Biochemistry and rheology of sputum in asthma

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In recent years there have been many advances in the epidemiology of chronic bronchitis and in the immunology and treatment of asthma but scant attention has been paid to a factor common to both diseases—the production of mucoid sputum. It is still not known why some men can smoke heavily all their lives without developing the hypersecretion of mucus which characterizes simple chronic bronchitis, nor why some patients with asthma have a profuse bronchorrhoea. Nor is it known why the hypersecretory state, once established, is so often, but not always, complicated by bacterial infection. Much has been written of the viscosity of sputum which does not stand up to measurement by modern techniques and with little knowledge of the part it plays in obstructive airway disease.

Neuraminic acid

Neuraminic acid is one of a group of sialic acids first described by Blix (1936) in bovine submaxillary gland mucus and by Klenk (1941, 1942) in the brains of children with Niemann-Pick disease or with amaurotic familial idiocy. It is widely distributed in all epithelial secretions and, together with ester sulphate, is responsible for the acidic properties of bronchial mucus gland secretion. It contributes from 2 to 6% of the dry weight of dialysed sputum with a mean concentration of 0.75 mg/ml of whole sputum in simple chronic bronchitis. It is also present in serum only to the extent of 0.82% of the dry weight but in similar concentration (0.66 mg/ml) to that found in sputum. The estimation of fucose in the sputum makes it possible to determine the predominance of bronchial mucus gland secretion or of serous exudate in the sputum since it is present in the former but not in serum (Keal, 1970).

Previous work had suggested the importance of neuraminic acid in determining the physical and biological properties of mucus. Gibbons (1959) and Gibbons & Glover (1959) related the changes in viscosity of bovine cervical mucus during the menstrual cycle to the neuraminic acid content—the viscid plug of pregnancy mucus having the highest level. Odin (1958) found that the viscid gel of pseudomyxomatous ovarian cysts contained 10% of neuraminic acid in the dry material compared with 2% in the less viscous material from pseudomucinous cysts.

The influenza virus and the Vibrio cholerae are rich sources of a neuraminidase. They are both organisms which attack mucosal surfaces and the influenza virus will cause haemagglutination (Hirst, 1941; Burnett, McCrea & Stone, 1946). This could be prevented by prior exposure of the virus to human serum or mucin with the liberation of neuraminic acid (Klenk, Faillard & Lempfrid, 1955; Odin, 1958). Marmion, Curtain & Pye (1953) isolated a glycoprotein which inhibited haemagglutination from the bronchial secretion of patients with chronic bronchitis and this was shown to contain 4% of sialic acid in the dry material (Howe, Rose & Schneider, 1957). Furthermore, Olitzki (1948) and Smith (1953) related the virulence-enhancing properties of mucus to three possible factors: the viscosity (now known to be related to the neuraminic acid content), the presence of particulate matter and to various polysaccharide components—this at a time when little was known of sialic acid in epithelial secretions. It is tempting to relate the presence of neuraminidase in viral and bacterial organisms to the neuraminic acid in bronchial mucosal gland secretion. Gottschalk (1960) concluded his monograph on the Chemistry and Biology of the Sialic Acids with the suggestion that the adaptive formation, by microbes inhabiting the respiratory tract, of enzymes splitting off the terminal sialic acid from sialo-mucoproteins may be considered the microbe’s answer to the host’s defence mechanism.

Sputum neuraminic acid in chronic bronchitis and its relation to viscosity

Since normal sputum is a contradiction in terms, the neuraminic acid content of mucoid sputum in simple chronic bronchitis was estimated and used as
a base-line for comparison with other diseases. A mean value was obtained for eight specimens from each of forty-eight patients in Newcastle collected over a period of 3 years. Over the same period Dr Alan Ogilvie at the Newcastle Bronchitis Centre graded the viscosity of some thirty specimens from each patient by an estimate of its ‘pourability’ (Table 1) and calculated a mean value for each patient.

