# Hypersecretion of Mucus Glycoproteins in Rat Airways Induced by Tobacco Smoke

Stephen J. Coles, PhD, Lionel R. Levine, BSc, and Lynne Reid, MD

**An animal model of chronic bronchitis has been produced *in vivo* by exposing rats for 6 weeks to tobacco smoke: the laryngeal and tracheal glands have been studied *in vitro* by organ culture to analyze glycoprotein precursor incorporation and glycoprotein secretion by individual cells, a feature not previously studied. In the hypertrophied glands produced by tobacco smoke exposure, the cellular rate of glycoprotein secretion was increased. The *in vivo* administration of phenylmethyloxadiazole (PMO) to rats exposed to tobacco smoke blocked this effect. *In vitro* analysis of glands from unexposed rats that received PMO showed that it modified cell function directly by reducing the rates both of glycoprotein discharge and of precursor incorporation into intracellular glycoproteins. (Am J Pathol 94:459–472, 1979)**

THE ANATOMIC STIGMATA of human chronic bronchitis, ie, goblet cell hyperplasia and submucosal gland hypertrophy, have been induced by exposure to tobacco smoke in several species of laboratory animals, including rabbits,[1] dogs,[2] lambs,[3] and rats.[4-7] Quantitative histochemical analysis of intracellular glycoprotein in goblet cells of rat airways [7] has shown that tobacco smoke causes a shift from the production of neutral to acid glycoprotein, the latter being mainly of the type containing neuraminidase-resistant sialic acid moieties.

The oxolamine derivative phenylmethyloxadiazole (PMO) has been shown to protect against the ciliostatic effect of tobacco smoke [8] and to exhibit anti-inflammatory properties.[9] Recent studies [6,7] have shown that PMO can prevent tobacco-smoke–induced goblet cell hyperplasia in rat airways, although it has no effect on the shift from neutral to acid glycoprotein or on tracheal gland hypertrophy.

The effect of tobacco smoke on the cellular rate of glycoprotein synthesis and secretion in mucus-secreting cells of the airways of experimental animals has not been analyzed, largely due to the lack of a suitable experimental system. Organ culture techniques combined with quantitative autoradiography have been used to analyze glycoprotein secretion by cells of the human bronchial gland [10-12] and have enabled comparison to be made between normal and hypersecreting airways.

The purpose of the present study was to modify and apply the organ

From the Department of Experimental Pathology, Cardiothoracic Institute, London, England.
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Address reprint requests to Dr. Stephen Coles, Department of Pathology, Children's Hospital Medical Center, 300 Longwood Avenue, Boston, MA 02115.
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culture technique of Sturgess and Reid [10] to provide an *in vitro* analysis of airway glycoprotein secretion in a model of chronic bronchitis produced *in vivo* by exposing rats to tobacco smoke. Morphometric and quantitative autoradiographic methods have been used to quantify changes in individual mucus-secreting cells. Experiments were also carried out to determine to what extent these changes are modified by phenylmethyloxadiazole.

## Materials and Methods

### Experimental Animals

Specific-pathogen-free, male, albino, Sprague–Dawley rats, weighing 150 to 200 g, were obtained from Olac and Anglia Laboratories (U.K.). Before use, 3 animals from each batch were killed and sections of trachea and of axial and lateral airways were examined for evidence of infection. Rats were considered unsuitable if more than two lymphocytic foci were present in their airways.[13]

### Morphologic Studies

The morphology of laryngeal and tracheal glands was studied in 10 rats which were killed by intraperitoneal administration of sodium pentabarbital (Nembutal). The upper airway between the epiglottis and mid trachea was carefully excised and fixed in phosphate-buffered, formal saline (pH 7.2) for 48 hours. The whole specimen was embedded in paraffin wax, and 4-$\mu$ transverse serial sections were cut from the epiglottis to the upper trachea. Sections were stained with hematoxylin and eosin for gland size measurements. For studies of gland structure, pieces of larynx and trachea were fixed in Karnovsky's fixative followed by postfixation in 1% osmium tetroxide. Tissue fixed in this way was dehydrated in graded alcohols and embedded in Epon. One-micron sections were prepared with a Sorvall MTB2 ultramicrotome and stained with toluidine blue.

