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Mucin Biosynthesis and Secretion in the Respiratory Tract

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The interface where most pulmonary toxicants initially encounter the respiratory tract lies at the luminal surface of the tracheobronchial mucosa and pulmonary alveoli. Providing the first barrier to injury from environmental agents, this luminal surface requires close scrutiny in a discussion of lung as a target organ. Not surprisingly, the luminal surface of the respiratory tract exhibits morphologic and biochemical features uniquely adapted to the maintenance of surface exposed to air and not shared by intestinal, genitourinary or other internalized epithelial surfaces. Most notably, a heavy blanket of mucus covers the surface of the tracheobronchial tree. This mucous blanket serves in preventing dehydration, trapping inhaled particles and microorganisms and in protecting against physical or chemical injury to the various surface epithelial cells. Propelled cephalad to the pharynx by the beat of underlying cilia, the migrating mucous blanket facilitates removal of foreign materials in a process referred to as mucociliary clearance. In addition, the respiratory epithelium, like other epithelia, possesses a prominent glycocalyx, rich in carbohydrate as its name implies, on the luminal aspect of the apical plasmalemma of all the cells. The mucous blanket mediating mucociliary clearance derives from secretion of cells in the surface epithelium of the trachea, bronchi and bronchioles and in submucosal glands of the trachea and bronchi. The glycocalyx on the apical plasmalemma originates, presumably, within the cell exhibiting the surface coat. The following discussion focuses first on normal production and secretion of the mucin on the surface of the respiratory epithelium. The morphologic basis for mucin production is described in terms of the various cell types contributing to glycoconjugate-rich respiratory secretions and the intracellular mechanisms for synthesizing and releasing the macromolecules. Lacking mucous secretory granules, the alveolar cells do not come into consideration. Biochemical and histochemical knowledge of the nature of the secretion as a whole and as a contribution from individual cell types is summarized along with information pertaining to the physiologic control of secretion. The effect of pulmonary toxicants on the normal secretory mechanisms receives final consideration.

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Morphologic Aspects of Mucin Production in the Respiratory Tract

Classification of Secretory Cells

Several different types of epithelial cells contribute to the mucinous secretion in the respiratory tract (*1–9*). In the tracheobronchial surface epithelium, mucous goblet-like cells, extending in man as far distally as do the cartilage plates and to a variable extent beyond, produce carbohydrate-rich secretion. Cells secreting glycoconjugate in glands of the lamina propria include those of serous tubules and demilunes, of mucous tubules and of glandular ducts. The serous tubules and demilunes empty into the mucous tubules which drain into the intralobular and extralobular ducts, lined in part at least by mucous goblet cells.

Viewed by electron microscopy, the cells in each of these three categories appear to be classifiable into subtypes on the basis of the number, size and shape of their secretory granules and the distribution and density of intragranular components with differing electron opacity. The presence or absence of a variably dense, amorphous core in secretory granules, for example, differentiates some from other cells in surface epithelium and mucous tubules. Serous cells vary from one another even more than mucous cells in the density and distribution of one to three different zones within the granule. Clara cells and secretory cells with sparse small granules populating the surface epithelium of the distal bronchioles display distinctive morphologic features which have been well defined in rat (*1,2,9*) but not in human bronchioles.

Cell Mechanisms for Biosynthesis and Secretion of Mucosubstances

The mechanisms underlying the synthesis of the protein core of mucous glycoproteins have not been studied in great detail, but are assumed to resemble the conventional process responsible for the secretion of secretory proteins. Protein synthesis in the ribosomes involves three phases: initiation, elongation and termination of the protein chain. The transport route of the developing protein core in glycoprotein-secreting cells does not differ, presumably, from that described for the pancreas. However, the rate of transport appears to be slow (*10*). The peptide chain moves within the cisternal space of the rough endoplasmic reticulum and migrates toward that of the smooth endoplasmic reticulum. Carbohydrates and sulfate are attached to the protein during passage through the granular reticulum and the Golgi area. Knowledge that the Golgi lamellae are a main site of sugar and sulfate attachment is based on evidence from ultrastructural, cytochemical and autoradiographic studies and on the finding that the activities of the glycosyltransferases are highest in subcellular fractions with characteristics of the Golgi elements (*11*). Once supplied with their sugar and sulfate components, the glycoproteins leave the Golgi cisternae inside vesicles budding from the Golgi lamellae. These vesicles fuse to form secretory granules which are released by a merocrine or apocrine process of exocytosis in response to secretory stimuli.

Mechanisms controlling the biosynthesis of glycoproteins involve the regulation of both protein and oligosaccharide synthesis (*12*). Genes control oligosaccharide sequences in an indirect fashion, by functioning as structural genes for the synthesis of glycosyltransferases and for the biogenesis of the complex membrane system within which the oligosaccharide assembly takes place (*11,12*). The competence of the final glycoconjugate as a unique structure is determined by the discrimination and specificity of the glycosyltransferases. Although mucous and serous cells have a similar synthetic pathway, the mucous cells show a slower uptake of ^{3}H-threonine by the Golgi apparatus and a slower turnover of granules than the serous cells (*13*). A number of factors of genetic, developmental, chemical and toxic nature can modify the biosynthesis of mucus glycoproteins, by affecting either the synthesis of the protein core of the glycoprotein molecule or the attachment of the sugar components of these molecules (see below).

Mucous glycoproteins are stored in secretory granules inside the epithelial cells, and there seems to be a gradual and orderly shift of layered generations of secretory granules toward the apex of the cell in preparation for merocrine secretion which occurs by a process called exocytosis. In this secretory process, the membrane of the secretory granule fuses with the plasma membrane and the granular content moves through the fusion opening into the lumen. Since new glycoproteins are synthesized continously and new secretory granules are added to those formed earlier, the cell has to remove granules from its store even in the absence of a secretory stimulus. In exocrine cells the redundant granules are secreted and, hence, fusions between the granules and the plasma membrane are observed even in unstimulated cells. Unstimulated secretion has also been reported in mucous cells, but the process by which the glycoproteins leave the cell may differ from what has been described for other types of secretory proteins. There seems to be a disruption of the apical rim of the plasma membrane and of the underlying cytoplasm through which the membrane-bound granule slips into the lumen where the membrane disintegrates and the contents become free (*14, 15*).

The frequency of fusion between secretory granules and the apical plasma membrane can be increased by secretory stimuli including hormones, neurotransmitters or drugs. The secretory cycles of mucous and serous cells apparently differ. Mucous cells appear to accumulate and discharge secretory macromolecules constantly while serous cells have distinct phases of accumulation and discharge (*16,17*).

