

A difference in the composition of bronchial mucus between smokers and non-smokers

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Kollerstrom, N., Lord, P. W., and Whimster, W. F. (1977). *Thorax*, 32, 155-159. **A difference in the composition of bronchial mucus between smokers and non-smokers.** The ratio of the amount of sulphated to sialic acid mucin (Su/Si) in the mucous glands of the human tracheobronchial tree has been investigated in seven smokers and seven non-smokers. The two mucins were studied in histological sections stained by the high iron diamine/Alcian blue pH 2.5 sequence and assessed by a point-counting method.

Su/Si was greater in the smokers than in the non-smokers, who were almost completely distinguished by this ratio. A decrease in the ratio with each generation of branching from the trachea down the inferior lingular bronchial segmental pathway was seen in both the smoking and non-smoking groups. Analysis of the logarithm of Su/Si showed the smoking group means to be 2.3 times that of the non-smokers at each generation, and over both groups the average decrease down successive generations was given by a factor of 0.9.

In the bronchial epithelium of rats exposed to cigarette smoke there is more sulphated mucin than in control animals (Jones *et al.*, 1973). We set out to see if the same were true of humans, but because the surface epithelium produces only a small amount of mucin and is rarely intact at postmortem examination, we confined our investigation to the subepithelial mucous glands.

The acid mucins synthesised by the cells of the bronchial glands may have sialic acid (neuraminic acid) groups on their side chains (Gibbons, 1963) or sulphate groups (Havez *et al.*, 1965); no other acid side chains have yet been identified. Lamb and Reid (1969) subdivided these two groups by dividing the sialomucin into a fraction sensitive to digestion by the enzyme sialidase (also called neuraminidase) and a fraction resistant to sialidase, and by dividing the sulphated mucin into a fraction which can be detected with stains for sulphated mucin, including high iron diamine, and the remainder which can be detected autoradiographically using ^{35}S in tissue culture.

In chronic bronchitis, which is often associated with cigarette smoking, there is said to be a higher proportion of sulphated mucin (de Haller and Reid, 1965; Lamb, 1969). Lev and Spicer (1965) suggested that the proportion of sulphated mucin was greatest in the tracheal glands and became less

in more distal glands, while in Lamb and Reid's (1972) data the proportion did not change. We therefore determined the ratio of sulphated to sialidated mucin (Su/Si ratio) in each generation from the trachea down the inferior lingular bronchus in smokers and non-smokers.

Material and methods

Fourteen left lungs were obtained *post mortem*, seven from non-smokers, six from cigarette smokers, and one from a pipe smoker (Table 1). The patients had died of diseases which did not involve the respiratory system, except for case 6, who had a carcinoma of the right lung.

The interval between death and postmortem examination varied between two and four days. Some autolysis was therefore inevitable, but in this country there is no way of obtaining bronchial tissues more quickly except by getting surgically resected specimens which are seldom available from non-smokers.

The entire airway from the trachea down the inferior lingular axial pathway to the eighth generation (G8) of dichotomous branching, counting the trachea as generation zero (G0), was dissected out. Transverse sections of tissue were taken from the middle of each generation from

Table 1 Details of the cases used to obtain the ratio Su/Si

| Case | Sex | Age | Smoking history | Mean Su/Si ¹ |
|-------------------------|-----|-----|-----------------|-------------------------|
| Cigarette smoker | | | | |
| 1 | M | 49 | 40/day | 0.72 |
| 2 | M | 56 | 40/day | 0.52 |
| 3 | M | 56 | 10/day | 0.37 |
| 4 | F | 71 | 20/day | 1.22 |
| 5 | M | 69 | 20/day | 0.63 |
| 6 | F | 73 | 10/day | 0.54 |
| Group mean | | | | 0.62 |
| Non-smoker | | | | |
| 7 | F | 64 | NS | 0.33 |
| 8 | M | 57 | NS | 0.22 |
| 9 | F | 62 | NS | 0.33 |
| 10 | F | 60 | NS | 0.21 |
| 11 | F | 49 | NS | 0.30 |
| 12 | M | 85 | NS | 0.27 |
| 13 | M | 68 | NS | 0.24 |
| Group mean | | | | 0.27 |
| Pipe smoker | | | | |
| 14 | M | 84 | 40 g/day | 0.31 |

¹Geometric mean, over generations.
NS = non-smoker.

G2 (lobar bronchus) to G8. For the trachea and main bronchus (G1) longitudinal sections of tissue about 12 mm long, which included cross-sections of two or three cartilage rings, were taken. Two such sections were taken from the middle part of the main bronchus, and three from each of the upper, middle, and lower trachea.

After routine processing and embedding in wax one section was taken from each block and stained with high iron diamine/Alcian blue at pH 2.6 (HID/AB) (Spicer, 1965) by which intracellular mucin was stained either dark brown (sulphated) or blue (sialidated). The sections were examined by light microscopy using $\times 25$ and $\times 40$ objectives and $\times 10$ eyepieces.

