ABSTRACT

JAMES M. SAMET. Role of Mucus in the Pulmonary Toxicology of Inhaled Pollutants. (Under the direction of Dr. Louise M. Ball)

Lung mucus is a complex airway secretion whose primary function as part of the mucociliary clearance system is to serve as a renewable and transportable barrier against inhaled particulates and toxicants. The rheologic properties necessary for this function of mucus are imparted by glycoproteins, or mucins. Some respiratory disease states e.g., asthma, cystic fibrosis and bronchitis are characterized by quantitative and qualitative changes in mucus biosynthesis that contribute to pulmonary pathology. Similar alterations in various aspects of mucin biochemistry and biophysics, leading to altered mucus rheology and hypersecretion, result from inhalation of certain air pollutants such as SO_2, O_3, NO_2 and cigarette smoke. The consequences of these pollutant-induced alterations in mucus biology are discussed in the context of pulmonary pathophysiology and toxicology.

TABLE OF CONTENTS

TABLE OF CONTENTS iii

ACKNOWLEDGEMENTS v

I. INTRODUCTION 1

II. MUCUS COMPOSITION 3

A. Water Content 4

B. Mucus Rheology 4

C. Biochemical Analysis 5

D. Mucin Peptides 6

E. Carbohydrate Content 7

III. FUNCTIONAL HISTOLOGY 9

A. Glycoprotein-Secreting Cell Types 9

B. Periciliary Fluid 11

C. Mucociliary Transport 11

IV. MUCIN BIOCHEMISTRY 16

A. Mucin Genes and Transcription 16

B. Glycoprotein Release 19

V. PHYSIOLOGIC AND PHARMACOLOGIC CONTROL OF MUCUS SECRETION 21

A. Neurogenic Control 21

B. Humoral Control 22

C. Mechanisms of Glycoprotein Release 22

D. Differential Control of Glycoprotein Release 22

VI. ROLE OF MUCUS IN DISEASE 24

A. Bronchitis 24

B. Cystic Fibrosis 25

C. Asthma 26

D. Sputum Analysis 27

E. Morphologic Changes in Disease 29

VII. EFFECTS OF AIR POLLUTANTS ON MUCUS STRUCTURE AND FUNCTION 30

A. Effects on Mucociliary Function 30

B. Physico-Chemical Alterations 31

C. Biosynthetic Alterations 32

D. Effects on Ciliary Fluid 33

E. Mechanisms of Hypersecretion 33

VIII. SUMMARY AND CONCLUSIONS 38

IX. REFERENCES 44

ACKNOWLEDGEMENTS

I wish to thank my advisor, Dr. Louise M. Ball, for her encouragement and caring guidance during my studies in the Department of Environmental Sciences and Engineering. I also would like to express my appreciation to Dr. Robert B. Devlin for his support and patience during the completion of my degree requirements. Additional thanks go to Dr. Lori Todd for her participation in my Master's Committee. Finally, I would like to acknowledge the generous and unfailing support of Dr. David J. Holbrook, Jr. during my years as a student in Chapel Hill.

This work was supported by ESO7126 and by The Center for Environmental Medicine and Lung Biology, University of North Carolina at Chapel Hill.

SECTION I

INTRODUCTION

The lung is a unique organ in that it represents a very large epithelial surface that is continuously exposed to the outside world. Unlike the skin, the lung cannot make use of multiple layers of relatively impermeable cells as a barrier to harmful substances in the outside environment because its very function, i.e., gas exchange, demands that there be a minimal thickness of gas permeable cell membranes between the airspace and the blood. Instead, the lung uses specialized secretions produced by the airways to provide a renewable and transportable protective layer to interact with, neutralize and remove inhaled toxic materials. Mucus is the main airway secretion with this function.

As discussed below, airway mucus is a viscous solution with defined physical and chemical properties that enable it to be transported out of the lungs by ciliated cells lining the airways. Thus airway mucus is part of the mucociliary clearance mechanism, also known as the mucociliary escalator, that continuously sweeps trapped or neutralized inhaled materials out of the airways. This system also provides a vehicle for the removal of alveolar macrophages, the principal resident phagocytic cell in the lung, whose function is to ingest microorganisms and other particulates that reach the alveolar space. Alterations in mucus biosynthesis, structure and function can occur as result of certain disease states and exposure to inhaled toxic compounds. As will be seen, these alterations can cause impaired pulmonary clearance and a number of conditions leading to and resulting from excessive mucus secretion.

The focus of this report is the role of mucus in the pulmonary toxicology of air pollutants. Included are sections describing current concepts in mucus biology, i.e., its biochemistry, biophysics and histology of mucus, as well as its function in normal and pathophysiology. This is followed by a review of effects on mucus biology that are relevant to the pulmonary toxicology of inhaled pollutants.

SECTION II

MUCUS COMPOSITION

The classic model of the airway mucociliary system depicts mucus as a layer of highly viscoelastic luminal secretion floating on a layer of fluid of low viscosity (the sol or periciliary fluid layer). Mucus from healthy subjects is difficult to obtain because, in the absence of trauma or disease, very little is produced by the lung (Silberberg, 1988, Thornton et al., 1990). Furthermore, even under carefully controlled conditions normal mucus contains significant quantities of cellular debris and airborne materials that confound analyses. Largely due to the lack of sufficient uncontaminated material available for study, the composition of normal human mucus is still uncertain (Phipps, 1984, Silberberg, 1988). Analysis of sputum from diseased subjects, normal sputum induced with hypertonic saline and recovered secretions from laryngectomized patients has yielded the consensus that mucus is 95 % water, 2 % glycoproteins, 1 % proteins, 1 % lipids and 1 % inorganic salts.

In contrast to normal airway secretions, mucus produced in the airways of patients with respiratory disease contains significant amounts of serum proteins. These proteins are believed to be responsible for alterations in the rheology of airway secretions characteristic of some pathologic conditions such as bronchitis and bronchiectasis (Phipps, 1984). Some studies have suggested that at least part of the lipid in intestinal mucus is in the form of fatty acids covalently attached to glycoproteins (Witas et al., 1983, Slomiany et al., 1984). Airway glycoproteins, however, appear to contain small amounts of fatty acids that are non-covalently associated with hydrophobic regions of these molecules (Hanson et al., 1988).

A. Water Content- Regulation of water content is crucial to maintaining viscoelasticity that is optimal for the transfer of momentum from the beating cilia of the airway epithelium to the mucus blanket of the mucociliary clearance system (Silberberg, 1988). A considerable fraction of the water in mucus is found bound to the macromolecules or physically trapped inside the interstices formed by the dissolved glycoproteins (Phipps, 1984). The amount of water in mucus is determined by the availability of water in the airway, which is tightly linked to the movement of the ions that make up the inorganic salts found in mucus.

The source of mucus water, and for that matter all airway water, is vascular transudation from the blood (Phipps, 1984). This transudation results, in part, from the difference in hydrostatic pressure between the vascular bed and the airspace. However, the main mechanism for moving water into the airway is provided by an osmotic gradient established by vectorial transport of ions into the lumen carried out by the airway epithelium. The tracheobronchial epithelium secretes Cl^- ions and absorbs Na^+ ions. Under normal conditions, the amount of Cl^- ion secreted exceeds the amount of Na^+ ion taken in, establishing an ionic gradient for net water secretion into the airway (Welsh, 1991). Water lost by evaporation during the humidification of inspired air also increases the osmotic gradient and, thus, the water movement into the airway lumen (Mautone and Cataletto, 1990). As will be discussed in later sections, the ability to maintain the proper ionic composition of airway secretions is essential to maintaining normal mucociliary clearance. A defect in the mechanisms that regulate ion balance in the airway lumen is the central pathogenic feature of cystic fibrosis (Welsh, 1991).

B. Mucus Rheology- In terms of both normal and pathophysiology, viscosity and elasticity are the most important physical properties of mucus, enabling it to be transported by ciliary movement (Sheehan et al., 1991). The principal determinant of the viscoelastic properties of mucus are the glycoproteins, or mucins. Mucins are structural and secretory products of all secretory epithelia, including the airway

epithelium. These glycoproteins are normally found as a dissolved, entangled network in mucus. When dissolved in purified form, mucins have been shown to recreate the rheologic properties of mucus (Silberberg, 1988). It is for these reasons that mucins and the factors that affect their structure and expression in health and disease have been extensively studied. Many modern methodological approaches to the study of the physicomechanical properties of this complex material have been developed, as recently reviewed (Braga and Allegra, 1988). They range from models that seek to mathematically describe and predict the behavior of mucins in solution (Braga et al., 1988), to nuclear magnetic resonance (Odeblad, 1988), two-phase gas-liquid flow (Clarke, 1988), magnetic rheometry (King, 1988) and sinusoidal oscillation methods (Braga, 1988).

C. Biochemical Analysis- Chemical analysis and description of mucins is also difficult because of the great complexity and heterogeneity of these molecules. Mucins are either acidic or neutral in character, depending on their content of acidic functional groups. These acidic functional groups can be either esterified sulfate or sialic acid. Sialic acid-containing mucins are further classified on the basis of their sensitivity to deglycosylation by the enzyme sialidase (neuraminidase). Thus based on this scheme, there are four identifiable groups of mucins: neutral, sulfated, and the sialidase sensitive and resistant varieties of sialic acid-containing mucins (Jones, 1977). Acid and neutral glycoproteins can be distinguished histochemically with Alcian blue-periodic acid Schiff stain, with which acidic glycoproteins stain blue and neutral ones appear red (Abraham, 1984). As will be discussed in later sections, this classification scheme is useful in evaluating mucin histochemistry changes that occur in a variety of disease processes.

Mucins are polydisperse and highly glycosylated peptide molecules approximately 700 amino acids in length with molecular weights varying from a few hundred thousand to more than a million daltons (Roussel et al., 1988). The classic

structural representation of mucin is that of the "bottle brush", which depicts hundreds of carbohydrate chains attaches to a peptide core (Lamblin et al., 1991). Typical methodology for the analysis of mucin (Carlstedt et al., 1983) involves extraction with a chaotropic agent such as 6 M guanidinium chloride in the presence of endogenous protease inhibitors. After low speed centrifugation to remove insoluble material, to the extractant is added a gradient forming salt such as CsCl. Gradient centrifugation permits the fractionation of the mucins according to their density. Characterization of mucin glycoproteins is accomplished by polyacrylamide gel electrophoresis with silver staining and analytical ultracentrifugation (Creeth, 1978, Sheehan et al., 1991). Electron microscopy is also widely used for the analysis of mucin glycoproteins and has revealed that these molecules are flexible random coils with lengths ranging from 0.2 to 5 um (Jensen et al., 1980, Rose et al., 1984, Sheehan et al., 1986), although variations between studies exists, apparently due to differences in sample preparation (Mikkelsen et al., 1985). Sample handling and preparation has a significant effect on the integrity of mucins analyzed by other methodologies as well. In addition to the mentioned use of protease inhibitors, the once standard high-shear extraction methods and covalent bond-breaking reagents such as dithiothreitol are now avoided (Sheehan et al., 1991).

D. Mucin Peptides- Until relatively recently, mucins were thought to have relatively conserved amino acid sequences (Silberberg 1988). However, recent evidence obtained using methodologies that preserve the protein core of these glycoproteins, as well as new gene sequence information, suggests that there are multiple and polydisperse peptides, or apomucins (Porchet et al., 1991). Protease-sensitive, non-glycoslylated regions are found at both or one end of the peptide and are rich in aspartate and cysteine (approximately 13-16). These cysteine residues form disulfide bonds within and between peptides and appear to dictate the folding of the assembled mucins (Silberberg, 1988). The glycosylated regions of mucin are protease-resistant segments

rich in serine and threonine residues linked to carbohydrate chains. Strictly speaking, these are the mucin glycopeptides (Lamblin et al., 1991).

E. Carbohydrate Content- Approximately 80 % of the weight of the mucin molecule is carbohydrate (Porchet et al., 1991), and it is these covalently-attached sugars that confer mucins with most of their polydispersity and structural complexity (Lamblin et al., 1991). These carbohydrates are arranged in chains, called O-glycans, that include fucose, galactose, N-acetylglucosamine; N-acetylgalactosamine and N-acetylneuramic acid. O-glycans are always linked to the mucin peptide at a serine or threonine residue by N-galactosamine that is base-labile (Lamblin et al., 1991). Reductive elimination frees the O-glycan from the peptide and produces chains that range in size from 1 (N-galactosamine) to 20 carbohydrate residues (Roussel et al., 1975). Free O-glycans are classified into 4 groups of increasing acidity: neutral, mainly sialylated, sulfated and highly sulfated (Roussel et al., 1975). Since each of these groups can be further subdivided chromatographically according to their size, 12 subgroups are obtained, only 3 of which (2 neutral and the smallest sialylated) have been studied extensively (Lamblin et al., 1991). To date, study of just these 3 subgroups has produced over 100 O-glycan species, 81 from one subject alone (Lamblin et al., 1991, Klein et al., 1988, Breg et al., 1988, Van Halbeek et al., 1988).