Chemical Nature of Mucous Secretions

Composition of Respiratory Secretions: Cystic Fibrosis and Noncystic Fibrosis Specimens Compared

The mucus secreted in the respiratory tract constitutes a mobile and protective barrier which provides a buffered, aqueous microenvironment to the airway surface. This film of mucus is primarily secreted by the submucosal tracheobronchial glands and, to a lesser extent, by the goblet cells of the surface epithelium (*5*). The main constituents of respiratory secretion are glycoproteins of high molecular weight (*6,18*), but other components including lysozyme, and immunoglobulins are also present (*19*).

Typically, mucus consists of about 1% of salts and other dialysable components, 0.5 to 1% of free protein, a similar proportion of glycoprotein and 95% or more of water. There is, in addition, a moderate amount of lipid in bronchial mucus (*20*). The biological properties of mucus are often altered in disease states and through various experimental manipulations in laboratory animals.

Both histochemical and biochemical evidence suggests that individual cell types in the airways are capable of producing structurally distinctive glycoproteins (*21,22*). The analysis of the composition of tracheobronchial secretions is complicated, however, by practical limitations in the accessibility to uncontaminated secretions from specific cell types and from individual portions of the respiratory tree. Tracheobronchial secre-

tions from healthy humans are difficult to obtain in adequate amounts for detailed chemical analysis and most studies on respiratory tract mucus have been carried out on expectorated sputum. Specimens obtained through lung lavage procedures and by fiberoptic bronchoscopy or endoscopy have also been analyzed as have secretions from nasal polyps and from tissue explants *in vitro*. Similar approaches have been used in animal species. Analyses were recently conducted in our laboratory of both lung lavage and sputum samples from cystic fibrosis (CF) and non-CF individuals, and their chemical composition was compared (*23,24*). The results of these studies on lavage samples are summarized in Table 1. Non-CF material had 48.9% carbohydrate, 33.8% protein and 12.3% lipid. The CF specimens contained significantly increased levels of total lipid, protein, phospholipid, neutral lipid, DNA and RNA. They also contained significantly increased levels of bound sulfate and were, therefore, more anionic in nature.

Table 2 illustrates the water content, macromolecular dry weight and the organic composition of sputa from CF and non-CF sources. Samples were obtained by postural drainage, by expectoration or by endotracheal aspiration during bronchoscopy. CF samples were found to be significantly less hydrated and to have higher macromolecular dry weight, protein, lipid, phosphatidylcholine and DNA content when compared to both purulent and nonpurulent sputum samples from non-CF individuals. Although the total carbohydrate component was also increased (Table 2), it actually comprised less of the macromolecular dry weight. Further studies indicated that the neutral and amino sugar molar ratios were similar in CF and chronic bronchitis sputum samples. However, the sialic acid and sulfate contents of the CF samples were significantly higher.

Table 1. Chemical composition of tracheobronchial secretions.

Component[a]	Composition, % of dry weight[b]	
	CF	Non-CF
Total protein	42.7	33.8
Total lipid	19.8	12.3
Phospholipid	9.41	5.7
PS	1.20	1.04
PI	0.04	> 0.01
PE	2.17	1.47
PC	5.62	3.18
Neutral lipid	8.02	6.97
Glycolipid	1.58	1.78
Total carbohydrate	31.1	48.9
D-Ribose	9.81	1.01
Ribose	2.12	.08
Mannose	1.11	3.28
Glucose	1.69	2.54
Fucose	1.88	5.82
Galactose	3.54	10.37
N-CHO	5.17	14.87
S.A.	5.77	10.93
High molecular weight glycoprotein	17.75	33.27
Protein	1	1
Fucose	0.49	1.21
Galactose	1.02	0.87
H-CHO	1.48	1.67
S.A.	1.71	1.54
SO_4^{2-}	0.51 (8.63–11.21%)	0.41 (4.91–7.72%)

[a] Abbreviations: PS, phosphatidyl serine; PI, phosphatidyl inositol; PE, phosphatidyl ethanoloamine; PC phosphatidyl choline; N-CHO, amino-sugars; S.A., sialic acid; SO_4^{2-}, sulfate.
[b] Data are means of eight CF and six non-CF samples.

Table 2. Chemical composition of nonpurulent, purulent and cystic fibrosis sputum.[a]

Constituent	Composition, mg/mL ± SD[a]		
	Nonpurulent[b]	Purulent	Cystic fibrosis
Water (mg/mL)	954±15	934±12	886±31*
Macromolecular dry weight	46±2.4	60±8.6‡	96±14.4†
Protein	12.1±1.1	19.9±4.6‡	44.4±18.2†
Lipid	10.6±0.8	18.1±2.7‡	32.6±3.8†
Phosphatidylcholine (% of total lipid)	27.1%	38.6%	51.0%†
Carbohydrate	9.2±1.4	12.3±1.4‡	14.3±2.4
Fucose (% of total sugar)	18.2%	17.0%	14.1%
Sialic acid (% of total sugar)	16.9%	21.4%	27.6%
DNA	0.008±0.002	0.820±0.28‡	2.730±1.12†
No. samples assayed	48	54	125
No. patients	31	47	21

[a] Unless otherwise designated.
[b] Nonpurulent sputum is defined as that having less than 0.025% DNA content (based on macromolecular dry weight).
* Significance of $p < 0.005$, Student t test, compared with nonpurulent and purulent water content.
† Significance of $p < 0.001$, Student t test, compared with nonpurulent and purulent sputum values.
‡ Significance of $p < 0.005$, Student t test, compared with nonpurulent sputum values.

Mucous Structure

Glycoproteins are polymeric substances consisting of carbohydrate covalently linked to protein. The units contain an average of 8 to 10 monosaccharide residues of five different types, including *L*-fucose, *D*-galactose, *N*-acetyl-*D*-glucosamine, *N*-acetyl-*D*-galactosamine and sialic acid (*18*). Carbohydrate units on glycoproteins are, therefore, relatively small and are frequently branched, have little or no repeating structure and do not contain hexuronic acid. The linkage between carbohydrate and protein in mucous glycoproteins is called *O*-glycosidic because it is through an oxygen atom (*6, 25*) in contradistinction to the N-glycosydic linkage in serum and membrane glycoprotein. The linkage sugar in mucus glycoprotein is usually *N*-acetylgalactosamine, which is joined to the hydroxyamino acids serine and threonine of the protein chain (*6,18,26*) as compared with the *N*-acetylglucosamine linkage to asparagine in serum and membrane glycoproteins. Most mucous glyco-

proteins contain over 50% carbohydrate. The physical and chemical characteristics of the mucous glycoprotein are determined, to a large extent, by its carbohydrate. The protein component of mucous glycoproteins is quite characteristic and different from that of plasma glycoproteins in having a high content of serine, threonine and proline, but only small amounts of aromatic and sulfur-containing amino acids (*18,22*).

