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Neuraminic acid content of sputum in chronic bronchitis

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The neuraminic acid content of sputum from 48 men with early chronic bronchitis has been estimated in samples collected over a period of three years. The results are compared with those from 29 advanced bronchitic patients and are related to the clinical features of both groups and to the physical and biological properties of the sputum. A seasonal variation in neuraminic acid content has been noted for the first time with higher levels during the winter months. Clinical assessment of sputum pourability correlated well with measured viscosity. The viscosity of mucoid sputum was related to its neuraminic acid content but also to the yield of dry macromolecular material. In the early bronchitic group whose sputum was assessed for purulence at monthly intervals pus was more often present in those men whose mucoid sputum contained higher levels of neuraminic acid. These findings are discussed in relation to the cause of exacerbations of chronic bronchitis.

The neuraminic acids are important constituents of epithelial secretions. The viscosity of bovine cervical mucus (Gibbons, 1959; Gibbons and Glover, 1959), of pseudomyxomatous gels (Odin, 1955), and of bronchitic sputum (Munies, Grubb, and Caliari, 1968) has been related to the neuraminic acid content. In the present report N-acetyl neuraminic acid (NANA) has been taken as a marker of acid glycoprotein in bronchitic sputum. In a prospective study of a group of early bronchitic subjects, in whom hypersecretion of mucus was the principal manifestation of disease without gross infection or airways obstruction, the variation in NANA content has been related to sputum viscosity and to the clinical features. The results are compared with those from a group of severe chronic bronchitic patients attending, or admitted to, hospital.

A correlation was also sought between the NANA content and the 'exacerbations' of chronic bronchitis since, at present, these are not well defined because there is inadequate knowledge of the events giving rise to them. Bacterial infection is by no means always present (Fisher *et al.*, 1969) and the role of viral infection is difficult to establish. Since most exacerbations occur during the winter months changes in atmospheric pollutants may play some part (Ogilvie, 1967). Lawther, Emerson, and O'Grady (1969) have suggested a growth-stimulating effect on the *Haemophilus influenzae* by aqueous extracts of atmospheric pollutants. The 'virulence-enhancing' or 'resistance-lowering' properties of mucus have been studied in great detail (Olitzki, Shelubsky, and Hestrin, 1946; Olitzki, Shelubsky, and Efrati, 1947; Olitzki, 1948; Smith, Gallop, and Stanley, 1952; Smith, 1953) before the advent of antibiotics swamped further interest. At that time the glycoprotein content of mucus was thought to be an important factor but little was then known of the neuraminic acids.

MATERIALS

EARLY CHRONIC BRONCHITIS Sputum specimens from 48 men were studied. These formed part of a larger group of early bronchitic subjects taking part in a 10-year prospective study from the Newcastle Bronchitis Centre (Ogilvie, 1967). They all had cough and sputum meeting the definition of simple chronic bronchitis (Medical Research Council, 1965) but had only presented to a doctor with some non-respiratory illness or with a 'chest cold', usually for the first time. They were all in regular employment and did not consider themselves ill in any way except for a smoker's cough or slight breathlessness. Nevertheless many were found to have significant airways obstruction or to suffer recurrent 'infective' episodes. Those admitted to the study all had an indirect maximum breathing capacity (MBC) between 50 and 75% of the predicted normal.

Sputum was collected during the first hour after waking at monthly intervals over the period November 1962 to April 1965. Each specimen was graded as

mucoid, mucopurulent, or purulent, and a measure of its viscosity was obtained by a grading of its 'pourability' according to the following criteria: grade 1—viscid sputum which adheres closely to the container when inverted; grade 2—viscid sputum which oozes slowly from the container; grade 3—viscid sputum which pours from the container but remains in one piece without fragmentation; grade 4—watery sputum pouring readily from the container but containing viscid particles. Each monthly specimen was also cultured but the presence of *H. influenzae* is the only bacteriological aspect considered here. The following clinical information was available for these subjects: age; occupation (25 were miners, 16 were in heavy industrial work, and 6 in sedentary occupations); smoking habits; duration of sputum production; MBC on entry to the study and after two and a half years, both expressed as a percentage of the predicted normal; the exacerbation scores. For the purposes of assessment and comparison, Ogilvie and his colleagues graded and scored the exacerbations experienced by their men in such a way that the number, severity, and duration of the exacerbations were reflected in the total score for a given period. This method takes no account of the cause of the 'exacerbation'—viral, bacterial, or environmental.

Of the monthly specimens of sputum assessed in Newcastle, seven or eight from each subject were submitted for chemical analysis. These were in November 1962, March, October, and December 1963, March and October 1964, and January and April 1965. All specimens of sputum for analysis reached the Newcastle Bronchitis Centre within 2 to 4 hours of production and after preliminary assessment were packed in vacuum flasks containing Cardice (−78° C) for transport and were later stored at −20° C.

LATE CHRONIC BRONCHITIS The group of late chronic bronchitics consisted of 29 patients either attending Dr. John Batten's bronchitis clinic at the Brompton Hospital or admitted to the hospital in exacerbation. These were, therefore, patients with sufficiently advanced disease to have been referred for specialist opinion or to require hospital admission. It should be stressed that the terms 'early' and 'late' in this context refer to the degree of disability and not to the duration of symptoms. Ogilvie (1967) found no relation between the duration of symptoms and the degree of disability. The results of sputum analysis were related to age, smoking habits, duration of symptoms, the peak expiratory flow rate (PEFR), and the presence of emphysema on the chest radiograph (Simon, 1964).

