (1960), in Australia, obtained negative results. Sommerville (1963) found serological evidence of infection with respiratory syncytial virus in exacerbations of chronic bronchitis in Glasgow during 21 months, but this finding was not repeated in subsequent years. Carilli *et al.* (1964) investigated

30 chronic bronchitics at a clinic in Washington, D.C., during eight months, at monthly intervals and during exacerbations. Fourfold antibody rises were observed to a number of viruses, in particular to respiratory syncytial virus. The overall rate was 52%, which is much higher than that found by any other worker.

Eadie *et al.* (1966) investigated exacerbations in 15 chronic bronchitics in Glasgow. They isolated a rhinovirus in 11 (23%) of 47 exacerbations. In 10 of the 11 isolations the exacerbation was associated with a "cold," which might have been responsible for the rhinovirus isolation. It is possible that the age distribution of their patients with rhinovirus isolations (mean age 43.0 years), which was very different from that of our patients (mean age 57.5 years), partly explains the difference between their findings and ours. Six of the seven patients from whom they isolated rhinoviruses were in close contact with children, and four of them were female (personal communication). In our series, which was more representative of the typical chronic bronchitic, young family contacts were rare.

Stenhouse (1967) isolated a rhinovirus from 8 (14%) out of 56 exacerbations studied in Sheffield. Some of these patients were seen at home and some at a clinic (personal communication). No information is available about the composition of the household in his patients. In each case the exacerbation was associated with an "acute respiratory illness," including "mild rhinorrhoea" in six of the eight instances. In our 56 fully investigated exacerbations rhinorrhoea preceded the exacerbation on four occasions and occurred as part of it on a further 27 occasions. Stenhouse obtained his specimens for viral isolation "10 days or less after the onset of the acute respiratory illness." In our investigation every effort was made to obtain specimens as soon after the first symptoms as possible. In the 56 fully investigated exacerbations 45 of the sputum specimens and 40 of the other specimens were obtained on the day of the first symptom or on one of the four subsequent days. Despite this our rhinovirus isolation rate was very low. The fact that Stenhouse used three cell lines sensitive to rhinoviruses, while we used only one, may be partly responsible for this difference. It cannot, however, be the only explanation, since our cell line showed itself susceptible to rhinovirus strains from other patients over the same period of time.

Ross *et al.* (1966) carried out a serological investigation over four years in Glasgow and Dumfries. Paired sera were collected during 125 exacerbations. A fourfold rise to a number of viral agents was found in 16% of the exacerbations, the commonest being influenza A. No rise to *M. pneumoniae* was found. In a subsequent study Stenhouse (1968) examined sera from his group of patients (Stenhouse, 1967) for viral and *M. pneumoniae* antibody rises. Of 16 significant rises in chronic bronchitics eight could be correlated with an exacerbation; influenza A was the commonest.

In the present study an infective agent was found in only 28 out of 63 exacerbations (Table V). Our findings do not suggest that viral and mycoplasmal infections, at least as demonstrated by present methods, play a large part in exacerbations of chronic bronchitis. It is possible that the use of newer techniques—for example, organ culture (Tyrrell and Bynoe, 1965)—may demonstrate closer association between chronic bronchitis and viruses.

Pneumococci and *H. influenzae* were certainly important. One or both were isolated in 30% of sputum specimens taken during exacerbations, but because of the use of antibiotics this may be an underestimate of the true prevalence. In 24 of a total of 34 exacerbations with negative bacteriological findings the patients were receiving antibiotics when the sputum specimen was taken. It is possible that in some of these exacerbations bacteria may have been a causal agent, but that the antibiotic the patient was receiving prevented their isolation. Pneumococci, in particular, are readily suppressed. Another possible cause of misleading bacteriological findings is that most of the sputum specimens were dispatched by post. May and

Delves (1964) found that the isolation rate of both pneumococci and *H. influenzae* from postal specimens was about half that from fresh specimens. Tests made from time to time in the Wellcome Laboratory, however, have shown no appreciable difference between the isolation rates of these organisms in fresh specimens and those retained in a cool place for 24 hours—which is in agreement with earlier work on the survival of pneumococci in sputum (White, 1938). In the present investigation it was hoped to reduce any possible error due to the use of postal specimens by careful examination of stained films of sputum.

In our "unexplained" exacerbations it is possible that atmospheric conditions may have played a part, but we have not been able to demonstrate a definite association, perhaps because pollution was generally low during the study. We suggest that the only hope of reliably testing an association would be to study a much larger number of patients. The work involved, both clinical and laboratory, in this small pilot study was considerable, and it would be very great if a large number of patients were studied in the same way.

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