Qualitative and quantitative studies in several laboratories have analyzed sputum and secretion from tracheal explants (*6,17,27–29*). The data suggest that there is heterogeneity with regard to acidity and molecular size of the carbohydrate side chains of acidic bronchial mucins from CF and chronic bronchitis patients (*22,30*). Various types of neutral and acidic (both sulfated and sialylated) glycoproteins have been described biochemically as well as histochemically in respiratory tract mucins from a variety of animal species (*8,31–34*).

Histochemical Observations

The mucous goblet cells of surface epithelium of trachea and bronchi in man produce sulfated glycoprotein in some areas and mainly sialylated glycoproteins or a mixture of both types in other areas as evidenced by light microscopic methods for differentiating and characterizing complex carbohydrates (*8*, unpublished observations). Sialidase digestion imparts staining to these cells by a method employing a peanut lectin-horseradish peroxidase conjugate to localize terminal galactose residues in glycoproteins (Fig. 1) (*35*). All goblets of human respiratory surface epithelium stain with the conjugate after but not before digestion with sialidase, demonstrating that the oligosaccharide side chains contain terminal sialic acid and penultimate galactose (unpublished observations). The sulfate esters evidenced histochemically in the goblet secretion

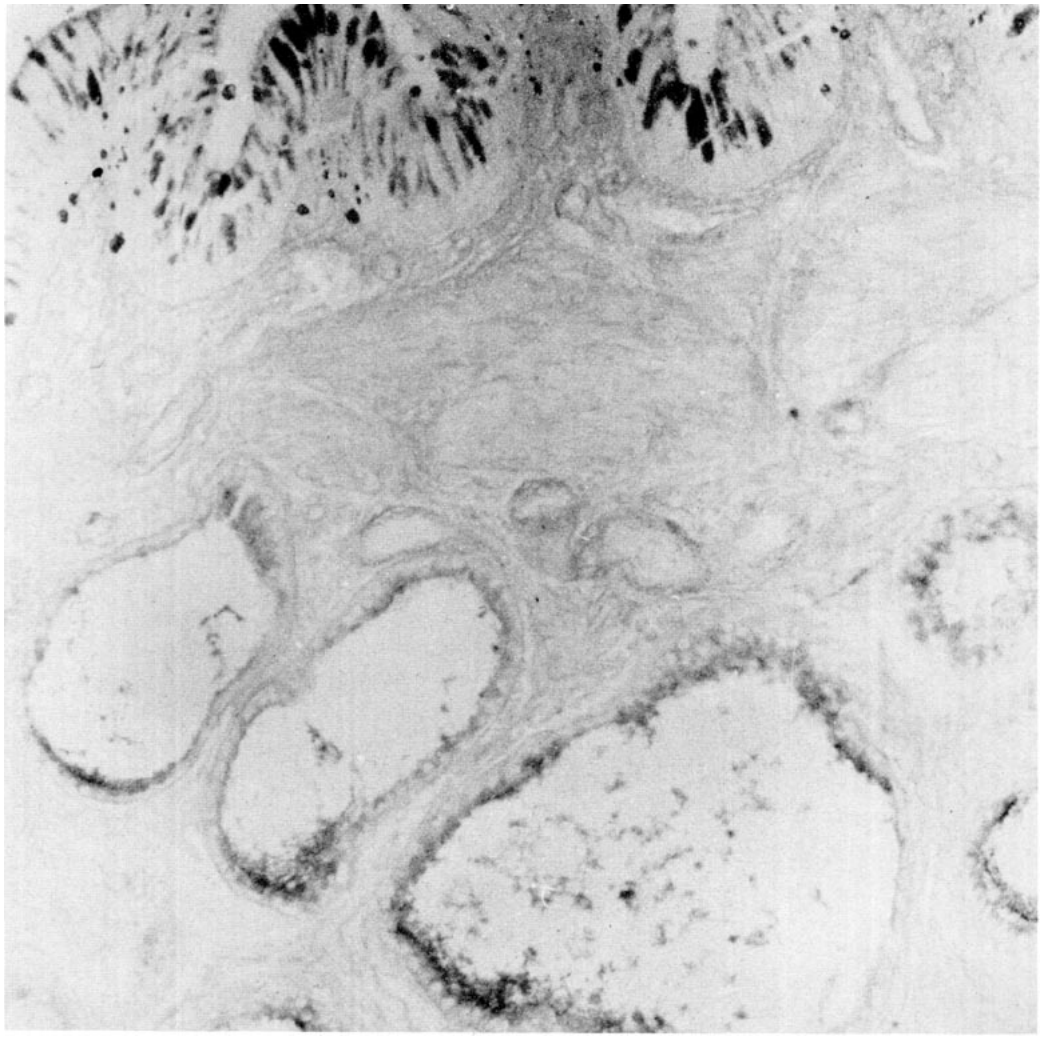

FIGURE 1. Human respiratory surface epithelium shows numerous intensely stained cells with apical globletlike accumulations of mucus. Unstained ciliated cells are not evident. Secretion of mucous goblets lacks staining without sialidase digestion but, as shown here, gains intense affinity for peanut lectin after digestion evidencing content of penultimate galactose residues under terminal sialic acid in the oligosaccharide side chains of the glycoprotein. Carnoy fixation, 6 hr. Sialidase digestion, peanut lectin-horseradish peroxidase stain. ×500.

apparently connect to internal residues of the glycoprotein side chains. Species variation is evident in that goblet cells normally form almost exclusively a nonsulfated sialomucin in the rat (*9*) and sulfomucin in the dog (*8*).

Glands in the lamina propria display differences among the histologic cell types in reactivity demonstrative of glycoconjugate. Variability exists between species in reactivity of a given type of cell. In man, serous tubules and demilunes store neutral glycoprotein in granules of some cells and lightly sulfated glycoprotein in granules of other cells.

The light to moderately intense staining of serous cells for neutral or sulfated mucosubstance contrasts with the strong reactivity of some mucous tubule cells for sialomucin, others for sulfomucin and still others for a mixture of the two components (*4,8*). These different secretory products can be demonstrated by $^{35}SO_4^{2-}$ radioautography, by blue versus black staining with the high iron diamine–Alcian blue sequence or by differences in staining with this method or the Alcian blue–periodic acid Schiff sequence with or without prior sialidase (*36*). Lack of staining with the peanut lectin–horseradish peroxidase conjugate before sialidase and strong reactivity after digestion reveals that the secretion throughout mucous cells in human tracheobronchial glands contains terminal sialic acid and penultimate galactose in the oligosaccharide side chains of the secretory glycoprotein. Taken together, the basic dye methods and the lectin procedures indicate that all the mucous tubule cells produce sialylated glycoprotein and this complex carbohydrate is sulfated to a moderate or marked degree in some cells but not at all in others.

