Bronchial secretion from normal human airways after inhalation of prostaglandin F2α, acetylcholine, histamine, and citric acid

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Bronchial secretion from normal human airways after inhalation of prostaglandin F2α, acetylcholine, histamine, and citric acid. Sputum produced by normal subjects after inhalation of prostaglandin F2α, acetylcholine, histamine, and citric acid has been analysed. Prostaglandin F2α was the most effective of the drugs in promoting sputum production. The material expectorated after inhalation of prostaglandin F2α shows the characteristics of mucoid sputum from patients with chronic bronchitis. The apparent viscosity and the concentration of marker substances for bronchial glycoprotein was in the lower part of the range found in mucoid chronic bronchitic sputum. The concentration of marker substances for serum glycoproteins and tissue fluid transudate were below the range found in chronic bronchitis, indicating that, in disease states, in addition to bronchial mucus there is a marked tissue fluid transudate component. Sputum produced after inhalation of acetylcholine and histamine contained relatively more tissue fluid transudate than sputum produced after inhalation of prostaglandin F2α. Sputum produced after inhalation of prostaglandin F2α is of special value in indicating the nature of secretion from normal airways.

Sputum, that is, bronchial fluid mixed with saliva, is generally available only in disease states. The rheological properties and chemical composition of sputum from patients with mucus hypersecretion—chronic bronchitis, bronchiectasis, cystic fibrosis, or asthma—have been well documented (Lopez-Vidriero et al., 1977) but very little is known about bronchial secretion from normal subjects.

Various methods have been used to obtain bronchial fluid from normal subjects. Some are known to increase serum transudation by an osmotic effect (Bickerman et al., 1958) while in others the small amount of secretion is flushed out with loss of the volumetric baseline (Turgeon et al., 1971; Falk et al., 1972).

During experiments carried out on healthy subjects to assess the bronchoconstrictor effect of prostaglandin F2α it was noticed that, after inhaling the drug, the subjects coughed and expectorated for about 20 minutes. This sputum seemed to justify further analysis since it represented secretion from the bronchial tree of a healthy subject.

In the present study administration of prostaglandin F2α (PGF2α) and other drugs has been used to obtain secretions from the normal bronchial tree. The effect of other known bronchoconstrictor and irritant agents—acetylcholine, histamine, and citric acid—has also been investigated to compare the effect of these drugs with that of PGF2α.

Bronchial glycoprotein contains relatively large amounts of fucose, N-acetyl neuraminic acid (NANA), and sulphate but no mannose, while serum glycoproteins are rich in mannose and NANA and contain little or no fucose or sulphate. Fucose and sulphate have therefore been used as marker substances of bronchial glycoproteins and NANA of both bronchial and serum glycoproteins. Levels of albumin indicate the serum transudate component present in sputum.

Material and methods

Two studies were carried out 12 months apart. Twelve normal subjects without clinical evidence of chronic bronchitis (Medical Research Council, London, UK).
The apparent viscosity of saliva and its chemical constituents fell within the range previously reported (Keal, 1971; Keal and Reid, 1972; Spiro et al., 1975), except in one subject in whom dry weight, NANA, and fucose concentrations were higher but viscosity was within the normal range. The levels of albumin, IgA, and IgG were also within the range found in normal saliva (Salvaggio et al., 1973).

**Bronchoconstrictor Effect**
Small decreases occurred in PEFR after inhalation of PGF2α (mean percent reduction 10%, range 1–32%), acetylcholine (mean percent reduction 7%, range 1–21%), histamine (mean percent reduction 7%, range 4–36%), and citric acid (mean percent reduction 4%, range 4–10%).

**Sputum Production**
The material expectorated was considered to be sputum if the level of viscosity and concentration of the marker substances of bronchial glycoprotein were above that of the saliva of the same individual and fell within the range found in mucoid sputum from patients with chronic bronchitis (Keal, 1971; Keal and Reid, 1972; Lopez-Vidriero et al., 1973).

