

Bronchial secretion from normal human airways after inhalation of prostaglandin F_{2α}, acetylcholine, histamine, and citric acid

M. T. LOPEZ-VIDRIERO, I. DAS, A. P. SMITH,¹ R. PICOT, AND L. REID²

From the Cardiothoracic Institute, Brompton Hospital, London SW3, UK

Lopez-Vidriero, M. T., Das, I., Smith, A. P., Picot, R., and Reid, L. (1977). *Thorax*, 32, 734–739. Bronchial secretion from normal human airways after inhalation of prostaglandin F_{2α}, acetylcholine, histamine, and citric acid. Sputum produced by normal subjects after inhalation of prostaglandin F_{2α}, acetylcholine, histamine, and citric acid has been analysed. Prostaglandin F_{2α} was the most effective of the drugs in promoting sputum production. The material expectorated after inhalation of prostaglandin F_{2α} 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 F_{2α}. Sputum produced after inhalation of prostaglandin F_{2α} 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 F_{2α} 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 F_{2α} (PGF_{2α}) 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 PGF_{2α}.

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,

Present addresses:

¹Llandough Hospital, Penarth, Glamorgan, UK.

²Children's Hospital Medical Center, Harvard Medical School, Boston, Massachusetts, USA

1965) aged from 21 to 52 years took part. One subject was a throat clearer, which can be taken as evidence of a lesser degree of mucus hypersecretion (Gregg, 1968); two subjects were recovering from a cold, and these results are mentioned separately. Five subjects were smokers. Five subjects took part in the two studies.

The two studies were carried out in the course of one day between 1000 and 1400 hours. Before inhaling any drug each subject was asked to produce 5–10 ml of saliva, and the best of three successive expiratory peak flow measurements was recorded (PEFR, Wright Peak Flow Meter); saliva samples were collected also at the end of the second study. Each subject inhaled 10 large breaths of an aerosol of the following drugs in order: prostaglandin F_{2α} tromethamine salt 500 μg/ml, histamine acid phosphate 3 mg/ml, acetylcholine chloride 18 mg/ml, and citric acid 20% solution. When the effects of the previous inhalation had disappeared and the PEFR had returned to baseline values, each successive drug was inhaled from a Bennett respirator nebuliser (60% particles less than 2 μ) adjusted to nebulise on inhalation only. In the first study citric acid was omitted and the drugs were given in the order indicated; in the second all drugs were given in random order save citric acid, which was given last.

Sputum produced after inhalation of the drugs was expectorated into a cardboard container. The viscosity of either sputum or saliva was measured immediately on a cone and plate Ferranti-Shirley viscometer (Charman and Reid, 1972). The remainder of the material was frozen and stored at -20°C for chemical analysis, which included dry macromolecular weight, N-acetyl neuraminic acid (NANA), fucose, mannose, and sulphate (Gibbons, 1955; Warren, 1959; Antonopoulos, 1962; Das *et al.*, 1974). Radial immunodiffusion estimation of albumin immunoglobulin A (IgA), immunoglobulin G (IgG), and transferrin was carried out in sputum and saliva samples from the second study (Mancini *et al.*, 1965).

Results

Inhalation of the drugs was followed by cough and tightness of the chest but no other unpleasant effects were observed.

Since no significant difference emerged between the results of the two studies, the results of both have been analysed together.

SALIVA

The apparent viscosity (at a shear rate of 1350s⁻¹), dry weight, and chemical constituents of saliva produced at the end of the second study were within the range found in saliva produced before inhaling

the drugs. 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 PGF_{2α} (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, PGF_{2α} was the most effective in producing sputum. After PGF_{2α}, 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 PGF_{2α}, 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 PGF_{2α} 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

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 PGF_{2a}, acetylcholine, histamine, and citric acid

