THE VISCOSITY OF BRONCHIAL SECRETION

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SUMMARY

1. The viscosity of sputum in patients with chronic bronchitis has been measured with the Weissenberg rheogoniometer, a cone and plate system, using oscillatory movement at low shear rates.

2. A characteristic pattern is described not previously reported for sputum or any other biological fluid.

3. Sputum viscosity decreased steadily with increasing shear rate; over a narrow range of shear rates a 'notched plateau' was shown. The destruction of this plateau by higher frequencies of oscillation was attributed to breakdown or rearrangement of the gel structure.

4. The effects of time on viscosity measurement, presumably caused by drying, are also described.

A key to the understanding of the natural function of bronchial secretion and its role in disease lies in the study of its rheological properties. One of the most fundamental of these is viscosity. Sputum has been described as a visco-elastic semi-solid or gel, behaving as a non-Newtonian fluid (White & Elmes, 1960). The viscosity of a non-Newtonian fluid varies with the shear rate applied. Most reports describe a marked reduction of sputum viscosity after testing at higher shear rates (Goldfarb & Buchberg, 1964; Lieberman, 1968), presumably due to a breakdown of the molecular arrangement.

Of the methods used to measure sputum viscosity the rotational viscometer has proved the most acceptable. Blanshard (1955) advocated a cone and plate rotational viscometer to measure sputum viscosity at constant shear rates. This method has the added advantage for biological fluids that accurate measurement can be made with small volumes. Baldry & Josse (1968) have suggested, without providing details, that their cone and plate viscometer does not give repeatable results. The Weissenberg rheogoniometer, a sophisticated cone and plate...
viscometer, has given repeatable results for sputum in the experiments reported here as well as on a wide range of fluids (Davies, 1966; Walters, 1968). This instrument offers lower shear rates than other cone and plate viscometers and differs from them in that oscillatory as well as rotational movement can be applied.

Comparison of sputum specimens can be based on 'apparent' viscosity, that is, the viscosity measured at an arbitrarily chosen shear rate (Palmer, 1960; Bruce & Kumar, 1968). The shear rate may be chosen because of its supposed physiological significance, for example, the force exerted by ciliary movement (Goldfarb & Buchberg, 1964). Denton (1960) and Lieberman (1968) studied a range of shear rates using rotational testing but did not give their results in absolute units of viscosity. Lieberman (1968) used sputum that had been deep frozen although Denton (1960) had reported that freezing caused reduction of viscosity, a finding which we have confirmed.

In the present studies the Weissenberg rheogoniometer has been used to measure the viscosity of freshly produced sputum, examined without any pre-treatment. It was the original intention to include rotational testing but it was found that rotation caused the specimen to slide away from the centre of the cone. This did not occur with oscillatory movement which has the additional advantage that it might be expected to minimize structural breakdown of the complex molecules. This oscillation was applied through a small angle and over a range of low frequencies to further reduce the possibility of breakdown. Absolute values of viscosity have been calculated in poises for each of a range of oscillatory frequencies. A pattern of variation in viscosity has been found not previously described for a biological fluid.

MATERIALS AND METHODS

Materials

Seven sputum specimens were studied from six patients. Sputum was collected over their first waking hour, starting roughly at 07.00 hours. Some collections included sputum coughed up on several occasions during the hour. As more than one experiment was performed on each sputum collection the term 'specimen' is used to refer to the whole collection and 'aliquot' to the part used for individual estimations of viscosity.

All patients studied had obstructive chronic bronchitis (Medical Research Council, 1965) and were in hospital because of an 'exacerbation' of shortness of breath. At the time of collection all sputum specimens were mucoid and free from pus, and some patients were still receiving antibiotic treatment. All patients had been heavy cigarette smokers and were smoking, though rather less than previously, at the time of these tests. Two patients (C.G. and H.B.) also suffered from asthma and were receiving treatment by corticosteroids. Two patients (C.G. and F.H.) produced such a large volume of sputum (over 150 g/day) that they were considered to have bronchorrhoea. The clinical details of the patients are given in Table 1.

Methods

Specimens were kept at room temperature until tested since chilling had been found to cause a reduction in the viscosity of sputum. The tests were generally started within 2 hr and completed within 4 hr of collection.

In the Weissenberg rheogoniometer, the specimen is placed between a lower conical and an upper flat platen (Fig. 1); the lower platen oscillates through a small angle when driven through
a 60-speed gear box and a sine wave generator; in these experiments the angle was maintained at $2.4^\circ$. The upper platen is suspended by a torsion bar from a fixed mount and maintained in a central position by an air bearing. Transducers are fitted to indicate the movement of both platens. The signals from the transducers are fed to a suitable recorder; measurements are made of the amplitude of both waves and of the phase difference between the two waves.