### Smoking Experiments

The effect on the size and secretory activity of tracheal and laryngeal glands of tobacco smoke, administered alone or with phenylmethyloxadiazole, was analyzed in 40 rats which weighed approximately 200 g at the start of the experiment. PMO (3-phenyl-5-methyl-1,2,4-oxadiazole) was provided by Professor T. Dalhamn (Uppsala, Sweden) and was suspended in 4% methylcellulose (Methocel) in physiologic saline for injection. Standard commercial cigarettes of 100% Virginia tobacco were obtained from H. M. Customs and Excise. Analysis of cold-trap condensate of the cigarette, smoked to a 10-mm butt, gave values of 20.56 mg tar and 2.83 mg nicotine per cigarette. [14] The pH of the smoke was 4.4. The animals were divided into groups (10 per group) and each group was subjected daily to one of the following regimens: a) tobacco smoke exposure, b) tobacco smoke plus PMO (20 mg/kg, intraperitoneally), c) PMO alone (20 mg/kg, intraperitoneally), and d) physiologic saline (2 ml/kg, intraperitoneally [control group]). Rats in Groups a and b were exposed to smoke from 25 cigarettes per day for 6 weeks in aluminum cabinets fitted with an automated smoking system.[15] Details of this system have been described previously.[6] The animals were weighed daily throughout the exposure period and were killed 16 hours after the last exposure.

### Organ Culture

Immediately after sacrifice, the larynx and upper trachea of 5 rats in each group were removed and washed in culture medium (Medium 199, BDH Biochemicals Ltd, U.K.) to remove blood and adherent mucus strands. From each specimen, complete rings of

larynx and trachea, approximately 2 mm deep, were cut from the region between the distal aspect of the dorsal cricoid cartilage and the second tracheal cartilage. These explants were placed on pieces of sterile lens tissue on perforated aluminum rafts in sterile Petri dishes [16] beneath which enough culture medium was pipetted (approximately 2 ml) to moisten the lens tissue. The explants were then incubated in a humidified atmosphere of 95% oxygen/5% carbon dioxide at 37 C in air-tight Perspex chambers. After equilibration for 1 hour, the medium was replaced with fresh medium containing 25 $\mu$Ci/ml $^3$H-threonine (specific activity, 8.21 Ci/mM; Radiochemical Center, Amersham, U.K.) and the explants were pulse-labeled for 30 minutes. The explants were then washed with fresh medium and reincubated in "chase" medium, containing no label, for 1, 2, 3, or 4 hours. In studies of mucous cell secretory rate, explants were chased for 90 minutes either in unlabeled medium or medium containing 10 $\mu$g/ml acetyl-$\beta$-methyl choline bromide (methacholine bromide) or 5 $\mu$g/ml atropine sulfate (Sigma Chemical Co., U.K.). At the end of the chase period, the explants were fixed in buffered formal saline, processed, and embedded in paraffin wax. Sections 4-$\mu$ thick were cut and stained with PAS [17] to visualize intracellular glycoprotein. Autoradiography was carried out by the stripping-film technique [18] using Kodak AR-10 stripping film and an exposure time of 7 days at 4 C. After exposure, the autoradiographs were developed in Kodak D-19 developer at 18 C, dried, and mounted with coverslips for microscopic examination.

