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Pharmacology of Airway Secretion*

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I. Introduction

THE INTERFACE presented by the lungs to the external environment is large. Multiple body defenses protect the lungs against inhalation of noxious materials (122). One group of these defenses is predicated upon the secretion of protective substances by airway cells. In addition to mucous glycoproteins, water, and electrolytes, these substances also include immunoglobulins, proteinases, antiproteinases, and bactericidal and bacteriostatic agents (22). Additionally, the tracheobronchial cells elaborate a number of neurohumoral agents which may serve to modulate airway secretion. During the past decade, knowledge concerning the cellular mechanisms of normal and pathological airway secretion and the effects of pharmacological agents on this secretion has grown at a rapid rate. A variety of experimental model systems have been utilized to understand airway secretory mechanisms. However, often these systems have yielded divergent results. The models have employed different animal species and various portions of the airways. At present, only an incomplete picture of airway secretory function has emerged. The purpose of this review is to describe what is known concerning this secretory function and to delineate the effects of various pharmacological agents on known mechanisms of secretion. Initially, I will describe the cellular structure of airway epithelium which may be of pertinence to understanding airway secretion. The complexity and variability of this structure are most likely responsible for the divergent information which has been derived from these differing experimental models used to study secretion. I will also delineate the physiological and pathophysiological mechanisms which

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may regulate airway secretion prior to discussing the effects of pharmacological agents.

II. Cellular Structure of Airway Epithelium

This manuscript will briefly outline cellular structure with an emphasis on those aspects of importance to an understanding of airway secretory mechanisms.

In mammals, 13 cell types occur in the surface epithelium, nine cell types occur in submucosal glands which open into the airways, and several other cell types are found in the submucosal connective tissue of airways (29). Not all cell types are present in each species, and the distribution of cell types varies depending upon the generation and portion of the airway involved (29). Furthermore, aging and both pharmacological and pathological agents can alter the type and distribution pattern of airway cells (29). Because of the complexity of airway tissue, knowledge concerning the secretory properties of many of the individual cell types is indirect or postulated based upon cellular appearance.

A. Surface Epithelial Structure

In general, the surface structure of trachea and bronchi of mammals consists of a pseudostratified columnar epithelium which is composed of ciliated, globlet, intermediate, basal, as well as a variety of less common cell types (29).

1. Ciliated cells. The predominant cell type which lines the airway lumen is the ciliated cell (29). In addition to approximately 250 cilia, the apical membranes of these cells contain microvilli and fine cytoplasmic processes (29, 60, 138). The main function of these cells is to propel airway secretions toward the mouth in the defense mechanism of mucociliary clearance (29). By virtue of the number of ciliated cells and their apical membrane structure, these cells may also be important either in the secretion or absorption of water by airways (84, 150). Water movement is mediated by active ion transport and probably plays a key part in regulating the depth of periciliary fluid in which the cilia beat (124).

2. Goblet cells. Goblet cells, present in the surface epithelium, are 5-fold less numerous than ciliated cells (60, 138). In normal human mucosa, there are approximately 6800 goblet cells per mm^2 (54). These cells contain secretory granules which stain positively with both alcian blue and periodic acid-Schiff stain (PAS), indicating the presence of acidic mucous glycoproteins (111). Normally, there are more goblet cells overlying cartilage than the membranous portion of the airways (29, 157), and their number increases in a craniocaudal direction in both portions of the airway (157). Chronic airway infection is associated with a generalized increase in goblet cell number (83, 155). In mammals, these cells are not under nervous control (29); they have a continuous base-line secretion of the apocrine type of acidic mucins (29). With certain nonspecific stimuli, e.g., chemical irritants, they increase their secretory activity (see below).

3. Other surface cells. Other cell types in the surface layer include basal and intermediate cells. These cells, which are thought to be differentiating into ciliated and goblet cells (29), have no known secretory activity (29). However, intermediate cells may function in protein transport (29). Less frequently occurring cells in this layer include: epithelial serous cell; brush cell; K cell; oncocyte; special type cell; nonciliated bronchiolar secretory (Clara) cell; lymphocyte; globule leukocyte; and neuroepithelial bodies. The oncocyte and special type cell have no known secretory function and will not be considered further (29).

The epithelial serous cell has been identified in rat trachea and bronchi (80). It has similar appearance and staining characteristics to serous cells found in submucosal glands (80) and may contribute to the production of the periciliary fluid layer (80). As in glandular serous cells, this cell appears to contain lysozyme and to produce the secretory component of IgA (29).

Brush cells are present in the tracheobronchial surface epithelium of a number of species, including rat (137), mouse (71), guinea pig (78), pig (16), and possibly human (163). These infrequently found cells contain filament bundles which occur throughout the cytoplasm and a dense population of apical microvilli (29). Although not definitely known, based upon the presence of numerous microvilli, an absorptive function has been postulated for this cell type (29).

K cells have been identified in the tracheobronchial tree of humans (64), mouse (56), and rat (45, 115). The cells resemble the Kultschitzky cell found in the gastrointestinal tract (29). The cell has the appearance of a neurosecretory cell and contains characteristic secetory granules of several different types (70). Hage (70) described three subclasses of K cells based on different morphological features of the secretory granules. Intraepithelial nerve axons have been identified in the close proximity of K cells and have been described as sensory (85), cholinergic (93), or adrenergic (56). The secretory function of K cells remains unknown and may differ depending upon the generation of airway (29). These cells may produce amines or kinins (29, 96), which possibly have an effect on airway smooth muscle tone (29) or upon the secretory responses of other airway cells.

Nonciliated bronchiolar secretory cells or Clara cells are usually found in abundance in the terminal bronchiolar epithelium (29); however, in certain smaller rodents, e.g., mouse (72) and rat (80), they are more numerous in proximal airways and even the trachea. These cells have been identified in numerous species including humans (18), mouse (72), pig (16), rat (80), dog (62), and llama (29). The cells are characterized by a lack of cilia, a columnar shape, a round or finger like apical process, and a large vacuolated droplet (29). Although there is a general consensus that they are secretory, the product of the nonciliated bronchiolar cell is not definitely known

and has been the source of considerable controversy (29). Most authors believe that the cell is involved in some aspect of the secretion of the surface active layer which is present in bronchioles and alveoli and perhaps the periciliary fluid in larger airways (29).

Lymphocytes are present in the surface epithelium and presumably function as they do in the rest of the body (29). Globule leukocytes are similar in appearance to mast cells (29). They are occasionally identified in the surface epithelium, as well. The function of these cells is presumed to be similar to subepithelial mast cells (see below).

Neuroepithelial bodies are intraepithelial corpuscles composed of several tall, nonciliated cells arranged in parallel, forming a corpuscle within the epithelium (97). They occur throughout the entire airway (97) and have been identified in the cat (77), mouse (77), rabbit (94), rat (147), and human (20). Their role is not known, but based upon structure, they are presumed to have neuroreceptor function (95). They are well innervated with both afferent and efferent fibers and contain intracytoplasmic dense-cored granules which contain serotonin (93). These cells may be sensitive to inhaled particles or to oxygen or carbon dioxide tension of the inhaled gas (29). It has been postulated that they may mediate a number of possible functions including hypoxia-induced pulmonary vasoconstriction, regulation of smooth muscle tone, regulation of airway secretion, and control of pulmonary hemodynamics.

B. Submucosal Gland Structure

The complexity of structure of the submucosal glands varies with species (29). In certain rodents, e.g., the rat and mouse, glands are rudimentary or even nonexistent (29, 66, 68). In humans and other mammals such as the cat, dog, and pig, the glands are complex structures (29). Three regions of the human submucosal gland can be identified: ciliated duct; collecting duct; and secretory tubules (113).

1. Ciliated duct. The ciliated duct contains cells similar to surface epithelium. In humans, the duct extends into the gland approximately 350 μm from the surface of the lumen. Presuambly these cells have similar secetory function to those cells found in the surface epithelium. The probable primary function of these cells is to move the glandular secretory product onto the airway surface via ciliary beating (113).

2. Collecting duct. The collecting duct cells are tall and columnar in shape (29). Based upon their large numbers of mitochondria and sparse PAS-positive secretory granules, these cells may be important in adjusting water and ion content of the glandular secretory product (113). The cells probably do not secrete mucins (29).

3. Secretory tubules. The secretory tubules branch off the collecting ducts. These tubules are lined by mucous and serous cells. The mucous cell is columnar and is filled with secretory granules which stain positively with both alcian blue and PAS (29). The granules flatten the nucleus against the base of the cell (29). Mucous cells secrete acidic mucous glycoproteins (136). Serous cells have densely packed spherical membrane-bound secretory granules which stain with PAS. These cells are presumed to secrete neutral mucins (29). Like the surface serous cells, these cells may also be involved with both IgA and lysozyme secretion (29). There is both morphological and physiological evidence that the mucous and serous cells are under cholinergic, adrenergic, and noncholinergic nonadrenergic nervous control (see below).

