Preliminary Communications

Goblet Cell Increase in Rat Bronchial Epithelium after Exposure to Cigarette and Cigar Tobacco Smoke

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Summary: Both cigar and cigarette tobacco produce an increase in the number of goblet cells in the rat trachea and intrapulmonary airways over a six-week period. The increase in goblet cells is similar for the two types of tobacco; in both it is proportional to increase in dose and greatest in the proximal intrapulmonary airways.

Introduction

Increase in the goblet cell number in the tracheobronchial tree of experimental animals has been produced by a wide range of irritants—formol saline (Florey, Carleton, and Wells, 1932), sulphur dioxide (Reid, 1963 ; Lamb and Reid, 1968), chlorine (Elmes and Bell, 1963), and nitric oxide (Freeman and Haydon, 1964). However, the only report of an increase in goblet cells in animals exposed to tobacco smoke (Mellors, 1958) failed to give quantitative data.

With the wide interest in the relation of smoking to the human disease of chronic bronchitis it seems surprising that so few investigations have been made into the relevant biological effects in animals. In particular there has been only one investigation into the comparative effects of cigar and cigarette tobacco smoke on animals (Passey and Blackmore, 1967 ; Lamb, 1967). Using levels of cigar and cigarette exposure of 30 to 40 a day, these workers showed that cigarette smoke produces more squamous metaplasia than cigar smoke. Localized increase in goblet cells was seen, but counting these cells was unsatisfactory as they were absent in areas of squamous metaplasia.

The purpose of the present study was to establish the increase in goblet cell number at different levels of the bronchial tree produced by various doses of cigarette and cigar tobacco smoke. To avoid severe metaplastic lesions three dose levels lower than that used by Passey and Blackmore (1967) were chosen for investigation.

Materials and Methods

Male rats (Anticimex—Sweden) descended from pathogen-free stock were used.

Exposure

Cigarettes were made from either cigarette or shredded cigar tobacco, using the same paper for each. The cigarette tobacco was flue-cured as used in a commonly smoked English cigarette ; the cigar tobacco was a naturally cured leaf. The average weight of a cigarette was 1.4 g. and of a cigar 1.0 g. These two types of cigarette are subsequently referred to as "cigarettes" and "cigars" respectively.

Animals were exposed to tobacco smoke in aluminium cabinets fed with smoke by the Wright Autosmoker, set to smoke one cigarette in 6 to 10 minutes with four puffs per minute. As the aim was to expose the animals to smoke from the same weight of tobacco over the same period of time a higher number of cigars were smoked continuously by the Autosmoker, while the fewer cigarettes were smoked over the same period with occasional 10-minute gaps. The animals were exposed to the tobacco smoke for six weeks, on five days in the week, for periods up to three and a half hours a day according to dosage. Two exposure cabinets were used, and to obviate any difference between them each "smoked" cigars and cigarettes on alternate days. The machines were cleaned each day.

Three doses of cigarette were investigated—5, 10, and 20 a day, as well as the equivalent doses of cigars. The experiments described here were carried out during two separate periods of six weeks' exposure (see Table). In exposure A control animals were compared with those exposed each day either to the smoke of 10 cigarettes or to that of equivalent cigars. In exposure B control animals and those exposed to the smoke of either 5 or 20 cigarettes or equivalent cigars were studied : that is, the same control animals were used for the 5- and 20-cigarette exposure. In all, 55 animals were used, the mean weight of the 25 in exposure A being 231 g. and of the 30 in exposure B 150 g.

Control animals were kept alongside "smoked" animals except during the smoking period, when they were kept in an adjoining room. Apart from the exposure period all rats received Plowco diet 86 and water as wished. Animals were killed by intraperitoneal injection of 1 to 2 ml. of Nembutal solution (pentobarbitone sodium *B.P.*, 60 mg./ml.) on the day after the last exposure to smoke—that is, about 20 hours after removal from the smoking-chambers.