### Morphometry of Submucosal Glands

Measurement of mucous tubule diameter, mucous cell height, and collecting duct diameter were made on 4-$\mu$-thick sections, stained with hematoxylin and eosin, of the larynx and upper trachea of 5 animals from each group. With a 8× measuring eyepiece graticule and 40× objective lens, the following measurements were made: a) mucous tubule diameter measured basement membrane to basement membrane at the minimum diameter of a mucous acinus to prevent error due to oblique sectioning, b) mucous cell height measured as the distance between the basement membrane and cell apex at the same position as in Group a, and c) collecting duct diameter measured at the minimum diameter of the duct. For each rat, laryngeal and tracheal glands were analyzed separately from three sections of laryngeal and two sections of tracheal gland. The average value for each measurement was calculated for each experimental group, and the group mean and standard error were determined. In addition, the frequency distributions of the variously sized mucous tubules were compared by $\chi^2$ analysis.

### Assessment of Secretory Rate

The rate at which labeled glycoprotein was secreted by mucous cells of the tracheal and laryngeal glands was determined by a modification of the secretory index (SI) method used by Sturgess and Reid [10] to measure secretory rate in the human bronchial gland. Autoradiographs of explants were viewed by light microscopy with a 100× oil immersion objective. Since individual mucous cells could not be distinguished, a squared eyepiece graticule was used to superimpose squares on the mucous acini so that the luminal surface was divided into segments (each segment approximately 10 $\mu$ wide). For each segment the presence or absence of silver grains at or on the luminal surface was recorded and the SI was calculated as the proportion of segments with luminal grains, ie, discharging labeled glycoprotein. For each explant, the SI was calculated from counts of 150 to 200 segments; explants providing less than this number of counts were not used for quantification. F-test analysis of variance of SI values revealed that there was no significant difference between SI values obtained from a) laryngeal and trancheal glands, b) different regions of the laryngeal glands, and c) different animals subjected to the same experimental regimen. For this reason, the mean SI for a group was based on individual explants rather than individual animals. In the present study, the mean for a group is based on 10 to 15 explants obtained from 5 rats.

### Assessment of $^3$H-Threonine Incorporation

The incorporation of $^3$H-threonine into mucous cells of tracheal and laryngeal glands was quantified by measuring, in autoradiographs, the density of silver grains overlying these cell types. All measurements of grain density were made by reflectance microscopy [19,20] using a Vickers M74 reflectance microscope. Reflectance measurements were made with a 100× oil immersion objective of areas of mucous cell cytoplasm of uniform size (30 sq $\mu$). Since the density of silver grains overlying the mucous cell changes during its secretory cycle,[12] measurements were restricted to those mucous cells having grains only in the basal aspect of the cell. Between 20 and 50 measurements were made per explant, and the mean grain density per explant was calculated. From each value, a background reflectance reading was subtracted (measured as the mean of 10 reflectance readings over areas of the autoradiographs away from the tissue section). To enable comparison of grain density values between batches of autoradiographs, a standard preparation of a light-fogged autoradiograph was prepared with each batch. Reflectance values of the experimental material in arbitrary photometer units (g) were expressed as a fraction of the value for the standard preparation (G) providing as the unit of grain density the dimensionless fraction g/G.[21]

## Results

### Morphology of Tracheal and Laryngeal Glands

Most of the submucosal gland in the upper trachea of the rat is between the distal edge of the vocal folds and the mucosa between the second and third tracheal cartilage rings. The laryngeal gland occurs almost entirely in the mucosal layer overlying the cricoid cartilage from its proximal aspect in the dorsal wall of the airway to its distal aspect in the ventral wall. At the level of the first tracheal cartilage ring, the laryngeal gland disappears and is replaced by discrete tracheal glands which diminish in size and frequency distally and are situated in the ventral wall of the airway between the cartilage rings.