The histochemical results appear consistent with production of a single glycoprotein containing a variable number of sulfate esters in all the mucous goblet cells of the surface epithelium of respiratory tract in man but do not conclusively demonstrate this thesis. Except for variable sulfation the mucosubstance could be similar also throughout the cells of the mucous tubules aside from extent of sulfation secretion in the surface goblet cells generally resembles that in mucous cells of the glands. However, the gland mucous cells differ somewhat from those of surface epithelium in staining a more purple shade with the Alcian blue–periodic acid sequence method, indicating a greater abundance of *vic*-glycol-containing hexose in the glandular mucous cells. The abundant serous cells of tracheobronchial glands, on the other hand, appear to secrete one or more different mucosubstances with a comparatively low carbohydrate content and containing relatively sparse sulfate esters in some and no sulfates in other cells. Determining whether the serous and other types of secretory cells each produce more than one macromolecular species will require development of histochemical procedures with greater specificity. A challenging problem for further inquiry concerns relating the different secretions in the several histologic sites to the three major mucous glycoproteins isolated by DEAE-cellulose chromatography from human lung lavage fluid (*28*).

In addition to the histochemical localization of mucosubstances an immunoperoxidase-bridge procedure for immunocytochemical demonstration of antigens in tissues has demonstrated lysozyme in lung. This low molecular weight, cationic glycosidase has been localized to secretory granules of tracheobronchial serous cells and type II pneumocytes (*37,38*). The biologic significance of lysozyme in these secretions presumably pertains to its capacity to hydrolyze amino sugar linkages in bacterial walls or, conceivably, in secretory glycoproteins and its potential for forming electrostatic complexes with anionic mucosubstances.

Employing cytochemical methods for visualizing mucosubstances at the ultrastructural level has confirmed and extended the light microscopic findings in rat trachea (Figs. 2–4) (*9*). The microscopic cytochemical approach has shown, in addition, the presence of glycoconjugate in secretory granules of cells such as the Clara cells where it was not otherwise detectable (Fig. 5). The electron microscopic cytochemistry shows quantitative differences not evident by light microscopy in carbohydrate content between individual cells of a given histologic type (Figs. 2–4). Whether these also reflect qualitative differences remains uncertain. The ultrastructural methods demonstrative of *vic*-glycol-containing hexose, or of carboxyl or sulfate groups, also provide evidence for variability in intragranular distribution of glycoconjugate among different cells of the same type. They show, moreover, differences in glycoprotein content among the secretory granule population of a single cell profile, indicating granule heterogeneity within a cell in human respiratory tract (unpublished observation). Ultrastructural staining with cationic reagents has revealed, in addition, the distribution of complex carbohydrates in zones within secretory granules and, on occasion, distinguished zones of heavy, light and no reactivity within a granule profile. The question here arises whether a different degree of staining with the basic reagent in two zones of a single secretory granule demonstrates qualitative or quantitative differences in content of acid groups in the glycoproteins in the two zones.

Ultrastructural staining of complex carbohydrates also discloses a heavy mucosubstance-rich coat on the luminal surface of the apical plasmalemma of most surface epithelial cells in the respiratory tract (Figs. 3 and 4) (*9*). This glycocalyx varies quantitatively and qualitatively on different cells. The complex carbohydrate in the glycocalyx coating the cilia, for example, lacks demonstrable *vic*-glycol-containing hexoses although showing staining for carboxyls, presumably in neuraminic acid. The apical microvilli of the ciliated and neighboring secretory cells, on the other hand, exhibit cytochemically reactive glycoprotein with *vic*-glycol-containing hexoses and carboxyl (Figs. 3 and 4). The cilia glycocalyx, thus, appears different cytochemically from luminal glycocalyx elsewhere in the respiratory tract. A unique coat on the cilia could be envisioned as