	Saliva n=15	PGF _{2a} n=13	Acetylcholine n=9	Histamine n=4	Citric acid n=4
Viscosity					
Mean	0.02	0.17	0.08	0.19	0.07
SE	0.07	0.06	0.02	0.06	0.02
Range	0.01-0.03	0.11-0.84	0.04-0.14	0.06-0.34	0.03-0.14
Dry weight					
Mean	4.01	6.93	5.25	6.11	8.24
SE	0.39	0.75	0.80	1.60	2.60
Range	1.70-6.80	2.43-12.0	2.22-9.83	3.30-8.92	5.53-13.37
NANA ($\mu\text{mol/ml}$)					
Mean	0.18	0.16	0.49	0.37	0.87
SE	0.02	0.07	0.12	0.13	0.34
Range	0.06-1.52	0.16-1.0	0.22-1.20	0.16-0.61	0.20-1.30
Fucose ($\mu\text{mol/ml}$)					
Mean	0.75	2.54	1.85	2.04	5.60
SE	0.80	0.27	0.26	0.44	3.50
Range	0.26-1.52	1.09-3.57	0.61-3.0	1.21-2.70	2.01-2.60
Sulphate ($\mu\text{mol/ml}$)					
Mean	0.33	0.73	0.67	0.51	1.32
SE	0.12	0.07	0.08	0.15	0.40
Range	0.21-0.57	1.14-0.21	0.51-1.12	0.31-0.81	0.61-2.50
Mannose ($\mu\text{mol/ml}$)					
Mean	0.18	0.23	0.24*	0.18*	0.43*
SE	0.04	0.02	0.13	0.13	0.28
Range	0.06-0.41	0.08-0.41	0.11-0.37	0.18-0.19	0.15-0.72

*n=2.

mannose content of sputum produced after inhalation of PGF_{2a}, 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 (Spira *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 PGF_{2a} 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 PGF_{2a}, 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 PGF_{2a} and

acetylcholine, the absolute levels of chemical constituents and viscosity were nearly identical.

SERUM PROTEINS AND IMMUNOGLOBULINS

The results of albumin, IgA, IgG, and transferrin levels in saliva and sputum produced after PGF_{2a}, acetylcholine, and histamine are given in Table 2. The amount of sputum produced after citric acid was not sufficient for radial immunodiffusion analysis.

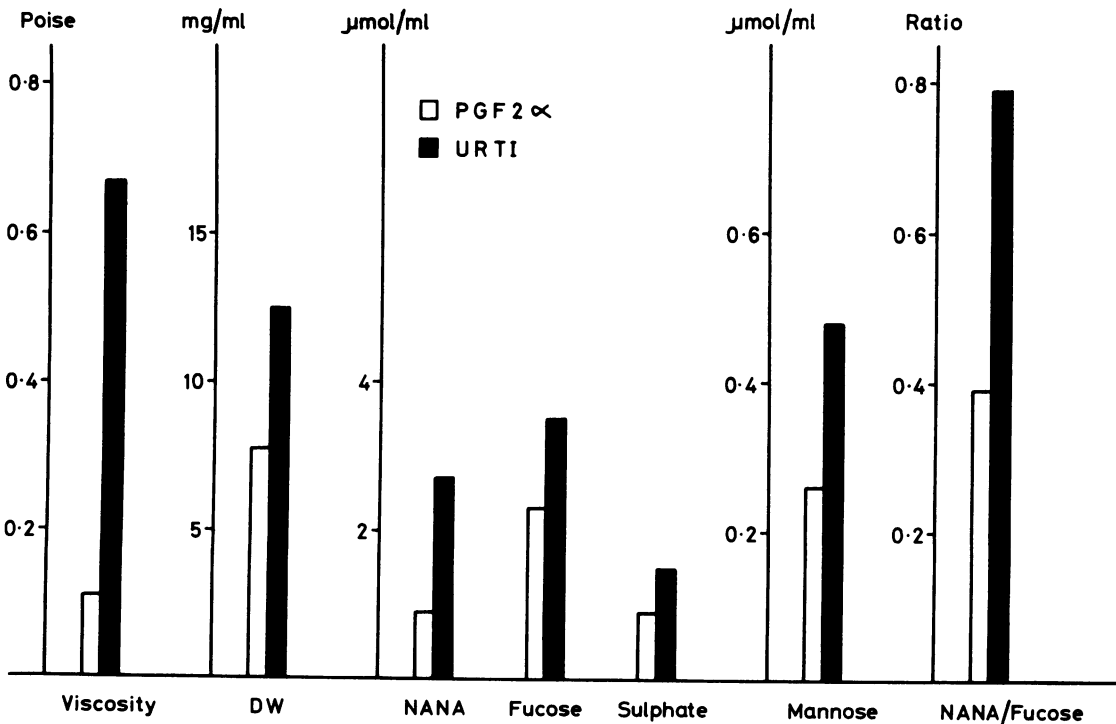
The albumin concentration in sputum produced after inhalation of any of the drugs was higher than that in saliva. The highest levels of albumin were seen in sputum produced after inhaling histamine and acetylcholine. In five out of seven subjects who produced sputum after inhalation of PGF_{2a} albumin levels fell within the range (4-18 mg/100 ml) reported for normal bronchial fluid (from bronchial washings) (Falk *et al.*, 1972) and in the other two subjects fell within the range for mucoid chronic bronchitis.