METHODS

PRELIMINARY TREATMENT OF SPUTUM After removal from deep freeze, specimens were thawed rapidly by standing the containers in cold, running tap water. Whenever the total volume allowed, 5 ml aliquots were taken for dialysis, without prior treatment of the sputum, and the yield of macromolecular solids per millilitre of sputum was obtained. For analysis material was suspended in distilled water to which was added one drop of ficin solution and one drop of cyanide activator and was incubated overnight at 37° C.

ESTIMATION OF NEURAMINIC ACID The estimation of NANA was by the thiobarbituric acid method of Warren (1959), which estimates only free neuraminic acid and requires preliminary enzyme degradation or acid hydrolysis. In this study the total NANA was estimated as a percentage of the dry material. From the biochemical studies three indices are therefore available for evaluation in relation to the clinical information: the yield of dry macromolecular material per millilitre of sputum (DW mg/ml); the estimated NANA as a percentage of the dry weight (NA% DW); and the calculated concentration of NANA per millilitre of sputum (NA mg/ml).

MEASUREMENT OF SPUTUM VISCOSITY In the present study sputum viscosity was assessed by its fluidity or 'pourability' which has been shown to correlate with measured viscosity. At the time no method was available for the storage and transport of sputum which did not affect its viscosity (Elmes and White, 1954). Since then a method of rapid freezing with liquid nitrogen followed by rapid thawing has been shown not to affect the measurement of sputum viscosity on a Ferranti–Shirley cone and plate viscometer (Charman, personal communication, 1971). To relate pourability to viscosity, fresh mucoid sputum from hospital patients was tested within half an hour of its production. It was possible to estimate grades of pourability intermediate between those defined by Ogilvie, and independent readings by two observers did not differ by more than one grade on this extended scale. Readings of apparent viscosity at 900 sec^{-1} were taken on the Ferranti–Shirley cone and plate viscometer (McKennell, 1960) without preliminary treatment of the sputum (Sturgess, 1970).

The comparison between the grades of pourability and measured viscosity is shown in Figure 1. The correlation coefficient (0·8155, $P=<0·001$) is highly significant and, though lacking the precision of the instrumental method, the classification of sputum into pourability grades appears to be a useful method of grading viscosity in field studies (Keal, 1970a).

RESULTS

RELATIONSHIP OF DRY WEIGHT TO NANA CONTENT OF SPUTUM The Newcastle subjects formed a homogeneous group with disease due primarily to hypersecretion of mucus uncomplicated by gross infection, severe airways obstruction, or specific underlying disease. They therefore offer the nearest approach to what might be called 'normal' sputum and the results of analysis for this group may be used as a baseline for comparison with other disease groups (Keal, 1970b). In Figs 2, 3, and 4,

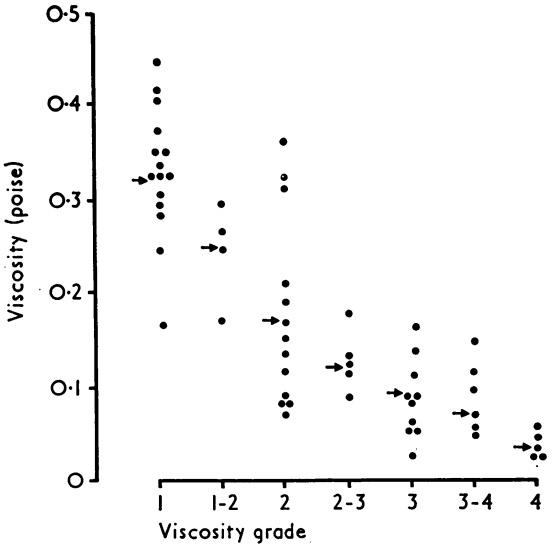

FIG. 1. *Apparent viscosity of mucoid sputum at* 900 sec^{-1} *related to viscosity grade* (r=0·8155, P=<0·001).

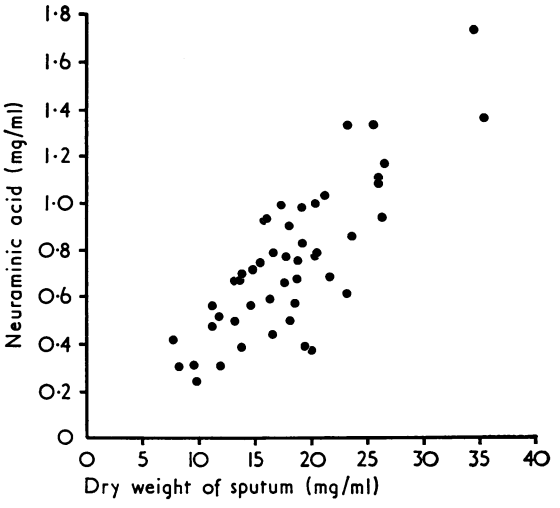

FIG. 3. *Yield of macromolecular material from mucoid sputum related to concentration of NANA in sputum* (r=0·8112, P=<0·001).

the three values of dry weight, percentage, and concentration of NANA are compared, each value quoted being the mean for the seven or eight specimens of sputum from each patient. With few exceptions the sputum was mucoid.

