

Bronchorrhoea—separation of mucus and serum components in sol and gel phases

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ABSTRACT Bronchorrhoea sputum separates into two phases—sol and gel—at low speed centrifugation (5000 g), although higher speed (160 000 g) is required to obtain complete separation. Markers of mucus glycoprotein and serum transudate have been estimated in sputum, sol, and gel in five cases of bronchorrhoea associated with chronic bronchitis, asthma, or alveolar cell carcinoma. In all samples markers of both mucus glycoprotein and serum component were present in each phase. The concentration of serum markers was similar in both phases suggesting that it is to the serum that mucus glycoprotein is added. Since the volume of sol is greater, the total of serum components is higher in the sol than in the gel although, in total, a considerable amount was present in the gel. The fucose/sulphate ratio suggests that the glycoprotein in the sol is relatively more sulphated than is that of the gel, indicating that they may represent secretion from different cell types.

The pattern of separation of two serum markers, IgA and transferrin, is different from that of albumin. Whether this is because they are locally produced or because they are selectively bound to mucus glycoprotein in the gel is not clear.

Bronchorrhoea is a condition characterised by the production of more than 100 ml sputum in 24 hours. It can be idiopathic or associated with diseases such as chronic bronchitis (Kourilsky, 1960; Calin, 1972), asthma (Keal, 1971; Lopez-Vidriero *et al*, 1973), or alveolar cell carcinoma (Wood, 1943; Kenamer, 1951; Storey *et al*, 1953; Schools and Ray, 1961; Spiro *et al*, 1975).

Bronchorrhoea sputum includes a large amount of froth, often increases in viscosity with time, sometimes separates spontaneously into two phases, and has low concentration of mucus glycoprotein (Keal, 1971; Lopez-Vidriero *et al*, 1973; Spiro *et al*, 1975). These earlier studies showed that the concentration of markers of mucus glycoprotein is low compared with that in mucoid chronic bronchitis sputum and that the serum transudate component, assessed only by N-acetyl neuraminic acid (NANA)/fucose ratio is relatively high. Detailed analysis of serum components has been reported only in bronchorrhoea sputum associated

with alveolar cell carcinoma (Warfringe, 1955; Gernez-Rieux *et al*, 1963; Asselain *et al*, 1969).

Ryley and Brogan (1968) by using high speed centrifugation (118 000 g) separated sputum from patients with chronic bronchitis or asthma into two phases—a continuous or sol phase and a disperse or gel phase. But even at this high speed the phases were incompletely separated. They carried out extensive qualitative and quantitative studies of the serum components present in the sol phase of sputum from patients with chronic bronchitis, asthma, or cystic fibrosis (Ryley and Brogan, 1968; Ryley 1970; Brogan *et al*, 1971; Ryley 1972; Ryley and Brogan, 1973; Brogan *et al*, 1975) and in one paper they described the high molecular weight components of the disperse or gel phase (Ryley and Brogan, 1968). Roberts (1974) using relatively low speed ultracentrifugation (45 000 g for 90 minutes) obtained a similar degree of separation into sol and gel phases, and at this speed the gel was almost free of serum proteins. The four sputum samples studied were all from patients with bronchorrhoea, either idiopathic or associated with chronic bronchitis. These results suggest that the separation of bronchorrhoea sputum re-

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quires a lower centrifugal force than chronic bronchitis, asthma, or cystic fibrosis sputum.

The purpose of the present study was to establish the speed required to obtain complete separation of bronchorrhoea sputum into sol and gel phases and to study the nature of sputum in bronchorrhoea associated with various diseases—chronic bronchitis, extrinsic asthma, intrinsic asthma, alveolar cell carcinoma, and the constituents of the sol and gel phases.

