

A study of plasma proteins in the sol phase of sputum from patients with chronic bronchitis

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ABSTRACT We have studied the sputum/serum protein concentration ratios from 23 patients with bronchitis both in the stable clinical state and during acute chest infections. During the stable state there was a significant negative correlation ($2 P < 0.005$) between the ratio and protein size. The ratios of IgG, IgA, C₃, and alpha₁-antichymotrypsin were significantly displaced from this relation suggesting local production in the lung. IgM was found in all samples and alpha₂-macroglobulin in 55% of non-infected samples which may be the result of local production rather than transudation from serum, because of their larger size. During acute chest infections the albumin content of sputum rose from a mean sputum/serum ratio of $0.83 (SE \pm 0.08) \times 10^{-2}$ to $13.77 (SE \pm 3.21) \times 10^{-2}$ suggesting increased transudation from the blood. In the presence of increased transudation, local production of protein appears to be less significant.

The role of immunoglobulins and proteolytic enzyme inhibitors in protecting the lung from damage is far from clear. In particular there have been conflicting reports concerning the presence of these proteins within the bronchial secretions and whether they are locally produced in the lung.

There are five possible ways in which a protein may enter the bronchial secretions (fig 1). Mechanisms one and two show the production of proteins unique to the bronchial secretions by epithelial cells and submucous glands respectively.

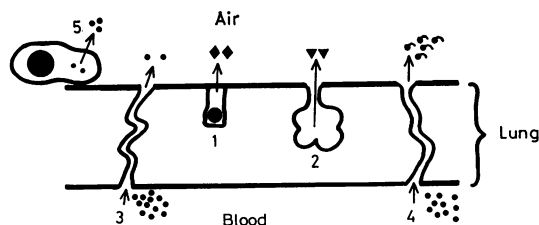


Fig 1 Possible sources of proteins within bronchial secretions. 1=Production by epithelial cells; 2=Production by submucous glands; 3=Passive diffusion from serum; 4=Diffusion and alteration within secretions; 5=Production by plasma cells or macrophages.

These proteins would be found in the bronchial secretions but not in the serum; an example is the low molecular weight bronchial inhibitor of elastase (Tegner, 1978). Mechanisms three, four, and five relate to proteins also found in serum. These proteins may passively diffuse from serum (3), become structurally altered within the secretion exemplified by the addition of secretory piece to IgA (4), or be present as the result of passive diffusion together with local production by plasma cells or macrophages (5). Mechanisms 4 and 5 would result in higher concentrations of the protein in the bronchial secretion than could be explained by passive diffusion alone.

Burnett *et al* (1976) studied the diffusion of plasma proteins into amniotic fluid and showed that the protein concentrations were inversely related to their molecular sizes (fig 2). Protein A has a fluid to serum ratio greater than albumin and thus by convention shows evidence of local production or retention. Proteins B and C have ratios similar to or less than albumin. They are larger molecules, however, and their ratios are higher than would be expected for their size. This also suggests local production or retention.

The present study was designed to clarify the origin of plasma proteins in sputum by measuring their sputum to serum ratios and taking into

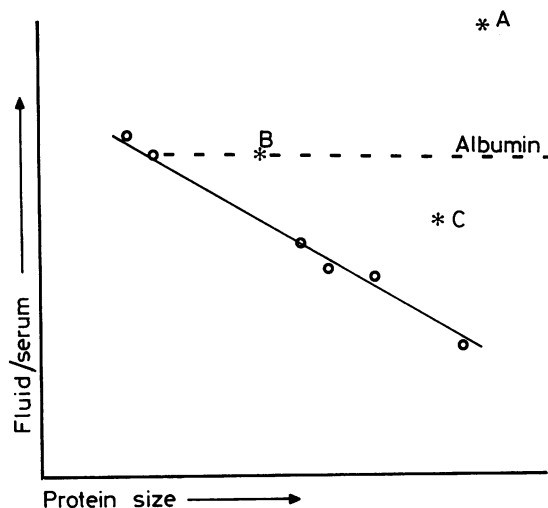


Fig 2 Diagrammatic relation between protein size (Stoke's radius, nm) and fluid to serum concentration. Proteins present by simple fluid diffusion (○) and those actively secreted or locally produced (*).

account their molecular sizes. We wished to determine whether any proteins were present in greater concentrations than would be expected by simple diffusion from serum thereby suggesting local retention or production.