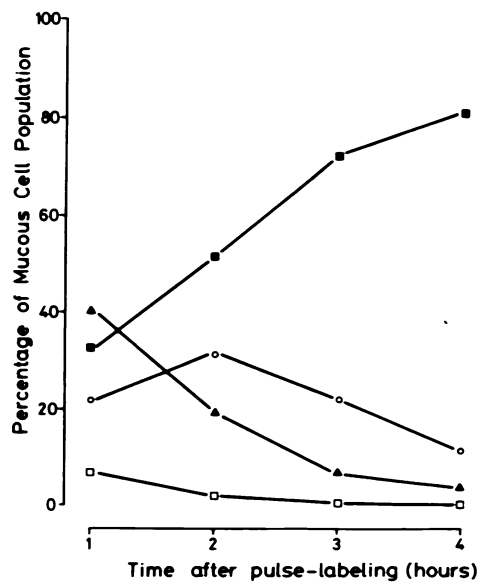
In structure, the laryngeal and tracheal glands are of the compound tubulo-acinar type and resemble the human bronchial gland [22] in that they consist of a main collecting duct and typical mucous and serous secretory tubules.

Collecting ducts occurred only in the mid and lower regions of the laryngeal gland and were more frequently seen in the latter, although their mean diameter was similar at both levels (117.4 ± 6.9 $\mu$ and 108.7 ± 8.4 $\mu$, respectively). The number of mucous tubules at each level was in direct proportion to the area of mucosa occupied by gland and was greatest (mean = 27.1 acini/section) in the mid region of the laryngeal gland. Mucous tubule diameter and mucous cell height were similar at each level in the laryngeal gland (mean = 48.7 ± 3.9 $\mu$ and 12.21 ± 0.33 $\mu$, respectively) but were smaller in the tracheal gland (35.9 ± 3.0 $\mu$ and 10.24 ± 0.36 $\mu$, respectively). In studies of gland size changes, laryngeal and tracheal glands were thus analyzed separately.

In this study, quantification of the size and secretory activity of serous tubules was not carried out since in humans the major changes associated with gland hypertrophy occur in the mucous cell population.[23] Serous cells were examined routinely in all experiments, however, and, when appropriate, qualitative changes were recorded.

### Secretory Activity in Organ Culture

Autoradiographic localization of $^3$H-threonine in mucous cells at different times after pulse-labeling indicated that label was initially incorporated into the basal part of the cell in the region around the nucleus. After incorporation, the label moved up the cell, within secretory granules, to the cell apex from which it was ultimately discharged into the tubule lumen. A similar pattern of labeling has been described for mucous cells of the human bronchial gland.[10,12] In mucous cells of the rat tracheal and laryngeal glands, fixed at the same time after pulse-labeling, silver grains were not located in each cell at the same level, indicating that the secretory cycles of mucous cells in the same gland are not synchronous. The proportions of the mucous cell population with grains at different intracellular locations at various intervals after pulse-labeling are illustrated in Text-figure 1. As early as 1 hour after pulse-labeling, $^3$H-threonine was incorporated into glycoprotein and, in 73% of the mucous cell population, was in the apical region of the cells. Of these cells, 32% could be seen discharging labeled glycoprotein. By contrast, in human bronchial




TEXT-FIGURE 1—Position of labeled glycoprotein in mucous cells 1 to 4 hours after pulse-labeling. Explants from control animals were pulse-labeled with $^3$H-DL-threonine for 30 minutes, chased with Medium 199, and fixed in formal saline at 1, 2, 3, and 4 hours after pulse-labeling. Autoradiographs were prepared as described in *Materials and Methods*. The percentage of mucous cells with grains localized to each of the following intracellular regions was assessed: basal region (*open squares*), basal and middle regions (*triangles*), apical region (*circles*), and apical region and cell surface (*solid squares*).

gland 1 hour after pulse-labeling, less than 10% of the mucous cell population is discharging labeled glycoprotein.[10] Since the rate of secretion of glycoproteins was evidently faster than that of the human bronchial gland, the secretory index method of quantifying secretory rate of the latter [10] was inappropriate since this method was based on measuring the proportion of mucous cells discharging label 4 hours after addition of the pulse. In the rat, since 81% of mucous cells were discharging labeled glycoprotein 4 hours after pulse-labeling (Text-figure 1), a secretory index based on a 2-hour incubation was more appropriate.