Structurally, there are 3 parts to an O-glycan: the core (not to be confused with the peptide core), the backbone and the periphery (Hounsell and Feizi, 1982). The core is simply the first one or two sugar residues that are bound to the N-acetylgalactosamine. The most common core is galactose B1-3 N-acetylgalactosamine, which consists of a galactose residue bound at its 1 position to the 3 position of N-acetylgalactosamine via a B linkage. This core may be further substituted by N-acetylglucosamine linked B1-6 to the N-acetylgalactosamine, or, alternatively, by N-acetylneuraminic acid linked by an A2-6 bond to the N-acetylgalactosamine. The later occurrence forms a sialylated core (Lamblin et al., 1991, Hounzel and Feizi, 1982).

O-glycan backbones consist of disaccharide's of galactose and N-acetylglucosamine, several of which can be linked to the core at the galactose or N-acetylglucosamine. These disaccharide units may be added to the core linearly or can branch using B1-3 and B1-6 linkages on galactose residues of the backbone. The periphery contains the sugar residues fucose, galactose, N-acetylglucosamine, N-acetylgalactosamine and N-acetylneuraminic acid, added via A-linkages or, alternatively, sulfate substituted on the backbones (Lamblin et al., 1991).

Thus, mucin O-glycans alone can vary extensively in their overall structure, based on the possible permutations of the cores with the many different backgrounds and possible peripheries. When one considers that the mucus glycoproteins consist of peptide chains covered in sections with this vastly heterogeneous spectrum of O-glycans, it can be readily appreciated that these are very large and highly complex molecules.

SECTION III

FUNCTIONAL HISTOLOGY

A. Glycoprotein-Secreting Cell Types- The lungs of a typical healthy adult secrete mucus at a rate of 0.1 to 0.3 ml/kg body weight per day. Lung mucus is produced and secreted by several different cell types. Perhaps the most specialized cell for this task is the goblet cell, found mainly scattered among ciliated cells in the trachea and, in fewer numbers, in the bronchi (Wheater et al., 1979). Goblet cells are the only example of a unicellular gland in mammals, their main secretory product being mucin. As its name implies, this is a vessel-shaped cell with an expanded apical end that is filled with glycoprotein droplets, called mucigen droplets. These droplets are secreted from the cell as the membrane surrounding individual droplets or coalesced groups of droplets fuses with the plasma membrane, spilling their contents out of the cell. Under certain stimuli, goblet cells are capable of secreting all of their granules at once (Fawcett, 1986). Goblet cells secrete mainly acidic glycoproteins (Mautone and Cataletto, 1989).

Clara cells are the most prevalent cell type found in the small airways and are capable of differentiating into other cell types of airway epithelium such as goblet cells (Sleigh et al., 1988). Clara cells are known contributors to the sol, or periciliary, fluid layer and are also the principal site of xenobiotic metabolism in the lung (Mautone and Cataletto, 1989). Serous cells are another type of glycoprotein-secreting cell. These cells produce mainly neutral glycoproteins which are found packaged in distinct, electron-dense granules (Forrest and Lee, 1991). Interestingly, serous cells appear to secrete N-glycosylated glycoproteins (Aubert et al., 1991), a type of glycoprotein related to but distinct from the O-glycans. Serous cells are known to be capable of

responding to inhaled irritants by undergoing a form of metaplasia to become goblet cells (Jefferey and Reid, 1975, Sleigh et al., 1988).

Serous cells are arranged within the terminal portion of the acini (an acinus is a rounded exocrine secretory unit) of submucosal glands (Fawcett, 1986). The approximately 5000 submucosal glands found under the airway epithelium between the trachea and the subsegmental bronchi are the major source of mucus in the normal lung (Mautone and Cataletto, 1989). The proximal terminus of the submucosal glands are lined by mucus-secreting cells. These cells are packed with confluent, electron-lucent mucigen granules (Fawcett, 1986). Mucus acini in submucosal glands secrete mainly glycoprotein, while serous acini also secrete antibacterial compounds and antiproteases (Jeffery, 1991). The size of submucosal glands increases when the airway is exposed to environmental irritants such as tobacco smoke (Fawcett, 1986).

Morphometric studies on airway goblet cells and gland cells have shown that mucus is secreted from these cells as droplets 1-2 um in diameter (Wu and Carlson, 1991). The droplets are believed to be made up of concentrated glycoproteins, which in a matter of seconds absorb several hundred-fold their weight and volume in water drawn from the periciliary fluid (Verdugo, 1984). As the glycoprotein droplets swell, they begin to form plaques or "rafts" of mucus that move over the periciliary fluid. As the diameter of the airways increases, these rafts coalesce into larger islands of mucus, to eventually form a sheet of mucus (Iravani and van As, 1972).

All secreted mucus is eventually transported out of the airways to the top of the trachea where it is swallowed. This process is referred to as the mucociliary apparatus or mucociliary function. It is estimated that 10 ml of mucus reaches the top of the trachea in a healthy adult every day (Toremalm, 1960). The mucus layer over the airway epithelium is approximately 5-10 um thick and flows towards the trachea at a rate of 4.2 to 7 mm per minute (Casarett, 1960). Mucus flow at the trachea is between 7 and 25 mm per minute (Mautone and Cataletto, 1990). Factors affecting the rate of

mucus flow include the thickness of the periciliary fluid layer and the relative humidity of inspired air.

B. Periciliary Fluid- Surprisingly little is known about the periciliary fluid layer. It is believed to be an epithelial cell exudate (Widdicombe, 1984), 6 um in depth (Mautone and Cataletto, 1990), having low viscosity and an ionic content that is tightly maintained by the movement of sodium and chloride by the airway cells (Nadel et al., 1979). The periciliary layer in the distal airways and respiratory bronchioles probably contains some surfactant and other alveolar components (Sleigh, 1991). A phospholipid layer appears to exist between the periciliary fluid layer and the mucus sheet, and may serve to lower the surface tension between the two layers (Yoneda, 1976). The thickness of the periciliary fluid layer provides a low viscosity medium between the cell surface and the mucus layer in which the cilia can beat and propel the mucus blanket (Sleigh et al., 1988).

The relative humidity of inspired air determines the rate of evaporation of fluid from the mucus sheet. This effect is most evident in the upper airways and the trachea, where incomplete humidification of inspired air is more likely (Man et al., 1979). The effect that the evaporative loss that takes place in the airways has on the depth of the periciliary fluid layer is not known (Mautone and Cataletto, 1990).

C. Mucociliary Transport- Another key determinant of the rate of mucus flow is the ciliary beat frequency. The movement of secreted mucus is carried out by the predominant cell type in the airways, the ciliated airway epithelial cell. Ciliated cells in the airway are arranged in close juxtaposition, forming a field of cilia, interrupted by islands of goblet cells and the openings of mucus glands (Wheater et al., 1979). The cilia beat in a synchronized, wavelike manner to propel the mucus towards the trachea. Each ciliated cell has 200 to 300 uniformly spaced cilia about 6 um in length (Mautone and Cataletto, 1990). The cilia project into the periciliary fluid, with only their tips embedded in the mucus layer. Each cilium is 0.25 um in diameter and is composed of

an axoneme surrounded by a special process of the plasma membrane called the ciliary membrane (Wheater et al., 1979, Rhodin, 1966). The axoneme itself is made up of 9 interconnected doublets of microtubules surrounding a pair of central microtubules. The axoneme is anchored via the basal body to the network of microtubules of the cell cortex. The basal body is composed of 9 interconnected triplet microtubules. Each microtubule is made of tubulin protofilaments of tubulin, a self-assembling cellular protein. The microtubule doublets are linked to each other via arms of dynein, a protein with ATP-ase activity (Fawcett, 1986). The length of the cilium of the respiratory epithelium is a compromise between the bioenergetic advantages offered by a longer dynein arm and the required stiffness imparted by minimal length (Sleigh, 1991).

During ciliary movement, the axoneme microtubule doublets slide past each other. The binding of ATP to dynein releases and shortens the arm linking the doublets. As the ATP is hydrolyzed, the linkage between the doublets reforms at a more proximal site (Fawcett, 1986). This cycle of attachment and reattachment of dynein arms between microtubule doublets is repeated many times during each ciliary beat. The beating of groups of neighboring cilia is synchronized into "metachronal waves" as a result of a similarity in viscous forces experienced by the cilia at a given location (Sleigh, et al., 1988). A similar self-regulating mechanism is responsible for enlisting the number of beating cilia necessary to keep mucus flowing at a steady rate. A given patch of mucus is propelled by a group of many cilia beating in a coordinated fashion. As the movement of the cilium is slowed down by the inertia of the slower-moving mucus blanket, other cilia catch up with it and transfer their energy to the mucus as well. The net effect is that the slower the mucus blanket moves over a given area of the epithelium, the greater the number of synchronized cilia propelling it (Sleigh et al., 1988).

Due to its high glycoprotein content, mucus behaves as a non-Newtonian fluid, with elastic properties that cause it to temporarily absorb energy by changing its shape

(Sleigh, 1991). The amount of time elapsed between the deformation of mucus and its recoil to its original shape is the relaxation time, which has been measured to be approximately 30 seconds for mucus (Gilboa and Silberberg, 1976). Efficient transfer of energy from the beating cilia to the viscoelastic mucus layer requires that the ciliary beat frequency be faster than the relaxation time (Sleigh, 1991). This requirement is easily met since typical ciliary beat frequencies range from 7 cycles per second in the peripheral airways to 25 cycles per second in the trachea (Sleigh, 1977). This variation in ciliary beat frequency is reflected in the above mentioned increased rate of mucus flow that takes place in the trachea. The surface area over which mucus flows in the peripheral airways has been calculated to be approximately 70 square meters, compared to 0.6 square meters in the trachea (Mautone and Cataletto, 1990). This means that the proportionate volume of mucus reaching the trachea is increased over 100-fold with respect to the airways. Thus higher tracheal ciliary beat frequencies are needed to prevent mucus accumulation and clogging of the airways.

Studies on the regulation of ciliary beat frequency have suggested a dual control mechanism. A direct neurohormonal effect seems to be mediated by B-adrenergic stimulus and transduced via cAMP, with an ultimate effect on the axoneme itself (Sanderson and Dirksen, 1989). A second, possibly independent, effect is based on mechanical stimulation of the cilia by foreign particles or by mucus itself. (Sleigh, 1991, Sanderson and Dirksen 1989). Calcium flux experiments have shown that mechanostimulation of ciliary activity may involve the opening of calcium channels and the elevation of intracellular calcium concentrations (Sleigh, 1991). It is clear that many stimuli of mucus secretion also stimulate mucus transport, i.e., increase ciliary beat frequency. Whether the ciliary response to these stimuli is secondary to the presence of increased amounts of mucus on the cell or is independent from it is an issue that has yet to be resolved (Sleigh, 1991).

In addition to optimized ciliary beat frequencies, efficient mucus mobilization requires proper contact between the cilia and the mucus blanket. Under normal conditions, the cilia beat while completely immersed in periciliary fluid, with only the tips of the cilia coming in contact with the mucus blanket (Fawcett, 1986). Each ciliary beat consists of a propulsive stroke, a rest phase and a preparatory stroke. During the propulsive stroke the cilium reaches its maximum extension, causing the ciliary crown to penetrate the mucus layer and transfering momentum to it (Sleigh, 1991, Sleigh et al., 1988). The rest phase may merely reflect a metabolic recovery period or serve as a reserve period that enables increased beat frequency when needed (Mautone and Cataletto, 1990). Ciliary movement during the recovery period is backwards and downwards, with a clockwise rotation in a plane parallel to the cell surface (Sleigh, 1991, Sleigh et al., 1988). The combined effect of the different phases of the ciliary beat is to propel the mucus unidirectionaly, with a minimal amount of energy spent repositioning the cilium for the next stroke. As mentioned earlier, the thickness of the periciliary layer provides the critical distance that allows optimal ciliary contact with the mucus layer. The cilium itself appears to have a regulatory effect on the depth of the periciliary fluid. If the fluid is too deep, excess fluid may be swept away by ciliary beating. When the layer is too shallow, ciliary contact with the mucus layer will be stronger and this may stimulate ionic transport by the ciliated epithelium which in turn results in increased fluid secretion into the airway (Sleigh, 1991).

Several techniques for the measurement of mucus clearance rates have been devised over the years, as recently reviewed by Schlesinger, 1990. In their simplest form, these methods involve monitoring the movement of endogenous or artificial markers (inhaled, blown in or placed on the airway) from the periphery toward central areas of the respiratory tract. Artificial markers commonly used include pollen, Teflon disks, dyes, colored beads or technetium-99-radiolabeled iron particles. Detection methods depend on the type of marker used, but typically involve bronchoscopy, serial

sampling or imaging with a gamma camera. An elegantly simple method for measuring mucus velocity in the nose is the saccharine test, in which the time elapsed before a subject is able to taste a saccharine tablet placed in his nose is measured (Schlesinger, 1990, Sleigh et al., 1988).