### Table 1. Clinical details

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Sputum (g/day)</th>
<th>Duration of sputum production (years)</th>
<th>Steroid therapy</th>
<th>Additional diagnosis</th>
<th>Radiographic appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.C.</td>
<td>M</td>
<td>59</td>
<td>20</td>
<td>12</td>
<td>-</td>
<td></td>
<td>Widespread emphysema</td>
</tr>
<tr>
<td>C.G.</td>
<td>M</td>
<td>63</td>
<td>150</td>
<td>12</td>
<td>+</td>
<td>Asthma and bronchorrhoea</td>
<td>Normal</td>
</tr>
<tr>
<td>H.B.</td>
<td>F</td>
<td>59</td>
<td>90–120</td>
<td>30</td>
<td>+</td>
<td>Asthma</td>
<td>Localized emphysema</td>
</tr>
<tr>
<td>B.R.</td>
<td>M</td>
<td>72</td>
<td>Tr–30</td>
<td>6</td>
<td>-</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F.L.</td>
<td>M</td>
<td>68</td>
<td>30</td>
<td>20</td>
<td>-</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>F.H.</td>
<td>M</td>
<td>45</td>
<td>150</td>
<td>11</td>
<td>-</td>
<td>Bronchorrhoea</td>
<td>Localized emphysema</td>
</tr>
</tbody>
</table>

**Fig. 1.** Diagram to illustrate the details of the cone and plate, the drive and torsion bar. $P =$ platens, $T =$ torsion bar, $L =$ lever from which movements of the torsion bar are recorded, $B =$ air bearing. Reproduced, by permission of the Editor, from *Federation Proceedings* (1966), 25, 1069.
Between six and twelve frequencies were investigated on each specimen; at each frequency at least five cycles of oscillation were recorded. The lowest frequency selected, 0:01 sec\(^{-1}\), was that which gave sufficient recordings within 20 min; the highest frequency, 2:5 sec\(^{-1}\), was that which gave sine wave recordings without interference from the natural frequency of the torsion head. During each cycle of oscillation the platen came to rest at the limit of its movement; the shear rate reached a maximum as the platen passed through its mid position. In these circumstances, the fluid was subjected to a shear rate which varied during each cycle of oscillation from 0 sec\(^{-1}\) to 0:029 sec\(^{-1}\) at a frequency of 0:01 sec\(^{-1}\); at a frequency of 1:0 sec\(^{-1}\) it varied from 0 sec\(^{-1}\) to 2:02 sec\(^{-1}\).

To minimize humidity variation, the experiments were performed in a room at constant temperature (19:5 ± 1°) with the platens enclosed in a small chamber. The platens chosen were of 5 cm diameter and 2° cone angle; in most experiments the torsion bar was \(\frac{1}{10}\) in. diameter and the natural oscillation frequency was about 3:5 sec\(^{-1}\).

Both ultraviolet and pen recorders may be used with the rheogoniometer. In these experiments the pen recorder was selected as it is more suitable for low frequency recordings. The signal to the recorder was fed through a filter which cut out 'noise' with frequencies greater than 1 sec\(^{-1}\). Over the range of frequencies studied it was necessary to make one change in the paper speed of the recorder; this change was effected at the same frequency during each experiment and occasioned a delay of about 5 min.

The precautions taken during these experiments to eliminate errors due to the recording apparatus were similar to those taken in the earlier experiments of Palfrey & White (1968). Individual observations were carried out separated by the shortest possible time interval.

Calculations. The viscosity was calculated from the equation

\[
\eta = A \cdot B \cdot \frac{\Delta_t}{\Delta_i} \cdot \frac{1}{\sin \phi} \text{ dyne sec cm}^{-2}
\]

after the manner of Palfrey & White (1968). The quantity \(A\) is dependent on the shape of the platens and torsion bar and is therefore constant throughout each experiment. The quantity \(B\) depends on the frequency of oscillation and the natural oscillation frequency of the torsion head; \(B\) decreased as the frequency of applied oscillation was raised but was always positive in these experiments. The quantity \(\Delta_t\) is the amplitude of the movement at the torsion head transducer, measured in thousandths of an inch; \(\Delta_i\) is the corresponding measurement at the input transducer. The angle \(\phi\) (in degrees) represents the phase difference between the movement of the two platens.