Methods

Twenty-three patients with chronic bronchitis (MRC, 1965) aged 48–75 were studied. Sputum was collected over a four-hour period and a 10 ml sample of blood was taken during this time. The sputum was separated into sol and gel phase (the latter being discarded) by ultracentrifugation (100 000 g) for 90 minutes. The blood was allowed to clot, was centrifuged, and the serum was removed. Samples of serum and sol phase of sputum were stored at -20°C until analysed.

Three of the patients were studied during an acute chest infection, but the remainder were in a stable clinical state (at least two weeks after an infection).

PROTEIN MEASUREMENTS

Twelve proteins were measured in each sputum and serum sample (albumin, orosomucoid, transferrin, ceruloplasmin, haptoglobin, α_1 -antitrypsin (α_1 -AT), α_1 -antichymotrypsin (α_1 -ACh), α_2 -macroglobulin (α_2 -M), C₃, IgA, IgG, and IgM). The immunoglobulins were measured by radial immunodiffusion (Mancini *et al*, 1965) and the remaining proteins by rocket immunoelectrophoresis (Laurell, 1966). All results were expressed as a percentage of a pooled reference serum (Immunodiagnostic Research Laboratory, Department of Immunology, University of Birmingham). The

sputum to serum ratio was then calculated for each protein in each sample. Albumin was taken as the reference protein and the sputum/serum ratio for each protein was divided by the albumin result. This gave a value for albumin of 1 in each sample, and the remaining protein ratios were corrected for albumin.

STOKES RADII

The Stokes radii for most proteins were taken from Burnett *et al* (1976). Albumin, haptoglobin, α_2 -M, and IgM were taken from Felgenhauer (1971, 1974). The relation between sputum/serum protein ratios and Stokes radii was determined using the least squares method for linear regression for five proteins. Albumin, orosomucoid, transferrin, ceruloplasmin, and haptoglobin were chosen because they have a range of Stokes radius from 3.45–5.0 nm and have not been metabolically implicated in lung disease. Their presence in the sputum was assumed to be the result of passive diffusion alone.

The regression line was used to estimate the expected sputum/serum ratios of the other proteins for their Stokes radius by interpolation. The expected value was then compared to the observed value using the standard error of difference of means.

Results

All 12 proteins were found in each specimen except α_2 -M which was undetectable ($<200 \mu\text{g/l}$) in nine of the non-infected patients. Its ratio in these patients was therefore taken as zero.

The sputum/serum ratios of all the proteins

corrected for albumin are summarised in table 1 for the non-infected patients (divided into smokers and non-smokers) and for the patients with active chest infection. At the bottom of the table is the actual albumin sputum/serum ratio (multiplied by 10^2 for convenience). In the non-infected patients this ranged from 0.45 to 1.37 but was much higher in the three patients with infection as shown in fig 5 (7.53, 15.29, and 18.50). No significant difference was found between the smokers and non-smokers for the sputum/serum ratio of any protein.

Mean sputum/serum concentrations of proteins corrected for the albumin result (shown at bottom). Values in parentheses ± 1 SE

Protein	Smokers (n=10)	Non-smokers (n=10)	All patients (n=20)	Present infection (n=3)
Albumin	1.0	1.0	1.0	1.0
Orosomucoid	0.60 (0.07)	0.78 (0.11)	0.69 (0.07)	0.67 (0.14)
Transferrin	0.96 (0.09)	1.00 (0.08)	0.98 (0.06)	0.95 (0.08)
Caeruloplasmin	1.11 (0.20)	0.88 (0.12)	0.97 (0.12)	0.16 (0.06)
Haptoglobin	0.41 (0.06)	0.49 (0.09)	0.45 (0.08)	0.51 (0.34)
α_1 -AT	1.64 (0.19)	1.39 (0.07)	1.52 (0.10)	0.82 (0.05)
α_1 -ACh	6.64 (1.86)	3.18 (0.40)	4.91 (1.01)	1.21 (0.23)
α_2 -M	0.44 (0.32)	0.31 (0.19)	0.37 (0.18)	0.40 (0.09)
IgG	1.71 (0.25)	1.55 (0.24)	1.67 (0.17)	0.70 (0.10)
IgA	17.97 (3.65)	18.01 (3.54)	17.99 (2.46)	1.80 (0.34)
IgM	3.29 (0.65)	2.96 (0.97)	3.13 (0.57)	0.84 (0.37)
C ₃	1.43 (0.21)	1.31 (0.29)	1.37 (0.18)	0.60 (0.02)
Albumin (sputum/serum $\times 10^2$)	0.84 (0.12)	0.82 (0.10)	0.83 (0.08)	13.77 (3.21)