Histological Preparation

The trachea and lungs were fixed by inflating with buffered formol saline till the lung margins were rounded. The specimens were fixed on pieces of card to maintain the tracheal length as in the body. One block was cut longitudinally down the trachea and one through the left lung to obtain an ideal section, including the left main airway and its five major branches. Sections were cut at 4μ thickness and stained with haematoxylin and eosin, and, to show up goblet cells, with the combined Alcian blue and periodic acid Schiff stains (A.B./P.A.S.).

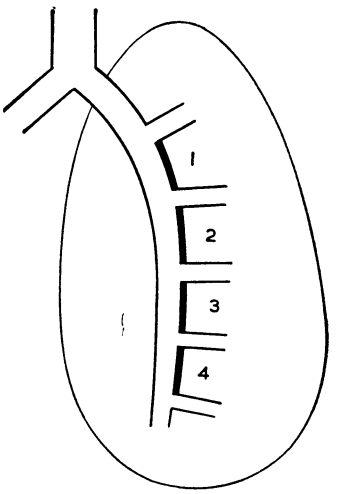

FIG. 1.—Diagram showing the four levels of the intrapulmonary airways at which goblet cells were counted.

The goblet cells were counted continuously along the trachea for 20 consecutive high-power fields, each field being about 0.3 mm. in length. Goblet cells in the left lung were counted continuously over parts of the main airway lying between the

major branches (levels 1–4, see Fig. 1). The number of goblet cells for each site was recorded as the mean count per high-power field.

RESULTS

FAILURE TO GAIN WEIGHT

The general condition of all animals was good throughout the experiment. Though the rats appeared to be little affected by the highest exposure dose of cigarettes, the exposed rats failed to gain weight as much as the others (see Table). The weight

Effect on Animals' Weight of Exposure to Tobacco Smoke

Daily Exposure Dose	No. of Rats	Mean Weight (g.) at End of Experiment (Range)	Weight Difference (as % of Control Weights)
		Exposure A	
Controls	8	362 (355–375)	
10 cigarettes ..	9	326 (310–345)	10
Equivalent cigars ..	8	317 (305–325)	12·4
		Exposure B	
Controls	6	298 (280–325)	
5 cigarettes ..	6	291 (280–300)	2·3
Equivalent cigars	6	280 (270–300)	6
20 cigarettes ..	6	252 (245–270)	15·4
Equivalent cigars	6	271 (260–275)	9

of the animals exposed either to five cigarettes or the equivalent cigars was not significantly different from the normal. Animals exposed to either 10 cigarettes or the equivalent cigars showed a similar weight gain, which was significantly less than that of the controls. This difference from the controls was significant ($P<0.01$ for cigars and <0.05 for cigarettes). In animals exposed to 20 cigarettes or the equivalent cigars there was also failure to gain weight. In addition the animals exposed to this dose of cigarette smoke weighed significantly less than those exposed to equivalent cigar smoke ($P<0.01$).

HISTOLOGICAL EFFECTS

At the end of the six weeks' exposure to tobacco smoke the only histological changes in the tracheal bronchial epithelium were a thickening of the epithelium and the increase in the number of goblet cells. The cilia appeared normal and intact throughout the airways, and there was no evidence of the transitional or squamous metaplasia found with more severe exposures to cigarette smoke (Lamb, 1967). None of the animals showed any rat bronchiectasis or lymphocytic cuffing of the bronchi.

GOBLET CELL INCREASE

The goblet cell counts are summarized in Fig. 2. Animals exposed either to five cigarettes or to the equivalent cigars all show increased goblet cell counts in the trachea and more proximal airways but not at the periphery (Fig. 2). At levels 1 and 2 the increase is significantly different from the control values ($P<0.01$); at level 3 the slight increase gives no statistical difference between the control and smoking animals. Even the results for the cigar animals, which are higher than for the cigarette, are not statistically significant ($P<0.5$). The goblet cell counts at level 4 are the same as the control values. After exposure to 10 cigarettes or the equivalent cigars goblet cell counts were significantly higher at all levels than in the control group ($P<0.01$). There was no difference, however, between the effect of cigar and cigarette smoke at any level, the overlap in results being nearly complete. The increase in the goblet cell number is greatest in the trachea and proximal airway levels.