Of the drugs tested, PGF2α was the most effective in producing sputum. After PGF2α, sputum was expectorated by all except one subject, and another subject failed to produce sputum in the first study but did so in the second. Seven out of 12 subjects produced sputum after inhalation of acetylcholine, four out of 12 after histamine, and three out of nine after citric acid. Sputum production was not related to smoking history, to the order in which the drugs were given, or to the degree of bronchoconstriction.

**Viscosity**
The results of apparent viscosity of sputum produced after inhalation of PGF2α, acetylcholine, histamine, and citric acid are shown in Table 1. Apparent viscosity was higher in sputum than in saliva, and levels were within the lower part of the range found for mucoid sputum from patients with chronic bronchitis (Charman and Reid, 1972; Charman, 1973).

The mean apparent viscosity of sputum produced after inhalation of PGF2α and after histamine was similar and both were higher than that of sputum produced after inhalation of acetylcholine or citric acid but the difference did not reach significance. Student t test and Mann-Whitney non-parametric U test were applied to test these differences. None was significant even at the 0.05 level.

**Dry Weight and Chemical Constituents**
The dry weight, NANA, fucose, sulphate, and...
mannose content of sputum produced after inhalation of PGF2α, acetylcholine, histamine, and citric acid are given in Table 1. Whereas absolute levels of dry weight, NANA, fucose, and sulphate fell within the lower part of the range of mucoid sputum from patients with chronic bronchitis (Spiro et al., 1975), those of mannose were below this range after each of the drugs tested.

The levels of NANA, fucose, sulphate, and dry weight were found to be significantly higher in sputum produced after inhalation of PGF2α than in saliva (NANA: r = 6.6, p < 0.001; fucose: r = 6.1, p < 0.001; sulphate: r = 5.8, p < 0.01; dry weight: r = 3.5, p < 0.01). After inhalation of acetylcholine and histamine it was only fucose that achieved significance (r = 4.0, p < 0.01 respectively). When sputum produced after inhalation of PGF2α, acetylcholine, histamine, and citric acid were compared for dry weight and chemical constituents, no statistically significant difference emerged.

It was of interest that, in three of the subjects who took part in both studies and on two occasions produced sputum after inhalation of PGF2α and acetylcholine, the absolute levels of chemical constituents and viscosity were nearly identical.

Table 1  Mean values, standard error of mean, and range of viscosity, dry weight, NANA, fucose, sulphate, and mannose of saliva and sputum produced after inhalation of PGF2α, acetylcholine, histamine, and citric acid