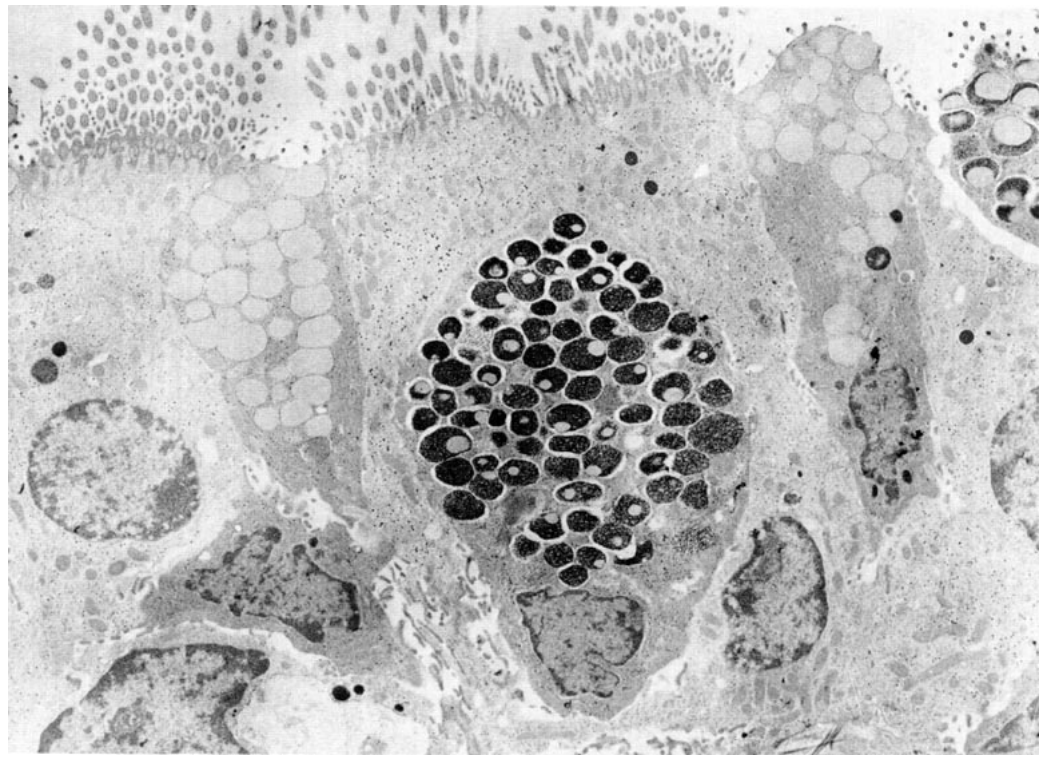

FIGURE 2. Surface epithelium of rat trachea composed of serous and ciliated cells. Granules in some serous cells exhibit a thin rim of carbohydrate-rich material and those of other cells contain no complex carbohydrate. A cell at the far right encloses granules with a moderately thick rim or cap containing well stained complex carbohydrate rich in periodate-reactive hexoses. Several types of serous cells have been distinguished on the basis of distribution of a carbohydrate-rich peripheral component (*9*). A mucous-type cell containing granules with glycoprotein-rich, heavily stained matrix and an unreactive, eccentric nucleoid occupies the center of the field. Mucous cells, too, can be subdivided on the basis of the amount and distribution of glycoconjugates stained by cytochemical methods (*9*). Periodic acidthiocarbohydrazed-silver-proteinate (PA-TCH-SP) stain. ×5000.

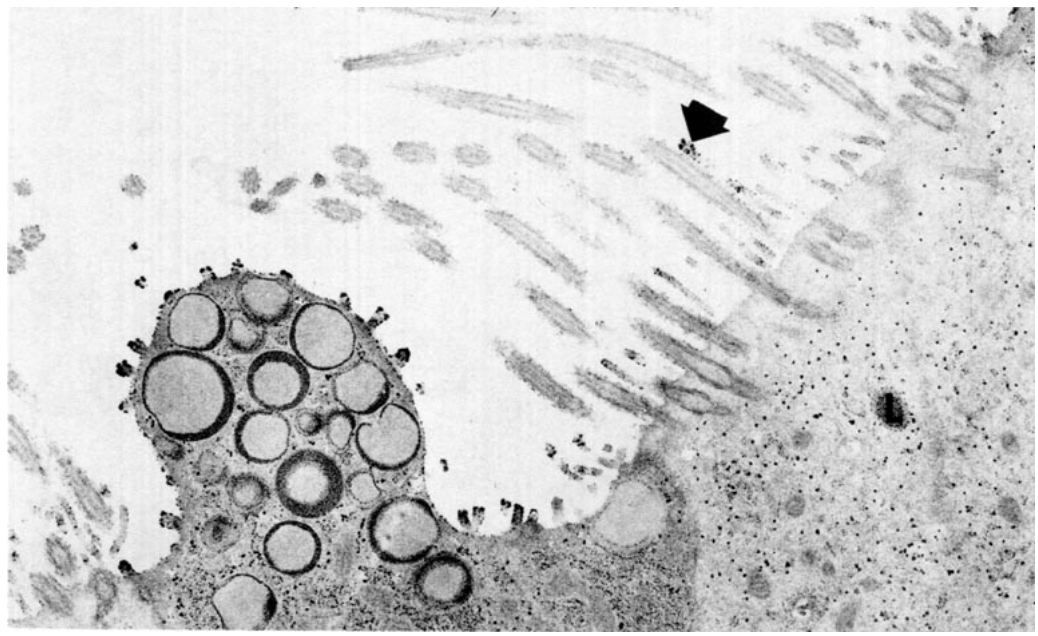

FIGURE 3. A serous cell at the left exhibiting stored secretory granules with a moderately thick rim of stained glycoprotein contrasts with serous cells on either side of the central ciliated cells. The latter serous cells contain granules with only a thin glycoprotein-positive rim or a cortex with a thin inner and outer band of reactivity. The apical plasmalemmae exhibit staining for glycoprotein, but the lateral plasmalemmae and tight junctions lack reactivity. Tips of the microvilli show more intense staining. Microvilli (arrow) of the ciliated cells at the center differ from the cilia in possessing a strongly stained glycocalyx. Lysosomes (L) in the ciliated cells reveal staining as do glycogen particles scattered throughout the cytoplasm. PA-TCH-SP stain. ×10,000.

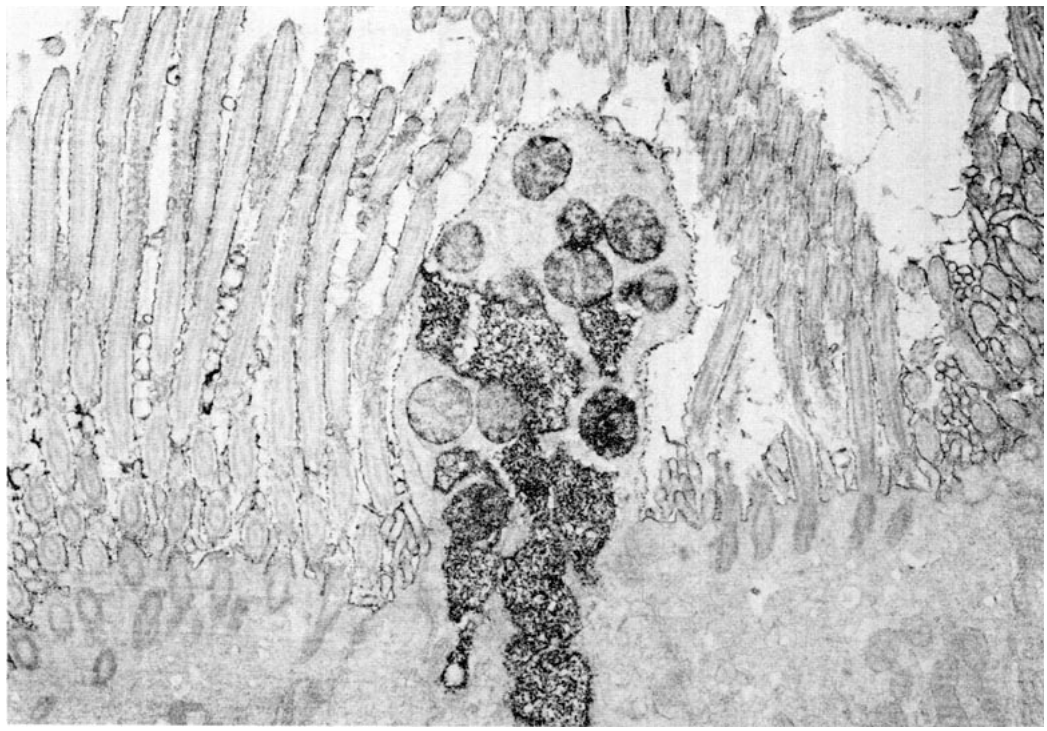

FIGURE 4. Rat tracheal surface epithelium displays strong staining demonstrative of abundant carboxyl or sulfate groups throughout the matrix of secretory granules in the protruding cytoplasm of the mucous cell. Glycocalyx covering the surface of the cilia, microvilli on the ciliated cell and the apical plasmalemma of the mucous cell all evidence strong affinity for the basic reagent. The strong basophilia in the glycocalyx covering the ciliated cells contrasts with the lack of periodate reactivity indicative of hexoses with *vic*-glycol groups. (cf. Fig. 3). Dialyzed iron stain. ×12,500.

having significance for the lubricating properties and the protection against interciliary adhesion and friction required for the synchronous beating of these closely spaced, greatly elongated structures. This glycocalyx conceivably constitutes a vulnerable site for unfavorable action of pulmonary toxicants or microbial enzymes, but little is known concerning pathologic alteration of the surface coat of cilia.