#### Effect of Tobacco Smoke and PMO

No pathologic changes, other than those reported below, were observed in any experimental group. The lungs of animals exposed to tobacco smoke showed no evidence of bronchiectasis or consolidation; in the airways, no epithelial stripping, loss of cilia, or squamous metaplasia was seen. The degree of leukocyte infiltration was as low in exposed as in control animals.

#### Changes in Gland Size

In animals exposed to tobacco smoke, there was a significant increase in the mean diameter of mucous tubules in both tracheal ($P < 0.05$) and laryngeal ($P < 0.001$) glands (Table 1). The lumens of mucous tubules in tobacco-smoke–exposed animals, unlike the controls, were often filled with frothy, PAS-positive material presumably derived from adjacent mucous and/or serous cells. Mucous tubule hypertrophy induced by tobacco smoke seemed, at least in part, due to an increase in the size of individual mucous cells (Table 1; $P < 0.001$ in both tracheal and laryngeal glands). In exposed animals, the proportion of cells replete with secretory

Table 1—Effect of Tobacco Smoke, With or Without PMO, on Dimensions of Tracheal and Laryngeal Glands

| Gland | Regimen | Tubule diameter (μ) | Cell height (μ) | Collecting duct diameter (μ) |
|---|---|---|---|---|
| Laryngeal | Control | 50.1 ± 2.5 | 12.43 ± 0.31 | 124.7 ± 6.8 |
| | Tobacco smoke | 69.7 ± 2.9 | 16.71 ± 0.34 | 176.5 ± 9.6 |
| | Tobacco + PMO | 59.1 ± 1.6 | 14.97 ± 0.24 | 146.9 ± 6.7 |
| | PMO | 55.9 ± 2.1 | 14.01 ± 0.38 | 135.6 ± 6.9 |
| Tracheal | Control | 38.4 ± 2.9 | 10.61 ± 0.27 | |
| | Tobacco smoke | 52.6 ± 3.4 | 13.32 ± 0.30 | |
| | Tobacco + PMO | 45.7 ± 2.9 | 11.89 ± 0.27 | |
| | PMO | 37.2 ± 3.9 | 11.23 ± 0.21 | |

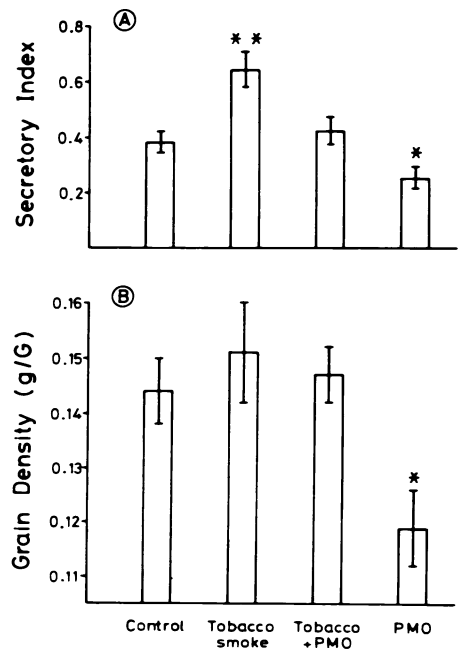
Gland size quantification based on study of 5 rats per group as described in *Materials and Methods.* Values given are means ± SE.

material was evidently considerably lower than in the controls, presumably as a direct result of increased cellular secretory rate (see below). In addition to mucous cell changes, tobacco smoke exposure caused a significant increase in the size of collecting ducts (Table 1; $P < 0.001$); some ducts in excess of 400 $\mu$ were present.