RESULTS

The pattern of results was similar for all specimens studied. Figs. 2–5 illustrate the results obtained with the pen recorder for one patient (R.C.).

The ratio \(\Delta_t/\Delta_i\). The formula for calculating viscosity includes the ratio \(\Delta_t/\Delta_i\) and the manner in which this varies with the frequency of oscillation is illustrated in Fig. 2. The curves PQ and RS represent two series of measurements using separate aliquots of one specimen. For the curve PQ the first measurement was made at a low frequency of 0:01 sec\(^{-1}\); for each subsequent measurement the frequency of oscillation was increased by a factor of approximately 2:5, the final measurement being made at a frequency of 2:5 sec\(^{-1}\). Thus the curve PQ
Viscosity of bronchial secretion was obtained at increasing frequencies. The curve RS was obtained at similar frequencies but these were applied in descending order. Both curves show that the value $\Delta_T/\Delta_i$ increases as the oscillation frequency rises and both approximate to a straight line.

At the lowest frequency tested (0.01 sec$^{-1}$) the value of $\Delta_T/\Delta_i$ is smaller if the test was carried out at the beginning of the series (P) than at the end of the series (S). Similarly at the highest frequency (2.5 sec$^{-1}$) the first value of the series (R) was lower than the last in the series (Q). It follows that the line PQ must cross the line RS at an intermediate point; this cross-over is observed in this instance at a frequency of 0.08 sec$^{-1}$.

Angle $\phi$. The variation of the angle $\phi$ with the frequency of applied oscillation is illustrated in Fig. 3. For this quantity the curves PQ and RS coincide closely and there is no cross-over. The value of $\phi$ increases from the lowest frequency, 0.01 sec$^{-1}$, to a maximum of 0.06 sec$^{-1}$ and then decreases to a minimum of 0.14 sec$^{-1}$; from this point it rises steeply and is continuing to rise at the highest frequency tested 2.5 sec$^{-1}$, where it has attained a value of approximately 100$^\circ$.

The formula used in calculating the viscosity requires the sine of the angle $\phi$. The relation between sin $\phi$ and the frequency of oscillation shows a pattern similar to that for $\phi$, and is not separately illustrated.

Viscosity. The variation of viscosity with frequency of oscillation is illustrated in Fig. 4. The curves PQ and RS were obtained at increasing and decreasing frequencies respectively. Over the range studied, viscosity decreases with increasing frequency. The curve PQ was obtained from estimations at increasing frequencies and gives an initial viscosity of approximately 300 poises at a frequency of 0.01 sec$^{-1}$, decreasing to 60 poises at 0.06 sec$^{-1}$. Between frequencies of 0.06 sec$^{-1}$ and 0.15 sec$^{-1}$ there is no apparent alteration in viscosity; thereafter the viscosity decreases steadily to a value of 9 poises at the highest frequency studied, 2.5 sec$^{-1}$. The range of frequency over which there is no change in viscosity corresponds to the peak obtained when the angle $\phi$ is plotted against frequency (Fig. 3).
With the same oscillation frequencies, applied in descending order, the viscosity plot, RS, is approximately a straight line; the viscosity increases from about 5 poises at the highest frequency to 400 poises at the lowest frequency. Unlike PQ, there is only a minimal change in slope of the curve between frequencies 0.06 sec\(^{-1}\) and 0.15 sec\(^{-1}\). Both curves, PQ and RS, show the cross-over effect already described for \(\Delta T/\Delta t\) at a corresponding frequency of oscillation, 0.09 sec\(^{-1}\).

At a given frequency the viscosity was higher when measured towards the end of a series of tests than when measured early in the series. The difference between the two values suggests that drying of sputum has occurred to give higher viscosity.

**Time and viscosity.** The effect of time on sputum viscosity is further illustrated by the results on one aliquot presented in Fig. 5. Curve PQ represents a series of tests carried out from low to high frequency and separated by minimal time intervals. At Q the aliquot of sputum was left between the platens, without oscillation, for 1 hr; the measurements represented by RS were then performed on the same aliquot, over the same range but proceeding from the highest to the lowest frequency. The dotted line QR, at a frequency of 1.0 sec\(^{-1}\), represents an increase in viscosity from 16 poises to 160 poises over a period of 1 hr. At the lowest frequency, 0.01 sec\(^{-1}\),
the viscosity is approximately 580 poises at the beginning of the series (P) compared with 47 000 poises at the end of the series (S); at all intermediate frequencies the values on the plot PQ are lower than on RS. The dotted line represents a ten-fold increase in viscosity in 1 hr when the specimen was left between the platens without oscillation. The divergence of PQ and RS as they are traced towards lower frequencies shows an eighty-fold increase in viscosity which may be attributed to the longer time intervals between the readings at P and S. This increase in viscosity with the passage of time may reflect drying, or may be due to some other change not yet characterized.