<table>
<thead>
<tr>
<th></th>
<th>Saliva</th>
<th>PGF2α</th>
<th>Acetylcholine</th>
<th>Histamine</th>
<th>Citric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viscosity</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Mean</td>
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<td>0.17</td>
<td>0.08</td>
<td>0.19</td>
<td>0.07</td>
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<tr>
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<td>0.06</td>
<td>0.02</td>
<td>0.06</td>
<td>0.02</td>
</tr>
<tr>
<td>Range</td>
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<td>0.11-0.84</td>
<td>0.04-0.14</td>
<td>0.06-0.34</td>
<td>0.03-0.14</td>
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<tr>
<td>Dry weight</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.01</td>
<td>6.93</td>
<td>5.25</td>
<td>6.11</td>
<td>8.24</td>
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<td>SE</td>
<td>0.39</td>
<td>0.75</td>
<td>0.80</td>
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<td>2.60</td>
</tr>
<tr>
<td>Range</td>
<td>1.70-6.80</td>
<td>2.43-12.0</td>
<td>2.22-9.83</td>
<td>3.30-8.92</td>
<td>5.53-13.37</td>
</tr>
<tr>
<td>NANA (μmol/ml)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean</td>
<td>0.18</td>
<td>0.16</td>
<td>0.49</td>
<td>0.37</td>
<td>0.87</td>
</tr>
<tr>
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<td>0.02</td>
<td>0.07</td>
<td>0.12</td>
<td>0.13</td>
<td>0.34</td>
</tr>
<tr>
<td>Range</td>
<td>0.06-1.52</td>
<td>0.16-1.0</td>
<td>0.22-1.20</td>
<td>0.16-0.61</td>
<td>0.20-1.30</td>
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<tr>
<td>Fucose (μmol/ml)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.75</td>
<td>2.54</td>
<td>1.85</td>
<td>2.04</td>
<td>5.60</td>
</tr>
<tr>
<td>SE</td>
<td>0.80</td>
<td>0.27</td>
<td>0.26</td>
<td>0.44</td>
<td>3.50</td>
</tr>
<tr>
<td>Range</td>
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<td>1.09-3.57</td>
<td>0.61-3.0</td>
<td>1.21-2.70</td>
<td>2.01-2.60</td>
</tr>
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<td>Sulphate (μmol/ml)</td>
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<tr>
<td>Mean</td>
<td>0.33</td>
<td>0.73</td>
<td>0.67</td>
<td>0.51</td>
<td>1.32</td>
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<td>SE</td>
<td>0.12</td>
<td>0.07</td>
<td>0.08</td>
<td>0.15</td>
<td>0.40</td>
</tr>
<tr>
<td>Range</td>
<td>0.21-0.57</td>
<td>1.14-0.21</td>
<td>0.51-1.12</td>
<td>0.31-0.81</td>
<td>0.61-2.50</td>
</tr>
<tr>
<td>Mannose (μmol/ml)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.18</td>
<td>0.23</td>
<td>0.24*</td>
<td>0.18*</td>
<td>0.43*</td>
</tr>
<tr>
<td>SE</td>
<td>0.04</td>
<td>0.02</td>
<td>0.13</td>
<td>0.13</td>
<td>0.28</td>
</tr>
<tr>
<td>Range</td>
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<td>0.08-0.41</td>
<td>0.11-0.37</td>
<td>0.18-0.19</td>
<td>0.15-0.72</td>
</tr>
</tbody>
</table>

*n = 2.

Discussion

The material expectorated after inhalation of PGF2α...
Effect of drugs on bronchial secretion from normal human airways

Table 2  Mean values, standard error of mean, and range of albumin, IgA, IgG, and transferrin of saliva and sputum produced after inhalation of PGF2α, acetylcholine, and histamine

<table>
<thead>
<tr>
<th></th>
<th>Saliva n=8</th>
<th>PGF2α n=7</th>
<th>Acetylcholine n=5</th>
<th>Histamine n=2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (mg/100ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>4.90</td>
<td>15.88</td>
<td>32.80</td>
<td>58.30</td>
</tr>
<tr>
<td>SE</td>
<td>2.65</td>
<td>4.40</td>
<td>21.10</td>
<td>35.70</td>
</tr>
<tr>
<td>Range</td>
<td>1.50-8.20</td>
<td>4.00-39.20</td>
<td>4.10-74.00</td>
<td>22.60-94.00</td>
</tr>
<tr>
<td>IgA (mg/100ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>6.41</td>
<td>6.03</td>
<td>7.70</td>
<td>5.30</td>
</tr>
<tr>
<td>SE</td>
<td>3.24</td>
<td>0.62</td>
<td>2.10</td>
<td>1.80</td>
</tr>
<tr>
<td>Range</td>
<td>2.40-11.40</td>
<td>1.80-8.50</td>
<td>3.50-9.20</td>
<td>3.50-7.10</td>
</tr>
<tr>
<td>IgG (mg/100ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>3.04</td>
<td>3.24</td>
<td>7.03</td>
<td>12.00</td>
</tr>
<tr>
<td>SE</td>
<td>1.79</td>
<td>1.85</td>
<td>2.10</td>
<td>6.40</td>
</tr>
<tr>
<td>Range</td>
<td>1.30-7.00</td>
<td>1.30-6.10</td>
<td>2.20-12.60</td>
<td>5.60-18.50</td>
</tr>
<tr>
<td>Transferrin (mg/100ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.32</td>
<td>1.20</td>
<td>1.90</td>
<td>3.20</td>
</tr>
<tr>
<td>SE</td>
<td>0.21</td>
<td>0.44</td>
<td>1.40</td>
<td>2.60</td>
</tr>
<tr>
<td>Range</td>
<td>0.00-0.60</td>
<td>0.00-3.60</td>
<td>0.00-4.60</td>
<td>0.00-5.80</td>
</tr>
</tbody>
</table>