4. Other glandular cells. Lymphocytes, mast cells, globule leukocytes, and K cells are occasionally found in the glandular acini (29). Also, myoepithelial cells are found between the basement membrane and the mucous, serous, and collecting duct cells (29). These cells probably serve to squeeze secretions out of the glands (29).

C. Subepithelial cells

Several cell types that are present below the airway epithelium are of potential importance to airway secretion. These include subepithelial mast cells and bronchus-associated lymphoid tissue cells. Mast cells appear to contain intracellular IgE within granules (29) and may function to transfer this IgE into the secretions which line the airways. These cells respond to specific antigens by releasing biogenic amines (29). Nodules of lymphoid tissue are present within the bronchial mucosa down to the level of the small bronchioles (29). The nodules are analogous to such gut-associated lymphoid tissue as the intestinal Peyer's patches (29). It appears that these cells are involved in immune processes that protect the organism from inhaled antigens. These nodules may provide B cells which produce IgA and T cells for cell-mediated reactions in the airways. The role of this tissue has been reviewed recently (19).

D. Variability of Airway Structure

The structure of airways is variable. Differences exist between species; there are also structure changes in response to pharmacological and pathological influences. The full spectrum and implications of these differences and changes in terms of secretory responses are not entirely known. However, it seems that certain of this variability may be of relevance and is described below.

1. Species differences in airway structure. Although most mammalian species have the type of cells outlined above, variability does exist in the relative numbers and distribution of airway cells. Several review articles delineate some of these differences (29, 68), the complete details of which are beyond the scope of this review. The difference in airway structure most important to secretory processes may be the relative complexity and distribution of submucosal glands and the presence or absence of Clara-like cells in larger airways. In several rodent species, the submucosal glandular structure is rudimentary (66, 68, 125, 126). In mouse, submucosal glands are

either rudimentary or absent (125, 126). Additionally, large airways differ from those of other mammalian species, in that goblet cells are nearly absent, and large numbers of Clara-like cells are present (125, 126). The larger airways of many rodents resemble more distal airways of other mammalian species (125, 126). This difference may serve to keep the relatively narrow airways of rodents free of obstructing mucin secretions (126).

2. Effects of pharmacological and pathological agents on airway structure. The relative proportion of airway cell types is not fixed. Rather, there is evidence that cell number and characteristics can change. For instance, Jones and Reid (82) showed in rat airways that both isoproterenol (injected for 6 days, 10 mg/100 g of body weight) and salbutamol (injected for 6 or 12 days, 10 mg/100 g of body weight) caused a change in both the number and staining characteristics of secretory cells located in the surface epithelium. In all instances, the number of cells staining positively for secretory granules (containing either acidic or neutral glycoproteins) increased (82). The increase was not uniform throughout the airways, and there was an alteration in the composition of secretory granules in the different secetory cells in response to the various agents (82). In this study the change in cell number and secretory type was in response to a pharmacological stimulus. Based upon this responsiveness, the authors speculated that, even in normal airways, there could be changes which reflect alterations due to normal physiological stimuli. The details of these putative normal alterations were not delineated. Vidic et al. (161) have shown that estrogen alters the epithelial secretory cells in rat trachea. They surgically removed the ovaries of female rats. For a period of 8 days, they injected an experimental group with 17β-estradiol (25 μg/day). A control ovariectomized group received no estrogen. On the ninth day, both groups were killed, and the tracheas were studied both histochemically and morphologically. Estrogen treatment caused a change in the secretory cell population. A significant decrease occurred in estrogen-treated rats in the number of large PAS-reactive secretory cells, while cells staining for both weakly acidic and neutral glycoproteins increased significantly. Morphological changes were present in the secretory cells consistent with an increased rate of synthesis and secretion by cells containing weakly acidic mucous glycoproteins (161). These alterations suggested that hormonal effects may play a role in altering the quantity and character of mucus glycoprotein secretion by airways. Nygren et al. (123) studied the effects of cholera toxin, dibutyryl cyclic adenosine monophosphate (cAMP), and prostaglandin E_1 on the morphological appearance of mouse intrapulmonary airway epithelium. All of these agents are known to increase intracellular cAMP. These materials were instilled intranasally into mice, in doses which were known to cause physiological responses. The airways were examined for morphological changes. The instillations resulted in an increased number of mucous cells in the airways relative to control. Breuer et al. (30) showed that human neutrophil elastase, a proteinase released into mucous secretions during the inflammatory response, caused a metaplasia of secretory cells in the airways of hamsters. In an earlier similar study in hamster employing instillation of porcine type III elastase, McDowell et al. (112) suggested that the apparent increase in secretory cells was due to an accumulation of secretion granules within the cells rather than due to conversion of undifferentiated cells to secretory cells or the production of new cells by mitosis. Cholera toxin is a bacterial product, and prostaglandins and elastase are released during the inflammatory response. This morphological responsiveness to these agents raised the possibility that pathological agents and diseases might result in cellular alterations which are of significance to airway secretion.

A number of studies are compatible with this hypothesis. Pathological agents, such as viruses (36, 42, 154), bacteria (42, 75, 118), and cigarette smoke and other irritants and pollutants (17, 34, 50, 55, 61, 69, 79, 81, 90, 102, 112, 134, 153), also caused an increase in the secretory cells of the airways, morphological changes which may alter the secretory responses. Further, a number of disease states, including chronic bronchitis (34, 52, 155, 158), cystic fibrosis in its later stages (35, 98, 151), and asthma (149), are characterized by hyperplasia of both submucosal gland and goblet cells in the airways.

E. Conclusions from Morphological Studies

It is important to consider a number of features of airway morphology when describing the pharmacology of airway secretion. (*a*) Multiple cells may be responsible for the airway secretory product. (*b*) There are species and regional differences in the distribution of airway cells. This variability is increased further by the apparent morphological alterations which occur in response to pharmacological and pathological agents and perhaps in response to normal physiological stimuli. Such a change may be reflected in alterations in airway secretion. In considering the pharmacology of airway secretion, it is necessary to be aware of these confounding factors.

III. Physiological Regulation of Airway Secretion

A knowledge of normal mechanisms which regulate airway secretion is prerequisite to understanding the pharmacology of this secretion. In this portion of the review, I will provide an overview of normal mechanisms of airway secretion. Available information largely relates to the function of submucosal glands and surface epithelial cells of the airways and their secretion of mucin and electrolytes. The mechanisms which control the secretion of submucosal glands are more fully characterized than either the secretory function of surface epithelial cells or other subepithelial cells.

A. Physiology of Submucosal Glandular Secretion

1. Nervous control of submucosal glandular secretion. There are both anatomical (12, 51, 53, 91, 103, 117, 120, 159) and physiological (26, 48, 49, 67, 89, 119, 135, 160) data to indicate that glandular secretion is under nervous control. The known secretory products of submucosal glands include acidic and neutral mucous glycoproteins, IgA, and lysozyme (22). There is also indirect evidence that serous cells of the submucosal glands may secrete sodium and chloride which, by forming osmotic gradients, may contribute to water secretion by the glands (63). This possibility has been challenged by Corrales et al. (ref. 43; see below).

2. Anatomical studies indicating nervous innervation of submucosal glands. In an early study, Larsell (91) described nerve terminations present in bronchial mucous glands of rabbit. More recently, utilizing fluorescent histochemical techniques, Mann (103) studied the innervation of the bronchial tree in five mammalian species. He demonstrated the presence of catecholamine-containing nerve fibers in the vicinity of bronchial glands in adult sheep, but was unable to do so in calf, goat, pig, rabbit, or fetal sheep. Acetylcholinesterase-containing fibers were observed in association with bronchial glands of sheep and goat, but not in calf, pig, or rabbit. Similarly, El-Bermani and Grant (53) demonstrated in large bronchi of the rhesus monkey that acinar cells of glands were surrounded by acetylcholinesterase-positive nerves. In a quantitative study, Murlas et al. (117) examined the distribution of adrenergic and cholinergic axon varicosities to serous and mucous cells of cat tracheal glands. They examined all varicosities that could be identified within 10 μm of the glands. They found that 90% were cholinergic, and 10% were adrenergic. They observed no differential innervation of serous and mucous cells. Employing an immunofluorescent technique, Uddman et al. (159) demonstrated the presence of nerves which contained vasoactive intestinal peptide (VIP). These nerves were present throughout the upper respiratory tract of cat, rabbit, and guinea pig. Nerves were most numerous in cat and less so in rabbit and rarely present in guinea pig. In all cases the authors describe fine varicose VIP nerves forming plexa around submucosal glands. Similarly, Dey et al. (51) described the presence of VIP nerves in the vicinity of bronchial glands of dogs, cats, and humans.