Analysis of notched pattern. In the range of oscillation frequencies where viscosity values depart from the straight line to form a plateau, detailed measurements were made at the smallest frequency intervals possible with the Weissenberg rheogoniometer. Results from another patient (C.G.) are shown in Fig. 6. In this case, PQ represents a series of estimations performed at ascending frequencies from 0-01 sec \(^{-1}\) to 1-5 sec \(^{-1}\) with numerous estimations between 0-06 sec \(^{-1}\) and 0-25 sec \(^{-1}\). In general, viscosity shows a steady fall from the lowest frequency studied 0-01 sec \(^{-1}\), to the highest frequency, 1-5 sec \(^{-1}\), except over the range 0-06–0-2 sec \(^{-1}\). Up to a frequency of 0-06 sec \(^{-1}\) the viscosity decreases from 650 poises to 180 poises, rises to a peak of 420 poises at a frequency of 0-08 sec \(^{-1}\), then falls to a value of 215 poises at 0-15 sec \(^{-1}\) before a second sharp rise in viscosity, to 340 poises, between 0-15 and 0-20 sec \(^{-1}\). After this point there is a steady fall in viscosity to 6 poises at the highest frequency studied, 1-5 sec \(^{-1}\).

It is perhaps relevant that between the observations at frequencies of 0-06 and 0-08 sec \(^{-1}\) it was necessary to change the speed of the recorder, which involves a delay of about 5 min. This corresponds to the first sudden increase in viscosity on the curve PQ.
Another series of estimations on a separate aliquot of this patient's sputum was performed over the same oscillation frequency range but in descending order from 2.5 to 0.01 sec\(^{-1}\) (RS). This plot approaches a smooth curve and does not show the irregular pattern of PQ. A similar change in recorder speed and consequent delay occurs at the same point on the curve RS without any marked increase in viscosity.

With large increments in frequency a plateau was seen in the plot of viscosity. A reduction in the size of the increment used to study specimens from patients F.L. and H.B. revealed a single notch in the plateau. With further reduction in the increment size on patients B.R. and C.G. a double notch was revealed. The region of the plateau and notching always occurs within an identical frequency range (between 0.06 and 0.20 sec\(^{-1}\)) and is independent of absolute viscosity values for the sputum. The viscosity values range between 60 and 1000 poises in this region in the cases studied.

_Sputum variation._ Sputum has a range of viscosity values, dependent on the shear rate applied, characteristic of a non-Newtonian fluid. Three frequencies of oscillation, 0.01, 0.1 and 1.0
Viscosity of bronchial secretion

Viscosity of bronchial secretion have been selected at which to compare absolute values of viscosity. For each patient the results were obtained from one sputum aliquot and are given in Table 2.

At each of the chosen frequencies there is a very large variation in the viscosity of sputum when different patients are compared, e.g. at a frequency of 0.01 sec\(^{-1}\) the lowest value is 285 and the highest 800 000, a 3000-fold variation. In spite of the variation in absolute viscosity values, all these sputa gave a plateau in the viscosity plot similar to that shown in Figs. 5 and 6.

![Viscosity plot](image)

**FIG. 6.** Viscosity (poises) plotted against oscillation frequency (sec\(^{-1}\)). PQ and RS are derived from different sputum aliquots (from a different patient to that represented in Figs. 1–4). The plot PQ was obtained at increasing oscillation frequency and shows a complex pattern, largely lost in RS, obtained at decreasing frequencies. Between frequencies 0.06 and 0.25 sec\(^{-1}\) measurements were made at the smallest intervals permitted by the rheogoniometer giving a more detailed study than that shown in Fig. 4. (Case C.G.).

**DISCUSSION**

**Notched plateau.** A characteristic notched plateau has been found in the viscosity plot for all sputa examined. This is independent of the absolute level of viscosity and is always seen over the same narrow range of frequencies. The notched plateau occurred over the same frequency range in the four sputa in which tests were made at sufficiently small frequency intervals; even in the other two a plateau was apparent over the same frequency range although the intervals were too large to reveal notching. This pattern was less obvious when studied at descending frequencies, the characteristic notching being completely removed and the plateau markedly reduced after testing at higher oscillation frequencies.