Fig. 1  Absolute levels of viscosity and marker substances in sputum produced by a subject after inhalation of PGF2α and during an upper respiratory tract infection (URTI).
shows the characteristics of sputum in that the apparent viscosity and the concentration of marker substances of bronchial glycoprotein—fucose, sulphate, and NANA—are similar to those found in mucoid sputum from patients with chronic bronchitis although they fall within the lower part of the range. In all subjects, except two with high levels of albumin, concentrations of mannose, IgG, and albumin—markers of serum transudate—were below the range reported for mucoid sputum from patients with chronic bronchitis. It appears that in bronchitis transudation is a significant component of sputum.

This is supported by the fact that, in two subjects who inhaled PGF2a when recovering from a cold, mannose concentration was similar to that of mucoid sputum from patients with chronic bronchitis. In one subject who developed an upper respiratory tract infection a few months after the study and was able to produce sputum spontaneously, levels of marker substances of bronchial glycoprotein and of serum transudate were found to be higher than in the sputum the subject produced at the time of the PGF2a administration, suggesting that even during a cold both airways secretion and serum transudate are increased. (Figs. 1 and 2).

![Graph showing absolute levels of albumin, IgG, and IgA in sputum produced by a subject after inhalation of PGF2a and during an upper respiratory tract infection (URTI).](image)

A variation was observed between subjects in the absolute levels of marker substances of bronchial glycoprotein in sputum produced after inhalation of PGF2a. This is perhaps to be expected since histochemical studies carried out on normal human bronchi (Lamb and Reid, 1972; Jones and Reid, 1973) have shown a wide variation between individuals in the different types of acid glycoprotein secreted.

The bronchial fluid expectorated after inhalation of each drug may represent secretion previously discharged into the airway from the secretory cells of the bronchial epithelium and glands; but it is likely that some drugs caused discharge of intracellular glycoprotein.

The possible mechanisms by which the drugs induced sputum production could be either an increase in bronchial secretion, contraction of bronchial smooth muscle and myoepithelial cells, and/or increased serum transudation.

From organ culture studies it is known that acetylcholine increases the secretory index and that histamine does not (Sturgess and Reid, 1972). Data are not yet available on the effect of prostaglandin F2a on the secretory index of human bronchi. The constrictor effect on human bronchial smooth muscle of acetylcholine, histamine, and citric acid has been demonstrated by several authors (Curry, 1946; Simonsson et al., 1967; Mathé et al., 1973; Smith, et al., 1975). Contraction of myoepithelial cells in a manner similar to bronchial smooth muscle could lead to expulsion of secretion from the secretory tubules (Meyrick and Reid, 1970).

The absence of evidence of serum transudation after PGF2a suggests that this drug is not inducing the production of sputum by an effect on the vascular bed. It is, therefore, probable that PGF2a induces sputum production by its effect on bronchial smooth muscle and myoepithelial cells; histamine, although having a similar effect on smooth muscle, in addition increases serum fluid transudate whereas acetylcholine leads to sputum production possibly by a combination of the three mentioned mechanisms.

The main value of this study has been to show that, after inhalation of PGF2a, enough sputum can be produced from the normal bronchial tree for chemical analysis and determination of viscosity. Furthermore, it has been shown that this sputum contains bronchial glycoprotein mixed with relatively little transudate.

References


Charman, J. (1973). Relevant controls of individual and
Effect of drugs on bronchial secretion from normal human airways

Lopez-Vidriero, M.


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