Additional factors affecting mucociliary transport include gravity and air flow (Mautone and Cataletto, 1990). Gravity can impair the efficiency of ciliary activity significantly when the thickness of the periciliary layer is greater than 10 um or when the fluid becomes less viscous due to dilution (Proctor, 1986). In large airways, turbulent airflow can contribute to mucus clearance (Proctor, 1986). This is in fact the mechanical basis for the cough reflex, the only alternative mechanism for removing mucus from the airways when mucociliary clearance is impaired (Mautone and Cataletto, 1990).

SECTION IV

MUCIN BIOCHEMISTRY

Given that glycoproteins are responsible for the physiologically relevant characteristics of mucus, it has naturally followed that research efforts directed at understanding the metabolic and genetic aspects of lung mucus biology have focused on mucins. Accordingly, the following section will consist of a review of current mucin biochemistry and molecular biology.

A. Mucin Genes and Transcription- Present understanding of the organization and regulation of genes coding for mucin peptides, or apomucins, is rudimentary, as this field is very much in its developing stages. Human tracheobronchial apomucin genes have been mapped by Aubert and colleagues to chromosomes 11p15, 13 and 3 (Nguyen et al., 1990, Porchet et al., 1991). In addition, a cystic fibrosis tracheal apomucin gene has been mapped to 11p13-11Ter (Gerard et al., 1990). The presence of multiple nucleotide sequence homologies between chromosomes 11 and 13 have hampered efforts to determine whether there are multiple apomucin genes on chromosome 11p15. However, at present, at least 2 or 3 other chromosomes are implicated as containing apomucin genes.

Only partial cDNA sequences of tracheobronchial apomucins have been published in the literature to date. Aubert and co-workers screened a lambda-gt11 cDNA library from human bronchial mucosa with antibodies prepared against chemically denuded airway glycoproteins. Immunohistochemical studies carried out with these antisera showed specific labeling of goblet and mucous glands that was limited to the perinuclear area and did not include mucus granules, which contain the

glycosylated product (Aubert et al., 1991). Upon screening of the cDNA library with these antisera, the nucleotide sequences of positive clones were examined (Porchet et al., 1991). This work has so far produced 3 families of human tracheobronchial glycoprotein genes, suggesting that human airway apomucins are considerably more heterogeneous than once thought. The first family is described as consisting of repetitive sequences of 8 and 16 amino acids. This pattern of multiple repeated sequences appears to be a characteristic of apomucin genes. The second family contains two clones with near identical amino and carboxy termini that share perfect homology with 14 of a 22 amino acid stretch of a human tracheobronchial apomucin previously published by a separate group (Rose et al., 1989). Furthermore, this second family contains a third clone that has sequences coding for 30 uninterrupted hydroxy amino acids. The third family described by Aubert and associates features hydrophobic and hydrophilic regions arranged in alternating patterns. All of the tracheobronchial mucin cDNA sequences described by Aubert's group exhibit a repeated pattern consisting of a domain of hydrophilic amino acids flanked by a histidine-rich sequence and a proline-rich sequence. Presumably, these flanking sequences code for non-glycosylated an glycosylated regions of the peptide, respectively.

In addition to the extensive heterogeneity of apomucin genes, it is also evident that there is much variation at the transcriptional level. Northern hybridization of mucosal epithelium with mucin probes produces smears instead of discreet bands, indicating that there is extensive heterogeneity in the size of mucin mRNAs (Crepin et al., 1990, Perini et al., 1991, Jany and Basbaum, 1991). On the basis of sequence alignments and comparisons, Aubert and colleagues have reported that tracheobronchial mucin exons are relatively small and may be derived from complex alternative splicing (Porchet et al., 1991, Aubert, et al., 1991).

B. Glycosylation- As with any peptide, apomucin translation takes place in the rough endoplasmic reticulum and involves the initiation, elongation and termination of the

peptide product. From the cisternae of the rough endoplasmic reticulum, the peptide passes through the smooth endoplasmic reticulum into the lamellae of the Golgi apparatus. During this passage, initial glycosylation as well as the formation of disulfide bonds takes place. The Golgi lamellae are the site of most glycosylation and sulfation of the mucin peptide, as evidenced by cytochemical, histochemical and autoradiographic data (Phelps, 1978). Assembled mucin glycoproteins are then packaged inside vesicles formed from the Golgi lamellae. Mucin secretory granules are made up of coalesced vesicles, and migrate towards the apical aspect of the cell where they release their contents into the extracellular environment (Spicer and Martinez, 1984).

As presented by Phelps, 1978, glycosylation of the mucin peptide consists of two phases: synthesis of the oligosaccharide units and conjugation of these units to the peptide. The sugar molecules needed for the synthesis of the oligosaccharide units are derived from the hexose monophosphate shunt. Sugar molecules are activated at the expense of nucleotide triphosphates to form the following nucleotide sugar products which participate in the conjugation reactions directly: UDP-galactose, GDP-fucose, CMP-silate, UDP-N-acetylglucosamine and UDP-N-acetylgalactosamine. Sulphate is similarly activated via the synthesis of adenosine phosphosulphate which is then converted to the direct donor molecule, phosphoadenosine phosphosulphate (Phelps, 1978).

The conjugation reactions are carried out by a family of highly specific enzymes called glycosyltransferases. Although Ropp and associates, 1991, have succeeded in purifying and characterizing a specific B6N-acetylglucosaminyltransferase from bovine tracheal epithelium, little about these enzymes is currently known. Glycosyltransferases add the nucleotide sugars one at a time to the peptide at the hydroxyl group of serine or threonine residues on the peptide (Phelps, 1978, Silberberg, 1988). Because the number of hydroxy amino acids available is in large excess of the number that will be

glycosylated, the choice of a specific amino acid is one source of heterogeneity during O-glycosylation. The sequence of addition of sugars to the apomucin is another source of heterogeneity, and is determined by the availability of nucleotide sugars, the availability of individual glycosyltransferases, and an apparent set of rules that restrict the order in which sugars may be added. These rules are reflected in the ultimate structure of the O-glycans (See previous section). For instance as mentioned earlier, the first sugar to be added is always N-acetylgalactosamine. Some sugars can only be linked to a specific point on certain sugars already part of the O-glycan, while others allow two sites, permitting branching. Fucose and neuraminic acid are terminal sugar residues in glycoprotein oligosaccharide synthesis because no additional sugars may be linked to them, and thus their addition contributes of the size heterogeneity of oligosaccharide chains (Schachter, 1977, Silberberg, 1988).

To a yet undetermined degree, the amino acid sequence, length and composition of the apomucin itself appears to dictate the activity of the glycosyltransferases. Recent work by Brockhausen et al., 1990, has demonstrated that glycosyltransferases are able to recognize the peptide environment near the glycosylated amino acid of synthetic O-glycans. This work suggests that O-glycosylation sites have characteristic O-glycan core structures.

B. Glycoprotein Release- As reviewed by Verdugo, 1991, there have been several recent advances in our understanding of the complex processes that lead up to the release of mucins from the cell and the formation of the continuous mucus gel over the airway epithelium. The attachment of a secretory granule containing mucins in a condensed state to the plasma membrane at the apical end of the epithelial cell is the first step in mucin exocytosis. The expansion of a fusion pore on the plasma membrane leads to increased water and ionic permeability and release of the granule contents into the airway. Until relatively recently it was thought that membrane tension established by osmotic swelling of the secretory granule was responsible for the formation and

widening of the pore. However, recent evidence has shown that pore formation precedes, and can be uncoupled from, granule swelling (Verdugo, 1991).

The explosive rate of swelling undergone by mucins upon exocytosis cannot be accounted for by a simple osmotic process. Moreover, condensed mucin granules fail to decondense when placed in water free of ions (Fernandez et al., 1991). These and other findings have led to the "jack-in-the-box" model of glycoprotein exocytosis (Verdugo, 1991). This theory proposes that glycoprotein exocytosis is the result of rapid swelling of the glycoprotein polymer inside the secretory granule. While inside the granule, glycoproteins are in a condensed state that is maintained by the presence of a shielding species, such as calcium ion in the case of mucin. Increased permeability due to pore formation and widening results in the exchange of calcium for sodium inside the granule. This causes the mucin molecules to undergo a rapid phase transition to a hydrated, decondensed state (i.e., to swell) and be released.

Once released, airway mucins serve as the key rheologic assembly component of airway mucus. As pointed out by Verdugo, 1991, it is the ability of mucins to form entaglements rather than crosslinked networks allows mucus to expand in an unconstrained manner while absorbing water to form the mucus gel. Determinants of mucus hydration include water availability, the concentration of ions and polycations, and the pH of the airway liquid (Verdugo, 1991).

SECTION V

PHYSIOLOGIC AND PHARMACOLOGIC CONTROL OF MUCUS RELEASE

A. Neurogenic Control- Basal production and release of lung mucus in humans is believed to be spontaneous and independent of ennervation, since neurotransmitter antagonists and vagotomy have no effect on secretion (Gallagher, 1975). Furthermore, tracheobronchial explants continue to secrete mucus in vitro (Phipps, 1984). However, stimulation of mucin production above basal levels by submucosal glands is clearly under autonomic control. Parasympathetic control is evidenced by studies showing that electrical stimulation of the vagus nerve results in increased mucus secretion by submucosal glands. Vagal control of submucosal glands is cholinergic, since it can be reproduced with cholinergic agonists like pilocarpine and blocked with cholinergic antagonists such as atropine (Widdicombe, 1978).

There is also evidence of sympathetic control of airway mucus secretion via beta receptors (Widdicombe, 1978). Alpha and beta adrenergic sympathomimetic agents have specific effects on submucosal glands. Mucus and serous cells are stimulated through alpha receptors to secrete mainly fluid and some glycoprotein. In contrast, specific beta agonists target mucus cells to release chiefly glycoprotein. This implies that mucus composition is at least partly under autonomic control, and is thus susceptible to pharmacologic intervention (Phipps, 1984).

As reviewed by Phipps (1984), airway mucus secretion can also be stimulated via reflex mechanisms. Both fluid and glycoprotein secretion by submucosal glands can be increased by mechanical and chemical irritation of the airway epithelium. Reflex-

mediated release of glycoprotein from submucosal glands involves, but may not be limited to, sympathetic and parasympathetic ennervation.

B. Humoral Control- A variety of bioactive peptides and lipids also stimulate mucus secretion in the airways under certain experimental conditions. Histamine is effective in the cat and goose trachea (Widdicombe, 1978), but not human airways in vitro (Nadel et al., 1979), while substance P, kallidin are stimuli of the dog and rat trachea (Spicer and Martinez, 1984). Prostaglandins A1, E1, E2, F1alpha and F2alpha are also stimuli of mucin tracheal secretion (Parke, 1978, Spicer and Martinez, 1984). The potent phospholipid inflammatory mediator platelet activating factor also stimulates mucin secretion of tracheal explants in vitro (McManus and Deavers, 1989).

C. Mechanism of Glycoprotein Release- Very little is known about the cellular and biochemical mechanisms that mediate the release of glycoprotein in airway cells. As reviewed by Spicer and Martinez, 1984, there is evidence to suggest that these mechanisms are similar to those of other exocrine glands (Spicer and Martinez, 1984). Cyclic nucleotides have been shown to stimulate mucin release in rat tracheal explants (Spicer and Martinez, 1984), suggesting their role in a signal transduction mechanism. Similarly, the phosphodiesterase inhibitor theophiline causes glycoprotein secretion in human airways in vitro (Widdicombe, 1978). A calcium dependency, possibly with a role in signal transduction, has also been demonstrated in rat trachea stimulated with acetylcholine (Spicer and Martinez, 1984).

D. Differential Control of Glycoprotein Release- The study of selective release of glycoprotein from submucosal glands and goblet cells in the airways is complicated since both are present in airway tissue. Recently, studies of goblet cells in tissue culture have yielded insightful information, as reviewed by Kim, 1991. While stimulation of glycoprotein release from submucosal glands is neurogenic, goblet cells appear to be under local humoral control (Widdicombe, 1978). No efferent ennervation occurs above the level of the submucosal glands of the airway (Wheater et al., 1979).

Furthermore, direct neurogenic stimulation, such as stimulation of the vagus nerve does not alter mucin release in the goblet cell (Spicer and Martinez, 1984). Consistent with these findings, many effective submucosal autonomic agents that stimulate mucin release in explants have no effect on goblet cells in vitro. These include acetylcholine, norepinephrine and isoproterenol (Kim, 1991). This contrast in the pharmacology of mucin release by submucosal glands and goblet cells seems to extend to pro-inflammatory humoral mediators as well. For example, prostaglandins E2 and F2alpha have no effect on goblet cells in vitro, yet they induce mucus secretion in animal trachea (Spicer and Martinez, 1984, Kim, 1991).