A number of factors can be excluded as the cause of this pattern. The range of frequencies at which the plateau was seen did not relate to the natural frequency of the torsion head. A change in pattern might be caused by the presence of air bubbles, but this was avoided by careful loading of specimens. Occasional cells may be found even in mucoid sputum but as
such cells would be less than 100/mm³ in number and 10 μ diameter they would constitute less than 0-1% by volume. Such a proportion of cells would be unlikely to affect viscosity. Furthermore, any of these factors would be unlikely to produce the same effect at corresponding frequencies in all samples. The change in viscosity that occurs with change in recorder speed does not explain the plateau, since it is only present at increasing frequencies.

**General pattern.** In addition to demonstrating a notched plateau, these experiments have confirmed that sputum behaves as a non-Newtonian fluid, that is, its viscosity decreases with an increase of the shear rate at which it is tested. Synovial fluid is the only other fluid containing glycoproteins that has been studied in detail, and under identical conditions to those used here for sputum. It gives a smooth plot of viscosity against oscillation frequency (Palfrey & White, 1968), viscosity decreasing with increasing frequencies but without any plateau.

**Chemical basis.** Sputum is a secretion produced by the surface epithelium goblet cells and by the mucous and serous cells in the glands of the bronchial wall, mixed with saliva and tissue fluid transudate. The bronchial mucous and serous cells produce a range of acid glycoprotein including sialomucin, sulphomucin and sulpho-sialomucin (Reid, 1967; Lamb, 1969; Lamb & Reid, 1969). Other protein molecules have also been identified including serum proteins and those more specific to bronchial secretions (Havez et al., 1967). Gibbons & Roberts (1963) have recovered a small protein molecule from sputum which they suggest is linked to the side chains of the acid glycoprotein. The precise relation between these various proteins is not yet clear. Synovial fluid is reported to consist almost entirely of one type of acid glycoprotein, a hyaluronoprotein (Davies, 1969; Ogston & Stanier, 1950, 1953).

The overall reduction in sputum viscosity with increasing oscillation frequency is comparable to that seen in synovial fluid and probably represents a similar change in the glycoprotein. This may be realignment of the molecules, alteration in their shape or in their relation to associated water molecules. It is also possible that this change in viscosity may be caused by changes in the structure of the molecules or the number and site of bound ions.
The characteristic notched region found with sputum probably reflects the fact that, unlike synovial fluid, it is a mixture of secretions; more particularly it may reflect the variety of acid glycoproteins known to be present.

The absence of notching and the reduction of the plateau in sputum after testing at higher frequencies suggests that rapid oscillation causes a more dramatic change in the molecules than that described for a synovial fluid. Such a change is most likely to be a breakdown of a particular type of cross-linkage, which may be between the side chains of the acid glycoproteins or between these and other protein molecules. This suggestion is supported by the observation that the plateau occurs over a characteristic range of frequencies in all sputa tested.

**Viscosity and disease.** The wide range of sputum viscosity summarized in Table 2 emphasizes the variation found in disease, especially at lower shear rates; this has been independently reported (Sturgess, 1969). The sputum studied in the present tests came from patients with obstructive chronic bronchitis; two in addition suffered from asthma and two were being treated with corticosteroids. The significant feature of these results is that sputa from all these patients showed a similar notched plateau.

Keal has shown that in some cases of bronchorrhoea steroid therapy caused change in the chemical constituents of the acid glycoproteins in sputum (Keal, personal communication; Reid, 1967). Nothing is known of the effect of corticosteroids on sputum viscosity. It appears from histochemical studies that different diseases, with which hypersecretion of the bronchial glands is associated, are not characterized by abnormal constituents but by abnormal proportions of the usual constituents (Reid, 1967). The uniformity of the rheological pattern seems to support this.

**Viscosity and time.** Blanshard (1955) reported a three- to four-fold increase in sputum viscosity according to the degree of hydration of the patient. Denton (1960) demonstrated changes in viscosity that he associated with drying of sputum.

If the viscosity of sputum is measured at the same frequency, an increase of ten-fold has been observed after a delay of 1 hr. Over longer periods this increase may be as much as 100-fold. Inspection of the specimen shows that there is some evaporation from the periphery of the platens, but this evaporation is similar in extent to that seen in synovial fluid, where the resultant viscosity increase is less than 5%.

This time-dependent effect is greater than any previously described and is being further investigated. Preliminary results suggest that at a particular frequency the logarithm of the rate of increase of viscosity is linearly related to time measured from the start of testing. Drying may be the most relevant factor but experiments at controlled and selected humidities will be necessary to establish its true importance.

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