Administration of PMO to animals exposed to tobacco smoke partially prevented the increase in size of mucous tubules, mucous cells, and collecting ducts, although in this group all values were greater than the controls. In animals which had received only PMO, the size of mucous cells in laryngeal glands was significantly greater ($P < 0.05$) than in the controls, apparently the result of retention of intracellular secretory material, whereas mucous tubule and collecting duct diameters were not significantly different from those of controls.

### Changes in Secretory Activity

The SI values of mucous cells in the tracheal and laryngeal glands of rats exposed to tobacco smoke were significantly greater than those of control animals (Text-figure 2; $P < 0.01$), but the density of silver grains overlying mucous cells was similar in the two groups. Since the grain density is based on a measurement made per unit area of cytoplasm and since tobacco smoke caused a significant increase in cell size (see above),




TEXT-FIGURE 2—Effect of tobacco smoke and PMO on secretory rate and $^3$H-threonine incorporation into mucous cells. Explants of trachea and larynx from each experimental group (controls [n=16], tobacco smoke [n=17], tobacco smoke + PMO [n=14], and PMO alone [n=11]) were pulse-labeled with $^3$H-DL-threonine. Secretory index (**A**) and grain density (**B**) were measured as described in *Materials and Methods*. Values given are means ± SE. Values marked with *asterisks* differed significantly from control values: $^*P < 0.05$, $^{**}P < 0.01$ (by Student *t* test).

the absolute number of silver grains per mucous cell was evidently higher in the exposed than in the control animals.

Administration of PMO to rats exposed to tobacco smoke completely prevented the tobacco-smoke–induced increase in secretory rate of mucous cells (Text-figure 2), but the density of grains was similar to both control and tobacco smoke groups. As with the tobacco smoke group, the mucous cells of animals exposed to tobacco and PMO were larger than those of control animals (Table 1) and, thus, the amount of $^3$H-threonine incorporated into a mucous cell was probably slightly greater than in controls.

PMO administered alone caused a significant decrease both in secretory rate ($P < 0.05$) and $^3$H-threonine incorporation ($P < 0.05$) compared with the controls. Mucous cells after PMO administration were characterized by retention of secretory material, but since the low level of $^3$H-threonine incorporation suggested that glycoprotein synthesis was reduced, this effect was probably due to an effect of PMO on glycoprotein discharge.

### Effect of Parasympathomimetic Agents

In human airways, submucosal gland hypertrophy is accompanied not only by an increase in basal secretory rate [10] but also by enhanced sensitivity to parasympathomimetic stimulation and a reduction in the efficacy of cholinergic antagonists in blocking secretion.[11]

The results of a study of the effect of methacholine and atropine on the secretory rate of mucous cells in each experimental group are presented in Table 2. The SI values of mucous cells in explants of control animals were increased by 49% ($P < 0.01$) by administration of 10 $\mu$g/ml methacholine.

Table 2—Effect of Methacholine and Atropine, Added After Pulsing, on Secretory Index of Mucous Cells in Explants From Rats Exposed to Tobacco Smoke, With or Without PMO

| Regimen | Secretory index | | |
|---|---|---|---|
| | No drug | Methacholine (10 $\mu$g/ml) | Atropine (5 $\mu$g/ml) |
| Control (n = 6)* | 0.35 ± 0.04 | 0.53 ± 0.03 | 0.28 ± 0.04 |
| Tobacco smoke (n = 5) | 0.64 ± 0.05 | 0.91 ± 0.03 | 0.61 ± 0.04 |
| Tobacco + PMO (n = 5) | 0.36 ± 0.04 | 0.47 ± 0.05 | 0.32 ± 0.05 |
| PMO (n = 5) | 0.21 ± 0.03 | 0.29 ± 0.04 | 0.19 ± 0.06 |

* Values given in parentheses are the number of explants used for each determination. Geometric mean ± SE values are given. Secretory index was quantified as described in *Materials and Methods.*

In animals exposed to tobacco smoke, methacholine caused an increase in SI of 42% ($P < 0.01$), indicating that although basal secretory rate of mucous cells was increased, the sensitivity to stimulation with methacholine was not. Atropine, when added after pulsing to explants of control or exposed animals, did not reduce the secretory rate of mucous cells. In animals exposed to tobacco and PMO or to PMO alone, methacholine did not cause an increase in secretory rate of mucous cells, which suggests that PMO may antagonize the mechanism by which glycoprotein is discharged from mucous cells.