Release of glycoproteins by goblet cells in vitro can be stimulated by ATP, proteases, or by physicochemical manipulations, such as alteration in pH, mechanical stress, and hypoosmolarity (Kim, 1991). The response to ATP is receptor-mediated and specific. In contrast, glycoprotein release induced by physicochemical stimuli, such as pH changes, is believed to be the result of damage of the cell membrane. Hypoosmotic conditions are analogous to mechanical stress on the cell in that both types of stimulus seem to work through pressures exerted on the cells. Similarly, the effect of mast cell and neutrophil proteases (e.g., elastase, chymase and cathepsin G) is also non-specific, being the result of cleavage of membrane-bound glycoproteins as well as damage caused by hydrolysis of cell membrane proteins (Nadel, 1991, Kim, 1991).

SECTION VI

ROLE OF MUCUS IN DISEASE

Pathophysiologic changes involving lung mucus are a feature of many pulmonary diseases. These changes can be either qualitative, i.e., changes in mucus composition or structure, or quantitative i.e., changes in the amount of mucus in the lung. A change in mucus composition could be due to altered glycoprotein biosynthesis, electrolyte transport or water content. Alternatively, structural modifications can be the result of interactions between normal mucus and pathogens or reactive chemicals.

Typically, quantitative changes in lung mucus involve hypersecretion of airway mucin. Hypersecretion can be due to increased biosynthesis by goblet cells or submucosal glands, or to increased numbers of mucin-secreting cells brought about by faster cell division (hyperplasia) or by differentiation of non-secreting cells (metaplasia)(Robbins, 1981, Lundgren et al., 1990). Another mechanism leading to increased mucus volume in the lung is leakage of plasma components into the airspace as a result of increased vascular permeability (Lundgren et al., 1990). A brief review of pulmonary diseases in which some of these underlying mechanism contribute to the pathologic role played by mucus now follows.

A. Chronic Bronchitis- Bronchitis is basically an inflammatory disease of the airways characterized by persistent chronic cough with sputum production. Most patients with chronic bronchitis are smokers or ex-smokers (Wanner, 1984a). Microbial and viral infections, as well as urban and industrial air pollutants, are believed to exacerbate the condition, but are not likely initiators of bronchitis (Robbins, 1981). The pathologic

hallmark of bronchitis consists of hypertrophy and hyperplasia of submucosal glands, as well as hyperplasia and metaplasia of goblet cells (Robbins, 1981). Mucus hypersecreted in bronchitis is apparently normal in composition, although it can contain a higher content of plasma components which may inhibit ciliary activity (Wanner, 1984a, Lopez-Vidriero and Reid, 1978). Mucus from bronchitic patients is more viscous during flare-ups of the disease (Wanner, 1984a). In addition, persistent reductions in the rate of mucociliary clearance have been reported in patients with bronchitis (Wanner, 1984a).

B. Cystic Fibrosis- Cystic fibrosis is a disease that illustrates in compelling terms the importance of mucociliary clearance to normal lung function. The most common lethal genetic disease among caucasians (Welsh, 1991), cystic fibrosis is characterized by a systemic defect in exocrine gland secretion. Expression of the disease varies considerably, but sweat and mucus glands are involved most frequently. The most serious manifestation of the disease is the retention within the airways of abnormally viscous mucus (Robbins, 1981). The inability of the mucociliary system to remove this thick mucus, along with trapped microorganisms and debris, results in enlargement of airway caliber and persistent infection. Chronic infections with Staphylococcus aureus and Pseudomonas aeruginosa are responsible for the vast majority of the deaths in cystic fibrosis (Robbins, 1981).

As reviewed by Welsh, 1991, the basic defect in cystic fibrosis is an alteration in electrolyte transport by the airway epithelium which leads to insufficient hydration of glycoproteins. Airway epithelial cells secrete Cl^- into the airway and absorb Na^+ from the airway. Cl^- and Na^+ ions are first co-transported into the basolateral side of the cell in an electrically neutral process that allows intracellular transport of Cl^- against its concentration gradient. This process is made possible by the action of a Na^+/K^+ ATPase which exports Na^+ through the basolateral membrane of the cell, thus keeping its intracellular concentration low. This ATPase activity also moves K^+ into the cell,

which indirectly contributes to a favorable gradient for apical Cl^- secretion and Na^+ absorption through specific regulated channels. Many neuropeptides (e.g., bradykinin), hormones (e.g., aldosterone) and mediators (prostaglandins) influence the permeability of the Cl^- channel(s), apparently by increasing cAMP levels in the cell (Welsh, 1991).

Airway epithelial cells from cystic fibrosis patients secrete considerably less Cl^- ion and absorb more Na^+ through their apical membrane than cells from normal subjects (Welsh, 1991, Boucher et al., 1986). This is due, at least in part, to a defective cAMP-regulated Cl^- transport channel (Welsh, 1991, Davis, 1991). The putative gene responsible for this defect was identified in 1989 (Rommens et al., 1989, Riordan et al., 1989, Kerem et al., 1989). A 3-base pair deletion in the "cystic fibrosis transmembrane conductance regulator" (CFTR) gene codes for the deletion of a phenylalanine at amino acid position 508 of the protein. This single mutation in CFTR accounts for 70 % of cystic fibrosis genes (Kerem et al., 1989). The identity and function the CFTR gene product is still unknown. One possibility is that CFTR is a Cl^- channel that cannot be activated by cAMP. Alternatively, CFTR could be an integral or separate regulatory protein that interacts with the Cl^- channel (Davis, 1991). The biochemical complexity of cystic fibrosis pathogenesis demonstrates the great number and variety of possible susceptible targets that can lead to disruption of normal mucociliary function.

C. Asthma- Bronchial asthma is essentially a chronic respiratory disease that manifests itself intermittently as attacks of dyspnea (shortness of breath) and wheezing caused by bronchial spasms. Nearly all characteristics of the disease such as its severity, course, aggravating factors and frequency and duration of attacks varies widely among patients with asthma. Most patients have a familial predisposition to atopic disease. Several different types of asthma are recognized based on apparent etiologic factors. These include exercise asthma, cold air asthma and industrial (chemically-induced) asthma. (Robbins, 1981). While the basic mechanism of the disease is still poorly understood,

all asthma attacks seem to involve a predisposing airway hyperreactivity and the release of a battery of inflammatory mediators that cause bronchoconstriction and mucus hypersecretion (Robbins, 1980). A great deal of attention has been focused on the possible link between air pollution and an alarming rise in the number of cases of fatal asthma attacks (Whitelaw, 1991).

Gross pathology findings in the airways of victims of fatal asthma attacks (status asthmaticus) include the presence of a tenacious mixture of mucus, exudate, epithelial cells, lymphocytes and eosinophils (Jeffery, 1991). Mucoid impactions in the airways of asthmatics may be present even in the absence of infection (Anderson, 1990). Also evident are hyperplasia and hypertrophy of submucosal glands and hypertrophy, hyperplasia and metaplasia of goblet cells in peripheral airways (Wanner, 1984b, Robbins, 1981). The same histologic changes are reported in patients with stable asthma, but to a lesser degree (Wanner, 1984b). Mucus plugs and impaired mucociliary clearance may be found even in patients with mild asthma or during remission of the disease (Bateman et al., 1983, Pavia et al., 1985). In addition to hypersecretion, changes in mucus secretion in bronchial asthma include increased permeability to serum constituents and altered water transport into the airway (Wanner, 1984b).

Increased endothelial permeability is believed to mediate the airway edema observed in cases of fatal asthma. Similarly, increased epithelial permeability is likely to be responsible for the leakage of serum components into the airway, changes in the periciliary fluid layer which can lead to inhibition of ciliary function, and increased mucus volume in the lung (Wanner, 1984b, Lopez-Vidriero and Reid, 1978). In addition, changes in water transport by the airway epithelium, possibly mediated by histamine, have been reported in experimental asthma models and may also contribute to these effects (Wanner, 1984b).

D. Sputum Analysis- Mucus samples are often obtained for clinical analysis and diagnosis as expectorated sputum. Sputum is a chemically unstable mixture of mucus,

saliva, surfactant, cells and plasma constituents that is not present in the normal lung (Lopez-Vidriero and Reid, 1978). A further complication of inferences made from sputum analysis is the need to distinguish between purulent and non-purulent sputum. Purulent sputum is recovered from patients with an overt or underlying infection and usually has increased amounts of glycoprotein, DNA and a higher content of plasma constituents than non-purulent sputum (Lopez-Vidriero and Reid, 1978). In spite of these limitations, sputum samples can yield useful information about pathologic changes involving mucus production in disease. For instance, an increase in the N-acetylneuraminic acid:fucose ratio in the sputum is believed to reflect leakage of plasma constituents into the airway, which in turn, may be indicative of a relatively more severe inflammatory reaction in the airways. An elevated N-acetylneuraminic acid:fucose ratio is observed in sputum samples obtained from patients with asthma; however, the absolute concentration of these compounds is low. This suggests that plasma constituents contribute significantly to the increased volume of mucus present in asthmatic lungs. Sputum obtained from bronchitis patients, on the other hand, have high amounts of N-acetylneuraminic acid and fucose but the ratio is normal, indicating that the increased amounts of mucus produced in bronchitis is mainly the result of hypersecretion. In sputum from patients with cystic fibrosis the reverse is found, i.e., relatively normal amounts of N-acetylneuraminic acid and fucose but a high N-acetylneuraminic acid:fucose ratio. This suggests that in cystic fibrosis there is an inflammatory process that results in leakage of plasma components into the airway without hypersecretion of mucus (Lopez-Vidriero and Reid, 1978).

The DNA content of sputum is a similar marker of the severity of lung inflammation. Normal lung secretions and non-purulent bronchitis sputum do not contain detectable amounts of DNA. In contrast, purulent bronchitis sputum contains detectable amounts of DNA, and even non-purulent cystic fibrosis sputum contains

significant quantities. This finding may well attest to the relative severity of airway inflammation present in cystic fibrosis (Lopez-Vidriero and Reid, 1978).

E. Morphologic Changes in Disease: Although hypertrophy of submucosal glands is evident in both bronchitis and asthma, a relatively greater increase in mucus acini compared to serous acini occurs in bronchitis, but is not observed in asthma (Glynn and Michaels, 1960, Jeffery, 1991). Since the serous acini of submucosal glands secrete antibacterial and antiprotease compounds in addition to glycoprotein, the dilution of these components by an increased volume of mucus could result in diminished resistance to both infection and proteolytic attack in bronchitis (Jeffery, 1991).

The mechanism responsible for the development of submucosal gland hypertrophy and hyperplasia in asthma and bronchitis is presently unknown. However, neutrophil-derived proteases such as elastase have been implicated in goblet cell hyperplasia, as reviewed by Lundgren and associates, 1990. These authors have also proposed a possible mechanism wherein chemotactic lipid mediators such as leukotriene B4 are secreted by epithelial cells during the initial stages of airway inflammation. These mediators could recruit neutrophils into the airway which could then release elastase, causing goblet cell hyperplasia. In support of this scenario is the finding that glucocorticoids, which inhibit the synthesis of lipid inflammatory mediators, can prevent goblet cell hyperplasia induced by neutrophil products (Lundgren et al., 1988).

SECTION VII

EFFECTS OF AIR POLLUTANTS ON MUCUS STRUCTURE AND FUNCTION

Inhalation of a variety of ambient and occupational air pollutants is known to result in a number of untoward effects in the lung. These include changes in pulmonary function, diminished lung defense and impaired mucociliary clearance, as well as obstructive, inflammatory and neoplastic disease. These topics have been the subject of several excellent recent reviews (Gordon and Amdur, 1991, Mauderly and Samet, 1991, Cross and Halliwell, 1991, Graham, 1989, Koenig et al., 1989). This section will focus on specific effects of air pollutants on lung mucus function, secretion and biosynthesis, as they pertain to the development of pulmonary toxicology and disease.

A. Effects on Mucociliary Function- Impairment of mucociliary function is a consequence of exposure to a variety of air pollutants. The effects of inhaled toxicants on respiratory tract clearance mechanisms, was recently reviewed by Schlesinger, 1990. Essentially, mucociliary targets of inhaled air pollutants are the ciliated epithelium, the periciliary fluid and the mucus layer (Schlesinger, 1990).