The relationship between the weight of dry material and the percentage of NANA it contains is shown in Figure 2. There is a fourfold variation in the former and a threefold variation in the NANA but no significant relation between the two sets of values. Figure 3 shows a highly significant relationship between the dry weight of the sputum and the concentration of NANA per millilitre of sputum, and Fig. 4 shows a similar relationship between the percentage of NANA in the dry material and its concentration per millilitre of sputum. These results suggest that the sputum of

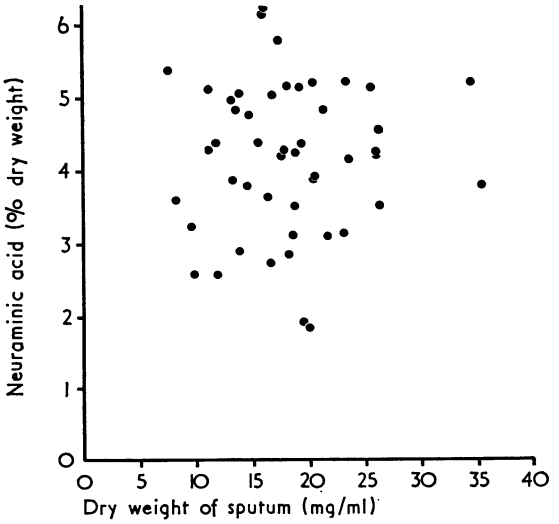

FIG. 2. *Yield of macromolecular material from mucoid sputum related to percentage of NANA it contains* (r=0·04, *NS*).

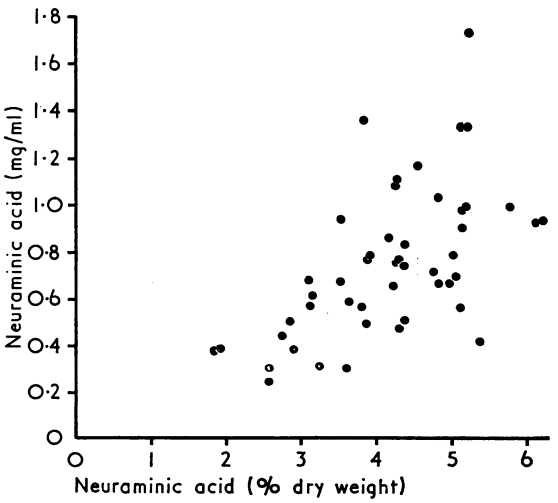

FIG. 4. *Percentage of NANA in dry macromolecular material of mucoid sputum related to concentration of NANA in sputum* (r=0·5829, P=<0·001).

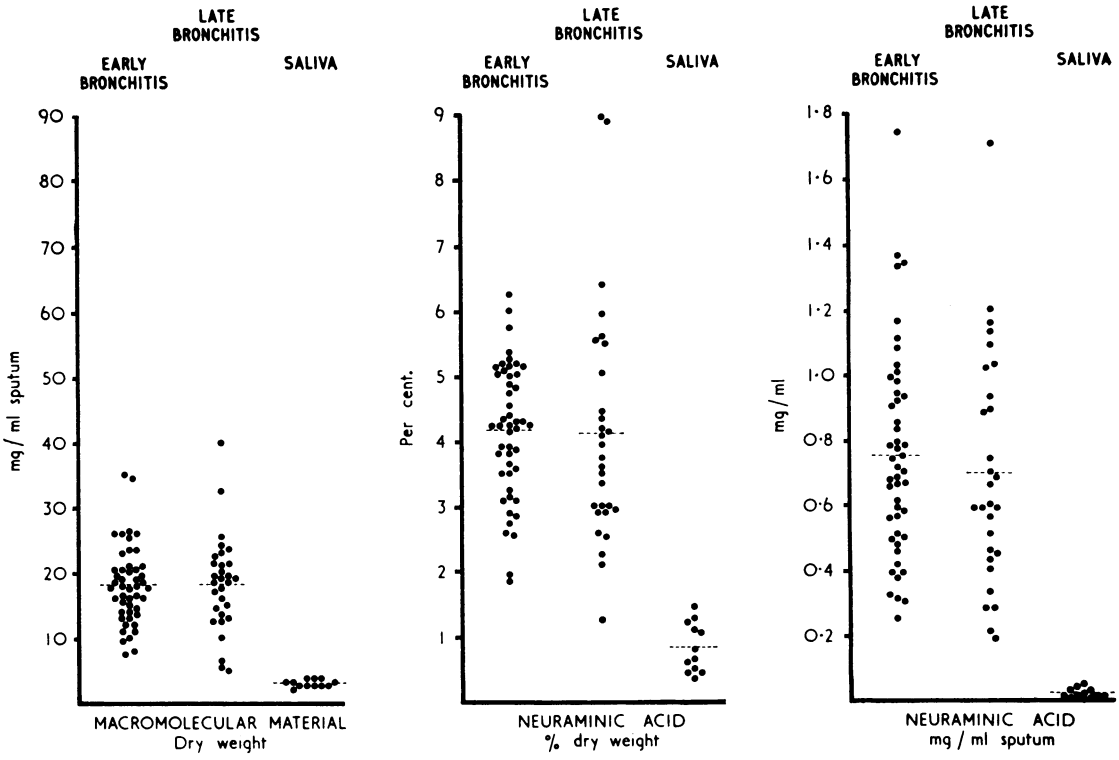

FIG. 5. *Dry weight and NANA content of mucoid sputum compared in early and late chronic bronchitis and with saliva.*

an individual patient may usefully be described biochemically by the yield of macromolecular material and the concentration of NANA per millilitre.