RELATION TO STOKES RADIUS

Non-infected patients

There is a significant inverse relation between the corrected sputum/serum ratios and Stokes radius for the five proteins mentioned earlier. The relation can be expressed as: sputum/serum ratio = $1.55 - 0.179 \times \text{Stokes radius}$ ($\text{SE} \pm 0.36$; $r = -0.29$; $t = 2.999$; $2P < 0.005$) (fig 3).

There are several proteins whose sputum/serum ratios lie outside this relation and some of them are significantly displaced (α_1 -ACh $P < 0.001$; IgG, $P < 0.01$; IgA, $P < 0.001$; IgM, $P < 0.001$; and C₃, $P < 0.05$).

Infected patients

The inverse relation was maintained in the three

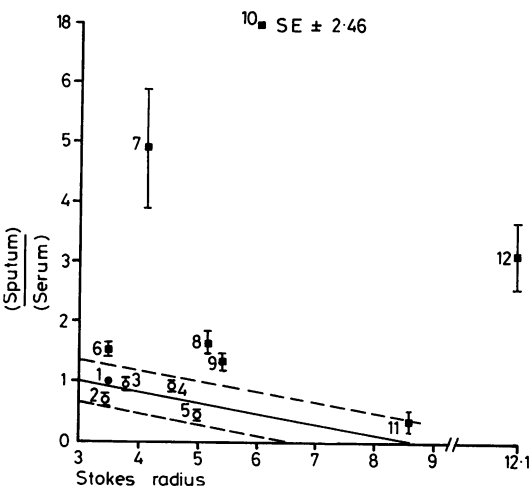


Fig 3 Sputum/serum concentration ratios corrected for albumin plotted against Stokes radius (nm). Regression line ± 1 SE (---) is drawn from albumin (●)—protein 1; orosomucoid—2; transferrin—3; caeruloplasmin—4; haptoglobin—5. Remaining protein (■) results are shown α_1 -antitrypsin—6; α_1 -antichymotrypsin—7; IgG—8; C₃—9; IgA—10; α_2 -macroglobulin—11; IgM—12. Bar lines are ± 1 SE.

patients who had active chest infection. Figure 4 shows, however, that most other proteins lie within this relation. Only IgA ($P < 0.001$) remained significantly displaced. The relation was: sputum/serum ratio = $2.10 - 0.356 \times \text{Stokes radius}$ ($\text{SE} \pm 0.28$; $r = -0.62$; $t \pm 2.849$; $2P < 0.02$).

The intercept is lower than in the non-infected patients. In the presence of inflammation larger proteins would be expected to leak into the secretions raising the intercept. Thus the finding of

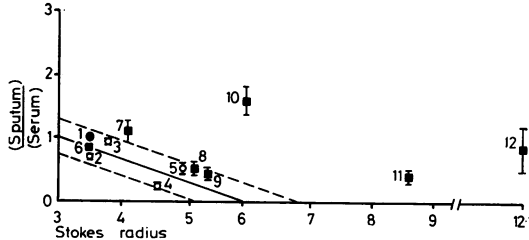


Fig 4 Sputum serum concentration ratios corrected for albumin plotted against Stokes radius in three patients with chest infection. Regression line ± 1 SE (---) is drawn from albumin (●)—protein 1; orosomucoid—2; transferrin—3; caeruloplasmin—4; haptoglobin—5. Remaining protein (■) results are shown α_1 -antitrypsin—6; α_1 -antichymotrypsin—7; IgG—8; C₃—9; IgA—10; α_2 -macroglobulin—11; IgM—12. Bar lines are ± 1 SE.