The histochemically evident differences between surface epithelial mucous cells, glandular mucous cells and serous cells emphasize again the longstanding questions concerning biologic significance of these cell types. The mucous blanket covering the cilia and mediating mucociliary clearance undoubtedly derives from a mixture of their secretions. Since glands are thought to produce a many times greater volume of secretion than surface epithelium (5), the goblet cells probably play a quantitatively minor role. Lying intermingled with neighboring ciliated cells, the surface goblet cells influence more directly, however, the secretion overlying the cilia. The upstream location of serous cells distal to mucous cells in the glands, the morphologic observation that serous cells empty into constricted intercellular canaliculi and the histochemical finding that the secretion of serous cells is comparatively low in carbohydrate and acidity support the view that the serous secretion is less viscous and serves in diluting and flushing more viscous secretion of downstream mucous cells through the ducts. The recent immunocytochemical demonstration of carbonic anhydrase selectively in serous cells of human bronchial glands (unpublished observations) suggests active ion and fluid transport by these cells and supports the view of their contributing a more hydrated, less viscous secretion compared with mucous glands. Myoepithelial cells underlying serous and mucous cells apparently facilitate, by their potential for contractility, the flow of macromolecule-rich, viscous secretion in ducts. Stasis of secretion in ducts have been evidenced in our observations on hypersecreting pathologic lung by the dilatation of ducts containing abundant inspissated secretion in the lumen and sparse stored secretion in the cells.

Physiological Control of the Secretion of Mucus in the Airways

Regulation by the Autonomic Nervous System

The resting secretion of respiratory tract mucus has never been accurately measured in healthy men. Patients with tracheostomies are said to secrete 10 to 15 mL/day. Resting secretion continues after vagotomy and is also observed in organ culture preparations of the

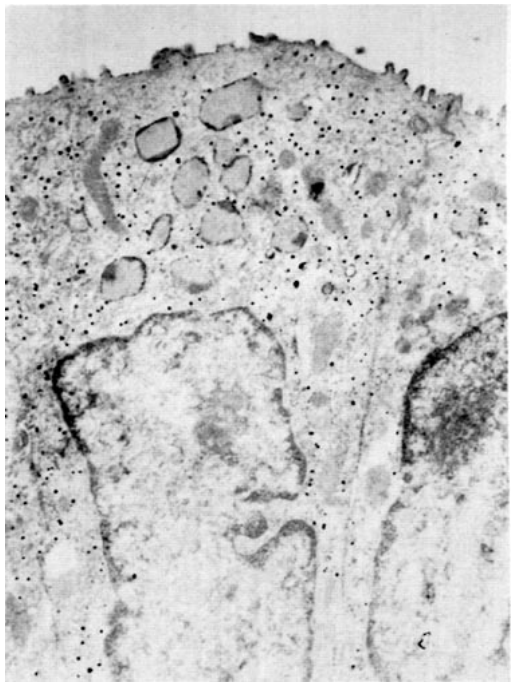

FIGURE 5. A Clara cell lining a terminal bronchiole in rat lung encloses several apical granules which contain a central unstained area with an angulated contour. Glycoprotein in the periphery of these granules stains strongly. In presumably more mature granules of other cell profiles, the unstained central material occasionally appears greatly elongated and glycoprotein encloses the crystalloid center in a configuration reminiscent of eosinophil crystalloid granules (*9*). The surface of the apical plasmalemma displays less reactivity than that of the secretory cells higher in the respiratory tract. (cf. Figs. 3 and 4). Its dialyzed iron affinity, however, compares favorably with that of the proximal surface epithelial cells. PA-TCH-SP stain. ×10,000.

trachea (*39*). Accordingly, it is thought that resting secretion proceeds independently of innervation. The autonomic nervous system, therefore, could be considered to increase the resting secretion. Electrical stimulation of the vagus nerves promotes secretion of mucus from the submucosal glands of the trachea (*39,40*). Stimulation of sympathetic nerves also promotes secretion of airway mucus in certain species, but not in others (*39,40*). The secretion induced by sympathetic stimulation is blocked by propranolol and mimicked by isoproterenerol, so the response is probably mediated by beta receptors (*39,40*). However, there is no evidence of sympathetic innervation of the mucus secreting tissues in human airways (*39*). It is not clear whether the composition of water content of the secretions elicited by parasympathetic and sympathetic stimulations are the same. Cultures of explanted human tracheobronchial tissues have been used to study mucus secretion *in vitro* and have demonstrated that a basal level of mucus secretion is present in both the mucous and serous cells of the submucosal glands. This basal level can be stimulated by parasympathomimetic drugs such as acetylcholine or pilocarpine and inhibited by cholinergic antagonists such as atropine (*41–43*). Sympathomimetic drugs or their inhibitors have no effect on mucus secretion *in vitro* (*29,41,43*).

Recent evidence has indicated, however, that in the cat trachea alpha-adrenergic agonists enhance ion-mediated fluid secretion and secretion of mucus, presumably from submucosal glands (*44*). The lack of effect of alpha-adrenergic agents on mucus secretion in some species may be attributable to absence or poor development of submucosal glands.

The response of mucous goblet cells or serous cells in the surface epithelium is less clear. Goblet cell secretion or changes in the cell's secretory capacity do not appear to be under nervous control in the human lung (*39,45*). Denervation of the lung has no effect on the number or the types of goblet cells, and these cells show no response to stimulation of the vagus nerve or to parasympathomimetic drugs or antagonists such as atropine (*43,46*). Nerve processes are not encountered by electron microscopy in the vicinity of surface epithelium as they are near submucosal glands (*9*). It has been suggested, therefore, that goblet cells respond only to local irritants or local reflexes (*32,39,42*). However, the physiological control of surface goblet cells needs to be re-evaluated in view of the finding that chronic administration of autonomic drugs such as pilocarpine and isoproterenol produces both a hyperplasia and a hypertrophy of the goblet cells in some species (*47,48*). Furthermore, with experimental animals *in vivo*, injections of sympathomimetic drugs into the trachea or the bloodstream increase the secretion of mucus in the airways. This is probably the result of the stimulation of beta receptors, since the response is prevented by propranolol (*32,39*).