In addition to the outright epithelial desquamation or destruction of cilia induced by high concentrations of SO_2, NO_2 and O_3 (Watanebe et al., 1973, Boucher, 1981), ciliary activity in the airway epithelium is susceptible to alteration by toxic agents. Agents that impair ciliary beat rate (i.e., induce ciliary dyskinesia or ciliostasis) include H_2SO_4, O_3 (Grose et al., 1980), SO_2, NO_2, ammonia, (Dalhamn and Sjoholm, 1963), wood dust, ammonia, cadmium, nickel, hairspray, (Pedersen, 1990) cigarette smoke (Kensler et al., 1963) formaldehyde (Morgan et al., 1986) and cadmium (Adalis et al., 1977). The mechanism of action of ciliostatic compounds can involve structural

damage of the cilium, as induced by NO_2 (Ranga and Kleinerman, 1981), or altered energy metabolism caused by heavy metals (Pedersen, 1990, Schlesinger, 1990).

In spite of these findings, the relevance of the ciliostatic effect of inhaled pollutants as a mechanism responsible for impaired mucociliary activity is thrown into question by two factors. First, as noted by Schlesinger and others, the dose of toxicant required to induce ciliostatic changes usually far exceeds that required to produce a reduction in mucociliary clearance (Schlesinger, 1990, Abraham, 1986). Second, a study conducted by Battista and colleagues, showed that 10 % of the ciliated epithelium in chicken trachea could carry out 30-50 % of particle transport activity seen in control animals, suggesting that ciliary activity is present in large excess of that needed for normal ciliary function (Battista et al., 1973). Thus changes involving mucus production and function may be more likely mechanisms of the impaired mucociliary clearance induced by inhaled toxicants (Abraham, 1986).

B. Physico-chemical Alterations- Another way in which an inhaled pollutant can alter mucociliary clearance is by altering mucus rheology by interacting with its constituents directly or by influencing its biosynthesis. An alteration of mucus rheology, such as a decrease or increase in viscosity, can diminish the efficiency with which energy is transferred from the beating cilia to the mucus blanket. As presented by Holma, 1989, variations in mucus pH, such as those caused by SO_2 can have a profound effect on mucus rheology. Although factors such as protein concentration and ionic strength also come into play, a reduction in pH generally increases mucus viscosity (Holma, 1989). Glycoproteins appear to be the principal acid-reactive component in mucus, and, in fact, have been demonstrated to be largely responsible for the buffering capacity of mucus (Holma, 1989).

Schlesinger, 1990, discusses chemical cross-linking of glycoproteins as a mechanism via which inhaled toxicants can alter mucus viscosity. Exposure to formaldehyde, a compound known to form chemical cross-links in proteins and nucleic

acids (Auerbach et al., 1977), reportedly results in increased mucus viscosity (Morgan et al., 1984). Similarly, reduced viscosity resulting from exposure to O_3 is proposed to be caused by a decrease in the number of chemical cross-links (Last, 1982, Schlesinger, 1990). However, Verdugo has recently argued that the rheologic properties of mucus are imparted not by cross-links between glycoprotein moieties, but by networks of entanglements between glycoprotein strands (Verdugo, 1991). Thus, while formaldehyde may indeed increase mucus viscosity by forming cross-links, the reduction in mucus viscosity may not be due to the destruction of existing cross-links between glycoproteins.

C. Biosynthetic Alterations- Certain inhaled toxicants induce qualitative changes in glycoprotein biosynthesis. Exposure to cigarette smoke, SO_2 or H_2SO_4 induces a shift in the type of glycoprotein secreted by the airway to produce a relatively more acidic mucin (Jones, 1977, Jones et al., 1978, Abraham, 1984, Schlesinger, 1990). This phenomenon is also seen in chronic bronchitis and cystic fibrosis, where the degree of sulfation of secreted glycoproteins is increased (Jones and Reid, 1978), suggesting that it is an adaptive response to cellular injury. With cigarette smoke, the increase in acidic mucin seen in submucosal glands and goblet cells is due to an increase in sialic acid-containing mucins (Jones, 1977). Normal rat tracheal epithelium preferentially secretes neutral glycoprotein, while in the peripheral airways there is a bias for the production of acidic glycoprotein. Acute exposure to cigarette smoke abolishes these regional differences within 24 hours, resulting in dominance by a cell that produces both acid and neutral glycoprotein throughout the rat respiratory tract. Sustained exposure to cigarette smoke eventually results in a population of cells that produces only acidic glycoprotein (Jones and Reid, 1978, Jones, 1977). Unlike goblet cell hyperplasia, the change towards secretion of acidic mucin is apparently not blocked by the anti-inflammatory compound phenylmethyloxadiazole (Jones, 1977). Whether increased secretion of acidic mucin is due to secretion of molecules with a higher acid content or

more molecules with acid moieties is unclear (Jones and Reid, 1978). Interestingly, induction of acid glycoprotein secretion was not observed using O_3 as a toxicant in a recent studies by Hotchkiss and associates, who found an increase in both acid and neutral glycoprotein in rat and primate nasal epithelium (Hotchkiss et al., 1991, Harkema, et al., 1987). Whether this reflects a difference in the response by nasal and tracheobronchial epithelium or the nature of the stimulus is not clear.

D. Effects on Ciliary Fluid- As discussed in a previous section, the depth of the periciliary fluid layer largely determines the quality of the interaction between the cilia and the mucus blanket over the airways. Optimal periciliary fluid depth and composition are necessary for efficient transfer of energy from the tips of the cilia to the mucus during the propulsive stroke, and for the recovery stroke to take place unimpaired (Sleigh, 1991). Periciliary fluid depth and composition can be affected by compounds that affect ion transport by the airway epithelium or increase epithelial permeability to serum components which can affect mucus rheology and secretion of glycoproteins (Schlesinger, 1990, Abraham, 1984a, Phipps et al., 1986). For example, in vitro exposure to O_3 results in increased Na^+ ion permeability across guinea pig airway epithelium (Stutts and Bromberg, 1987), while O_3 inhalation produces increased water and Cl^- ion secretion in sheep tracheal explants in vitro (Phipps et al., 1986). Similarly, O_3 inhalation results in increased epithelial permeability to macromolecules in humans (Kerhl, et al., 1987). Exposure to NO_2 caused increased mucosal permeability to large protein molecules in guinea pigs (Abraham, 1984). As discussed earlier, increased epithelial permeability to water and serum components is also a feature of the pathology of cystic fibrosis and asthma (See preceding section).

E. Mechanisms of Hypersecretion- Mucus hypersecretion is an important part of the sequelae induced by a number of toxic insults to the lung. Possible mechanisms of hypersecretion include increased release of existing glycoprotein stores, hypertrophy of submucosal glands, hyperplasia of goblet cells, metaplasia of non-secreting cells into

goblet cells, and a higher rate of mucin biosynthesis by goblet cells or cells in the submucosal glands.

Inhalation of some toxic compounds produces acute. mucus secretion into the airways. For example, NH_3, (Widdicombe, 1978), and O_3 (Abraham, 1984) induce glycoprotein discharge from glycoprotein-secreting cells in the airways. Exposure to acidic and alkaline media also stimulate mucin release, albeit secondary to cell membrane damage (Kim et al., 1989). Similarly, inhalation of dusts (e.g., charcoal or barium sulfate) provokes release of mucus from cat tracheal explants via a neurogenic reflex pathway as well as a direct stimulation of the mucosa (Abraham, 1984). Both myelinated and unmyelinated (C-fibers) neuronal pathways may be involved in the discharge of mucus in the airway (Phipps, 1984, Schlesinger, 1990). Eicosanoids such as leukotrienes C_4 and D_4 and hydroxyeicosatetraenoic acids, also been shown to mediate glycoprotein release from human airway explants (Marom et al., 1982, Marom et al., 1983), and may be involved in mucus secretion in response to toxicant exposure (Phipps et al., 1986). A study by Jones et al. showed that acute exposure of rats to cigarette smoke causes extensive degranulation of mucus-secreting cells, to the point that there is a transient decrease in the number of cells staining positive for glycoprotein content in the airways. In the same study, the anti-inflammatory phenylmethyloxadiazole did not prevent tobacco smoke-induced discharge of mucus-secreting cells (Jones et al., 1978). However, in a similar study the same drug was shown to be effective in preventing an increase in the basal rate of mucin discharge induced by tobacco smoke, possibly reflecting a difference in the time of administration of the drug between these studies (Coles et al., 1979). The balance between glycoprotein synthesis and release in secretory cells is reportedly altered as a result of chronic inhalation of tobacco smoke or SO_2. This is evidenced by a decrease in the number of intracellular glycoprotein granules in exposed cells, suggesting a higher rate

of secretion of glycoprotein granules relative to their storage time inside these cell (Jones and Reid, 1978, Spicer and Martinez, 1984).

An increase in the number of airway mucin-secreting cells can be induced by inhalation of SO_2 (Spicer et al., 1974), O_3 (Phipps et al., 1986), Cl_2 (Elmes and Bell, 1963), NO_2 (Freeman and Haydon, 1964) or cigarette smoke (Rogers and Jeffery, 1986). SO_2 and Tobacco smoke-induced hyperplasia have been studied as animal models of bronchitis. As reviewed by Spicer and Martinez, 1984, and Abraham, 1984, submucosal gland cell and goblet cell hyperplasia is readily apparent in the airways of dogs and rats chronically exposed to SO_2. The response of the goblet cell population in the bronchi and bronchioles of dogs exposed to SO_2 is characterized by an increase in both size and number (Spicer et al., 1974). The expansion of the goblet cells population is partially due to metaplasia of other epithelial cell types into goblet-like cells with enhanced mucus-secreting capabilities (Spicer and Martinez, 1984). Submucosal gland hypertrophy is apparently also due to both increased replication rate and a metaplastic change of serous cells into mucus cells (Spicer and Martinez, 1984, Jany and Basbaum, 1991). SO_2 inhalation in dogs has also been shown to induce higher rates of glycosyltransferase activity in lung homogenates, although it was not possible to determine whether this was merely a reflection of the histologic changes also observed in these animals (Baker and Sawyer, 1975). A recent study has tentatively suggested that there is an induction in mucin mRNA levels in the airways of rats chronically exposed to SO_2 (Jany and Basbaum, 1991).

Exposure to cigarette smoke also produces submucosal gland hypertrophy, goblet cell hyperplasia and evidence of serous-to-mucus cell metaplasia (Coles et al., 1975, Rogers and Jeffery, 1986, Jany and Basbaum, 1991). Tobacco smoke-induced hyperplasia of secretory cells can occur within hours of a single exposure and may also involve basal cells (Jones and Reid, 1978). Metaplastic changes in the airways of rats exposed to cigarette smoke are evidenced by increased rates of mitosis among basal

cells and serous cells but not mucus cells (Jany and Basbaum, 1991). Tobacco smoke-induced secretory cell hyperplasia in rats can be inhibited with indomethacin and steroidal anti-inflammatory drugs (e.g., dexamethasone, hydrocortisone), suggesting that cyclooxygenase products are involved (Rogers and Jeffery, 1986b). Mucolytic drugs such as N-acetylcysteine are also inhibitors of the hyperplastic response, perhaps by preventing glutathione depletion in cigarette smoke-exposed cells (Rogers and Jeffery, 1986a, 1986b).

O_3 exposure also induces hypertrophy of submucosal glands and hyperplasia of goblet cells (Phipps et al., 1986). In this study, the morphologic changes induced in the airways of sheep by chronic O_3 inhalation were correlated with decreased glycoprotein and increased water secretion. However, increased glycoprotein secretion with continued water secretion was observed following a period of recovery after exposure, suggesting that the initial decrease in glycoprotein secretion was due to depletion of mucin stores (Phipps et al., 1986). Similar kinetics i.e., decreased glycoprotein secretion followed by a rebound to increased secretion, was seen in rat tracheal explants from rats exposed to O_3 in vivo (Last et al., 1977).

It has been proposed that metaplasia may play a larger role in toxicant-induced hypersecretion than previously thought (Jany and Basbaum, 1991, Hotchkiss et al., 1991). Evidence for this hypothesis is based on studies showing increased numbers of goblet cell in areas of the lung where they are normally scarce or absent, such as the lung periphery (Lamb and Reid, 1968), and an increase in the population of mucus cells without an apparent change in the mitotic rate of these cells (Nygre et al., 1984). Hotchkiss and associates have demonstrated similar evidence of metaplastic changes responsible for the increased number of goblet cells in nasal epithelium from rats exposed to O_3 (Hotchkiss et al., 1991).

The possible role of inflammatory cell-derived proteases such as elastase in the development of metaplastic changes in the airway induced by tobacco smoke or SO_2

was recently reviewed by Jany and Basbaum, 1991 and Lundgren et al., 1990. A study by Christensen et al., 1977, showed that a single dose of pancreatic elastase causes an apparently irreversible goblet cell metaplasia in guinea pigs. In a more recent study, the steroidal anti-inflammatory drug dexamethasone was effective in inhibiting neutrophil elastase-induced goblet cell hyperplasia in rat trachea, suggesting the involvement of lipid mediators (Lundgren et al., 1988). Prostaglandin E1 has also been shown to induce increased numbers of mucus cells, without preceding DNA synthesis, in the airways of mice, a process apparently mediated through cAMP since the analog dibutyryl cAMP had the same effect (Nygren et al., 1984). These findings are intriguing in light of studies by Koren and colleagues showing that O_3 inhalation produces neutrophil infiltration into the airways, as well as increased levels of eicosanoids and neutrophil-derived elastase in bronchoalveolar lavage fluid from human subjects (Koren et al., 1989). Interestingly, no increase in elastase activity was found in this study. The authors suggest that this is possibly due to inactivation by antiproteases such as A-1-antitrypsin (Smith and Johnson, 1985), and also that microfocal secretion by neutrophils could permit some elastase to escape antiproteases (Witz et al., 1987, Koren et al., 1989).