Material and methods

Nine sputum samples were collected from five patients with bronchorrhoea; all produced more than 100 ml of sputum in 24 hours (table 1). Diag-

Table 1 Clinical features of cases of bronchorrhoea

Case No	Primary diagnosis	Sex	Age (yr)	Duration of symptoms	Sputum volume (ml)
1	Chronic bronchitis	M	54	1 month	150
2	Chronic bronchitis	M	43	1 week	112
3	Intrinsic asthma	M	44	2 years	120
4	Extrinsic asthma	M	42	2 weeks	110
5	Alveolar cell carcinoma	M	57	7 weeks	843

nosis of primary disease was based on the medical history of the patients before they developed bronchorrhoea. Diagnosis of chronic bronchitis was based on the MRC definition (1965), asthma on the Ciba definitions (1959, 1971), and alveolar cell carcinoma on sputum cytology.

Sputum samples were collected over a maximum period of three hours and centrifuged within 30 minutes of collection. All sputum samples were considered macroscopically as non-purulent, none was blood stained, and only one sputum sample (case 3) separated spontaneously into two phases.

ULTRACENTRIFUGATION

Four samples, two from case 3 and two from case 5, were centrifuged at low speed (5000 g) for 30 minutes at 4°C. Five sputum samples (cases 1, 2, 3, 4, and 5) were centrifuged at high speed 54 500 rpm (160 000 g) for 30 minutes at 4°C in a MSE Superspeed preparative ultracentrifuge using aluminium fixed angle rotor. At both speeds the sputum separated into two distinct phases—sol and gel. Our results are based on the sputum samples centrifuged at 160 000 g since at this speed a more complete separation is achieved, and this may allow comparison with other diseases for which high speed is necessary.

The gel phase was separated from the sol phase

and the wet weight of each phase determined. An aliquot from each phase was taken for radial immunodiffusion studies and the remainder was stored at -20°C for chemical analysis.

RADIAL IMMUNODIFFUSION STUDIES

An aliquot each of total sputum, sol, and gel phases was taken for estimation of albumin, transferrin, IgG, and IgA. The method of Mancini *et al* (1965) for quantitative analysis was followed; the radial immunodiffusion plates and antisera to serum albumin, transferrin, IgG, and IgA were obtained from Hoechst Pharmaceutical Inc (UK). (Transferrin had no cross-reactivity with lactoferrin). Total sputum and gel aliquots were homogenised by ultrasonication (Soniprobe-Dawe Instrument) using a microprobe. Samples were ultrasonicated for one minute while being cooled to prevent temperature rise.

CHEMICAL ANALYSES

Total sputum, sol, and gel phases were prepared for chemical analysis following the method previously described (Lopez-Vidriero *et al*, 1977a). Macromolecular dry weight was determined after dialysis. Chemical analyses included estimation of fucose (Gibbons, 1955), sulphate (Antonopoulos, 1962), and NANA (Warren, 1959).

Results

CHEMICAL CONSTITUENTS OF BRONCHORRHOEA SPUTUM

In total sputum, concentrations of macromolecular material, fucose, sulphate, and NANA were low (table 2) and fell within the levels previously reported in bronchorrhoea (Keal, 1971; Lopez-Vidriero *et al*, 1975; Spiro *et al*, 1975). Concentrations of albumin, transferrin, IgG, and IgA (not previously reported) were higher than in sputum produced after inhalation of prostaglandin F₂ α (Lopez-Vidriero *et al*, 1977b), and showed wide variation between patients (table 3). Concentrations of IgG and transferrin followed closely those of albumin, while concentrations of IgA were dissociated from albumin, suggesting that a large proportion of IgA is secretory.

VOLUME OF SOL AND GEL PHASES

Four samples were centrifuged at low speed (5000 g) and all separated into two distinct phases. The percentage separated as sol in case 3 (75%) and in case 5 (70%) was similar to that obtained at 118 000–120 000 g for chronic bronchitis, asthma, and cystic fibrosis sputum (Ryley and Brogan, 1968; Brogan *et al*, 1971, 1975). When

sputum samples from cases 3 and 5 were centrifuged at 160 000 g the percentage volume of sol increased to 91% and 95% respectively. The mean value of sol for the five cases studied at 160 000 g was 90% with a range of 86–95%.