3. Physiological studies indicating nervous regulation of submucosal glandular secretion. Several lines of evidence are consistent with the secretory responses of submucosal glands being under nervous control. These include studies on the neurohumoral receptors of glandular cells and experiments on the secretion of glandular products induced by nervous stimulation.

4. Neurohumoral receptors on glandular cells. Barnes and Basbaum (10) utilized autoradiography and radioligand binding to map adrenergic receptors of ferret tracheal submucosal glands. They found that the density of α-1 receptors on the glandular cells exceeded that of β receptors. Further, they found that the number of α-1 receptors was significantly greater on serous cells than on mucous cells. They suggested that the predominance of α-1 receptors on the serous cells may explain the watery secretion that is elaborated in response α-adrenergic stimulation of airways (160). Similarly Barnes et al. (11) demonstrated cholinergic receptors on submucosal gland cells of ferret trachea. In a more detailed study, Basbaum et al. (14) demonstrated the presence of approximately five binding sites/μm^2 for tritiated propylbenzilylcholine mustard, a muscarinic antagonist. Binding sites were on the basolateral membranes of gland cells. Serous and mucous cells did not differ in their receptor density. Marin and Culp (104) have characterized muscarinic cholinergic and adrenergic receptor characteristics in an isolated and disaggregated preparation of cat tracheal submucosal gland cells. Assuming that both mucous and serous cells have a similar density of receptors, they reported 42,000 muscarinic and 19,000 α-adrenergic receptor sites per glandular cell.

5. Studies involving direct stimulation of the nerves controlling glandular cells. In 1896, Kokin (89) described the nervous supply of the trachea of dogs and cats. By directly observing the surface of the exposed trachea which had been wiped dry, he was able to identify the formation of droplets of secretion which formed over openings of gland ducts. With electrical stimulation of nerves, he observed whether secretion from gland duct openings occurred. He came to the conclusions that (*a*) in dogs and cats, the superior laryngeal nerve contained secretory fibers for mucous glands of the larynx, and in dogs for the upper and middle part of trachea, as well, (*b*) the inferior laryngeal nerve of cat, but not dog, contained secretory fibers for mucous glands of trachea and the lower part of the larynx, (*c*) stimulation of secretory fibers of one side of the neck increased the secretory activity of the contralateral side (inhibitable by atropine, raising the possibility of reflex control of glandular secretion), and (*d*) the vagus nerve carried secretory stimuli. Recently, these studies were extended by two new techniques to define glandular secretion in vivo (48, 49, 67, 160). Davis et al. (49) coated exposed upper trachea of dogs with finely divided tantalum powder. The powder served to contain secretion from individual gland ducts in small elevations in the tantalum layer which were called "hillocks." By observing the rate of appearance and change in size of hillocks, these workers studied the effect of capsaicin injected into the bronchial artery. This agent was used as a method for stimulating C-fibers. Injection caused an increased glandular secretion which could be abolished by blocking vagal nerve transmission, by either cooling or by vagal section. The authors suggested that bronchial C-fibers furnished the afferent arm of an airway secretory response. In a

second new technique employing micropipettes, Ueki et al. (160) collected secretory product from individual gland ducts in vivo in exposed cat trachea. Stimulation of the vagus nerve increased, while blockade decreased, the secretory rate of the glands. In further studies employing the micropipette method, German et al. (67) demonstrated that reflex stimulation of the tracheal glands occurred in response to irritation of the stomach.

B. Secretory Physiology of the Surface Epithelium of the Airways

The factors that regulate normal secretion of surface airway cells are an area of current research interest. Although intraepithelial axons have been found in association with a variety of surface cells, there is little to indicate that these nerves play a secretory motor function (29). Specifically there is no evidence in mammals that goblet cells are under nervous control (29). At present little is known concerning the normal regulation of airway secretion. Recently, it has been suggested that the activities of the surface epithelium are regulated by the release of local mediators, e.g., prostaglandins or histamine, by airway cells (74). This possibility is attractive but as of yet not proven. A number of investigators have explored the mechanisms of ion and water secretion in airway epithelium. This secretory mechanism has been well characterized in a variety of species and different airway generations. Most of the ion transport activities of airways have been attributed to unspecified cells of the surface epithelium (63). Because the ciliated cell is the predominant cell type in surface epithelium, most investigators have suggested that this cell type is responsible for the majority of ion transport across airways (63).

1. Ion and water transport in airway surface epithelium. Recently, several reviews have outlined the ion transport properties of airway epithelium (48, 63, 120). An overview of studies concerning the ion transport properties of airway epithelium follows. Melon (112a) was apparently the first to study the ion transport properties of airways. He suggested that rabbit trachea actively absorbed sodium. Olver et al. (124) modified Ussing's short circuit technique (160a) and studied the ion transport properties of the posterior membranous portion of dog trachea in vitro. Utilizing radioactive chloride and sodium, they measured the unidirectional fluxes of these ions across the trachea and showed that, under short circuit conditions (i.e., in the absence of an electrochemical gradient acting across the trachea), there was a net secretion of chloride and a net absorption of sodium ion. Furthermore, the fluxes of chloride and sodium accounted for all of the simultaneously measured short circuit current (an indication of the total active ion transport of the tissue). Subsequently, several studies showed that the secretion of chloride by dog tracheal epithelium depended upon the presence of sodium in the bathing medium (5, 109, 174). By comparison to similar active transport processes in other tissues, a model (63) was suggested to explain this active transport (see fig. 1). According to this model, intracellular sodium is pumped out of the cell in exchange for potassium by a Na-K-ATPase located on the submucosal surface of the epithelial cell. There is a coupled entry of sodium and chloride across the submucosal surface driven by the gradient for sodium, created by the sodium-potassium pump. Thus, sodium enters the cell down its concentration gradient, while the entry of chloride is linked to this sodium entry. Thus, chloride accumulates within the cells against a concentration gradient because of the favorable gradient for sodium created by the sodium-potassium pump. Intracellular chloride concentration is thereby increased. The increased chloride conductance across the luminal cell membrane relative to the submucosal cell membrane accounts for a net movement of chloride toward the lumen.

This model is consistent with the findings that have been reported for dog (5, 109, 174), cow (90a, 162), human (87), and sheep trachea (130). As predicted by the model, chloride secretion in these epithelia is dependent upon the presence of sodium in the bathing medium (5, 109, 174). In dog trachea, ouabain binds preferentially to the submucosal membrane (173). Welsh (166) utilized barium ion, an inhibitor of potassium conductance, in dog tracheal epithelium and demonstrated by intracellular microelectrodes an inhibitable potassium conductance across the submucosal membranes of tracheal cells. A study employing chloride-sensitive microelectrodes showed that the intracellular chloride activity of canine tracheal epithelium was 47.2 mM, i.e., 30.1 mM above the electrochemical equilibrium for chloride across the apical membrane of the cells (146a). Agents known to block coupled sodium chloride entry ("loop" diuretics; 109, 162, 168, 170, 173a), Na-K-ATPase (ouabain; 162, 174), or increase chloride conductance (cAMP or epinephrine; 146a, 148) have responses that are predicted by this model (see below).

However, not all airway epithelium has chloride secre-

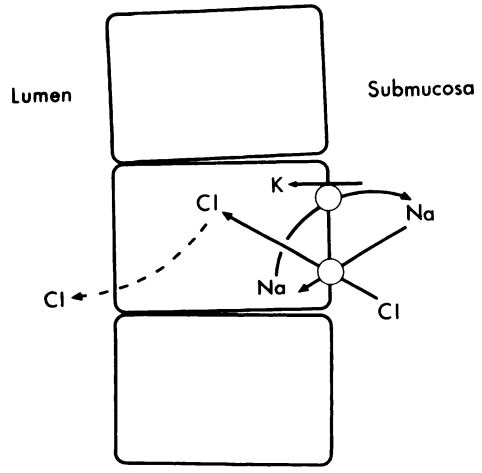

FIG. 1. Model to explain ion secretion by airway epithelium. *Rectangles* represent airway cells. Model is described in text.

tion under base-line secretory conditions. Airways smaller than the trachea have a predominant sodium absorption (27, 28). In some species such as the rabbit, sodium absorption also occurs under base-line conditions in the trachea (27). However, where tested these epithelia appear to have a similar mechanism for electrolyte secretion as predicted by the model. Sodium absorption and a lack of chloride secretion may simply relate to a relatively higher conductance of the submucosal membrane for chloride (see below).