DRY WEIGHT AND NANA CONTENT OF SPUTUM COMPARED IN EARLY AND LATE CHRONIC BRONCHITIS AND WITH NORMAL SALIVA The dry weight and NANA content of the sputum from 48 early bronchitics and 29 late bronchitics are shown in Figure 5. These are mean values for seven or eight specimens from each of the early bronchitics and individual specimens for the late bronchitic group and are compared with the values for normal saliva. It can be seen that even moderate contamination of the bronchial fluid by saliva will contribute very little to the weight of dry material, and as the percentage of NANA in the dry material is low in saliva it will contribute relatively even less to the concentration of NANA per millilitre of sputum. A possible source of error would be the volumetric dilution of bronchial fluid by large amounts of saliva but in practice this does not seem to occur.

SEASONAL VARIATION IN DRY WEIGHT AND NANA CONTENT OF MUCOID SPUTUM IN EARLY CHRONIC BRONCHITIS As this study progressed it was evident that in many subjects very consistent results were obtained (Fig. 6). In other cases there appeared a seasonal variation in all three values, most apparent in the NANA as a percentage of dry weight (Fig. 7). This pattern was seen in many more patients over the first two winters but was lost in the third when the October values were either the same or higher than those of the previous March. A 'batch effect' due to variation of analytical technique was excluded. Comparison of all three values for individual patients (Fig. 8) shows a concomitant rise at some stage during the winter. In this and other similar cases all specimens of sputum analysed were macroscopically mucoid, and these changes were seldom associated with clinical exacerbation of disease. The changes are quite unlike those due to the occasional presence of pus in the sputum (Fig. 9) when the rise in dry weight is balanced by a fall in the percentage of NANA. In this case in the following winter there is a rise in all three values in mucoid sputum.

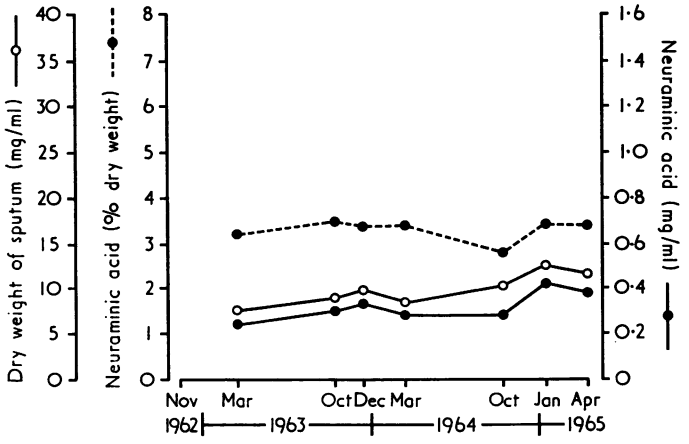

FIG. 6. *Serial analysis of mucoid bronchitic sputum in one subject over a period of 2½ years illustrating consistency of results.*

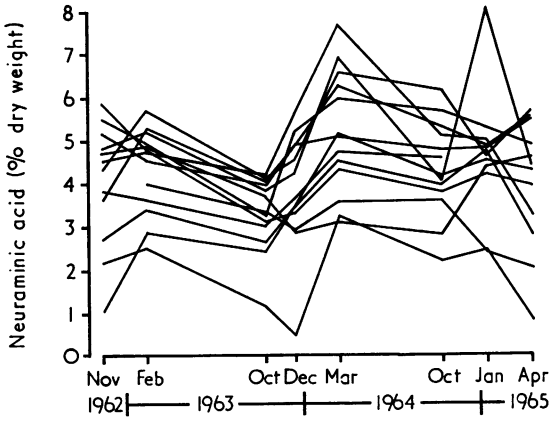

FIG. 7. *Seasonal variation of NANA as a percentage of dry weight in 16 of 48 subjects studied.*

SPUTUM VISCOSITY, DRY WEIGHT, AND NANA CONTENT In the group of early bronchitics a mean value for the dry weight and NANA content was obtained from the seven or eight specimens of mucoid sputum analysed for each patient, and a mean pourability grade was calculated for the 25 to 30 specimens of sputum assessed for each patient (Figs 10 and 11). The regression coefficient between the pourability grade and the concentration of NANA (r=0·334) is significant at the 2% level and that between pourability grade and dry weight (r=0·283) at the 5% level. There was no correlation between pourability grade and the percentage of NANA in the dry material (r= 0·192).

In order to investigate further the relationship of both dry weight and NANA content to the viscosity of mucoid sputum partial correlation coefficients were calculated holding one factor constant. The results suggest that the dry weight and NANA content of mucoid sputum are interdependent in their relationship to the viscosity.

DRY WEIGHT AND NANA CONTENT OF SPUTUM RELATED TO AGE Early and late bronchitics are compared with respect to the dry weight and NANA content of sputum and to age (Table I):

TABLE I

DRY WEIGHT AND NANA CONTENT OF MUCOID SPUTUM RELATED TO AGE

	Age	No. of Patients	Dry wt. (mg/ml)	NANA	
				% DW	mg/ml
Early bronchitics	30–40	16	19·1	4·014	0·753
	41–50	20	16·8	4·247	0·7
	51–55	11	19·7	4·386	0·866
Late bronchitics	47–55	8	15·5	3·955	0·658
	56–65	7	18·0	3·648	0·671
	65+	6	15·9	4·633	0·631

no significant trend is seen but the dry weight and NANA concentration are generally lower in the late bronchitic group.