These results indicate that the pattern of separation into two phases and the percentage volume of sol at low and high speed are independent of the primary disease and are a special feature of bronchorrhoea sputum.

Markers of mucus glycoprotein—fucose and sulphate—were present in both sol and gel, although their concentration was significantly higher in gel than in sol (table 3) as was that of NANA. Fucose/sulphate ratio was found to be lower in the sol than in the gel phase, suggesting that the mucus glycoprotein in the sol phase is relatively more sulphated. Although most of the mucus glycoprotein separated in the gel, a considerable amount was also present in the sol, and in one case (case 3) it contained more fucose and sulphate than the gel.

Serum components were present in both phases, the concentration of albumin and transferrin being higher in sol than gel, although the difference did not reach significance. Concentrations of IgG and IgA were similar in both phases. Total amounts of albumin, transferrin, IgG, and IgA were calculated. The amount of serum components was considerably higher in sol than gel. The albumin/fucose ratio was 40 times higher than in gel, indicating that most of the serum component separates in the sol phase while mucus glycoprotein is in the gel.

Albumin/IgG ratio was similar in both phases and showed small variance between samples. By contrast albumin/transferrin and albumin/IgA ratios were higher and showed considerable variance; in some samples the ratios were lower suggesting that in those cases transferrin and IgA had a higher binding capacity to the mucus glycoprotein.

When separate samples were compared differences within a disease and among diseases emerged (table 4). The two chronic bronchitis sputa differed from each other in the concentration and distribution of serum components. Sputum from case 2 contained more transudate and the serum component separated in the sol was qualitatively similar to that separated in the gel. By contrast in case 1 both IgG and IgA had selectively separated into the gel and were perhaps strongly bound to the mucus glycoprotein. These differences reflect different clinical stages of the disease; case 2 was studied during the first week while case 1 was studied at a late stage—one month after onset.

The two asthmatic cases differed from each other not only in the concentration and distribution of serum transudate but also in the distribution and nature of the mucus glycoprotein. In case 3 (intrinsic asthma) mucus glycoprotein markers were present in similar amounts in both phases while in case 4 (extrinsic asthma) they were considerably higher in the gel, suggesting that in case 3 the mucus glycoprotein secreted is considerably more soluble than in case 4. The fucose/sulphate ratio in the case of extrinsic asthma was similar in both phases suggesting that the mucus glycoprotein had a common source.

The case of extrinsic asthma differed from the two cases of chronic bronchitis in that most of the mucus glycoprotein separated in the gel phases. By contrast the case of intrinsic asthma was closer to the two cases of chronic bronchitis, particularly in the distribution and nature of mucus glycoprotein. Similar findings have been reported for sputum in a large number of samples (Lopez-Vidriero and Reid, 1978).

In case 5 (alveolar cell carcinoma) serum transudate was the major constituent of sputum, and its composition was also identical in both phases.

Discussion

Bronchorrhoea sputum whether associated with chronic bronchitis, asthma, or alveolar cell carcinoma presents certain special features, some of which have already been reported for total sputum (Keal, 1971; Lopez-Vidriero *et al*, 1975; Spiro *et al*, 1975). The work presented here adds information on the effect of low and high speed ultracentrifugation on the separation of this sputum into sol and gel phases and the distribution of mucus glycoprotein and serum components in each phase.