Discussion

The material expectorated after inhalation of PGF_{2a}

Table 2 Mean values, standard error of mean, and range of albumin, IgA, IgG, and transferrin of saliva and sputum produced after inhalation of PGF₂ α , acetylcholine, and histamine

	Saliva n=8	PGF ₂ α n=7	Acetylcholine n=5	Histamine n=2
Albumin (mg/100ml)				
Mean	4.90	15.88	32.80	58.30
SE	2.65	4.40	21.10	35.70
Range	1.50-8.20	4.00-39.20	4.10-74.00	22.60-94.00
IgA (mg/100ml)				
Mean	6.41	6.03	7.70	5.30
SE	3.24	0.82	2.10	1.80
Range	2.40-11.40	1.80-8.50	3.50-9.20	3.50-7.10
IgG (mg/100ml)				
Mean	3.04	3.24	7.03	12.00
SE	1.79	1.85	2.10	6.40
Range	1.30-7.00	1.30-6.10	2.20-12.60	5.60-18.50
Transferrin (mg/100ml)				
Mean	0.32	1.20	1.90	3.20
SE	0.21	0.44	1.40	2.60
Range	0.00-0.60	0.00-3.60	0.00-4.60	0.60-5.80

Fig. 1 Absolute levels of viscosity and marker substances in sputum produced by a subject after inhalation of PGF₂ α 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 PGF_{2α} 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 PGF_{2α} administration, suggesting that even during a cold both airways secretion and serum transudate are increased. (Figs. 1 and 2).

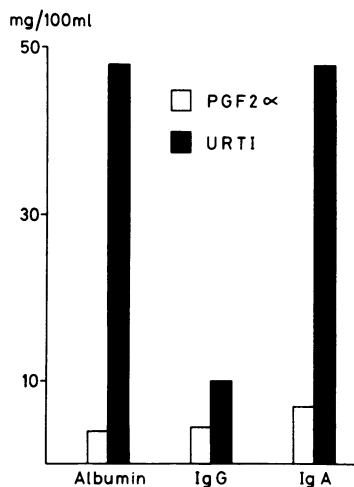


Fig. 2 Absolute levels of albumin, IgG, and IgA in sputum produced by a subject after inhalation of PGF_{2α} and during an upper respiratory tract infection (URTI).

A variation was observed between subjects in the absolute levels of marker substances of bronchial glycoprotein in sputum produced after inhalation of PGF_{2α}. 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 F_{2α} 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 PGF_{2α} suggests that this drug is not inducing the production of sputum by an effect on the vascular bed. It is, therefore, probable that PGF_{2α} 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 PGF_{2α}, 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

- Antonopoulos, C. A. (1962). A modification for the determination of sulphate in mucopolysaccharides by the benzidine method. *Acta Chemica Scandinavica*, **16**, 1521–1522.
- Bickerman, H. A., Sproul, E. E., and Barach, A. L. (1958). Aerosol method of producing bronchial secretions in human subjects; clinical technic for detection of lung cancer. *Diseases of the Chest*, **33**, 347–362.
- Charman, J. (1973). Relevant controls of individual and