TABLE 1. The grading of sputum viscosity by its 'pourability'

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Viscid sputum which adheres closely to the container when inverted</td>
</tr>
<tr>
<td>2</td>
<td>Viscid sputum oozing slowly from the container when inverted</td>
</tr>
<tr>
<td>3</td>
<td>Viscid sputum pouring from the container but remaining in one piece without fragmentation</td>
</tr>
<tr>
<td>4</td>
<td>Watery sputum pouring readily from the container but containing viscid particles</td>
</tr>
</tbody>
</table>

This concept of ‘pourability’ was subsequently related to the measurement of apparent viscosity using a Ferranti-Shirley rotational viscometer on fresh specimens of sputum from a number of hospital patients with a variety of lung diseases. The correlation is shown in Fig. 1 and Table 2.

Though lacking the precision of the instrumental method the estimation of the ‘pourability’ appears to be a valid indication of sputum viscosity in field studies.

The relationship between the viscosity of mucus and its neuraminic acid content was confirmed for sputum and is shown in Fig. 2 for the group of forty-eight patients with simple chronic bronchitis.

Further evidence of the relationship between the neuraminic acid content and the viscosity of sputum is seen in the similar patterns of Figs. 3 and 4 showing these values obtained for single specimens of sputum from patients with simple chronic bronchitis, asthma and bronchorrhoea and for saliva. The range of values and the standard deviation is greater in asthma than in simple chronic bronchitis; much lower values for viscosity and neuraminic acid are obtained in the sputum of patients with bronchorrhoea (vide infra) but, in every case, they are above those for saliva. The estimation of neuraminic acid or of viscosity serves to distinguish a true bronchorrhoea from excessive salivation in those patients in whom the distinction may be clinically difficult.

In the Newcastle group of forty-eight patients with simple chronic bronchitis there was no relationship between the neuraminic acid content of the

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**Fig. 1.** The correlation of ‘pourability’ grading with measured viscosity.

**Fig. 2.** The relationship of sputum viscosity estimated by the ‘pourability’ to the neuraminic acid content of the sputum (P < 0.02).

**TABLE 2.** The correlation of sputum pourability with apparent viscosity measured on the Ferranti-Shirley rotational viscometer

<table>
<thead>
<tr>
<th>'Pourability' grade</th>
<th>Number of specimens</th>
<th>Apparent viscosity (centipoises)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>15</td>
<td>32</td>
</tr>
<tr>
<td>1-2</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>2-3</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>3-4</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>
sputum or its pourability and the degree of airways obstruction present on entry to the study or to the patients' subsequent deterioration.

It may be noted in passing that the sputum of patients with cystic fibrosis does not have a high viscosity (Fig. 3). As the sputum in these cases was usually purulent the neuraminic acid content (Fig. 4) cannot be compared with that in the other conditions with mucoid sputum.

**Asthma with bronchorrhoea—the effect of steroid therapy**

In chronic bronchitis or in asthma it is unusual for patients to produce more than 25 ml of sputum in 24 hr (Miller, Tinker & Fletcher, 1965; Ashcroft, 1965) but occasionally either condition may be associated with a profuse bronchorrhoea—defined in this study as more than 100 ml of sputum daily. These formed a convenient group in which to study the day-to-day variation in the neuraminic acid content of the sputum during treatment with steroids, given primarily for the relief of the airways obstruction. Some fifteen cases have been studied and the following reports are illustrative of the different results obtained.

**Case 1** (Fig. 5). This shows the changes in the neuraminic acid content of the sputum in a man of 30 with intrinsic asthma and bronchorrhoea producing 200 ml of mucoid sputum daily. The initial level of neuraminic acid was low (2% of the dry weight) and rapid improvement of ventilatory capacity was associated with a reduction of sputum volume and a rise in the neuraminic acid content, both as a percentage of the dry weight and in the concentration per ml of sputum. There was no change in the yield of dry material per ml of sputum.

**Case 2** (Fig. 6). This shows the results of sputum analysis for a woman of 38 with extrinsic asthma and bronchorrhoea who also experienced infective episodes. The wheezing and bronchorrhoea had persisted after antibiotics and routine bronchodilators and prednisone was therefore used. The initial level of neuraminic acid is again low and an improvement in ventilatory capacity was again associated with the abolition of sputum and an overall rise in the neuraminic acid content. There was again little change in the dry weight of the sputum except on the third day when pus was again present in the sample. The effect of this is seen as a rise in the yield of dry material with a corresponding fall in the percentage of neuraminic acid it contained.