Animals exposed to either 20 cigarettes or the equivalent cigars showed significant increase in goblet counts at all levels ($P<0.01$). At each level the increase in goblet cell counts is similar for the two types of tobacco smoke, the increase being greatest in the trachea and proximal intrapulmonary airways.

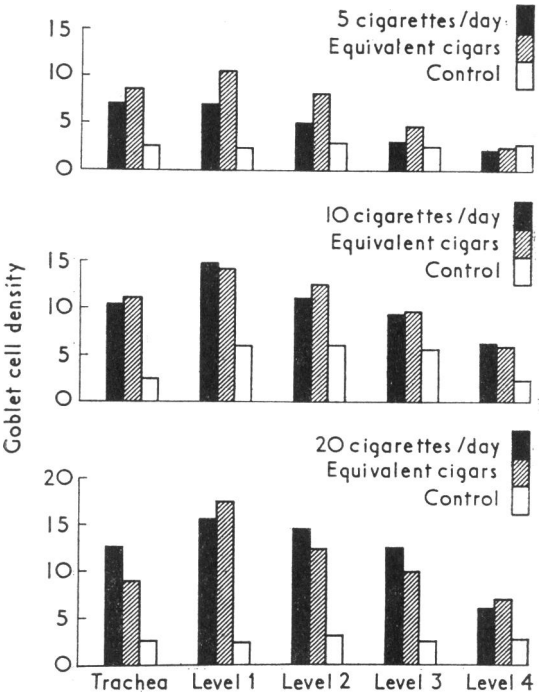

FIG. 2.—Goblet cell density (number of goblet cells/high-power field) in the tracheal and four intrapulmonary airway levels in rats exposed to 5, 10, and 20 cigarettes/day made of either cigarette tobacco (black) or cigar tobacco (shaded). The controls (open) for the 5- and 20-cigarette dose groups are the same. The exposure to tobacco gives a dose-related increase in goblet cells: the increase, at any dose, is similar for cigars and cigarettes.

As the animals used for exposure to 5 and 20 cigarette/day are from the same batch, and therefore compared with the same controls, the effect of dose can be precisely assessed. There is a marked dose effect. The results in the 10 cigarette/day batch are between those for the 5 and 20 cigarette/day groups, though the controls for those groups have slightly higher values.

HISTOCHEMICAL CHANGE

In a previous experiment with sulphur dioxide as the irritant (Lamb and Reid, 1968) an alteration in the histochemistry of the acid glycoprotein produced by the goblet cells was associated with the increase in goblet cell number. This change from a sialomucin to a sulphomucin was seen in those animals exposed to 10 cigarettes but not those exposed to 5 or 20 cigarettes. It appears that this histochemical change can be produced by exposure to cigarette smoke and is not associated only with sulphur dioxide. At present we cannot explain the absence of this effect with tobacco smoke in the younger animals.

DISCUSSION

The increase in goblet cell number in the rat bronchial tree produced by tobacco smoke is similar to that produced by other irritants, and confirms the importance of external factors in the production of an increase of goblet cells, one of the characteristic findings in chronic bronchitis (Reid, 1954). The dose-related increase in goblet cell count further illustrates the value of this technique of studying the effect of bronchial irritants (Reid, 1963).

The cigarette tobacco used in this investigation was flue-cured Virginian tobacco, and that in the cigars a naturally cured leaf. Although these two production methods produce tobaccos of different chemical composition, the present results indicate

that, despite these differences, the biological response of the bronchial epithelium to both was similar if assessed by goblet cell increase.

This similarity is in striking contrast to the difference in mitotic counts produced at higher doses. Daily exposure to smoke from more than 30 cigarettes produced so many mitoses that goblet cell counts as here described were not possible: there was no such excess from exposure to cigar smoke (Lamb, 1967 ; Passey and Blackmore, 1967). It has been shown, using sulphur dioxide as the irritant, that the alteration in goblet cell number does not parallel the severity of epithelial damage as measured by the mitotic count (Lamb and Reid, 1968).