#### Serous Tubules

Quantitative examination of serous tubules indicated that neither their size nor secretory activity was affected by tobacco smoke. In animals which received only PMO, however, the density of silver grains overlying serous cells appeared lower than that of either control or tobacco-smoke–exposed animals.

## Discussion

The combined organ culture and quantitative autoradiographic method developed by Sturgess and Reid [10] to analyze glycoprotein secretion at the cellular level in the human bronchial gland has here been applied to assess some of the functional changes which accompany laryngeal and tracheal gland hypertrophy induced in rats by tobacco smoke exposure. The similarities, both structural and physiologic, that have been demonstrated between the rat tracheal/laryngeal gland and the human bronchial gland commend this species as suitable for investigating the pathogenesis of chronic bronchitis. In confirmation of previous studies,[4,7] tobacco smoke exposure for several weeks has been shown to induce tracheal gland hypertrophy in rats; not previously reported was the finding that tobacco smoke also induces an increase in the basal rate of mucus glycoprotein secretion by laryngeal and tracheal glands. The anti-inflammatory agent PMO has been shown to antagonize both tobacco-smoke–induced gland hypertrophy (partially) and hypersecretion (completely). The findings of the PMO study may have clinical implications but also have permitted analysis of some of the functional features of mucus hypersecretion induced by tobacco smoke exposure.

In tracheal and laryngeal glands of control rats, the percentage of mucous cells discharging labeled glycoprotein in unit time was approximately twice that of the normal human bronchial gland.[10,12] In short-term cultures of human bronchial gland, basal secretory rate is determined largely by the level of endogenous acetylcholine,[12] although this does not

seem to be the case in the rat gland since atropine failed to reduce basal secretory rate.

In this study as in others,[2,4,7,24] tobacco smoke was shown to cause an increase in gland size, and, in agreement with the findings of Jones and coauthors,[7] this enlargement was due to an increase in the size of all mucous tubules, with the appearance of tubules larger than those seen in the control animals. This general hypertrophy seemed, at least in part, due to an increase in the size of individual mucous cells. The contribution of mitosis to gland hypertrophy was not assessed in the present study, although Lamb and Reid [25] demonstrated a small but significant increase in the number of mitoses in gland acini of rats exposed to sulfur dioxide for more than 1 week, and mitosis of the basal cell compartment has been shown to contribute significantly to goblet cell hyperplasia in the airway surface epithelium of rats exposed to tobacco smoke.[26] The number of mucous cells in the gland could also increase by transformation of serous cells, as occurs in the airway surface epithelium in response to irritants.[26]

The increased basal rate of glycoprotein secretion that accompanies tobacco-smoke–induced gland hypertrophy in rats is similar to that observed in the hypertrophied glands of patients with chronic bronchitis.[10,12] A difference emerges in that increased glycoprotein secretion from the hypertrophied human bronchial gland is accompanied by an increase in sensitivity to stimulation with parasympathomimetic agents and tolerance to atropine blockade. In the present study, no evidence of enhanced sensitivity to mathacholine stimulation was observed in mucous cells of tobacco-smoke–induced hypertrophied glands. In addition, atropine did not reduce basal secretory rate in the rat, unlike its action in the human bronchial gland.