**Case 3** (Fig. 7). This shows the results of sputum analysis in a man of 50 with intrinsic asthma and bronchorrhoea. The peak expiratory flow rate rose with steroid treatment from 110 to 400 l/min but there was little reduction in the volume of sputum.
In contrast to the two previous cases the initial level of neuraminic acid at 4% of the dry weight was nearer to the mean value found in simple chronic bronchitis and did not change significantly with treatment. This patient was bronchoscoped and the neuraminic acid content of the bronchial aspirate (BR in Fig. 7) is seen to be near that found in the sputum, confirming that any admixture with saliva has little effect on the results of analysis.

Case 4 (Fig. 8). This illustrates the findings in a man of 37 with intrinsic asthma and bronchorrhoea. The initial level of neuraminic acid is again high and, although the forced expiratory volume in 1 sec rose from 1350 to 3200 ml and there was some reduction in sputum volume, he continued to produce 50 ml of sputum daily and there was little change in the neuraminic acid content.

Case 5 (Fig. 9). This shows, in contrast to the earlier cases, the results of steroid treatment in a man of 64 with severe chronic bronchitis and bronchorrhoea. The initial level of neuraminic acid is again high; there was no reduction of airways obstruction or of sputum volume and no change in the neuraminic acid content of the sputum.

Fig. 5. Male, age 30. Intrinsic asthma. The changes in sputum volume, dry weight and neuraminic acid content during treatment with prednisone.

Fig. 6. Female, age 38. Extrinsic asthma and bronchitis. The changes in sputum volume and neuraminic acid content during treatment with prednisone. The effect of pus in the sputum on day 3 is seen as an increase in dry weight with a fall in the percentage of neuraminic acid.

Though these cases are by no means typical of asthma, in which sputum is usually scanty, the results suggest that airways obstruction may be relieved by steroid therapy independently of the sputum volume and in spite of changes in the neuraminic acid content of the sputum which are known to be associated with an increase in the viscosity of the sputum. They must therefore throw some doubt on the part played by the viscosity of the sputum in causing airways obstruction and on the role of drugs whose sole demonstrable effect is in the reduction of sputum viscosity in vitro. The squamous metaplasia of the bronchial epithelium (Messer, Peters & Bennett, 1960) and the hypertrophy of muscle (Dunnill, Massarella & Anderson, 1969) found in asthma are more likely causes of mucus retention and plug formation.

The initial levels of neuraminic acid may be some guide to the results to be expected from steroid therapy and the subsequent changes suggest that the mode of action lies in the reduction of transudate rather than in any change in the bronchial mucosal
gland secretion. The finding of a high level of neuraminic acid suggests the presence of bronchial mucosal gland hypertrophy due to associated bronchitis.

**The neuraminic acid content of bronchial casts in asthma**

In the course of this study opportunity was taken to determine the dry weight and neuraminic acid content of bronchial casts from two patients with quite different clinical pictures. The results are shown in Table 3 and are compared with the mean values for the mucoid sputum of simple chronic bronchitis.

**Table 3. The dry weight and neuraminic acid content of bronchial casts and of mucoid bronchitic sputum**

<table>
<thead>
<tr>
<th></th>
<th>Dry weight (mg/g wet weight)</th>
<th>Neuraminic acid (% of dry weight)</th>
<th>Neuraminic acid (mg/g wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case A</td>
<td>15.4</td>
<td>2.33</td>
<td>0.36</td>
</tr>
<tr>
<td>Case B 1</td>
<td>235</td>
<td>4.115</td>
<td>9.67</td>
</tr>
<tr>
<td>2</td>
<td>211</td>
<td>2.984</td>
<td>6.296</td>
</tr>
<tr>
<td>3</td>
<td>336</td>
<td>4.905</td>
<td>16.6</td>
</tr>
<tr>
<td>Bronchitic sputum</td>
<td>18.2</td>
<td>4.17</td>
<td>0.75</td>
</tr>
</tbody>
</table>