Ultracentrifugation was carried out without pretreating sputum with urea, EDTA, or disulphide cleaving agents; thus the macromolecular material separated in the sol phase represents the soluble component as it is present normally in bronchial fluid. It is important to point out that in our study sputum was collected over a short time, maximum three hours, and was centrifuged within 30 minutes of collection, whereas in other studies sputum was collected over 24 hours (Ryley and Brogan, 1968; Brogan *et al*, 1971; Ryley and Brogan, 1973; Brogan *et al*, 1975) and enzymatic degradation would have already altered the physical and chemical properties of sputum before centrifugation. Roberts (1974) kept the sputum samples at -20°C before centrifuga-

Table 2 Concentration of markers of mucus glycoprotein and plasma components in whole sputum (mean, standard deviation, and range)

	Dry weight (mg/ml)		Fucose (μmol/ml)		Sulphate (μmol/ml)		NANA (μmol/ml)		NANA/fucose ratio		Fucose/sulphate		Albumin (mg/100 ml)		Transferrin (mg/100 ml)		IgG (mg/100 ml)		IgA (mg/100 ml)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Mean	8.1	2.7	1.7	1.0	0.5	0.2	1.2	1.2	0.7	0.3	3.5	2.6	81.7	90.3	6.6	6.1	36.4	30.7	12.6	5.2
SD	2.7	1.0	1.0	0.2	0.2	0.3	1.2	1.2	0.3	0.4	2.6	1.0	15.1	236.0	0.2	14.5	4.7	76.8	4.6	17.7
Range	5.2-10.8	0.3-2.9	0.3-3.4	0.3-0.9	0.3-0.9	0.3-0.9	0.3-3.4	0.3-0.9	0.4-1.1	0.4-1.1	1.0-7.1	1.0-7.1	15.1-236.0	15.1-236.0	0.2-14.5	0.2-14.5	4.7-76.8	4.7-76.8	4.6-17.7	4.6-17.7

Table 3 Concentration of markers of mucus glycoprotein and plasma component in sol and gel phases (mean and standard deviation)

	Dry weight (mg/ml)		Fucose (μmol/ml)		Sulphate (μmol/ml)		NANA (μmol/ml)		NANA/fucose ratio		Fucose/sulphate		Albumin (mg/100 ml)		Transferrin (mg/100 ml)		IgG (mg/100 ml)		IgA (mg/100 ml)		Albumin/fucose ratio		Albumin/transferrin ratio		IgG ratio		IgA ratio	
	Sol	Gel	Sol	Gel	Sol	Gel	Sol	Gel	Sol	Gel	Sol	Gel	Sol	Gel	Sol	Gel	Sol	Gel	Sol	Gel	Sol	Gel	Sol	Gel	Sol	Gel	Sol	Gel
Mean	4.3	59.2	0.6	16.5	0.2	2.7	0.5	10.0	0.9	0.7	4.5	10.6	85.8	66.4	6.8	4.8	28.8	29.0	22.0	19.1	28.8	0.5	46.5	69.1	5.6	5.2	5.8	13.2
SD	0.6	26.0	0.4	7.9	0.1	2.2	0.4	7.8	0.3	0.5	4.8	11.2	91.5	69.7	6.3	4.5	32.3	22.5	21.9	17.5	52.3	0.6	69.1	107.9	6.3	5.9	7.6	24.9

Table 4 Concentration of markers of mucus glycoprotein and plasma component in whole sputum, sol, and gel phases for individual cases