IV. Pathophysiology of Airway Secretion

Pathological conditions have been shown to alter the morphology of airway secretory tissues (see above). Therefore, it has been postulated that airway secretion may be altered in response to these conditions. A limited number of studies have been performed which address this hypothesis. Again, available information largely relates to the function of submucosal glands and surface epithelial cells of the airways and their secretion of mucin and electrolytes.

A. Effects of Air Pollutants and Irritants on Airway Secretion

The effects of several pollutants and irritants, including cigarette smoke, on airway secretion have been investigated.

Last and Kaizu (92) exposed rats to 0.8 ppm ozone for variable lengths of time. They then removed the trachea and incubated explants for about 24 h with either $^{35}SO_4$ or [^{3}H]glucosamine in order to radiolabel the mucin. To assess mucin secretion, they collected trichloroacetic acid-precipitable proteins and quantitated the amount of radiolabel present. Using this technique, they showed that exposure to ozone initially decreased the rate of secretion compared to control rats during the first 2 to 3 days; however, the secretion rate returned to control after a period of 5 to 10 days. After 30 days, the rate of secretion was significantly increased. The initial decrease was dose related, with no change being noted at concentrations of 0.2 and 0.4 ppm. Preliminary studies with bonnet monkeys indicated that both 0.5 and 0.8 ppm ozone caused an increased mucin secretion (92), raising the possibility of species differences. These workers also studied the effects of combinations of pollutants on this secretion in rats. A combination of sulfuric acid (1.1 mg/m^3) and ozone (0.5 ppm) aerosol exposure resulted in a stimulation of secretion at both 3 and 14 days of exposure. Peatfield and Richardson (129) measured mucin secretion from tracheal segments of anesthetized pathogen-free cats. These investigators employed both charcoal and barium sulfate powder. The particles were either insufflated directly into the tracheal segment or mixed with the air which the animal inhaled. In both cases, these dusts caused an increase in mucin secretion. Based upon results obtained after vagotomy and with atropine and propranolol, these authors concluded that dusts stimulate mucins by both reflex and local mechanisms. Similarly, Richardson et al.(141) showed that both ammonia and cigarette smoke increased mucin secretions by both local and reflex mechanisms. Welsh (167) employed Ussing's short-circuit technique to determine the effect of cigarette smoke on ion transport properties of dog tracheal epithelium. He bubbled whole cigarette smoke through the solution which bathed the tracheal tissue. He observed that exposure to the luminal surface of the trachea to cigarette smoke resulted in an inhibition of the rate of chloride secretion with minimal effect on sodium absorption. Filtering out the particulates from the smoke minimized the effect.

B. Effect of Bacterial Infection on Airway Secretion

Although several studies have delineated morphological changes that occur in airways as a result of infection (see above), minimal quantitative information is available concerning the effects of such infection on airway secretion. One such study that may be of relevance is that of Adler et al. (2). These workers analyzed the effects of cholera toxin on the secretion of mucin by guinea pig tracheal explants. In concentrations of toxin between 5 and 50 μg/ml, they found a dose-dependent increase in mucin secretion.

C. Effect of Various Airway Diseases on Airway Secretion

As alluded to above, most of the information about the pathology of airway secretion relates to studies of the altered morphology of airways in diseased states. Some information is available concerning the actual pathophysiology which occurs with airway disease. The responsiveness of diseased tissue (i.e., cystic fibrosis, bronchitis, and bronchiectasis) to mucin secretogogues related primarily to glandular hypertrophy, rather then to a definite alteration in the mechanism of mucin secretion (152). Minor alterations have been observed in the biochemical composition of airway secretions of patients with various diseases including asthma, bronchitis, bronchiectasis, and cystic fibrosis (22, 32, 58, 101). Because of the complexity involved in the formation of airway secretions, it is difficult to assess the pathophysiological mechanisms responsible for these changes. Coles and Reid (41), did define some potential differences in the biosynthesis of mucins in patients with chronic bronchitis. They examined precursor uptake into explants from patients with chronic bronchitis and normals and found accelerated uptake of threonine and glycoprotein synthesis in explants from patients with bronchitis compared to control.

Certain potential pathological mechanisms have also been described in several other studies. For instance, Phipps et al. (130) studied the effect of in vitro challenge utilizing *Ascaris suum* antigen of tracheal tissue from allergic sheep. They found that the antigen (25 μg/ml) increased mucin secretion. Transiently, the antigen also

decreased chloride net flux. This was followed by a net secretion of chloride and sodium. These responses could be greatly but not completely reduced by pretreatment with cromolyn (10^{-4} M). The authors suggested that the cromolyn-sensitive effects were due to mediators released during the allergic response. Several studies have examined the effect of serum from patients with cystic fibrosis on both mucin secretion and also on ion transport properties. While Moriarty et al. (116) were unable to distinguish between the effects of control sera and sera collected from patients with cystic fibrosis on mucin secretion or ion transport properties of rabbit tracheal explants, Boat et al. (23) found that the initial response of mucin release of rabbit tracheal explants to cystic fibrosis sera was greater than control sera. Likewise, Rudick et al. (142) working with cell cultures from hamster trachea showed that sera from cystic fibrosis patients increased secretory rates of mucin relative to sera from normals. Serum contains multiple substances, and it is not clear which exact components of cystic fibrosis serum that are responsible for the possible pathological effects. Nor is it clear that the alterations are related to the pathogenesis of the disease. Recently, Knowles et al. (88) examined the ion transport properties of nasal tissue excised from patients with cystic fibrosis and from normal subjects. They found that tissue from cystic fibrosis patients had a relative impermeability to chloride ions. Based on similarities of nasal to lower airway tissues, this abnormality probably also is present in the lower airways of patients with cystic fibrosis as well (86).

V. Pharmacological Regulation of Airway Secretion

Effects of a number of different pharmacological agents on airway secretion have been examined. These will be surveyed in this portion of the review.

A. Effect of Cholinergic Agents on Airway Secretion

As outlined above, there is ample evidence to indicate that submucosal glandular secretion is under cholinergic nervous control. Cholinergic agonists, antagonists, and anticholinesterase agents have also been shown to have important effects on glandular secretory mechanisms.

In general, the effects of cholinergic agonists seem to simulate the effects of cholinergic nervous stimulation (see above). For instance in a study utilizing dog trachea, in vivo, coated with tantalum powder, hillocks formed in response to vagal stimulation and also in response to i.v. acetylcholine. These responses could be blocked by pretreatment with atropine (46). Likewise, in an in vitro study utilizing cat trachea (132), in which secretions were collected from individual gland duct openings, bethanechol, a cholinergic agonist, increased the secretory rate. The threshold concentration for stimualtion was 10^{-6} M. Dose-response characteristics were not measured. The secretions were isotonic with the bathing medium with the exception of sulfur content. The author postulated without documentation that the source of sulfur was mucous glycoproteins. He suggested that cholinergic stimulation resulted in both an increase in electrolyte and water secretion, as well as the secretion of mucins (132). Coles (37) employed histochemical and autoradiographical techniques to determine a secretory index for human bronchial submucosal glands. He showed that methacholine, a cholinergic agonist, in an unspecified concentration promoted the discharge of secretory granules, while atropine blocked this response. Physostygmine, an acetylcholinesterase inhibitor, also increased the secretory index in a dose-related manner, in concentrations between 10 and 1000 μg/ml, in both mucous and serous cells of submucosal glands. Sturgess and Reid (152) employed a similar technique and showed that acetylcholine had a similar dose-related effect on the secretory index, while parasympatholytic agents such as atropine and hyoscine decreased the index. Basbaum et al. (15) performed a morphological analysis of structural changes in serous cells of submucosal glands of ferret trachea. They showed that methacholine (10^{-5} M) decreased the number of secretory granules in serous cells relative to control. This response could be blocked by atropine. Tom-Moy et al. (156) used lysozyme, a bacteriolytic enzyme found in the secretory granules of serous but not mucous cells, as a marker for serous cell secretion in ferret tracheal submucosal glands. She showed that bethanechol (10^{-5} M) stimulated lysozyme release by these cells. Quinton (133) showed that bethanechol (10^{-4} M) induced vacuolation in serous cells of submucosal glands of cat trachea. This response could be blocked with atropine. Based upon the blocking of the formation of these vacuoles by bathing fluid deficient in calcium or bicarbonate and by the ability of ouabain to prevent vacuole formation, Quinton suggested that vacuolation was associated with transport of ions by the cells. He also postulated that this vacuolation was associated with the stimulation of the fluid component of secretion. In studies of whole dog trachea in vitro (105), acetylcholine increased in a dose-related manner the secretion of both chloride and sodium toward the tracheal lumen. A threshold response was observed at a concentration of 5×10^{-10} M acetylcholine. The pattern of response could not be explained in terms of a single acetylcholine receptor acting according to simple Michaelis-Menten kinetics. The possibility of two independent receptors with differing affinities was postulated. The responses to acetylcholine could be prevented by atropine in a concentration 1000-fold less than the concentration of acetylcholine. By virtue of the cholinergic innervation of submucosal glands, this secretion has been attributed to submucosal glandular cells (63). Others have contested active ion transport by these cells in response to secretogoguge (43). Corrales et al. (43) reported evidence that secretion of ions may be secondary to an osmotic gradient created by the release of

unspecified osmotically active components of secretory granules into the relatively unstirred layers of submucosal glandular ducts.