DRY WEIGHT AND NANA CONTENT OF SPUTUM RELATED TO SMOKING HABITS In the early bronchitics no relationship is apparent between sputum analysis and smoking habits (Table II). In the late bronchitics the NANA values appear to increase with the number of cigarettes smoked. The correlation coefficient between smoking and NANA as

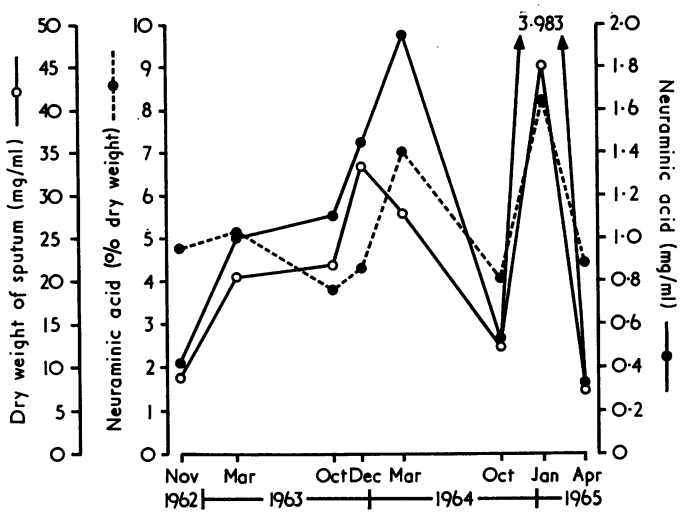

FIG. 8. *Concomitant rise in dry weight and NANA content of mucoid sputum from one subject during two successive winters.*

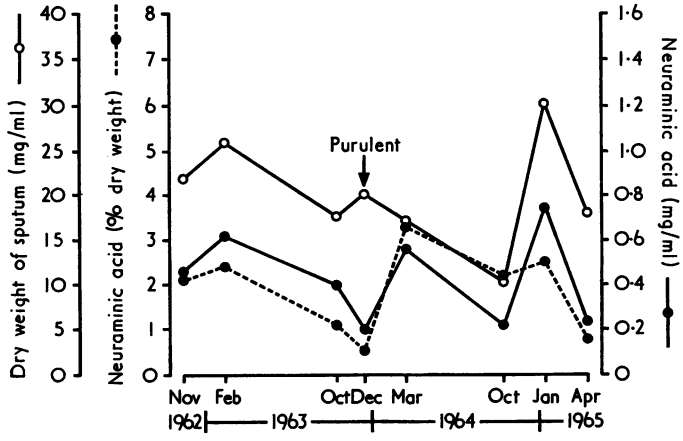

FIG. 9. *Effect of pus in one specimen of sputum during winter* 1963/64, *showing a rise in dry weight with reduction in NANA. Changes in mucoid sputum are seen in preceding and following winters.*

TABLE II

DRY WEIGHT AND NANA CONTENT OF SPUTUM RELATED TO SMOKING HABITS

	No. of Cigs.	No. of Patients	Dry wt. (mg/ml)	NANA	
				% DW	mg/ml
Early bronchitics	30–40	2	18·5	4·287	0·791
	5–15	18	17·1	4·268	0·698
	16–25	18	19·9	3·931	0·825
	25+	9	17·1	4·359	0·73
Late bronchitics	5–15	5	16·2	3·332	0·57
	16–25	9	18·9	3·8	0·708
	25+	4	14·9	4·487	0·685

a percentage of dry weight is significant at the 2·5% level; with the NANA concentration in the sputum it is not significant.

DRY WEIGHT AND NANA CONTENT OF SPUTUM RELATED TO MBC ON ENTRY TO STUDY AND TO ITS SUBSEQUENT DETERIORATION The results of sputum analysis are related to the maximum breathing capacity on entry to the study, expressed as a percentage of the predicted normal, and to its subsequent deterioration expressed as a per-

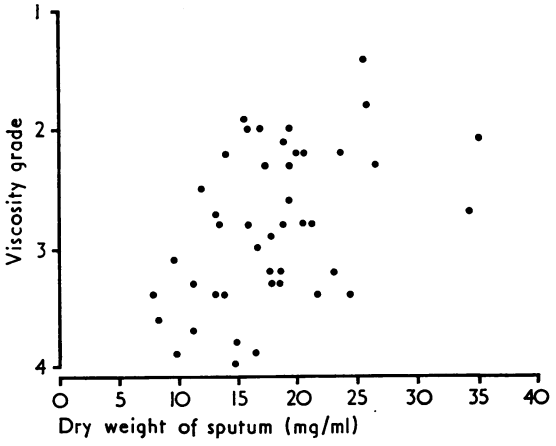

FIG. 10. *Viscosity grade related to dry weight of mucoid bronchitic sputum. Each point represents mean viscosity grade of about* 30 *specimens of sputum and mean dry weight of* 7 *or* 8 *estimations from each subject* (r=0·283, P = <0·05).

centage of the initial value (Table III). Greater initial impairment of MBC and greater subsequent deterioration appear to be associated with higher levels of NANA as a percentage of the dry weight although the regression coefficient is not significant (r=0·18).