SECTION VIII

SUMMARY AND CONCLUSIONS

Lung mucus is essentially a glycoprotein solution produced by the airways, whose function is to serve as a dynamic, replenishable barrier against inhaled particulate and gaseous contaminants in the lung. Key to the role of mucus as a component of both lung defense and pathology are its viscosity and elasticity which, under normal conditions, permit mucus to act as an efficient "biologic conveyor belt", removing trapped and dissolved materials that enter the lung with inspired air. These rheologic properties of lung mucus are imparted by glycoproteins synthesized and released by specialized cells in the airways.

Mucins are complex, heavily glycosylated peptide macromolecules whose biosynthesis and secretion appear to be under close regulatory control, yet able to respond quantitatively and qualitatively to alterations in lung homeostasis, such as those resulting from respiratory disease or inhalation of toxic materials. An example of such a biosynthetic response is the increased secretion of acidic glycoproteins seen in patients with chronic bronchitis or cystic fibrosis, and in animals exposed to cigarette smoke, SO_2 or H_2SO_4. Since changes in the pH of mucus are associated with alterations in its rheology, it is tempting to speculate that increased mucus acidity is behind the increase in mucus viscosity and reduced mucociliary clearance that are also associated with these diseases and exposures.

The finding of changes in mucus pH are also intriguing in light the fact that Holma, 1989, has suggested a link between inflammatory airway diseases such as asthma and alterations in the pH of mucus in the airway resulting from inhalation of

acid aerosols. Individuals producing mucus with low pH or low buffering capacity , e.g., some asthmatics and smokers, may have an elevated risk for developing untoward respiratory sequelae with exposure to acid aerosols. In addition to alterations in mucus rheology, acidification of mucus may lead to increased epithelial permeability, and could therefore be a mechanism leading to the airway edema seen with asthma (Holma, 1989).

Increased epithelial and endothelial permeability to serum constituents is a hallmark of inflammation in any tissue and a pathologic feature of cystic fibrosis and asthma (Robbins, 1981). Leakage of serum proteins and fluid is also thought to produce changes in mucus rheology and affect the periciliary fluid layer. One possible effect of these alterations is impaired mucociliary transport (Schlesinger, 1990). Another consequence of the presence of serum components in the airway is the propagation of inflammatory reactions that could potentially involve dozens of known inflammatory mediators and cytokines.

The eicosanoids are one class of inflammatory mediators strongly suspected of being involved in the pulmonary response to toxic insult, possibly including those affecting mucus physiology. These oxidized derivatives of arachidonic acid are produced in response to a wide variety of cellular perturbations by a myriad of cells types, apparently each able to produce a characteristic spectrum of these compounds. Eicosanoids such as leukotrienes C4 and D4 and hydroxyeicosatetraenoic acids, as well as the related lipid inflammatory mediator platelet activating factor, are known stimuli of glycoprotein release (Marom et al, 1982, 1983, McManus and Deavers, 1989) whose levels have been shown to increase in cells exposed to O_3 (Madden et al., 1991, Samet et al., 1992). In addition bronchoalveolar lavage fluid from subjects exposed to O_3 contain elevated levels of various eicosanoids (Koren et al., 1989). Thus it is possible that these bioactive lipids act as mucus secretagogues in response to toxicant inhalation.

An equally important role of eicosanoids may be to signal the recruitment of immune cells into the airways during the early stages of inflammation. Inhalation of some pollutants e.g., O_3, results in the recruitment of neutrophils into the airways. Similarly, increased numbers of lymphocytes and eosinophils are found in airway secretions of asthmatics (Robbins, 1981). Several eicosanoids, most notably leukotriene B4, are potent chemotaxins for neutrophils (Lundgren et al., 1990), while platelet activating factor administration produces neutrophil and eosinophil infiltration into the airways (McManus and Deavers, 1989). These effects are relevant to the role of mucus in the toxicology of inhaled toxicants vis a vis reports demonstrating morphologic changes induced by mast cell and neutrophil proteases.

The development of hyperplasia and metaplasia of glycoprotein-secreting airway tissues is a phenomenon that certain respiratory diseases and inhaled toxicants have in common. The hypertrophy of submucosal glands and hyperplasia of goblet cells that is seen in bronchitis, cystic fibrosis and asthma is also induced by inhalation of O_3, SO_2 and tobacco smoke. Increased secretory capacity is the major mechanism responsible for mucus hypersecretion in the lung, and it is likely a cause of increased mucus volumes produced in response to chronic toxicant inhalation.

The mechanism via which hyperplastic and metaplastic changes are induced in the lung are unknown. However, there is growing evidence that implicates the cationic protease elastase in these responses, as reviewed by Lungren and associates, 1989, 1990. Direct instillation of elastase is known to produce goblet cell hyperplasia in the lungs of rodents. In addition, airway secretions from cystic fibrosis patients and subjects exposed to O_3 contain elevated levels of elastase which, if active, could participate in the generation of morphologic changes leading to hyperplasia and mucus hypersecretion in the airway (Nadel, 1991, Koren et al., 1989). The fact that hyperplasia induced by neutrophil-derived products in rats can be inhibited by steroidal

antiinflammatory agents suggests that, here too, lipid inflammatory compounds such as eicosanoids could act as mediators of pathologic changes induced by inhaled pollutants.

Recently, much attention has been placed on the role of metaplasia in morphologic changes leading to hypersecretion in disease states and as a consequence of toxic insults (Jany and Basbaum, 1991, Hotchkiss et al., 1991). The hypothesis is that the increase in numbers of glycoprotein-secreting cells in disease or following toxicant inhalation is an adaptive response to airway injury that largely involves differentiation of non-secreting cells. Evidence supporting this notion is based on histochemical findings of increased goblet cell populations in areas of the lung where they are normally rare and in the absence of changes in the mitotic rate of cells in the airway. Preliminary work also indicates that exposure of rats to SO_2 can result in mucin gene expression changes that presumably would be required in order for non-secreting cells to transform into mucin-secreting cells (Jany and Basbaum, 1991).

As reviewed in this report, the targets of toxicants on mucus biochemistry and physiology are numerous, and it is certain that new ones will be identified as a result of ongoing research. The role of mucus as a mediator of lung injury is less clear, as the induction of acute and chronic mucus hypersecretion by environmentally relevant concentrations of pollutants needs to be established. One exception is cigarette smoke, for which clear evidence demonstrating the relationship between tobacco smoke-induced alterations in airway morphology, mucus hypersecretion and bronchitis already exists. It seems particularly important to determine whether pollutant-induced mucus hypersecretion contributes to the alarming rise in the number of asthma deaths that has been reported in recent years.

Many other important issues surrounding the role of mucus in the pulmonary toxicology of inhaled pollutants remain unresolved. A few are listed below.

- One basic question involves whether mucin gene expression can truly be induced by toxicant exposure. If so, is this induction part of a metaplastic response or independent from it?

- Our understanding of mucus biophysics also needs enhancing. Is mucus a cross-linked mucin polymer, an entangled mucin network or both? What is the true composition and function of periciliary fluid?

- The role of inflammatory mediators in the generation and progression of inflammatory processes in the lung is one of the most exciting and promising areas of investigation in lung biology and pulmonary medicine. What mediators are involved in mucus hypersecretion induced by toxicant inhalation?

- For practical reasons, in most studies with inhaled pollutants the exposures are acute and at high dose. What are the effects of the more relevant chronic exposures to low dose? Similarly, What are the effects of exposure to low levels of multiple pollutants?

In summary, essentially all aspects of lung pathophysiology involving toxicant-induced alterations in mucus structure and function can be viewed in the context of the role of mucus in a stereotypical, generalized response of the lung to cellular injury. Based on our current understanding of lung physiology, it is clear that the lung can secrete mucus in response to perceived environmental challenges such as irritation of the airway mucosa. This response can be described as graded in that it seems to be proportional to the magnitude of the stimulus and its duration. Thus a minor or brief irritant exposure may produce a localized and transient discharge of mucus from existing stores in a section of the lung, while a stronger or chronic irritation may result in a full blown response involving a long-lasting or permanent increase in the secretory capacity of the lung as a whole.

One teliologic rationale of this response is that it is an effort by the lung to neutralize and remove the offending stimulus in order to minimize tissue injury. It is somewhat ironic then that this protective mechanism of the lung can itself be both a

target of inhaled pollutants and a potential source of injury to the lung. From the point of view of the organism, the distinction between injury resulting from effects of the toxicant on mucus biochemistry and physiology and that resulting from the adaptive secretory response to the toxicant may well be academic.

SECTION IX

REFERENCES

Abraham, W.M. (1984). Effects of Inhaled Materials on Airway Secretions. Atmospheric Pollutants and Cigarette Smoke. *Semin. Respir. Med.* 5:324-328.

Abraham, W.M., Sielczak, M.W., Delehunt, J.C., Marchette, B. and Wanner, A. (1986). Impairment of Tracheal Mucociliary Clearance But Not Ciliary Beat Frequency by a Combination of Low Level Ozone and Sulfur Dioxide in Sheep. *Eur. J. Respir. Dis.* 68:114-120.

Adalis, D., Gardner,D.E., Miller, F.J., and Coffin, D.L. (1977). Toxic Effects of Cadmium on Ciliary Activity Using a Tracheal Ring Model System. *Environ. Res.* 13:111-117.

Anderson, W.M. (1990). Mucoid Impaction of Upper Lobe Bronchi in the Absence of Proximal Bronchiectasis. *Chest.* 98:1023-1027.

Aubert, J.P., Porchet, N., Crepin, M., Duterque-Coquillaud, M., Vergnes, G., Mazzuca, M., Debuire, B., Petitprez, D. and Degand, P. (1991). Evidence for Different Human Tracheobronchial Mucin Peptides Deduced from Nucleotide cDNA Sequences. *Am. J. Respir. Cell Mol. Biol.* 5:178-185.

Auerbach, C., Moutschen-Dahmen, M. and Moutschen, J. (1977). Genetic and Cytogenetical Effects of Formaldehyde and Related Compounds. *Mut. Res.* 39:317-362.

Baker, A.P. and Sawyer, J.L. (1975). Glycosyltransferases in Human Respiratory Tissue. *Bioch. Med.* 14:42-50.

Bateman, J.R.M., Pavia, D., Sheahan, N.F., Agnew, J.E., and Clarke, S.W. (1983). Impaired Tracheobronchial Clearance in Patients with Mild Stable Asthma. *Thorax.* 38:463-467.

Battista, S.P., Denine, E.P. and Kensler, C.J. (1972). Restoration of Tracheal Mucosa and Ciliary Particle Transport Activity After Mechanical Denudation in the Chicken. *Toxicol. Appl. Pharmacol.* 22:59-69.

Bergofsky, E.H. (1991). The Lung Mucosa: A Critical Environmental Battleground. *Am. J. Med.* 91:4S-10S.

Boucher, R.C. (1981). Mechanisms of Pollutant-Induced Airways Toxicity. *Clin. Chest Med.* 2:377-392.

Boucher, R.C., Stutts, M.J., Knowles, M.R., Cantley, L. and Gatzy, J.T. (1986). Na+ Transport in Cystic Fibrosis Respiratory Epithelia. Abnormal Rate and Response to Adenylate Cyclase Activation. *J. Clin. Invest.* 78:1245-1252.

Braga, P.C. and Allegra, L.. *Methods in Bronchial Mucology*. Raven Press, New York, 1988.

Braga, P.C. Sinusoidal Oscillation Methods. In: *Methods in Bronchial Mucology*. P.C. Braga and L. Allegra, Eds. Raven Press, New York, 1988. Pp. 63-72.

Braga, P.C., Allegra, L. and King, M. Mathematical Analysis of Dynamic Measures. In: *Methods in Bronchial Mucology*. P.C. Braga and L. Allegra, Eds. Raven Press, New York, 1988. Pp. 85-90.

Breg, J., Van Halbeek, H., Vliegenthart, J.F.G., Klein, A., Lamblin, G. and Roussel, P. (1988). Primary Structure of Neutral Oligosaccharides Derived from Respiratory-Mucus Glycoproteins of a Patient Suffering from Bronchiectasis, Determined by Combination of 500 MHz 1HNMR Spectroscopy and Quantitative Sugar Analysis. *Eur. J. Biochem.* 171:643-654.