Numerous other studies have evaluated the response of cholinergic agents on the release of mucous glycoproteins without specifically delineating the cellular source of the mucins, i.e., glandular *versus* surface secretory cells (33, 59, 65, 73, 144). These studies likewise indicate that cholinergic agents increase secretion of mucins by airways. A single study has evaluated the effect of anticholinesterases on bronchial secretion (44). These workers collected free-flowing secretions from the cephalad portion of the tracheas of anesthetized rabbits, cats, or monkeys. In four cats, sarin, an irreversible anticholinesterase, was injected s.c. in doses of 12 to 25 μg/kg at half-hour intervals until general symptoms occurred, i.e., convulsions, tachypnea, or respiratory arrest (usually a total dose of 50 to 60 μg/kg). The rate of fluid collection increased with the onset of symptoms. Maximum fluid flow was approximately twice base line. Similar results were observed in rabbits and in a single monkey. While injection of acetylcholine alone produced erratic results, a combination of sarin plus acetylcholine slightly augmented, above the effect of sarin alone, the flow of bronchial fluid in rabbits and a single monkey, and more predominantly in a single cat. In two rabbits, N-*p*-chlorophenyl-N-methyl carbamate of *m*-hydroxy phenyltrimethylammonium bromide, a substance which inhibits the "true" cholinesterases, in doses of 0.2 mg/kg and 0.5 mg/kg, resulted in augmentations of bronchial fluid rates of 10- and 26-fold, respectively.

B. Effect of Adrenergic Agents on Airway Secretion

Adrenergic agents have an apparent effect on both surface epithelial cells as well as submucosal glandular elements. This responsiveness exhibits species differences.

1. Effect of adrenergic agents on submucosal glandular secretion. As noted above, anatomical studies indicated that submucosal glandular cells are also supplied by adrenergic nerve endings (117). The density of these nerves in the vicinity of the gland cells in the cat is considerably less than cholinergic nerves (117). In both cat and ferret these cells have been shown to possess α-adrenergic receptors, albeit in lesser density than cholinergic receptors (11, 13, 14, 104). β-Adrenergic receptors while present on glandular cells are quite sparse (10).

Pharmacologically, adrenergic agents have a varied effect on glandular secretion. In several studies, adrenergic agents elicited a secretory response from submucosal glands (131–133, 156, 160) while in other studies these agents failed to stimulate a secretory response (21, 33, 121, 152).

In whole pieces of trachea from cats, Phipps et al. (131) showed that both phenylephrine (10^{-5} M) an α-adrenergic, and terbutaline (10^{-5} M), a β-adrenergic agent, increased the secretion of precursor radiolabeled (^{35}S) mucous glycoproteins. The stimulatory effect of phenylephrine exceeded that of terbutaline (147 ± 24% *versus* 43 ± 7%), and both responses were specifically blocked by appropriate antagonists. Dose-response characteristics were not defined. Liedtke et al. (100) examined the effect of β-adrenergic agents on mucin secretion from cat tracheal explants. They also found that β-adrenergic agents stimulated mucin secretion. At maximal concentrations (1 to 3 × 10^{-4} M) the efficacy of the catecholamines for increasing radiolabeled mucous glycoprotein release was as follows: l-epinephrine = l-isoproterenol > l-norepinephrine > dobutamine = terbutaline. In the presence of phentolamine, an α-adrenergic antagonist, the 50% effective concentrations (EC_{50}) for epinephrine and norepinephrine were 3.9 and 1.4 μmol, respectively. These investigators also showed that intracellular cAMP increased in response to β-adrenergic stimulation, and they suggested that this was a mediator of the mucin secretory response (100). Ueki et al. (160) showed that phentolamine, an α-adrenergic agonist, increased the flow of fluid from the duct of the submucosal glands of cat trachea. Further, Tom-Moy et al. (156), utilizing lysozyme as a marker of serous cell secretion from ferret trachea, found that both phenylephrine and terbutaline increased lysozyme release, but in relatively lesser degrees than cholinergic stimulation. Likewise, Quinton (133) showed that vacuoles formed in the cat serous cells in response to α- and β-adrenergic agonists. Again, the order of responsiveness to vacuole formation was cholinergic > α-adrenergic > β-adrenergic agonist.

In other studies, adrenergic agents did not elicit secretory responses (21, 33, 121, 152). In the case of geese, rabbits, and guinea pigs, this has been attributed to the rudimentary glandular structures present in these species (121). In other cases, the poor secretory responses have been attributed to a failure of the agent to reach submucosal glandular structures due to vasoconstriction (121). In human bronchial explants adrenergic agents failed to elicit lysozyme secretion (21). The reason for this species variation remains not entirely clear.

2. Effect of adrenergic agents on surface epithelial secretion. While adrenergic axons do not appear to be present in the surface epithelium of mammalian species (10, 140), studies show that receptors for both α- and β-adrenergic agents are present. Barnes and Basbaum (10) in radioligand studies involving pieces of whole ferret trachea showed that, in surface epithelium, the density of β-receptors exceeded the density of α-receptors.

Several investigations have defined the effects of adrenergic agents on ion transport (6, 47, 131, 146, 169). The type of cell affected by these agents has not been determined. Many have attributed the responses to surface epithelial cells, most likely the ciliated cell. Employing Ussing's short-circuit technique with pieces of dog trachea, Davis et al. (47) showed that terbutaline, a

β-adrenergic agonist, resulted in a dose-related increase (in concentrations of 10^{-7} M to 10^{-5} M) in the secretion of chloride but not of sodium. The effect of terbutaline could be inhibited by propranolol (10^{-6} M). These experiments utilized the posterior membranous portion of the tracheal epithelium of the dog—a portion which is relatively free of submucosal glands (personal observation). The experiments were performed under short-circuit conditions, i.e., in the absence of a transepithelial electrochemical gradient. The effect of adrenergic agents on canine trachea was confirmed and expanded by Al-Bazzaz and Cheng (6) who found a similar pattern of ionic secretion in response to these agents. The order of responsiveness of dog tracheal epithelium was isoproterenol > epinephrine > norepinephrine > phenylephrine. This order of responsiveness suggested that the chloride secretory response was more sensitive to β-adrenergic stimulation than to α-adrenergic stimulation. In studies performed under open-circuit conditions in cat trachea, Phipps et al. (131) showed that terbutaline (10^{-5} M) increased chloride but not sodium secretion. Several studies have utilized conventional microelectrodes to examine the effect of epinephrine on the permeability of the luminal and submucosal membranes of the surface epithelial cells of dog trachea (63, 146, 164–166, 169). These studies showed that epinephrine increased the rate of electrogenic chloride secretion (as indicated by an increase in short-circuit current) and also decreased both the luminal and submucosal cell membrane resistances of surface epithelial cells (63, 146, 164, 169). The decrease in luminal resistance related to an increase in chloride conductance of the luminal cell membrane; the submucosal resistance change probably was due to an increased potassium conductance at the submucosal cell membrane (146, 166). Demonstration of these events in surface epithelial cells supports further the contention that these cells are responsive to adrenergic stimuli. In further support of this concept, surface intracellular cAMP levels increased in response to β-adrenergic agents (148). This nucleotide probably mediates the secretory responses in these cells (63). β-Adrenergic agents may also release endogenous prostaglandins from surface epithelial cells, and these substances may in turn play a role in the ion transport secretory responses (4, 63).

C. Effect of Active Polypeptides on Airway Secretion

Several different polypeptides have been described which play a regulatory role in secretory processes in a number of different organs. These include circulating hormones and also neurotransmitters which may be active in noncholinergic, nonadrenergic nervous transmission. There is evidence that submucosal glands are supplied by noncholinergic, nonadrenergic nerve endings (51, 159). In an in vitro preparation of ferret trachea, Borson et al. (25) utilized electric field stimulation and the radiolabeled precursors of mucous glycoproteins to study the nervous regulation of submucosal glandular secretion. They found that, in addition to cholinergic and both α- and β-adrenergic regulation of the secretion of $^{35}SO_4$-labeled macromolecules, there was also nonadrenergic, noncholinergic nervous regulation as well.