DRY WEIGHT AND NANA CONTENT OF SPUTUM RELATED TO FREQUENCY WITH WHICH PUS WAS PRESENT IN SPUTUM The mean neuraminic acid level for each patient is that of his mucoid bronchial secretion. During the two and a half years of this study 30 monthly specimens of sputum were assessed by Ogilvie for the presence of pus. It appears from Table IV and Figs 12 and 13 that pus was more often present in the sputum of those patients whose mucoid bronchial

TABLE III

DRY WEIGHT AND NANA CONTENT OF SPUTUM RELATED TO MBC ON ENTRY AND TO ITS SUBSEQUENT DETERIORATION

	No. of Patients	Dry Weight (mg/ml)	NANA	
			% DW	mg/ml
MBC % predicted normal on entry				
Better than 70	7	18·9	3·63	0·733
60–70	14	16·8	4·19	0·663
50–59	27	18·6	4·3	0·798
MBC % deterioration				
Less than 10	22	17·9	3·933	0·732
10–20	13	19·9	4·267	0·819
More than 20	13	16·6	4·331	0·723

TABLE IV

DRY WEIGHT AND NANA CONTENT OF MUCOID SPUTUM RELATED TO FREQUENCY OF FINDING PUS IN MONTHLY SPECIMENS OF SPUTUM

Percentage of Specimens Containing Pus	No. of Patients	DW (mg/ml)	NANA	
			% DW	mg/ml
Less than 20	6	12·4	4·369	0·539
21–30	13	14·8	4·329	0·637
31–40	11	19·9	4·096	0·8
More than 40	18	21·4	4·042	0·866

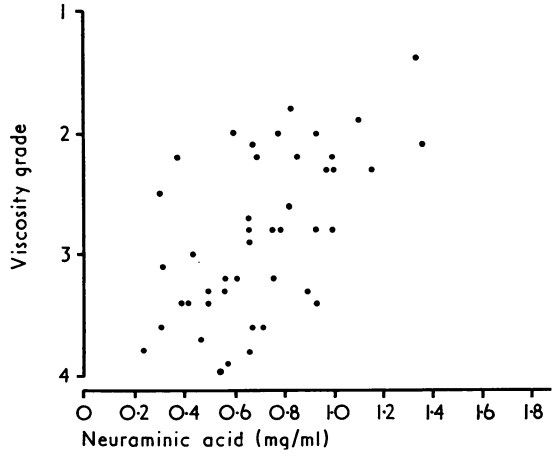

FIG. 11. *Viscosity grade related to NANA concentration in mucoid bronchitic sputum. Each point represents mean viscosity grade of about* 30 *specimens of sputum and mean NANA content of* 7 *or* 8 *estimations from each subject* (r=0·334, P=0·02).

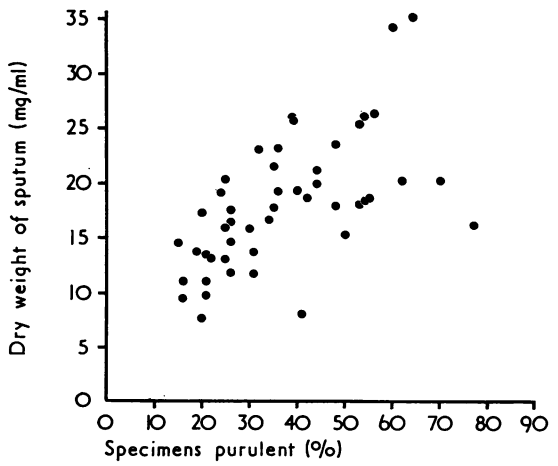

FIG. 12. *Mean dry weight of* 7 *or* 8 *specimens of mucoid sputum from each subject related to percentage of* 30 *monthly specimens which contained pus* (r=0·608, P=0·001).

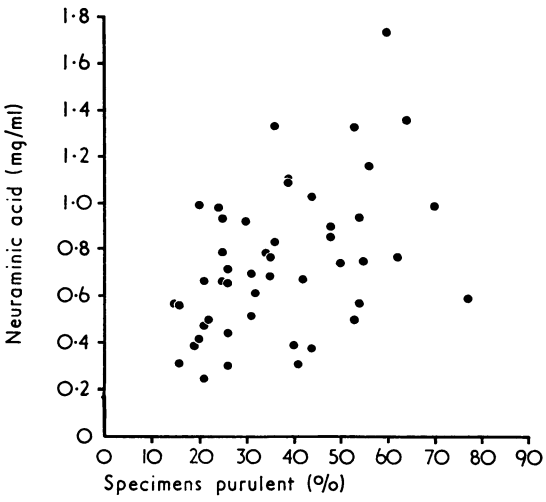

FIG. 13. *Mean NANA concentration of* 7 *or* 8 *specimens of mucoid sputum from each subject related to percentage of* 30 *monthly specimens which contained pus* (r=0·447, P=0·01).

secretions gave a higher yield of dry macromolecular material (r=0·608, P=<0·001) and contained a higher concentration of NANA (r=0·447, P=<0·01). There was no reciprocal relationship with the percentage of NANA in the dry material as would be expected if these changes were due to even small amounts of pus or cellular debris in the sputum analysed (vide Fig. 9).