Brockhausen, I., Moller, G., Merz, G., Aderman, K. and Paulsen, H. (1990). Control of Mucin Synthesis: The Peptide Portion of Synthetic O-Glycopeptide Substrates Influences the activity of O-glycan core 1 UDPgalactose: N-Acetyl-alpha-galactosaminyl-R beta3-Galactosyltransferase. *Biochemistry*. 29:10206-10212.

Carlstedt, I., Lindgren, H., Sheehan, J.K., Ulmsten, U. and Wingerup, L. (1983). Isolation and Characterization of Human Cervical-Mucus Glycoproteins. *Biochem. J.* 211:13-22.

Casarett, L.J. (1960). Physical and Physiological Factors Controlling the Fate of Inhaled Substances. II. Retention. *Health Phys.* 2:379.

Christensen, T.G., Korthy, A.L., Snider, G.L. and Hayes, J.A. (1977) Irreversible Bronchial Goblet Cell Metaplasia in Hamsters with Elastase-Induced Panacinar Emphysema. *J. CLin. Invest.* 59:397-404.

Clarke, S.W. Two-Phase Gas-Liquid Flow. In: *Methods in Bronchial Mucology*. P.C. Braga and L. Allegra, Eds. Raven Press, New York, 1988. Pp. 125-134.

Coles, S.J., Levine, L.R. and Reid, L. (1979). Hypersecretion of mucus Glycoproteins in Rat Airways Induced by Tobacco Smoke. *Am. J. Pathol.* 94:459-472.

Creeth, J.M. (1978). Constituents of Mucus and their Separation. *Brit. Med. Bull.* 34:17-24.

Crepin, M., Porchet, N. and Aubert, J.P. (1990). Diversity of the Peptide Moiety of Human Airway Mucins. *Biorheology*. 27:471-484.

Cross, C. and Halliwell, B. Biological Consequences of General Environmental Contaminants. *The Lung: Scientific Foundations*. R.G. Crystal and J.B. West, Eds. Raven Press, New York, 1991. Pp. 1961-1974.

Dalhamn, T. and Sjoholm, J. (1963). Studies of SO2, NO2 and NH3: Effect on Ciliary Activity in Rabbit Trachea of Single in vitro Exposure and Resorption in Rabbit Nasal Cavity. *Acta Physiol. Scand.* 58:287-292.

Davis, P.B. (1991). Molecular and Cell Biology of Cystic Fibrosis. *J. Appl. Physiol.* 70:2331-2333.

Elmes, P.C. and Bell, D. (1963). Effects of Chlorine Gas on the Lungs of Rats with Spontaneous Pulmonary Disease. *J. Pathol. Bact.* 86:317-330.

Fawcett, D.W. *A Textbook of Histology*. Saunders, Philadelphia, 1986.

Fernandez, J.M., Villalon, M. and Verdugo, P. (1991). Reversible Condensation of Mast Cell Secretory Products in vitro. *Biophys. J.* 59:1022-1027.

Forrest, J. B. and Lee, R.M.K.W. The Bronchial Wall: Integrated Form and Function. In: *The Lung: Scientific Foundations*. Raven Press, New York, 1991. Pp. 729-740.

Gallagher, J.T., Kent, P.W., Passatore, M., Phipps, R.J. and Richardson, P.S. (1975). *Proc. R. Soc. London*, B, 192:49-76.

Gerard, C., Eddy, R.L. and Shaws, T.B. (1990). The Core Polypeptide of Cystic Fibrosis Tracheal Mucin Contains a Tandem Repeat Structure. *J. Clin. Invest.* 86:1921-1927.

Gilboa, A. and Silberberg, A. (1976). In Situ Rheological Characterization of Epithelial Mucus. *Biorheology*. 13:59-65.

Glynn, A. and Michaels, L. (1960). Bronchial Biopsy in Chronic Bronchitis and Asthma. *Thorax* 15:142-153.

Gordon, T. and Amdur, M.O. Responses of the Respiratory System to Toxic Agents. In: *Toxicology: The Basic Science of Poisons*. 4th edition. M.O. Amdur, J. Doull and C. D. Klaassen, Eds. Pergamon, New York, 1991. Pp. 383-406.

Graham, J.A. (1989). Review, Discussion, and Summary: Toxicology. *Env. Health Persp.* 79:191-194.

Hansson, G.C., Sheehan, J.K. and Carlstedt, I. (1988). Only Trace Amounts of Fatty Acids Are Found in Pure Mucus Glycoproteins. *Arch. Biochem. Biophys*. 266:197-200.

Harkema, J.R. and Hotchkiss, J.A. (1991). In Vivo Effects of Endotoxin on Nasal Epithelial Mucosubstances: Quantitative Histochemistry. *Exp. Lung Res.* 17:743-761.

Holma, B. (1989). Effects of Acid on Airway Mucus and Its Consequences for Health. *Env. Health Perspect.* 79:109-113.

Hotchkiss, J.A., Harkema, J.R. and Henderson, R.F. (1991). Effect of Cumulative Ozone Exposure on Ozone-Induced Nasal Epithelial Cells and Secretory Metaplasia in Rats. *Exp. Lung Res.* 15:589-600.

Hounzell, E.F. and Feizi, T. (1982). Gastrointestinal Mucins. Strucutres and Atigenicities of their Carbohydrate Chains in Health and Disease. *Med. Biol.* 60: 227-232.

Iravani, J. and van As, A. (1972). Mucus Transport in the Tracheobronchial of Normal and Bronchitic Rats. *Pathol.* 106:81-93.

Jany, B. and Basbaum, C.B. (1991). Modification of Mucin Gene Expression in Airway Disease. *Am. Rev. Respir. Dis.* 144:S38-S41.

Jeffery, P.K. (1991). Morphology of the Airway Wall in Asthma and in Chronic Obstructive Pulmonary Disease. *Am. Rev. Respir. Dis.* 143:1152-1158.

Jeffery, P.K. and Reid, L. Ultrastructure of the Airway Epithelium and Submucosal Gland During Development. In: *Lung Biology in Health and Disease.* Vol. 6: Development of the Lung. Hodson, W.A. Ed. Marcel Dekker, New York, 1977.

Jenssen, A.O., Harbitz, O. and Smidsrod, O. (1980). Electron Microscopy of Mucin from Sputum in Chronic Obstructive Bronchitis. *Eur. J. Respir. Dis.* 61:71-76.

Jones, R. and Reid, L. (1978). Secretory Cell Hyperplasia and Modification of Intracellular Glycoprotein in Rat Airways Induced by Short Periods of Exposure to Tobacco Smoke, and the Effect of the Antiinflammatory Agent Phenylmethyloxadiazole. *Lab. Invest.* 39:41-49.

Jones, R.. Modification of Mucus in Animal Models of Disease. *In: Proceedings of International Symposium on Mucus in Health and Disease.* M. Elsten and D.V. Parke, Eds. Plenum, New York, 1977. Pp. 397.

Kensler, G.J. and Battista, S.P. (1963). Components of Cigarrete Smoke with Ciliary Depressant Acitivity. *N. Engl. J. Med.* 269:1161-1168.

Kerem, B.S., Rommens, J.M., Buchanan, J.A., Markiewicz, D., Cox, T.K., Chakravarti, A., Muchwald, M. and Tsui, L.C. (1989). Identification of the Cystic Fibrosis Gene: Genetic Analysis. *Science.* 245: 1073-1080.

Kim, K.C. (1991). Biochemistry and Pharmacology of Mucin-like Glycoproteins Produced by Cultured Airway Epithelial Cells. *Exp. Lung Res.* 17:533-545.

Kim, K.C., Nassiri, J. and Brody, J. S. (1989). Mechanisms of Airway Goblet Cells Mucin Release: Studies with Cultured Tracheal Surface Epithelial Cells. *Am. J. Respir. Cell Mol. Biol.* 1:137-143.

King, M. Magnetic Microrheometer. In: *Methods in Bronchial Mucology.* P.C. Braga and L. Allegra, Eds. Raven Press, New York, 1988. Pp. 73-84.

Klein, A., Lamblin, G., Lhermitte, M., Roussel, P., Breg, J., Van Halbeek, H. and Vliegenthart, J.F. (1988). Primary Structure of Neutral Oligosaccharides Derived from Respiratory-Mucus Glycoproteins of a Patient Suffering from Bronchiectasis, Determined by Combination of 500 MHz 1HNMR Spectroscopy and Quantitative Sugar Analysis. *Eur. J. Biochem.* 171:631-642.

Koenig, J.Q., Covert, D.S. and Pierson, W.E. (1989). Effects of Inhalation of Acidic Compounds on Pulmonary Function in Allergic Adolescent Subjects. *Env. Health Persp.* 79:173-178.

Koren, H.S., Devlin, R.B., Graham, D.E., Mann, R., McGee, M.P., Hortsman, D.E., Kozumbo, W.J., Becker, S., House, D.E., McDonell, W.F. and Bromberg, P.A. (1989). Ozone-Induced Inflammation in the Lower Airways of Human Subjects. *Am. Rev. Respir. Dis.* 139:407-412.

Lamb, D. and Reid, L. (1968). Mitotic Rates, Goblet Cell Increase and Histochemical Changes in Mucus in Rat Bronchial Epithelium During Exposure to Sulphur Dioxide. *J. Pathol. Bact.* 96:97-111.

Lamblin, G., Lhermitte, M., Klein, A., Houdret, N., Scharfman, A., Ramphal, R. and Roussel, P. (1991). *Am. Rev. Respir. Dis.* 144:S19-S24.

Last, J.A. Mucus Production and the Ciliary Escalator. In: *Mechanisms in Respiratory Toxicology.* Vol. I. H. Witschi and P. Nettesheim, Eds. CRC Press, Boca Raton, FL, 1982. Pp. 247.

Last, J.A., Jennings, M.D., Schwartz, L.S. and Cross, C.E. (1977). Glycoprotein Secretion by Tracheal Explants Cultured from Rats Exposed to Ozone. *Am. Rev. Respir. Dis.* 116:695-703.

Lopez-Vidriero, M.T. and Reid, L. (1978). Bronchial Mucus in Health and Disease. *Brit. Med. Bull.* 34:63-74.

Lundgren, J.D., Kaliner, M., Logun, C. and Shelhamer, J. (1988). Dexamethasone Reduces Rat Tracheal Goblet Cell Hyperplasia Produced by Human Neutrophil Products. *Exp. Lung Res.* 14:853-863.

Lundgren, J.D., Kaliner, M.A. and Shelhamer, J.H. (1990). Mechanisms by which Glucocorticosteroids Inhibit Secretion of Mucus in Asthmatic Airways. *Am. Rev. Respir. Dis.* 141:S52-S58.

Madden, M.C., Eling, T.E., Dailey, L.A. and Friedman, M. (1991). The Effect of Ozone Exposure on Rat Alveolar Macrophage Arachidonic Acid Metabolism. *Exp. Lung Res.* 17:47-63.

Man, S.F.P., Adams, G.K., III, and Proctor, D.F. (1979). Effects of Temperature, Relative Humidity, and Mode of Breathing on Canine Airway Secretions. *J. Appl, Physiol.* 46:205-212.

Marom, Z., Shelhamer, J.H., Bach, M.K., Morton, D.R. and Kaliner, M. (1982). Slow Reacting Substances, Leukotrienes C4 and D4, Increase the Release of Mucus from Human Airways in vitro. *Am. Rev. Respir. Dis.* 126:449-451.

Marom, Z., Shelhamer, J.H., Sun, F. and Kaliner, M. (1983). Human Airway Monohydroxyeicosatetraenoic Acid Generation and Mucus Release. *J. Clin. Invest.* 72:122-127.

Mauderly, J.L. and Samet, J.M. General Environment. In: *The Lung: Scientific Foundations.* G. Crystal and J.B. West, Eds. Raven Press, New York, 1991. Pp.1947-1960.

Mautone, A.J. and Cataletto, M.B. Mechanical Defense, Mechanisms of the Lung. In: *Pulmonary Physiology.* E.M. Scarpelli, Ed. Lea and Febiger, Philadelphia, 1990. Pp. 192-214.

McManus, L.M. and Deavers, S.I. (1989). Platelet Activating Factor in Pulmonary Pathobiology. *Clin. Chest. Med.* 10:107-118.

Mikkelsen, A., Stokke, B.T., Christensen, B.E. and Elgasaeter, A. (1985). Flexibility and Length of Human Bronchial Mucin Studied Using Low-Shear Viscometry, Birefringence Relaxation Analysis and Electron Microscopy. *Biopolymers*. 24:1683-1704.

Morgan, K.T., Patterson, D.L. and Gross, E.A. (1984). Frog Palate Mucociliary Apparatus: Structure, Function, and Response to Formaldehyde Gas. *Fund. Appl. Toxicol.* 4:58-66.