	1 (Chronic bronchitis)			2 (Chronic bronchitis)			3 (Chronic bronchitis)			4 (Intrinsic asthma)			5 (Extrinsic asthma)			6 (Alveolar cell carcinoma)		
	Sputum	Sol	Gel	Sputum	Sol	Gel	Sputum	Sol	Gel	Sputum	Sol	Gel	Sputum	Sol	Gel	Sputum	Sol	Gel
Fucose	(μmol/ml)	1.03	0.54	20.70	2.40	0.40	14.70	2.90	1.20	11.50	1.70	0.67	27.90	0.30	0.12	7.73	0.12	7.73
Sulphate	(μmol/ml)	0.50	0.30	3.60	0.34	0.30	0.60	0.55	0.10	0.55	0.89	0.10	5.70	0.30	0.20	3.20	0.20	3.20
NANA	(μmol/ml)	0.71	0.26	6.30	0.90	0.30	4.30	3.44	1.10	21.00	0.81	0.61	15.50	0.30	0.16	3.10	0.16	3.10
Albumin	(mg/100 ml)	15.1	15.0	17.0	86.1	97.0	121.0	29.4	30.0	23.0	41.8	47.0	9.9	236	240	161	240	161
Transferrin	(mg/100 ml)	2.8	3.0	3.0	11.6	11.7	10.8	0.2	0.2	0.1	3.9	4.0	3.3	14.5	15.0	5.0	14.5	5.0
IgG	(mg/100 ml)	8.1	6.6	30.0	38.4	33.0	58.4	4.7	5.0	2.3	76.8	82.5	42.0	14.4	14.7	12.5	14.4	12.5
IgA	(mg/100 ml)	10.7	10.0	22.0	4.6	5.0	2.1	15.7	15.0	23.0	17.7	20.0	3.3	54.2	60.0	45.0	54.2	45.0

tion, and it is known that freezing and thawing solubilises bronchial secretion (Masson and Heremans, 1973) and therefore can influence the pattern of separation into two phases as well as the distribution of mucus glycoprotein.

A special feature of bronchorrhoea sputum is that the speed required to obtain two distinct phases is extremely low. At 5000 g 75% of sputum separates as sol; Ryley and Brogan (1968) reported similar separation for chronic bronchitis and asthma sputum at 118 000 g. When sputum was centrifuged at 160 000 g only 20% more volume of sol was separated from the gel. These results suggest that bronchorrhoea sputum is more easily separated into two phases; this is likely to be due to the high water content of bronchorrhoea sputum.

Although serum is mainly separated in the sol phase, a considerable amount is also present in the gel. All markers estimated were present in both phases but their proportion, particularly IgA and IgG, varied among diseases and within disease. In cases 2, 4, and 5 the distribution and concentration of IgA followed that of albumin, suggesting that most of it was of serum origin rather than secretory. On the other hand, in cases 1 and 3 IgA was selectively separated in the gel and did not follow the distribution of albumin indicating that it was more strongly associated to the mucus glycoprotein and perhaps of secretory origin.

Markers of mucus glycoprotein—fucose and sulphate—were present in both phases and although the concentration was significantly higher in gel in all cases, a considerable amount was separated in the sol particularly in sputum from patients with chronic bronchitis or intrinsic asthma in whom submucosal gland hypertrophy and increase in number of goblet (mucous) cells are characteristic pathological findings. In the case of extrinsic asthma where typical changes of chronic mucus hypersecretion are not expected to occur (Keal and Reid, 1975), almost all the mucus glycoprotein separated in the gel phases. Brogan *et al* (1971) found that the concentration of ethanol-precipitated carbohydrate in the sol phase was greater in patients with chronic bronchitis than in those with asthma. Perhaps the gel phase represents mainly gland secretion.

In sputum mucus glycoprotein is present both in soluble and gel form and it may be that these have different origins. Histochemical and electron microscopy studies have shown that although mucous and serous cells of submucosal glands and surface epithelium have certain features in common, there are differences to support the idea that their secretory product is different. Serous

cells have higher degree of sulphation per unit of radioactive glucose uptake (Lamb and Reid, 1969; Jeffery and Reid, 1977) and the uptake of ^3H -threonine and ^3H -glucose is lower than in mucous cells suggesting that the secretion is either more diluted or that the glycoprotein synthesised is less complex (Meyrick and Reid, 1970). In our study of human secretion the mucus glycoprotein separated in the sol phases was found to be more sulphated and therefore may derive from different cell types.

The results presented here indicate that the distribution of mucus glycoprotein and serum components in sol and gel is based not only on the proportion of each component in total sputum but also on the nature and degree of interaction or cross-linking between mucus glycoprotein molecules and between mucus glycoprotein and plasma type glycoproteins, particularly those locally produced in the airways.

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