DRY WEIGHT AND NANA CONTENT OF SPUTUM RELATED TO FREQUENCY OF FINDING *H. influenzae* IN SPUTUM AND TO EXACERBATION SCORES No correlation, such as that found between NANA levels and the frequency of finding pus in the sputum, was found between NANA and the frequency with which *H. influenzae* was cultured from the sputum nor with the exacerbation scores. Table V, however, was derived from the information given by Ogilvie for the 48 patients whose sputum was analysed and relates the exacerbation scores for each patient to the percentage of his monthly specimens of sputum which contained pus. There is no correlation, and these figures support the suggestion that exacerbations of chronic bronchitis are not always associated with acute bacterial infection but are not incompatible with the finding that bacterial overgrowth producing pus occurs more often in those patients with a high NANA level in their mucoid sputum.

TABLE V

EXACERBATION SCORES RELATED TO PERCENTAGE OF MONTHLY SPUTUM SPECIMENS CONTAINING PUS

Exacerbation Score	No. of Patients	Percentage of Specimens Containing Pus
0– 50	18	35·7
51–100	13	44·0
101–200	10	35·0
>200	7	35·1

DRY WEIGHT AND NANA CONTENT OF SPUTUM RELATED TO DURATION OF SYMPTOMS AND TO PRESENCE OF EMPHYSEMA There was no correlation between the chemical results and the occupation of the Newcastle men, nor with the duration or frequency of sputum production in either group. In the late chronic bronchitics there was no relation between the results of sputum analysis and the presence of emphysema on the chest radiograph.

DISCUSSION

Sputum is a complex mixture of mucous gland and goblet cell secretion, mucosal exudate and transudate, cellular debris and contributions from the upper respiratory tract. It cannot therefore be regarded as a simple biological fluid, and any conclusions to be drawn from its analysis must necessarily be related to a consideration of its source. This will be discussed in a further paper (Keal and Reid, in preparation). In a group of early, simple chronic bronchitics (Medical Research Council, 1965) it was anticipated that the sputum would consist mainly of bronchial mucous gland secretion with some contribution from the goblet cells and from saliva and might thus be taken as the nearest approach to 'normal' sputum, in itself a contradiction in terms, with which to compare other disease groups. The yield of macromolecular material from saliva is much less than that from sputum, and the percentage of neuraminic acid it contains is low (Fig. 5). It will therefore contribute little to the concentration of neuraminic acid in the sputum and is disregarded for the purpose of this study. This method of analysis provides, however, a useful distinction between excess saliva and a true bronchorrhoea in those patients in whom the distinction may be clinically difficult.

A neuraminidase is produced by many organisms and in particular by the influenza virus and by *Vibrio cholerae,* organisms which specifically attack mucosal surfaces. Marmion, Curtain, and Pye (1953) isolated from the bronchial secretion of patients with chronic bronchitis mucoproteins inhibiting the effects of neuramini-

dase and shown to contain 4% sialic acid (Howe, Rose, and Schneider, 1957).

The effects of influenza virus infection on the bronchial mucosa are well known (Hers, 1955) and the susceptibility to bacterial infection following influenza is well recognized. Gottschalk (1960), concluding his monographs on *The Chemistry and Biology of Sialic Acids and Related Substances,* suggested that the adaptive formation, by microbes inhabiting or invading the respiratory tract, of enzymes splitting off the terminal sialic acid from sialomucoproteins may be considered the microbes' answer to the host's defence mechanism.

Gibbons (1959) and Gibbons and Glover (1959) related the higher viscosity of pregnancy compared with oestrus bovine cervical mucus to the higher concentration of neuraminic acid it contained, other constituents being the same. This was also found in pseudomyxomatous gels (Odin, 1955) and in bronchitic sputum (Munies *et al.,* 1968). In the present study the relationship of viscosity to the concentration of neuraminic acid in mucoid sputum has been confirmed in a large group of patients—in spite of the fact that 'pourability' was used as a measure of viscosity and that, in each patient, the viscosity value is the mean of 30 specimens of sputum and the neuraminic acid is the mean of eight specimens. There is also, however, a similar correlation, not previously noted, between the sputum viscosity and the yield of macromolecular solids from the sputum. More recent work (Charman, 1971, personal communication), in which viscosity was measured on a cone and plate viscometer and chemical analysis was carried out on the same specimen, has confirmed these findings with a greater degree of significance. Munies *et al.* (1968) also measured viscosity and neuraminic acid on the same specimens and found a highly significant correlation between the two but did not measure the yield of macromolecular material. They did find, however, a peak value for neuraminic acid beyond which it remained constant while the viscosity continued to increase, suggesting that other factors besides the neuraminic acid affect the viscosity. In our cases all the sputum analysed appeared mucoid and it is unlikely that any increase in macromolecular material with viscosity was due to pus or cellular debris since this would cause a fall in neuraminic acid percentage (Fig. 9). It is possible that the degree of sputum hydration may also influence the dry weight and neuraminic acid concentration and hence the viscosity, but the association between viscosity and an increase in the percentage of neuraminic acid in the macromolecular material, while not statistically significant, suggests that alteration in sialomucoproteins is also a factor. This is supported by an earlier report (Keal, 1971a) that the percentage of neuraminic acid in the sputum of patients with asthma and bronchorrhoea may be changed by steroid therapy independently of the yield of macromolecular material.