Several experimenters have examined the effects of regulatory peptides on airway secretion. These studies have provided variable results. Baker et al. (8), utilizing canine tracheal explants incubated with radiolabeled glucosamine (a precursor primarily of mucous glycoproteins), studied the effect of several different polypeptides on the release of radiolabeled macromolecules. While kallidin was associated with an increase in the amount of radioactivity released in macromolecules, hexadimethrine, an inhibitor of kinin formation, decreased the amount. Substance P, physalaemin, and eledoisin all enhanced the secretion of radiolabeled macromolecules. The investigators also showed that kallidin, kallidin derivatives, glucagon, vasopressin derivatives, substance P, vasotocin derivatives, eledoisin-related peptide, and certain melittin derivatives could all enhance the activity of mucin galactosyltransferase, an enzyme important in the synthesis of mucous glycoprotein. These authors suggested that these various polypeptides may be important in the synthesis and/or release of respiratory glycoproteins, presumably from submucosal glands. Peatfield et al. (127), employing a similar system with ferret tracheal explants, found that VIP, possibly a neurotransmitter of nonadrenergic, noncholinergic nerves increased the output of radiolabeled macromolecules. Because of the paucity of goblet cells relative to submucosal glandular elements in ferret trachea, these workers attributed this output to the submucosal glands. On the other hand, Coles et al. (38) reported that VIP caused an inhibition in the secretion of radiolabeled macromolecules from "normal" human bronchial explants but not from bronchial mucosa obtained from patients with chronic bronchitis. In dog tracheal explants, VIP caused a dose-related increase in glycoprotein release. In canine trachea, these investigators found that substance P was a particularly potent secretogogue ($EC_{50} = 8.2 \times 10^{-10}$ M *versus* an $EC_{50} = 6.3 \times 10^{-7}$ M for methacholine). While physalaemin, eledoisin, and eledoisin-related peptide all increased radiolabeled glycoprotein release, albeit with less potency than substance P, bombesin had no effect on the release of glycoprotein from dog tracheal explants. Substance P caused an initial stimulation of secretion within 10 min, and this was followed by a 10- to 20-min period of inhibition of secretion. Coles et al. (38) postulated that substance P was causing the mucus present in the lumen of the gland to be discharged rather than to altering the synthesis of glycoprotein. The differences between Coles et al. (38) and the studies of Peatfield et al. (127) may relate to differences in technique and/or to true species variability. Al-Bazzaz et al. (7) examined the effect of substance P on the ion transport properties of short-circuited dog trachea. They found that 10^{-7} M

substance P increased net chloride secretion without effecting net sodium transport. They suggested that this neuropeptide may play a role in the regulation of ion transport.

D. Effect of Phosphodiesterase Inhibitors and Intracellular Cyclic Adenosine Monophosphate (cAMP) on Airway Secretion

β-Adrenergic agents, histamine, and prostaglandins of the E series, all increase intracellular cAMP levels in airway tissues (63, 99). Phosphodiesterase inhibitors cause an increase in intracellular cAMP (99, 100) by preventing its breakdown. In terms of airway secretion, phosphodiesterase inhibitors seemed to mimic the effects of β-adrenergic agonists. For instance Liedtke et al. (100) observed that both 0.25 mM 3-isobutyl-1-methylxanthine (IBMX) and 8-bromo-cAMP in concentration between 10^{-5} and 10^{-3} M increased the release of mucin from explanted cat tracheal tissues. In an autoradiographical study employing human bronchial explants, Whimster and Reid (172) demonstrated that, like dibutyryl cAMP (500 μg/ml), theophylline (2000 μg/ml) caused an increased discharge of mucin. Employing Ussing's short-circuit technique in dog trachea, Al-Bazzaz and Al-Awqati (5) evaluated the effect of theophylline (5 mM) on short-circuit current and chloride and sodium fluxes. They found that, like β-adrenergic agents, theophylline increased short-circuit current and the net flux of chloride toward the tracheal lumen. However, it failed to alter the sodium flux. These findings were confirmed by Welsh et al. (171), who also demonstrated that the increased chloride net flux mediated a secretion of water toward the tracheal lumen.

E. Effect of Cytoplasmic Calcium and Calcium Ionophores on Airway Secretion

Cytoplasmic calcium concentration is postulated to serve as a mediator of airway secretion (63). There is evidence that calcium ionophores, which increase the cytoplasmic calcium concentration, caused both mucin release as well as altered the ion transport properties of airway epithelium (see below). Other in vitro studies have investigated the effects of calcium concentration in the bathing medium on the secretion of mucins and upon the ion transport properties of the tissue. The results of these studies are variable, and it is not clear the exact role that calcium plays in airway secretion.

Al-Bazzaz and Jayaram (3) investigated the effect of A23187, a calcium ionophore, on ion transport properties of dog tracheal epithelium. A23187 (10^{-6} M) added to the solution bathing the submucosal side of the posterior membranous portion of dog trachea caused a significant increase in net chloride secretion and abolished net sodium transport. The cellular site of these actions was not delineated. Marin and Zaremba (108) studied the effect of calcium concentration in the medium bathing the posterior membranous portion of dog tracheal epithelium on the ion transport properties of the tissue. They observed no alteration in net fluxes of chloride and sodium measured under short-circuit conditions when the calcium concentration in the bathing medium was varied between 0 and 10 mM. Lack of calcium in the bathing medium resulted in a decrease in electrical resistance. This alteration was probably secondary to an increased permeability of the intracellular junctions due to a lack of calcium. Mian et al. (114), working with chicken tracheal mucosa, a tissue with only rudimentary submucosal glands, examined the effect of A23187 (2×10^{-5} M) on mucin secretion by this tissue. They found radiolabeled mucin secretion to be stimulated by the addition of the ionophore to either side of the trachea. Bogart et al. (24) found a similar result in rabbit tracheal epithelial explants, and Barbieri et al. (9), in canine tracheal explants. Marin et al. (107) found that removal of calcium from the medium bathing the posterior membranous portion of dog tracheal epithelium caused a decrease in radiolabeled mucous glycoprotein secretion. This portion of the trachea is relatively devoid of submucosal glands, and, thus, these results may reflect secretion from goblet cells in the surface epithelium. Barbieri et al. (9) found that prolonged (18 to 22 h) preincubation of canine tracheal explants in calcium-free bathing solution rendered secretion of mucin by the tissues unresponsive to methacholine stimulation. However, base-line secretion of mucin was slightly increased in tissues incubated without calcium. Coles et al. (39) also employed dog tracheal explants and observed an increase in the base-line secretion of mucin in the absence of calcium in the bathing medium. These authors proposed that calcium depletion increased the rate of flow of mucus from the duct lumina of the tracheal glands.

F. Effect of Prostaglandins and Their Antagonists and Other Arachidonic Acid Metabolites on Airway Secretion

Lung tissue when challenged by antigen in vitro released various prostaglandins into the medium, i.e. prostaglandin E_2, prostaglandin $F_{2\alpha}$, and prostaglandin D_2 (1, 143). These mediators have been implicated as being important in the allergic response (139). As noted above, prostaglandins of the E series increased intracellular cAMP (63). On the other hand, prostaglandin $F_{2\alpha}$ failed to alter intracellular cAMP (63). In many respects the effects of prostaglandins of the E series on airway secretion are similar to the effect of β-adrenergic agents. Other prostaglandins and arachidonic acid metabolites also have an effect on secretory function.

Richardson et al. (141) developed a method of collecting mucin in vivo from anesthetized cats. They fluid filled a segment of trachea and collected samples from this segment. Mucous glycoprotein in these animals was radiolabeled by either i.v. administration to the animal or instillation into the tracheal segment with radiolabeled precursors of mucous glycoproteins. The authors

observed that prostaglandins A_2, E_1, $F_{1\alpha}$, and $F_{2\alpha}$ all increased mucin output. Based on autoradiographic localization of the radiolabeled precursors, these investigators suggested that secretion was from submucosal glands rather than from the surface goblet cells. Yamatake and Yanaura (175) devised a technique to collect respiratory tract fluid from the top of the trachea in anesthetized dogs. They infused prostaglandins into the bronchial artery and showed that prostaglandin $F_{2\alpha}$ increased in a dose-dependent manner the volume of secretions collected, while prostaglandin E_2 did not effect secretory activity. This technique did not allow them to define the cellular source of the secretory product. Marom et al. (110) studied the effects of arachidonic acid and its metabolites on the release of radiolabeled mucous glycoproteins from explants of human airway tissue. They found that arachidonic acid and prostaglandins A_2, D_2, and F2α significantly increased mucous glycoprotein release, whereas prostaglandin E_2 significantly reduced release. Several lines of evidence in their studies suggested that lipoxygenase products of arachidonic acid agumented the release of mucin. For instance, they showed that nonsteroidal antiinflammatory drugs, like acetylsalicyclic acid and indomethacin, which inhibited the formation of prostaglandins but not the lipoxygenase products, still increased mucin release. However, nonspecific lipoxygenase inhibitors, like vitamin E, inhibited release. In additional studies, they also showed directly that various monohydroxyeicosatetraenoic acids and leukotrienes (145), lipoxygenase products, increased release. Likewise, in anesthetized cat, Peatfield et al. (128) showed that leukotriene C_4 stimulated mucin release. Working with human bronchial explants, Coles et al. (40) confirmed and extended these observations. They observed and characterized the dose-related effects of leukotrienes C_4 and D_4 on mucin release. These agents failed to effect lysozyme secretion. Based on the time course of secretion, these authors suggested that the effect of leukotrienes was primarily on release of mucins from submucosal glands rather than upon the biosynthesis of mucin. Rich et al. (139), utilizing preparations from human bronchi, also found that prostaglandins $F_{2\alpha}$ increased in a dose-dependent manner the secretion of radiolabeled mucin. The responses to prostaglandins of the E series were more variable and not significantly different from control.