The concentration of $^{3}$H-labeled glycoprotein in mucous cells at unit time after pulse-labeling was similar in control rats and rats exposed to tobacco smoke. A similar finding has been reported for the human bronchial gland, although studies with specific metabolic inhibitors have shown that hypertrophy of the human bronchial gland is accompanied by increases in the rates of precursor uptake and glycoprotein synthesis.[12] These apparently conflicting findings are not inconsistent since individual mucous cells are increased in size in the hypertrophied gland and, thus, the total amount of $^{3}$H-threonine incorporated per cell will be increased proportionately.

The mechanism of tobacco-smoke–induced bronchial gland hypertrophy and hypersecretion, either in the human smoker or in the experimental animal, is unknown. While in this study, some of the principle stigmata of chronic bronchitis, ie, gland hypertrophy and mucus hyper-

secretion, have been induced by a 6-week exposure to tobacco smoke, other changes, notably hypersensitivity to cholinergic agonists, were not. Recent observations [27] indicate that gland hypertrophy may develop in rats after only 2 weeks of exposure to tobacco smoke, while Jones [28] demonstrated goblet cell hyperplasia after only a few days of exposure. Thus, it may be that the late features of the human disease are more closely allied to functional changes in the various types of mucus-secreting cell than to increases in cell number and gland size, which evidently can occur soon after smoking is started.

The anti-inflammatory agent PMO antagonized the tobacco-smoke–induced increase in basal rate of mucus glycoprotein secretion by laryngeal and tracheal glands and partially prevented the increase in gland size. These results differ from those of Jones and coauthors,[7] who were unable to show any effect of PMO on the increase in gland size induced by tobacco smoke. In their study, however, PMO was administered as a component of the tobacco smoke and it did prevent the goblet cell hyperplasia induced by tobacco smoke. Since PMO either prevents or diminishes several of the changes characteristic of chronic bronchitis, there is some indication that anti-inflammatory agents might be of clinical importance in the prevention or control of sputum production. Preliminary studies of the better known, nonsteroidal, anti-inflammatory agent phenylbutazone indicate that it may have an effect similar to that of PMO.[29] The present and previous [6,7] studies suggest that the principal effect of PMO is prophylactic, and there is no indication that it would reverse cell hyperplasia or mucus hypersecretion when established. The question remains, however, whether it would be desirable to prevent the chronic mucus hypersecretion of chronic bronchitis, which is a response both to infection and irritation of the respiratory tract: what is usually regarded as a means of protection becomes a cause of disability.

Studies in organ culture of glands from rats exposed to PMO showed that, in mucous cells, it reduced basal secretory rate and $^{3}$H-threonine incorporation and sensitivity to stimulation with methacholine. PMO thus seems to antagonize membrane-mediated events in secretion, particularly glycoprotein discharge by exocytosis. A membrane-stabilizing effect has been described for PMO [30] and other anti-inflammatory agents,[31] all of which confer resistance to hypotonic hemolysis in erythrocytes. If PMO mimics other anti-inflammatory agents, it could also reduce the rate of glycoprotein synthesis.[32-35] Independent and unequal effects on different stages in secretion may account for the finding that mucous cells of rats exposed to PMO were larger and contained more secretory material than those of controls. Since in animals exposed to PMO, precursor incorpora-

tion was reduced by 17% while secretory rate was lowered by 35%, this apparent imbalance could result in retention of secretory material and eventual cell hypertrophy. Imbalances between different stages in secretion are not surprising since changing the rate of glycoprotein discharge has no short-term effect on the rate of synthesis.[12]

Of special interest is the finding that animals exposed to tobacco and PMO exhibited gland hypertrophy but not hypersecretion. By contrast, Jones and co-workers [7] showed that in the goblet cells of rats exposed to tobacco and PMO there was a shift to the production of acid glycoprotein without an increase in goblet cell number. Thus, it seems that in airway mucus-secreting cell types, functional changes such as increase in secretory rate or alteration in the type of mucus glycoprotein synthesized can occur independently of goblet cell hyperplasia and gland hypertrophy.

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