Role of Peptides and Prostaglandins

Recent evidence indicates that other humoral factors may be involved in the regulation of mucus secretion in the airways. Although histamine promotes secretion in the cat trachea in high doses, this mediator is inactive on mucus secretion of human airways *in vitro* (*45*). Both the dog and the rat trachea respond to certain peptides, such as substance P and kallidin, with an increased secretion of mucous glycoproteins (*49,50*). The rat trachea has also been recently shown in our laboratory to respond to prostaglandins E_1 and $F_{2\alpha}$ with an increased release of mucous glycoproteins. Table 3 shows the results of this experiment. Prostaglandins A_1, E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$ have also been shown to increase mucous glycoprotein secretion when applied directly to the cat trachea (*40*). Prostaglandin $F_{2\alpha}$ was also found to be the most effective stimulant of human sputum production (*40*).

Specific Cellular Responses to Stimulation

From studies on the secretion of mucus by cat trachea, it has been concluded that pilocarpine provokes

Table 3. Release of ^{3}H-labeled glycoproteins.

Stimulant	Release of glycoproteins, DPM[a]	
	Control	Reserpine
None (baseline)	464 ± 87	944 ± 127
Bradykinin	621 ± 62	1531 ± 144
Substance P	895 ± 101	2018 ± 193
Prostaglandin E_1	1109 ± 121	3778 ± 350
Prostaglandin F_2	1582 ± 298	7681 ± 325
Prostaglandin E_1 + F_2	2788 ± 236	12343 ± 548

[a] Data expressed as DPM ± SD released by tracheas 10 min following stimulant addition.

the release of sulfated glycoproteins while irritants produce glycoproteins with a low sulfate content and a high sialic acid content (*39,40*). The question arises concerning the cellular source of these different secretions. Conceivably the surface epithelial goblet cells, lacking an autonomic response mechanism account for the low sulfate, high sialic acid secretion induced by irritants. In absence of histochemical knowledge of glycoconjugate in the feline trachea, it can only be speculated that the cat resembles the rat where surface goblet cells produce nonsulfated, sialylated glycosubstance whereas mucous tubules of glands secrete sulfomucin (*9*). Low sulfate secretion could not derive from surface mucous cells in man and dog because surface goblets form sulfomucin in these species (*8*).

The secretions originating from the epithelial goblet cells have been compared biochemically with those of the submucosal glands in explants from the dog trachea that were denuded of the surface epithelium. It was concluded that goblet cell secretions are highly sulfated, an observation that was confirmed by autoradiography in double-label experiments with glucosamine and sulfate (*51*) and by histochemistry (*8*). Biochemical characterization of the lateral secretions indicated that the submucosal glands contain a mixture of acidic mucins and histochemical methods yield comparable information (*8*). The species differences indicate the difficulties in extrapolating findings from one species to another and caution against interpreting the cell source of a secretion induced in one species on the basis of knowledge of the type of secretion produced by the various cell types in another species.

Basic Mechanism of Secretion

The basic mechanism by which chemical mediators and drugs act to modify the secretion of mucus in the airways has been little investigated. In other exocrine glands such as the pancreas and the salivary glands, cyclic nucleotides and calcium have been implicated as intracellular mediators of the stimulus secretion coupling mechanism (*52,53*). There is some evidence, however, that the same mediators may be involved in the secretion of glycoproteins by the respiratory epithelium. Thus, addition of cyclic AMP promotes secretion by human airways in organ culture and theophylline also promotes secretion by inhibiting phosphodiesterases (*39*). Recent experiments in an isolated perfused preparation of the rat trachea conducted in our laboratory have also indicated that cyclic nucleotides of guanine and adenine promote glycoprotein secretion in this tissue and that their effects are additive (*54*). Calcium ions also seem to be involved in the secretory process. In the same experiments indicated above, substitution of calcium for other divalent cations in the incubation medium in which the isolated rat trachea was perfused resulted in a complete inhibition of the secretory response to cholinergic agents.

The possible relationship between glycoprotein secretion and the transepithelial transport of electrolytes and water in the airways has received little attention. The two processes may be intimately linked, however, as suggested by recent experiments in our laboratory which demonstrated that the enhanced secretion of glycoprotein induced in the perfused rat trachea by acetylcholine was abolished when Na^+ or Cl^- was substituted by other monovalent ions in the fluid bathing the preparation (*55*). The coupling between the two processes during the response to physiological regulators can have important implications, when disturbed, in the pathogenesis of the pulmonary manifestations of certain pulmonary diseases characterized by hypersecretion of physicochemical alterations of mucus. Increasing experimental evidence indicates, furthermore, that electrolyte transport is an important component of the secretory response in the airways and that it involves electrolyte fluxes of different extents and directions in the various segments of the airway epithelium (*45*).

Effects of Toxicants on Secretion of Mucus

Drugs

Experiments in our laboratory have indicated that the chronic administration of reserpine to rats produces a widespread exocrine gland disturbance similar in several ways to that observed in cystic fibrosis (*56–58*). In rat submandibular glands, for example, chronic reserpine treatment causes increased storage of secretory granules, and depletion of the granular reticulum and Golgi lamellae and acts to diminish the response to secretogogues (*59*). Reserpine also causes an enhanced secretion of mucous glycoproteins in the respiratory tract and an increased sensitivity of the secretory elements to stimulation with autonomic mediators (*47, 60*). The trachea of the reserpine-treated animals is not only more susceptible to autonomic mediators but also to other potential humoral mediators, including peptides and prostaglandins (*50*). The mechanism by which chronic reserpine administration may induce these changes in the secretory elements of the respiratory tract is not entirely clear at present. In other exocrine

tissues, this drug regimen produces a very marked direct toxic effect in addition to its effect on the catecholamine stores of the tissues, (*57,58*).