Loss of weight or failure to gain weight is usual in experimental animals exposed to tobacco smoke. This loss of weight is not usually attributable to the severity of pulmonary damage, and Elson and Passey (1963) produced evidence to suggest that the nicotine present in the tobacco smoke is the main cause of loss of weight.

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Medical Memoranda

Treatment of Severe Salicylate Poisoning by Forced Alkaline Diuresis

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Treatment of adult salicylate poisoning by forced alkaline diuresis is now well established. The case reported here illustrates the effectiveness of this form of treatment in very severe intoxication. The relative importance of diuresis and alkalinization in elimination of salicylate from the body is discussed.

CASE REPORT SUMMARY

A man aged 36 was admitted in coma to the London Hospital approximately one and a half hours after taking 300 0.3-g. aspirin tablets (90-g. of salicylate). Following immediate administration of 150 mEq of sodium bicarbonate his standard bicarbonate was still only 18 mEq/l. Further intravenous therapy consisted of rapid infusion, in rotation, of sodium bicarbonate, normal saline, and 5% dextrose. During the first 12 hours 14 litres of fluid were administered and urine output was 10.3 litres ; 190 g. of mannitol in solution was given to counter fluid retention.

Repeated estimations were made of blood salicylate, serum potassium, arterial *p*H, standard bicarbonate, and $Paco_2$. Urine *p*H and salicylate concentrations were measured hourly.

Fig. 1 shows the rise in arterial and urine *p*H and the rapid fall in blood salicylate level from 138 to 15 mg./100 ml. during the first 12 hours. Over the second 12-hour period intravenous fluids were administered at a much reduced rate. During the first period serum potassium fell to 2.8 mEq/l. despite administration of 230 mEq of potassium as intravenous potassium chloride. Preparations were made for haemodialysis, but this proved unnecessary.

DISCUSSION

Severe aspirin poisoning can be treated either by haemodialysis or by forced alkaline diuresis. Haemodialysis is an effective method of treatment and is of especial value in the circumstances of cardiovascular collapse or renal failure. It may be required if the blood salicylate continues to rise despite other treatment. The reports reviewed by Beveridge *et al.* (1964) show that 3 to 9 g. of salicylate may be removed during two to six hours of haemodialysis, resulting in a significant fall in blood level. The majority of patients in this review had high levels of salicylate (51 to 115 mg./100 ml.) and had taken the drug more than 12 hours before treatment was instituted. The present case shows that forced diuresis can result in excretion rates in excess of the above values. Moreover, haemodialysis requires special staff and apparatus which takes two to three hours to prepare.

Forced alkaline diuresis has the advantage that it is simple to carry out if carefully controlled, and may be started immediately the diagnosis is made. By giving a water load the dehydration commonly present in salicylate poisoning due

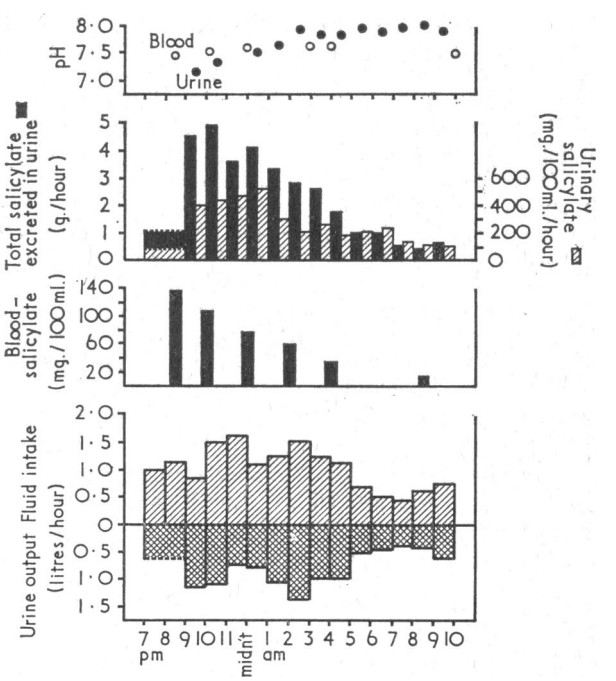

FIG. 1.—Biochemical observations during treatment.