Estimates of the areas of HID and AB staining mucins in the subepithelial mucous glands were obtained by counting the number of points superimposed on them by the intersections of an eyepiece graticule with a 0.5 mm square lattice. The ratio Su/Si was derived from the number of points appearing on HID (Su) and AB (Si) staining mucus. Su/Si ratios were obtained separately for each generation down the bronchial pathway in each case. The counts from the three trachea blocks at each level and from the two main bronchus blocks were pooled to obtain those Su/Si ratios. Su/Si ratios were obtained down to the fifth generation, but beyond this there were too few glands. Generally, whole sections were counted, but in the upper airways, where there was much gland tissue, randomly sampled gland clusters were counted. Autolysis made some areas

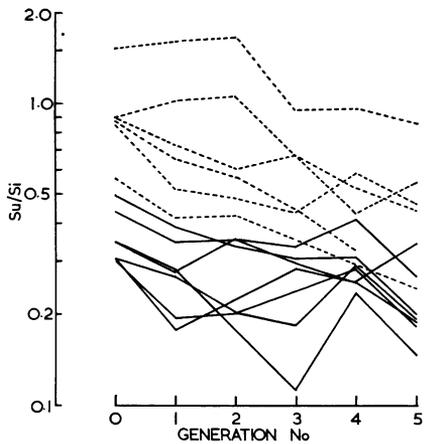
unsuitable for counting, but even where the surface epithelium had gone the glands usually remained compact and coherent.

Counts generally ranged between 100 and 1000 but occasionally, where the sulphate or sialic component was scarce, even at the higher magnification, counts less than 100 were accepted.

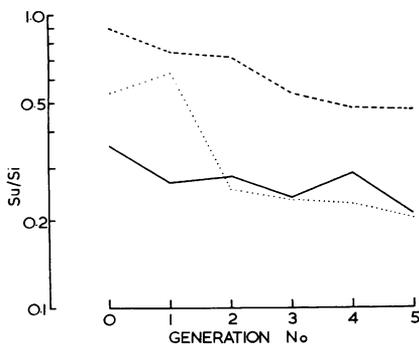
Results

The results are displayed in Fig. 1a,b and the overall means of the ratio Su/Si for each case are tabulated in Table 1. There were no significant differences between the three levels in the trachea, so the counts at the three levels were pooled to give Su/Si for the whole trachea (G0). The ratios from smoking and non-smoking groups (ignoring the pipe smoker) were almost completely separated at all levels down the bronchial pathway. Su/Si was significantly lower in non-smokers ($P < 0.001$), that is, there was relatively less sulphated mucin in non-smokers.

Analysis of variance (Table 2) of the logarithm of Su/Si, taking as factors both smoking habit and linear regression on airway generation number, showed a significant decrease ($P < 0.001$) between generations, taking all cases together (except the pipe smoker). The gradient of the \log_{10} of Su/Si on generation number was -0.045 ± 0.015 (regression \pm SE), which implies that there was a multiplicative factor in Su/Si of 0.9 between successive generations down the pathway. A non-significant interaction between the regression on generation number and the smoking groups in this analysis suggested no difference between regressions within the smoking and non-smoking groups. Because the number of cigarette smokers was not the same as the number of non-smokers, cases and smoking groups could not be included in the same analysis and so separate analyses of variance were performed on the smoking groups, taking as factors cases and linear regression on generation number. These showed significant variations between the cases within each group ($P < 0.01$), and also showed significant ($P < 0.001$) decreases in the \log_{10} of Su/Si with generation number, yielding regression coefficients of -0.060 ± 0.007 and -0.032 ± 0.009 per generation (regression \pm SE) for the smokers and non-smoker groups respectively—they were significantly different ($P < 0.05$, *t* test). These regression coefficients represent multiplicative factors in Su/Si of 0.87 and 0.93 respectively between successive generations down. Insufficient data precluded an analysis to test homogeneity among the individual regressions of \log_{10} Su/Si on generation number in each case, but there



(a)



(b)

Fig. 1 (a) *Su/Si* ratio in six cigarette smokers (----) and seven non-smokers (—) at each generation of branching down the inferior lingular axial pathway. *Su/Si* is plotted using a logarithmic ordinate. The pooled values for the trachea are plotted at generation 0. (b) Mean values of *Su/Si* for the cigarette smokers (----) and non-smokers (—) at each generation. The results for the pipe smoker are plotted on this graph (.....).

appeared to be more variation within the non-smoker group (Fig. 1a).

The patient with a carcinoma of the right lung (case 6) was a smoker and her results were within the smoking range. Exclusion of this case would not materially affect the results.