Morgan, K.T., Patterson, D.L. and Gross, E.A. (1986). Response of the Mucociliary Apparatus of F-344 Rats to Formaldehyde Gas. *Toxicol. Appl. Pharmacol.* 82:1-9.

Nadel, J.A. (1991). Role of Mast Cell and Neutrophil Proteases in Airway Secretions. *Am. Rev. Respir. Dis.* 144:S48-S51.

Nadel, J.A., Davis, B. and Phipps, R.J. (1979). Control of Airway Secretion and Ion Transport in the Airways. *Annu. Rev. Physiol.* 41:369-381.

Nguyen, V.C., Aubert, J.P., Gross, M.S., Porchet, N., Degand, P. and Frezal, J. (1990). Assignement of Human Tracheobronchial Mucin Gene(s) to 11p15 and a Tracheobronchial Mucin-Realted Sequence to Chromosome 13. *Hum. Genet.* 86:167-172.

Nygren, H., Lange, S. and Lonnroth, I. (1984). Development of Mucous Cells in Mouse Intrapulmonary Airways Induced by Cholera Toxin, Dibutyryl Cyclic AMP and Prostaglandin E1. *J. Exp. Pathol.* 65:549-556.

Parke, D.V. (1978). Pharmacology of Mucus. Brit. Med. Bull. 34:89-94.

Pavia, D., Bateman, J.R.M., Sheahan, N.F., Agnew, J.E. and Clarke, S.W. (1985). Tracheobronchial Mucociliary Clearance in Asthma: Impariment During Remission. *Thorax.* 40:171-175.

Pedersen, M. (1990). Ciliary Activity and Pollution. *Lung.* Suppl:368-376.

Perini, J.M., Vandamme-Cubadda, N. and Aubert, J.P., Porchet, N., Mazzuca, M., Lamblin, G., Herscovics, A. and Roussel, P. (1991). Multiple Apomucin Translation Products from Human Respiratory Mucosa mRNA. *Eur. J. Biochem.* 196:321-328.

Phelps, C.F. (1978). Biosynthesis of Mucus Glycoprotein. *Brit. Med. Bull.* 34:43-48.

Phipps, R.J. (1984). Production of Airway Secretions. *Semin. Respir. Med.* 5:314-318.

Phipps, R.J., Denas, S.M., Sielczak, M.W. and Wanner, A. (1986). Effects of 0.5 ppm Ozone on Glycoprotein Secretion, Ion and Water Fluxes in Sheep Trachea. *J. Appl. Physiol.* 60:918-927.

Porchet, N., Dufosse, J., Audie, J.P., Guyonnet Duperat, V., Perini, J.M., Nguyen, V. C., Degand, P. and Aubert, J.P. (1991). Strucural Features of the Core Proteins of Human Airway Mucins Ascertained by cDNA Cloning. *Am. Rev. Respir. Dis.* 144:S15-S18.

Proctor, D.F. Form and Function of the Upper Airways and Larynx. In: Handbook of Physiology. *The Respiratory System,* Vol. 3. Mechanics of Breathing. P.T. Macklem and J. Mead. American Physiological Society, Bethesda, 1986. P. 63.

Ranga, V. and Kleinerman, J. (1981). A Quantitative Study of Ciliary Injury in the Small Airways of Mice: the Effects of Nitrogen Dioxide. *Exp. Lung Res.* 2:49-56.

Rhodin, J.A.G. (1966). Ultrastructure and Function of the Human Tracheal Mucosa. *Am. Rev. Respir. Dis.* 93:1-15.

Riordan, J., Rommens, J.M., Kerem, B.S., Alon, N., Rozmachel, R., Grzetczack, Z., Zielenski, J., LAk, S., Plavsik, N., Chou, J.L., Drumm, M.L., Ianuzzi, M.C., Collins, F.S. and Tsui, L.C. (1989). Identification of the Cystic Fibrosis Gene: Cloning and Characterization of the Complementary DNA. *Science* 245:1066-1073.

Robbins, S.L., Angell, M. and Kumar, V. *Basic Pathology.* Saunders, Philadelphia, 1981.

Rogers, D.F. and Jeffery, P.K. (1986a). Inhibition of Cigarette Smoke-Induced Airway Secretory Cell Hyperplasia by Indomethacin, Dexamethasone, Prednisilone, or Hydrocortisone. *Exp. Lung Res.* 10:285-298.

Rogers, D.F. and Jeffery, P.K. (1986b). Inhibition by Oral N-Acetylcysteine of Cigarette Smoke-Induced "Bronchitis" in the Rat. *Exp. Lung Res.* 10:267-283.

Rommens, J.H., Ianuzzi, M.C., Kerem, B.S., Drumm, M.L., Meimer, G., Dean, M., Rozmahel, R., Cole, J.L., Kennedy, D., Hidaka, N., Zsiga, M., Buchwald, M., Riordan, J.R., Tsui, L.C. and Collins, F.S. (1989). Identification of the Cystic Fibrosis Gene: Chromosome Walking and Jumpimg. *Science Wash. DC.* 245:1059-1065.

Ropp, P.A., Little, M.R. Cheng, P.W. (1991). Mucin Biosynthesis: Purification and Characterization of a Mucin Beta6N-Acetylglucosaminyltransferase. *J. Biol. Chem.* 266:23863-23871.

Rose, M.C., Kaufman, B. and Martin, B.M. (1989). Proteolytic Fragmentation and Peptide Mapping of Human Carboxyamidomethylated Tracheo-Bronchial Mucin. *J. Biol. Chem.* 264:8193-8199.

Rose, M.C., Voter, W.A., Brown, C.F. and Kaufman, B. (1984). Structural Features of Human Tracheobronchial Mucus Glycoprotein. *Biochem. J.* 222:371-377.

Roussel, P. Lamblin, G., Degand, P., Walker-Nasir, E. and Jeanloz, R.W. (1975). Heterogeneity of the Carbohydrate Chains of Sulfated Bronchial Glycoproteins Isolated from aPatient Suffering from Cystic Fibrosis. *J. Biol. Chem.* 250:2214-2222.

Roussel, P. Lamblin, G., Lhermitte, M., Houdret, N., Lafitte, J.J., Perini, J.M., Klein, A. and Scharfman, A. (1988). The Complexity of Mucins. *Biochimie.* 70:1471-1478.

Samet, J.M., Noah, T.L., Devlin, R.B., Yankaskas, J.R., McKinnon, K., Dailey, L.A. and Friedman, M. (1992). Effect of Ozone on Platelet Activating Factor Production in Phorbol-Differentiated HL60 Cells, A Human Bronchial epithelial Cell Line (BEAS S6) and Primary Human Bronchial Epithelial Cells. Submitted.

Sanderson, M.J. and Dirksen, E.R. (1989). Mechanosensitivity and Beta-adrenergic Control fo the Ciliary Beat Frequency of Mammalian Respiratory Tract Cells in Culture. *Am. Rev. Respir. Dis.* 139:432-440.

Schachter, H. (1977). Control of Biochemical Parameters in Glycoprotein Production. *Adv. Exp. Med.* 89:103-129.

Schlesinger, R.B. (1990). The Interaction of Inahled Toxicants with Respiratory Tract Clearance Mechanisms. *Crit. Rev. Toxicol.* 20: 257-286.

Sheehan, J.K., Oates, K. and Carlstedt, I. (1986). Electron Microscopy of Cervical, Gastric and Bronchial Mucus Glycoproteins. *Biochem. J.* 239:147-153.

Sheehan, J.K., Thornton, D.J., Somerville, M. and Carlstedt, I. (1991). Mucin Structure. The Structure and Heterogeneity of Mucin Glycoproteins. *Am. Rev. Respir. Dis.* 144:S4-S9.

Silberberg, A. Models of Mucus Structure. In: *Methods in Bronchial Mucology.* P. C. Braga and L. Allegra, Eds. Raven Press, Ltd, New York, 1988. Pp. 51-61.

Sleigh, M.A. Mucus Propulsion. In: *The Lung: Scientific Foundations.* Crystal, R.G. and West, J.B.; Eds. Raven Press, New York, 1991. Pp.189-196.

Sleigh, M.A. The Nature and Function of Respiratory Tract Cilia. In: *Lung Biology in Health and Disease. Vol. 5. Respiratory Defense Mechanisms.* J.D. Brain, D.F. Proctor and L.M. Reid. Marcel Dekker, New York, 1977. P.277.

Sleigh, M.A., Blake, J.R. and Liron, N. (1988). The Propulsion of Mucus by Cilia. *Am. Rev. Respir. Dis.* 137:726-741.

Slomiany, A., Liau, Y.H., Takagi, A., Laszewics, W., and Slomiany, B.L. (1984). Characterization of Mucus Glycoprotein Fatty Acyltransferase from Gastric Mucosa. *J. Biol. Chem.* 259:13304-13308.

Smith, J. and Johnson, D.A. (1985). Human Bronchial Leukocyte Proteinase Inhibitor. *Biochem. J.* 225:463-472.

Spicer, S.S. and Martinez, J.R. (1984). Mucin Biosynthesis and Secretion in the Respiratory Tract. *Env. Health. Persp.* 55:193-204.

Spicer, S.S., Chakrin, L.W. and Wardell, J.R. (1974). Effect of Chronic Sulfur Dioxide Inhalation on the Carbohydrate Histochemistry and Histology of the Canine Respiratory Tract. *Am. Rev. Respir. Dis.* 110:13-24.

Stutts, M.J. and Bromberg, P.A. (1987). Effects of Ozone on Airway Epithelial Permeability and Ion Transport. Toxicol. *Letters.* 35:315-319.

Thornton, D.J., Davies, J.R., Kraayenbrink, M., Richardson, P.S., Sheehan, J.K. and Carlstedt, I. (1990). Mucus Glycoproteins from "Normal" Human Tracheobronchial Secretions. *Biochem. J.* 265:179-186.

Toremalm, N.G. (1960). The Daily Amount of Tracheobronchial Secretions in a Man. A method for Continous Tracheal Aspiration in Laryngectomized and Tracehotomized Patients. *Acta Otolaryngol.* 158:43-53.

Van Halbeek, H., Breg, J., Vlieghenthart, J.F.G., Klein, A., Lamblin, G. and Roussel, P. (1988). Isolation and Strucutral Characterization of Low-Molecular-Mass Monosialyl Oligosaccharides Derived from Respiratory Mucus Glycoproteins of a Patient Suffering from Bronciectasis. *Eur. J. Biochem* 177:443-460.

Verdugo, P. (1984).Hydration Kinetics of Exocytosed Mucins in Cultured Secretory Cells of the Rabbit Trachea: A New Model. *Ciba Found. Symp.* 109:212-225.

Verdugo, P. (1991). Mucin Exocytosis. *Am. Rev. Respir. Dis.* 144:S33-S37.

Wanner, A. (1984a). Bronchial Asthma. *Sem. Respir. Med.* 5:332-335.

Wanner, A. (1984b). Chronic Bronchitis. *Sem. Respir. Med.* 5:329-331.

Watanabe, S., Frank, R. and Yokoyama, E. (1973). Acute Effects of Ozone on Lungs of Cats. *Am. Rev. Respir. Dis.* 108:1141-1151.

Weitz, J.I., Huang, A.J., Landman, S.L., Nicholson, S.C., Silverstein, S.C. (1987). Elastase-Mediated Fibrinogenolysis by Chemoattractant-Stimulated Neutrophils Occurs in the Presence of Physiologic Concentrations of Antiproteases. *J. Exp. Med.* 166:1836-1850.

Welsh, M.J. Abnormal Chloride and Sodium Channel Function in Cystic Fibrosis Airway Epithelia. In: *The Lung: Scientific Foundations.* R.G. Crystal and J.B. West, Eds. Raven Press, Ltd., New York, 1991. Pp. 2073-2081.

Wheater, P.R., Burkitt, H.G. and Daniels, V.G. *Functional Histology*. Churchill Livingston, New York, 1979.

Whitelaw. W.A. (1991). Asthma Deaths. *Chest.* 99:1507-1509.

Widdicombe, J.G. (1978). Control of Secretion of Tracheobronchial Mucus. *Med. Bull.* 34:57-61.

Widdicombe, J.H. (1984). Fluid Transport Across Airway Epithelia. *Ciba Found. Symp.* 109:109-120.

Witas, H., Sarosiek, J., Aonu, M., Murty, V.L.N., Slomiany, A. and Slomiany, B.L. (1983). Lipids Associated with Rat Small-Intestinal Mucus Glycoprotein. *Carbohydr. Res.* 120:67-76.

Wu, R. and Carlson, D.M. Structure and Synthesis of Mucin. In: *The Lung: Scientific Foundations*. Raven Press, New York, 1991. Pp. 183.

Yoneda, K. (1976). Mucus Blanket of Rat Bronchus: An Ultrastuctural Study. *Am. Rev. Respir. Dis.* 114:837-842.