The chronic administration of isoproterenol or pilocarpine to rats has been shown to cause goblet cell hyperplasia and submucosal gland hypertrophy (*48*). Lung lavage samples from normal rats treated with these two agents for 12 days were analyzed in our laboratory and the results indicated that chronic isoproterenol administration caused a 2.5-fold increase in the lipid content, but no change in the protein or carbohydrate content of such samples. Chronic treatment with pilocarpine resulted, on the other hand, in 2.6-, 3.3- and 1.9-fold increases in the protein, lipid and carbohydrate contents of these samples (*47*). Rats previously treated with reserpine and then exposed to isoproterenol or pilocarpine for 12 days showed a greater sensitivity to the two drugs and increased contents of protein, carbohydrate and lipid in the lung lavage samples (*47*). Isoproterenol is thought primarily to affect glycoprotein synthesis, rather than discharge, whereas chronic pilocarpine causes an indirect effect on discharge (*48*). The specific effects of autonomic drugs and their antagonists on the various aspects of glycoprotein synthesis and secretion in the respiratory tract needs more careful examination. A particularly important question concerns the specific cell types that are affected by acute or chronic exposure to the different drugs.

Chemicals and Irritants

Many studies have shown that acute inhalation of chemical irritants increases the secretion of mucus or the glycoprotein output in the airways (*3,6,7,21,33, 61–63*). Repeated exposure of animals to chemical irritants such as sulfur dioxide, ammonia vapor and tobacco smoke causes structural and functional changes in the secretory elements of the airways.

Dogs exposed to chronic inhalation of sulfur dioxide, for example, display extreme hyperplasia of both mucous goblet cells in surface epithelium and submucosal glands (Fig. 6) (*64,65*). Mucous goblet cells increase in size and number in surface epithelium, suggesting transformation of ciliated cells into mucous cells or proliferation of undifferentiated cells selectively into mucous cells. Conversion of serous cells to mucous cells has also been proposed (*21,33,62*). Wide separation of ciliated cells by hyperplasia of mucous cells in these dogs could burden the mucociliary clearance, not only through increasing the mucous load from goblet cell secretion, but also by introducing gaps of nonciliated cells unable to participate in moving the mucus. Such a mechanism possibly explains the conspicuous adherence of luminal mucus to the epithelial surface and the polypoid organization of luminal mucous observed in the dog trachea after SO_2 irritation (*65*).

The effects of pulmonary toxicants are usually associated both with changes in the chemical composition of the secreted mucus involving the production of more acidic glycoproteins and with altered physical properties of this mucus (*21,33,62*). Hypertrophy of the surface goblet cells and submucosal glands during chronic exposure of dogs to sulfur dioxide is accompanied by increased activity of some of the glycosyl transferases in the respiratory tract (*64*). This increase could be attributed to the mucous cell hyperplasia evident histochemically.

The mechanisms responsible for the response of mucous-secreting cells to irritants have not been generally well analyzed but, presumably, involve both reflex and direct stimulation of the mucous-secreting cells. A further possibility is that chemical irritants cause release of glycoproteins from the surface layer of

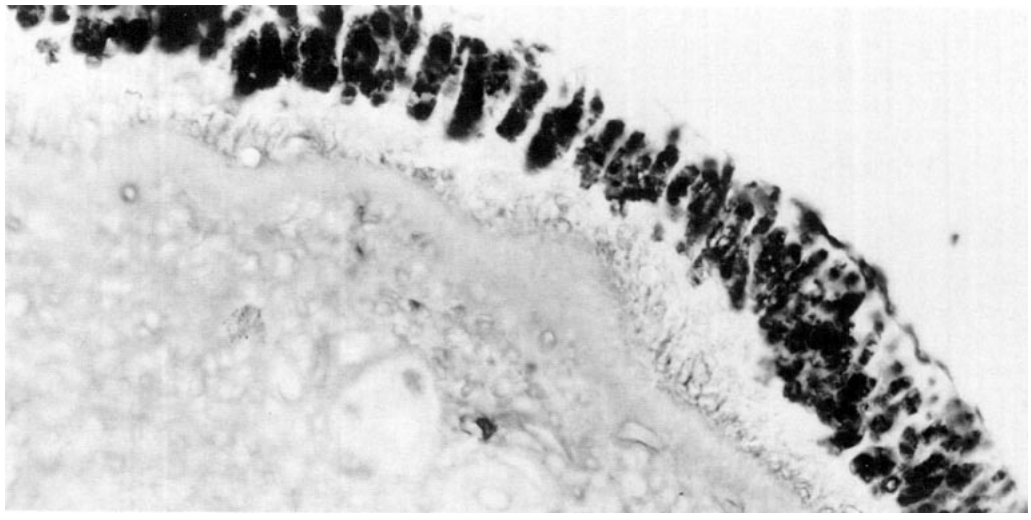

FIGURE 6. A bronchus from an SO_2 treated dog (*65*) shows hyperplastic mucous goblet cells in the overlying surface epithelium and hyperplastic dilated glands containing secretion stained for glycoprotein in the lamina propria below. Alcian blue-periodic acid Schiff stain. ×500.

ciliated epithelial cells, possibly the glycocalyx. In general, secretory cells that have undergone hypertrophy or hyperplasia tend to show an enhanced secretion of mucus and a hypersensitivity to stimulants. In the case of the mucous-secreting cells of the submucosal glands, the heightened secretion seems to be proportional to the degree of gland hypertrophy in the airway (*33,46*). There is evidence, moreover, that exposure to irritants also alters the balance of synthesis and discharge of mucus in the secretory cells (*33*). Thus, in SO_2-treated dogs, basophilia demonstrative of sulfate esters and periodic acid-Schiff staining demonstrative of hexoses in the hyperplastic surface goblet cells appeared decreased, indicating that an increased secretory rate resulted in decreased storage of secretion in apical granules of the irritant response cells (*65*). The same pattern of response is observed in certain chronic lung diseases such as cystic fibrosis, chronic bronchitis and bronchiectasis. In each case, there is gland hypertrophy, mucus hypersecretion and hyper-reactivity of the secretory elements to stimulation of parasympathomimetic agents (*13,46*).

Infection

Submucosal gland hypertrophy and changes in the acidity of glycoproteins are also observed in pigs with enzootic pneumonia induced by Mycoplasma (*21,33*). The proportion of acid glycoprotein (both sulfated and neuraminidase-sensitive sialylated glycoproteins) increases, and modifications of the intracellular granules, are evident ultrastructurally. Dogs hypersensitive to ascaris and chronically exposed to ascaris antigen evidenced luminal casts composed of sulfated glycoconjugate in ducts of tracheal and bronchial glands suggesting a residual stasis following prior hypersecretion (*66*). An increased pulmonary infiltrate of mast cells in these dogs was possibly related to a sensitivity state. Inoculation of rats with *Pseudomonas aeruginosa* embedded in agar beads has been shown to cause hyperplasia and metaplasia of goblet cells (*67*), but it is not known whether this results in alterations of mucous glycoproteins.

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