Al-Bazzaz et al. (4), using Ussing's short-circuit technique, tested the effect of prostaglandins $F_{2\alpha}$ and E_1 on the transport of chloride and sodium by dog tracheal epithelium. They observed that, like β-adrenergic agents, prostaglandin $F_{2\alpha}$ increased net chloride secretion without altering the absorption of sodium. On the other hand, prostaglandin E_1 increased net chloride secretion and decreased net sodium secretion. While indomethacin depressed base-line chloride secretion, it augmented the increase in chloride secretion in response to prostaglandin E_1. Prostaglandin E_1 increased the intracellular levels of cAMP; prostaglandin $F_{2\alpha}$ was without effect. The actual site of the cellular response to these agents was not defined. The cAMP levels were examined in surface cells scraped from the trachea. Thus, submucosal glandular cells may not have been sampled by this technique. The difference in ion transport response to the two different prostaglandins may reflect a difference in the site of action of the two agents, with E_1 affecting the surface cells and $F_{2\alpha}$ altering the transport in submucosal glandular cells. Langridge-Smith et al. (90a) showed that ion transport by bovine trachea was markedly altered by indomethacin, presumably on the basis of inhibition of the synthesis of endogenous prostaglandins by the tracheal cells. Indomethacin (10^{-6} M) increased sodium absorption and reduced chloride secretion, thereby reversing net transepithelial ion flow. Because these effects were reversed by prostaglandin E_1 and epinephrine, these authors suggested that the effect of indomethacin was upon prostaglandin synthesis rather than directly upon ion transport mechanisms.

G. Effect of Histamine and Antagonists on Airway Secretion

Histamine is released from mast cells in response to specific allergens. This release may play an important role in the allergic responses of airways. Liedtke et al. (99) showed that rabbit tracheal cells increased their cAMP contents in response to histamine. Several studies have examined the effects of histamine on both mucous glycoprotein secretion and ion transport of airways.

Chakrin et al. (33) incubated dog tracheal explants with radiolabeled precursor of mucous glycoprotein and noted that histamine failed to alter the secretion of radiolabeled mucin. However, Richardson et al. (141), utilizing the in vivo fluid-filled tracheal segment model (described above), delivered histamine as an aerosol into the lungs of the cat or directly as a solution into the tracheal segment. When delivered as an aerosol, histamine failed to elicit a mucin response but when added directly to the tracheal segment, it caused a marked increase in mucin release. The cellular site of this response was not defined. Marin et al. (106) employed Ussing's short-circuit technique and examined the effect of adding histamine to the submucosal bath on chloride and sodium transport across dog tracheal epithelium. They found a dose-related response of the short-circuit current to histamine which could be competitively inhibited by diphenhydramine, an H-1 antagonist, but not by burimamide, an H-2 inhibitor. Histamine increased net chloride secretion toward the tracheal lumen and decreased net sodium absorption. Vulliemin et al. (162) found a similar effect of histamine on the ion transport properties of cow trachea.

H. Effect of Diuretic Agents on Airway Secretion

Diuretic agents have been utilized in studies of the ion transport properties of airways as tools to better characterize the ion transport of these tissues.

Furosemide inhibited chloride transport in the kidney (31) and coupled sodium chloride transport in the intestine (76). Marin and Zaremba (109), utilizing Ussing's short-circuit technique, showed that furosemide added to the medium bathing the submucosal side of dog tracheal epithelium decreased net chloride secretion while augmenting net sodium absorption. These observations were extended by the study of Welsh (168). He showed that the response to furosemide occurred primarily when the agent was applied to the submucosal side of the tissue. Utilizing conventional microelectrode techniques, he showed further that furosemide had only minimal effects on the transepithelial and luminal membrane resistances, indicating that the primary effect of the agent was not an inhibition of chloride exit across the luminal cell membrane. Further, submucosal membrane resistance and electromotive force were not altered, suggesting an electrically neutral entry of chloride at the submucosal membrane. Calculation of intracellular chloride concentration indicated that furosemide decreased intracellular chloride. The results indicated that furosemide inhibited an electrically neutral entry of chloride across the submucosal membrane. These findings were felt to be compatible with the model of ion transport shown in fig. 1 (see above). Further studies of "loop" diuretics were performed by Widdicombe et al. (173a). They examined the effects of MK-196, bumetanide, piretanide, and furosemide on ion transport by dog tracheal epithelium. In all cases they found that these inhibitors of chloride transport caused a decrease in the unidirectional and net fluxes of chloride toward the lumen measured under short circuit conditions. They were unable to demonstrate an effect upon active sodium absorption. With the exception of MK-196 (which was equipotent), the drugs were more potent when added to the submucosal bathing medium. They also performed studies of sodium and chloride influx in isolated tracheal cells. The diuretics caused an approximately equal decrease in the sodium and chloride influx into the cells. Furthermore, removal of either sodium or chloride from the bathing medium caused a reduction in the influx of the other ion, indicating a linked Na-Cl entry process into the tracheal cells. Again, these finding are compatible with the model of ion transport shown in fig. 1.

Boucher et al. (27) observed that amiloride, a diuretic which inhibits sodium conductance, decreased slightly but not significantly the spontaneous potential difference across canine tracheal epithelium both in vivo and in vitro. Vulliemin et al. (162) noted that amiloride (10^{-4} M) added to the medium bathing the luminal side of short-circuited cow trachea resulted in an 80% decrease in net sodium absoption. Estep et al. (57), employing conventional microelectrodes, showed that the spontaneous luminal potential difference of dog tracheal epithelium was minimally hyperpolarized by amiloride. In indomethacin-treated tissues, Welsh et al. (169), utilizing dog tracheal epithelium in a similar experiment, observed a decrease in short-circuit current, hyperpolarization of the luminal membrane, and an increase in the transepithelial resistance. These finding were consistent with amiloride inhibiting the flux of sodium across the luminal cell membranes. The minimal hyperpolarization under spontaneous conditions suggested that the sodium conductance across the luminal membrane played, relative to chloride, a minor role in the maintenance of the luminal cell membrane potential (57).

I. Effect of Ouabain on Airway Secretion

Ouabain, a cardiac glycoside, is an inhibitor of Na-K-ATPase. As such, ouabain, has been used extensively as a tool to understand the potential mechanisms involved in airway secretion. There is evidence that ouabain affects both the ion transport properties of the surface epithelial cells and also that it alters the secretory properties of submucosal glandular cells.

1. Effect of ouabain on the secretion from surface epithelial cells of the airway. Widdecombe et al. (173) studied by autoradiography the binding of tritiated ouabain to pieces of dog tracheal epithelium. They found that there was specific saturable binding of ouabain which could be inhibited by high concentrations of potassium, metabolic inhibitors, low sodium, and low temperature. The binding was localized to the submucosal surfaces of both surface epithelial cells as well as gland cells. In further studies, Widdecombe et al. (174) showed that ouabain added to the submucosal bathing medium abolished the active transport of ions across dog tracheal epithelium. These findings were confirmed by Boucher et al. (28), who found similar results in both dog trachea and bronchi, and by Vulliemin et al. (162) in cow trachea. These findings were compatible with the model described above (see fig. 1).

2. Effect of ouabain on the secretion from submucosal glandular cells. Ouabain also has apparent effect on submucosal glandular cells. Coles (37) used human bronchial explants and an autoradiographical technique to examine the effects of ouabain on the secretory rate and incorporation of mucin precursors in serous and mucous cells. He found that ouabain decreased the incorporation of radiolabeled threonine and glucose into the secretory granules of mucous and serous cells. The secretory index for these tracer compounds was also decreased by ouabain. Neither incorporation nor secretion of glucosamine was affected by ouabain. Coles suggested that ouabain inhibits sodium-linked active incorporation of threonine and glucose and thereby inhibits the secretion from these cells. In a further study, Coles and Reid (41) showed that the uptake of these precursors was not ouabain sensitive in surface epithelial secretory cells. Quinton (133)

showed that the vacuolation of serous cells in response to phenylephrine was inhibited by ouabain, and Corrales et al. (43) showed that the output of precursor-labeled macromolecules from cat tracheal glands was prevented by ouabain. These studies all suggest that the activity of the sodium pump may be an important factor in macromolecular glandular secretion as well.