- disease variation in viscosity. *Bulletin de Physiopathologie respiratoire*, **9**, 429–432.
- Charman, J., and Reid, L. (1972). Sputum viscosity in chronic bronchitis, bronchiectasis, asthma and cystic fibrosis. *Biorheology*, **9**, 185–199.
- Curry, J. J. (1946). The action of antihistamine substances and other drugs on histamine bronchoconstriction in asthmatic subjects, *Journal of Clinical Investigation*, **25**, 792–799.
- Das, I., Lopez-Vidriero, M. T., and Reid, L. (1974). A simple sensitive enzymatic assay method for mannose in glycoproteins. Abstracts of Papers, *Federation of European Biochemical Societies 9th Meeting, Hungary*. p. 438.
- Falk, G. A., Okinaka, A. J., and Siskind, G. W. (1972). Immunoglobulins in the bronchial washings of patients with chronic obstructive pulmonary disease. *American Review of Respiratory Diseases*, **105**, 14–21.
- Gibbons, M. N. (1955). The determination of methylpentoses. *Analyst*, **80**, 268–276.
- Gregg, I. (1968). A study of the causes of progressive airways obstruction in chronic bronchitis. In *Current Research in Chronic Respiratory Disease*. 11th Aspen Conference, pp. 235–248. US Government Printing Office, Washington.
- Jones, R., and Reid, L. (1973). The effect of pH on Alcian Blue staining of epithelial acid glycoproteins. II. Human bronchial submucosal gland. *Histochemical Journal*, **5**, 19–27.
- Keal, E. E. (1971). Biochemistry and rheology of sputum in asthma. *Postgraduate Medical Journal*, **47**, 171–177.
- Keal, E. E., and Reid, L. (1972). Neuraminic acid content of sputum in chronic bronchitis. *Thorax*, **27**, 643–653.
- Lamb, D., and Reid, L. (1972). Quantitative distribution of various types of acid glycoprotein in mucous cells of human bronchi. *Histochemical Journal*, **4**, 91–102.
- Lopez-Vidriero, M. T., Charman, J., Keal, E., de Silva, D. J., and Reid, L. (1973). Sputum viscosity: correlation with chemical and clinical features in chronic bronchitis. *Thorax*, **28**, 401–408.
- Lopez-Vidriero, M. T., Das, I., and Reid, L. (1977). Airway secretion source, biochemical and rheological properties In *Respiratory Defense Mechanisms*, edited by J. D. Brain, D. F. Proctor, and L. Reid, pp 289–356. Marcel Dekker, New York and Basel.
- Mancini, G., Carbonara, A. O., and Heremans, J. F. (1965). Immunochemical quantitation of antigens by single radial immunodiffusion. *International Journal of Immunochemistry*, **2**, 235–254.
- Mathé, A. A., Hedqvist, P., Holmgren, A., and Svanborg, N. (1973). Bronchial hyperreactivity to prostaglandin F_{2a} and histamine in patients with asthma. *British Medical Journal*, **1**, 193–196.
- Medical Research Council (1965). Definition and classification of chronic bronchitis. *Lancet*, **1**, 775–779.
- Meyrick, B., and Reid, L. (1970). Ultrastructure of cell in the human bronchial submucosal glands. *Journal of Anatomy*, **107**, 281–299.
- Salvaggio, J., Lopez, M., Arquembourg, P., Waldman, R. W., and Sly, M. (1973). Salivary, nasal wash and sputum IgA concentrations in atopic and nonatopic individuals. *Journal of Allergy and Clinical Immunology*, **51**, 335–343.
- Simonsson, B. G., Jacobs, F., and Nadel, J. A. (1967). Role of autonomic nervous system and the cough reflex in the increased responsiveness of airways in patients with obstructive airway disease. *Journal of Clinical Investigation*, **46**, 1812–1818.
- Smith, A. P., Cuthbert, M. F., and Dunlop, L. S. (1975). Effects of inhaled prostaglandins E1, E2 and F2 alpha on the airway resistance of healthy and asthmatic man. *Clinical Science and Molecular Medicine*, **48**, 421–430.
- Spiro, S. G., Lopez-Vidriero, M. T., Charman, J., Das, I., and Reid, L. (1975). Bronchorrhoea in a case of alveolar cell carcinoma. *Journal of Clinical Pathology*, **28**, 60–65.
- Sturgess, J., and Reid, L. (1972). An organ culture study of the effect of drugs on the secretory activity of the human bronchial submucosal gland. *Clinical Science*, **43**, 533–543.
- Turgeon, P., Robert, J., and Turgeon, F. (1971). Études des immunoglobulines sériques et sécrétoires trachéo-bronchiques. *Union Medicale du Canada*, **100**, 232–239.
- Warren, L. (1959). The thiobarbituric acid assay of sialic acids. *Journal of Biological Chemistry*, **234**, 1971–1975.

Requests for reprints to: Professor L. Reid, Children's Hospital Medical Center, Harvard Medical School, Boston, Massachusetts, USA.