SEASONAL VARIATION OF NEURAMINIC ACID The seasonal variation of neuraminic acid concentration in the sputum was an unexpected finding not previously described. It is paralleled by changes in the yield of macromolecular solids and in the percentage of neuraminic acid which they contain. It is unlikely, therefore, that the winter rise is due to the addition of pus or cellular debris or to dehydration but rather to either an alteration in mucous gland activity or to an increase in transudate. One possible cause of this is seen in the variation in atmospheric pollutants in Newcastle during the period of the study (Fig. 14) when the winter rise might be expected to increase bronchial irritation. The possibility of an effect of viral infection cannot be assessed on the evidence available.

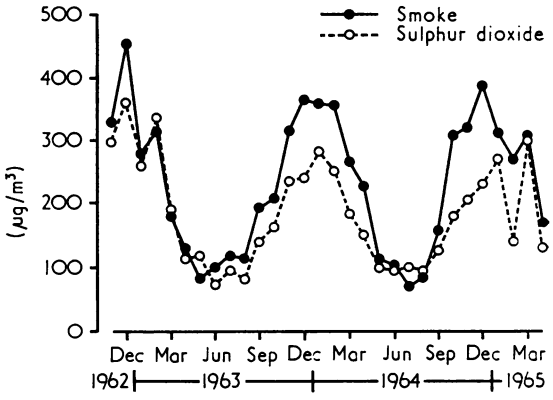

FIG. 14. *Seasonal variation in smoke and sulphur dioxide concentrations in Newcastle during period of this study.*

The consequences of the winter rise in the neuraminic acid content must remain speculative. On the evidence presented, an increase in the dry weight and neuraminic acid content would result in an increase in sputum viscosity. Does this lead to greater difficulty in expectoration and retention of secretions within the bronchial tree independently of bacterial infection? There is an increasing awareness that many 'exacerbations' of chronic bronchitis are not associated with either bacterial or viral infection. Fisher *et al.* (1969) found no evidence of an infective agent in 56% of 63

exacerbations studied in Edinburgh, and Semmens (personal communication, 1967) found no evidence of acute bacterial infection in over half of 60 chronic bronchitics admitted to hospital during an exacerbation.

Stuart-Harris (1971) states that hypersecretion of mucus resulting from environmental change allows organisms from the upper respiratory tract to lodge and multiply in the airways. While there is general agreement that pneumococci and *H. influenzae* are more often present in the sputum when it is purulent (May, 1953) and during exacerbations (Fisher *et al.*, 1969; Jenne *et al.*, 1970), *H. influenzae* can frequently be cultured from mucoid sputum during intermissions of the disease. Is the multiplication of *H. influenzae* in the lower respiratory tract during the winter months related to the increased neuraminic acid content of the sputum, to its effect on sputum viscosity, or to a combination of these factors? Reference has already been made to the 'virulence-enhancing' or 'resistance-lowering' properties of mucus and to the part played by the carbohydrate content.

In the present study there was a significant correlation between the neuraminic acid concentration and the percentage of the 30 monthly specimens which contained pus (Fig. 12; $P = < 0{\cdot}01$.) That is to say, the sputum is more often purulent in those patients who have a higher concentration of neuraminic acid in their mucoid sputum. The neuraminic acid may play some part in virulence enhancement by bronchial mucus by either the factor of increased viscosity or carbohydrate content.

SPUTUM VISCOSITY AND AIRWAYS OBSTRUCTION

While it is an attractive theory that the viscosity of sputum is related to the degree of airways obstruction or to the symptoms of cough and expectoration, it would be surprising if this were a close relationship in view of the complexity of the function of the mucociliary escalator and the many factors which may disturb it. Recently, bromhexine given by mouth has been shown to reduce the viscosity of mucoid sputum (Burgi, 1965; Hamilton, Palmer, and Gent, 1970) but evidence of benefit to the patient is inconclusive. Gent, Knowlson, and Prime (1969) found a small increment in ventilatory capacity during the winter months but not in the succeeding summer, while Langlands (1970) showed no change in the clinical condition or ventilatory capacity and Hamilton *et al.* (1970) found no change in these factors despite impressive changes in sputum volume and viscosity. It has been suggested that the greatest benefit may be obtained in the early stages of chronic bronchitis when hypersecretion of mucus is the predominant feature without gross infection or airways obstruction.

The subjects taking part in the Newcastle study were all 'early' bronchitics with a maximum breathing capacity greater than 50% of the predicted normal. The results of neuraminic acid estimation in this group do not suggest any relationship between sputum viscosity and either the degree of airways obstruction on entry to the study or its subsequent deterioration. There was no difference in the neuraminic acid content of the sputum in the 'late' severely disabled bronchitics compared with the earlier group.

We acknowledge the co-operation of Dr. Alan Ogilvie and his colleagues, who are carrying out a 10-year prospective study of a series of early bronchitics which will be published shortly, for the provision of sputum specimens and of certain information regarding their series. Dr. John Batten provided similar facilities for his patients. Professor B. Benjamin gave valuable statistical advice. This work was supported by the Tobacco Research Council and by the Medical Research Council and formed part of a thesis accepted for the degree of M.D. (London) (Keal, 1971b). The Ferranti-Shirley viscometer was provided for the department by the Cystic Fibrosis Research Trust.

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