The pipe smoker gave results which were among the cigarette smoking group in the two major airways, and which in the lower airways were within the non-smoking group (Fig. 1b).

Table 2 Analysis of variance for $\log_{10} (Su/Si)$ taking smoking group and regression on generation number as factors: tracheal sites pooled

| Source of variation | Degrees of freedom | Mean sum of squares | Variance ratio (over residual) |
|---|--------------------|---------------------|--------------------------------|
| Smoking group | 1 | 2.557 | 102.72 |
| Regression on generation number | 1 | 0.454 | 18.23 |
| Deviations from regression | 4 | 0.014 | 0.55 |
| Smoking \times regression | 1 | 0.043 | 1.71 |
| Smoking \times deviations from regression | 4 | 0.014 | 0.55 |
| Residual | 61 | 0.025 | |

The pipe smoker was excluded from the analysis.

Discussion

We have measured only the ratio of the counts from the two areas of staining so we are not able to say whether there were absolute increases or decreases in one or other of the components.

Our results, even in a small number of cases, show that there is significantly more sulphated acid mucin relative to sialomucin in the bronchial mucous glands of cigarette smokers compared with non-smokers. We have also confirmed a diminishing proportion of sulphomucin down the inferior lingular pathway from the trachea. Using the logarithm of the *Su/Si* ratio the decrease is approximately linear with generation number. These logarithmic relationships in *Su/Si* reveal

- (1) a factor of 2.3 (cf. Table 1) between the mean *Su/Si* of the smoking and non-smoking groups, which holds at all generations down the airway, and
- (2) a factor of about 0.9 between the mean *Su/Si* at successive generations down the airway, which is nearly the same for both the smoking and non-smoking groups separately, if not for individuals within groups.

The pipe smoking patient was a retired tin miner. There was no evidence of silicosis but there was marked emphysema. The *Su/Si* results were in the smoking range for the proximal generations but in the non-smoking range distally. It is possible that the pipe smoke differed from the cigarette smoke in affecting only the upper airways, but we do not know whether his habit was to inhale.

One might object that the groups were not homogeneous with regard to sex, but the *Su/Si* ratios in the two female smokers (both post-menopausal) and the four female non-smokers (three post-menopausal) did not differ significantly from those in the men.

The staining technique (HID/AB pH 2.5) allowed us to recognise sulphated mucin which had taken up high iron diamine and sialomucin which had subsequently taken up Alcian blue at pH 2.5. Lamb and Reid (1969) suggest that these uptakes are reasonably constant.

Our Su/Si ratios do nevertheless support reports of a shift towards a greater proportion of sulphated acid mucin in (1) the human subepithelial gland cells in bronchitis and in bronchiectasis (de Haller and Reid, 1965; Lamb, 1969), (2) the glands of pigs with enzootic pneumonia (Jones *et al.*, 1975), and (3) the epithelial goblet cells of rats exposed to sulphur dioxide (Lamb and Reid, 1968).

If the higher proportion of sulphated side chains of mucin in the glands of the upper airways was due to exposure to higher concentrations of inhaled irritants from the ambient air in non-smokers and from tobacco smoke in the smokers, this would be in accord with the smoker/non-smoker difference. Alternatively, the observed gradient in both groups may satisfy a need for mucus with different properties in airways of varying dimensions.

By what mechanism do the bronchial glands produce different mucins? Lamb (1969) claimed that the four types of mucin were evenly distributed throughout the bronchial tree and argued that this reflected 'overall control' of the type of mucus produced by the glands. To explain our finding of a Su/Si gradient down the bronchial tree the control mechanism would have to adjust for glands at different sites. If there was such control then the consistent difference between smokers and non-smokers, along the pathways, suggests that cigarette smoke has a systemic rather than a local effect. This would favour the idea that the control system is affected by soluble components, gaseous or particulate. Alternatively, the gradients observed in both smokers and non-smokers would be more readily explained by graded local responses to diminishing doses of irritant. Inhaled material is also trapped in the mucus, and as it is carried up the bronchi towards the glottis the more proximal airways are exposed to increasing amounts of it. Inspection of Table 1 indicates that there may be a dose effect, those who smoked more having a higher mean Su/Si ratio, which would support this theory.

Quantification of the types of mucin at well-defined sites within the bronchial tree has produced results which are not only amenable to statistical analysis but which may also in this case be used to construct a hypothesis, capable of mathematical formulation, for the mechanisms involved in the variations of the ratio of two types

of mucus. For example, it is of interest to note the exponential decrease in Su/Si reported here and to compare it with the exponential decreases in the radii and lengths of the airways (Horsfield and Cumming, 1968). If the Su/Si ratio was related to the rheological properties of the mucus, this could be used in constructing models of normal and abnormal mucus transport within the airways.

We believe the Su/Si ratio may prove to be a sensitive measure of response to inhaled irritant, including cigarette smoke.

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