emphysema (Eriksson, 1978). We have found evidence suggesting that α_2 -M and α_1 -ACh are locally produced or secreted into the bronchial fluids. Lung fibroblasts have been shown to manufacture α_2 -M in vitro (Kaighn and Prince, 1971) but information on α_1 -ACh is lacking. This enzyme inhibits cationic proteolytic enzymes from granulocytes by the formation of complexes (Ohlsson and Åkesson, 1976) and therefore may play an important part in protecting the lung from damage by these proteases. Whether α_1 -ACh is locally produced in the lung is not certain. Burnett (1975) found several samples of amniotic fluid had a higher fluid/serum ratio of α_1 -ACh than would be expected by diffusion. This may be explained by the presence of protein complexes which may alter the antigenic properties of α_1 -ACh resulting in its over-estimation and a broadened precipitation peak on two-dimensional electrophoresis. Ryley and Brogan (1973) measured α_1 -ACh using two-dimensional electrophoresis and commented that the precipitation peak was broadened compared to serum. We measured α_1 -ACh by radial immunodiffusion but have subsequently reviewed our samples using two-dimensional electrophoresis and found similar broadening of the α_1 -ACh precipitation peak to a variable degree (fig 6). This may, at least in part, explain the high sputum/serum ratios in our patients and merits further study.

There are several differences between the results in the non-infected and infected patients. The sputum/serum albumin ratio was much higher in the infected patients. Caution must be expressed in the interpretation of this finding because of small numbers and uncertainty about sample dilution by saliva. It is consistent, however, with increased protein transudation from serum due to bronchial inflammation.

The ratios of α_1 -ACh, IgG, and C₃ were not different from their predicted values in the infected patients and the IgA (fig 5) and IgM ratios were lower in relation to albumin. These findings are probably the result of increased protein transudation. Local production of protein is likely to be small and highlighted by the relative absence of protein from other sources (serum). When inflammation occurs, protein transudation would increase exceeding local protein production, thus decreasing the relative contribution from local production. The sputum/serum ratios would be reduced to values closer to those expected by diffusion from the serum. This is more likely to account for the low IgA ratios in infection than a reduction in local synthesis as suggested by Soutar (1977). A further study of the relative quantities of

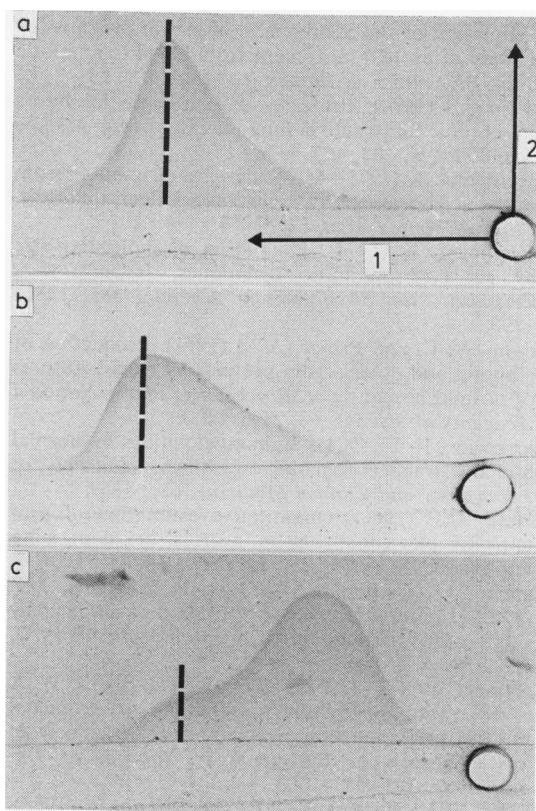


Fig 6 α_1 -ACh by two-dimensional electrophoresis. Protein samples placed in well and electrophoresed right to left in plain agar gel (1) and then vertically into gel containing monospecific antibody to α_1 -ACh (2). Plates a to c are different samples showing progressive broadening of α_1 -ACh precipitation peak as a result of increasing complex formation (see text). Vertical dotted line indicates mobility of serum α_1 -ACh.

IgA and secretory IgA in patients with and without active chest infection would be necessary to clarify this observation.

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