*Case A* was a man of 53 with intrinsic asthma who experienced wide variations of airways obstruction over short periods of time. From a state of relative comfort with a peak expiratory flow rate of over 200 l/min he would deteriorate into a state of asphyxia with a ‘silent’ chest. On these occasions he required urgent bronchoscopy in bed when numerous branching bronchial casts could be sucked out with some relief of airways obstruction sufficient to allow assisted ventilation to continue. In such circumstances there was clearly no time for dehydration to play much part in the formation of morphological plugs and the results of analysis of these gave figures for the dry weight and neuraminic acid content similar to those of mucoid sputum.

*Case B* was a schoolboy of 14 years with atopic asthma and eczema. He was admitted to hospital with a 3-weeks’ history of unproductive cough and of increasing breathlessness and cyanosis for 2 weeks. The breath sounds were poor but with only slight wheezing and the lung function tests showed a gross restrictive defect. There was no response to bronchodilators, mucolytic agents or steroids but his condition improved when he began to cough up large, branching bronchial casts. The analysis of these showed a very high dry weight but with a normal percentage of neuraminic acid in the dry material suggesting that dehydration of mucus in the bronchial tree over a long period of time played a large part in
The formation of these casts and was compatible with the history.

These two cases show that more than one mechanism may lead to the formation of bronchial casts. Dehydration of mucus in the bronchial tree may play some part but in the first case some other factor—such as a change in the glycoprotein linkages or an alteration in the calcium or electrolyte content affecting the viscosity or some other physical factor—must be involved.

The contribution of sputum analysis to the evaluation of bronchial lavage in asthma

Dr Norman Macdonald and his colleagues at Clare Hall Hospital kindly allowed me to study material from his patients with asthma treated by bronchial lavage. Sputum was collected for analysis on the 2 days preceding lavage and on the 2 subsequent days. The lavage fluid recovered was also obtained and the mucus extracted from it by centrifugation. Of about twenty patients studied the results of analysis for five are shown in Table 4, and the changes in ventilatory function are related to the percentage of fluid recovered, to the weight of mucus recovered and to previous steroid treatment in Table 5.

The values for the dry weight and neuraminic acid content of the sputum prior to lavage covered the same wide range as in the larger group of asthmatics shown in Fig. 4. They were not related to the degree of airways obstruction or to the benefit obtained from lavage. There was no consistent change in the dry weight or neuraminic acid content following lavage. The greatest immediate improvement in ventilatory function was seen in the two patients (M.P. and P.F.) from whom the greatest weight of mucus was removed by the procedure. These were also the two patients who had not had previous steroid treatment.

Conclusion

In these patients with asthma the estimation of neuraminic acid in the sputum serves as a marker of glycoprotein but does not in itself indicate the source of the sputum from bronchial mucosal glands or from transudate. The neuraminic acid content has been related to the viscosity of the sputum but the changes found in patients with asthma and bronchorrhoea during steroid therapy suggest that the viscosity of the sputum bears little relation to the degree of airways obstruction. The initial levels of neuraminic acid may predict the response to steroid treatment.

Table 4. The dry weight and neuraminic acid (NA) content of sputum before and after bronchial lavage, and of the mucus recovered by lavage

<table>
<thead>
<tr>
<th></th>
<th>Dry weight (mg/ml)</th>
<th>NA % dry wt</th>
<th>NA (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-L</td>
<td>LM</td>
<td>Post-L</td>
</tr>
<tr>
<td>T.B.</td>
<td>34:43</td>
<td>3:15</td>
<td></td>
</tr>
<tr>
<td>C.G.</td>
<td>8:49</td>
<td>21:05</td>
<td>10:58</td>
</tr>
</tbody>
</table>

Pre-L, sputum before lavage; LM, mucus separated from lavage fluid; Post-L, sputum after lavage.
and a high level is probably indicative of mucus gland hypertrophy due to associated chronic bronchitis.

Acknowledgments

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