VI. Conclusions

Airways are complicated tissues made up of multiple cell types. A major function of these tissues is the secretion of a complex protective airway fluid. This secretory process is controlled by multiple factors and as described above can be altered by physiological, pathophysiological, and pharmacological interventions. This review stresses the complexity and individual and species variability in airway secretion. Present knowledge only begins to describe the overall pharmacology of airway secretion. Before a complete picture of the pharmacology of airway secretion can emerge, we must learn the individual functions and the interactions between the multiple cell types of the airways. We must fully define the considerable species differences and understand how these differences affect the overall secretory mechanisms. Finally, we must understand how pathological mechanisms alter normal airway secretion and how these alterations influence the pharmacology of secretion.

REFERENCES

1. ADKINSON, N. F., JR., NEWBALL, H. H., FINDLAY, S., ADAMS, K., AND LICHTENSTEIN, L.M.: Anaphylactic release of prostaglandins from human lung in vitro. Am. Rev. Respir. Dis. **121:** 911–920, 1980.
2. ADLER, K. B., HARDWICK, D. H., AND CRAIGHEAD, J. E.: Effect of cholera toxin on secretion of mucin by explants of guinea pig trachea. Lab. Invest. **45:** 372–377, 1981.
3. AL-BAZZAZ, F., AND JAYARAM, T.: Ion transport by canine tracheal mucosa: effect of elevation of cellular calcium. Exp. Lung Res. **2:** 121–130, 1981.
4. AL-BAZZAZ, F., YADAVA, V. P., AND WESTENFELDER, C.: Modification of Na and Cl transport in canine tracheal mucosa by prostaglandins. Am. J. Physiol. **240:** F101–F105, 1981.
5. AL-BAZZAZ, F. J., AND AL-AWQATI, Q.: Interaction between sodium and chloride transport in canine tracheal epithelium. J. Appl. Physiol. **46:** 111–119, 1979.
6. AL-BAZZAZ, F. J., AND CHENG, E.: Effect of catecholamines on ion transport in dog tracheal epithelium. J. Appl. Physiol. **47:** 397–403, 1979.
7. AL-BAZZAZ, F. J., KELSEY, J. G., AND KAAGE, W. D.: Substance P stimulation of chloride secretion by canine tracheal mucosa. Am. Rev. Respir. Dis. **131:** 86–89, 1985.
8. BAKER, A. P., HILLEGASS, L. M., HOLDEN, D. A., AND SMITH, W. J.: Effect of kallidin, substance P, and other basic polypeptides on the production of respiratory macromolecules. Am. Rev. Respir. Dis. **115:** 811–817, 1977.
9. BARBIERI, E. J., BOBYOCK, E., CHERNICK, W. S., AND MCMICHAEL, R. F.: The calcium dependency of mucus glycoconjugate secretion by canine tracheal explants. Br. J. Pharmacol. **82:** 199–206, 1984.
10. BARNES, P. J., AND BASBAUM, C. B.: Mapping of adrenergic receptors in the trachea by autoradiography. Exp. Lung Res. **5:** 183–192, 1983.
11. BARNES, P. J., NADEL, J. A., ROBERTS, J. M., AND BASBAUM, C. B.: Muscarinic receptors in lung and trachea: autoradiographic localization using [^{3}H]quinuclidinyl benzilate. Eur. J. Pharmacol. **86:** 103–106, 1983.
12. BASBAUM, C. B.: Innervation of the airway mucosa and submucosa. Semin. Respir. Med. **5:** 308–313, 1984.
13. BASBAUM, C. B., BARNES, P. J., GRILLO, M. A., WIDDICOMBE, J. H., AND NADEL, J. A.: Adrenergic and cholinergic receptors in submucosal glands of the ferret trachea: autoradiographic localization. Eur. J. Respir. Dis. **64:** 433–435, 1983.
14. BASBAUM, C. B., GRILLO, M. A., AND WIDDICOMBE, J. H.: Muscarinic receptors: evidence for a nonuniform distribution in tracheal smooth muscle and exocrine glands. J. Neurosci. **4:** 508–520, 1984.
15. BASBAUM, C. B., UEKI, I., BREZINA, L., AND NADEL, J. A.: Tracheal submucosal gland serous cells stimulated in vitro with adrenergic and cholinergic agonists. Cell Tissue Res. **220:** 481–498, 1981.
16. BASKERVILLE, A.: Ultrastructural studies of the normal pulmonary tissue of the pig. Res. Vet. Sci. **2:** 150–155, 1970.
17. BASKERVILLE, A.: The development and persistence of bronchial-gland hypertrophy and goblet-cell hyperplasia in the pig after injection of isoprenaline. J. Pathol. **119:** 35–47, 1976.
18. BASSET, F., POIRIER, J., LE CROM, M., AND TURIAF, J.: Etude ultrastructurale de l'epithelium bronchiolaire humain. Z. Zellforsch. Mikrosk. Anat. **116:** 425–442, 1971.
19. BIENENSTOCK, J., CLANCY, R. L., AND PEREY, D. Y. E.: Bronchus associated lymphoid tissue (BALT): its relationship to mucosal immunity. *In* Immunologic and Infectious Reactions in the Lung, ed. by C. H. Kirkpatrick and H. Y. Reynolds, p. 29, Marcel Dekker, New York, 1976.
20. BLESSING, M. H., AND HORA, B. I.: Glomera in der lunge des menschen. Z. Zellforsch. Mikrosk. Anat. **87:** 562–570, 1968.
21. BOAT, T., AND KLEINERMAN, J.: Human respiratory tract secretions. II. Effect of cholinergic and adrenergic agents on in vitro release of protein and mucous glycoprotein. Chest **67:** 32s–34s, 1975.
22. BOAT, T. F., AND MATTHEWS, L. W.: Chemical composition of human tracheobronchial secretions. *In* Sputum: Fundamentals and Clinical Pathology, ed. by M. J. Dulfano, p. 243, C. C. Thomas, Springfield, Ill., 1973.
23. BOAT, T. F., POLONY, I., AND CHENG, P. W.: Mucin release from rabbit tracheal epithelium in response to sera from normal and cystic fibrosis subjects. Pediatr. Res. **16:** 792–797, 1982.
24. BOGART, B. I., CONOD, E. J., AND CONOVER, J. H.: The biologic activities of cystic fibrosis serum. I. The effects of cystic fibrosis sera and calcium ionophore A23187 on rabbit tracheal explants. Pediatr. Res. **11:** 131–134, 1977.
25. BORSON, D. B., CHARLIN, M., GOLD, B. D., AND NADEL, J. A.: Neural regulation of $^{35}SO_4$-macromolecule secretion from tracheal glands of ferrets. J. Appl. Physiol. **57:** 457–466, 1984.
26. BORSON, D. B., CHINN, R. A., DAVIS, B., AND NADEL, J. A.: Adrenergic and cholinergic nerves mediate fluid secretion from tracheal glands of ferrets. J. Appl. Physiol. **49:** 1027–1031, 1980.
27. BOUCHER, R. C., BROMBERG, P. A., AND GATZY, J. T.: Airway transepithelial electric potential in vivo: species and regional differences. J. Appl. Physiol. **48:** 169–176, 1980.
28. BOUCHER, R. C., STUTTS, M. J., AND GATZY, J. T.: Regional differences in bioelectric properties and ion flow in excised canine airways. J. Appl. Physiol. **51:** 706–714, 1981.
29. BREEZE, R. G., AND WHEELDON, E. B.: The cells of the pulmonary airways. Am. Rev. Respir. Dis. **116:** 705–777, 1977.
30. BREUER, R., CHRISTENSEN, T. G., LUCEY, E. C., STONE, P. J., AND SNIDER, G. L.: Quantitative study of secretory cell metaplasia induced by human neutrophil elastase in the large bronchi of hamsters. J. Lab. Clin Med. **105:** 635–640, 1985.
31. BURG, M., STONER, L., CARDINAL, J., AND GREEN, N.: Furosemide effect on isolated perfused tubules. Am. J. Physiol. **225:** 119–124, 1973.
32. CARUBELLI, R., SACHDEV, G. P., FOX, O. F., WEN, G., HORTON, F. O., AND ROGERS, R. M.: Chemical composition of pathological human tracheobronchial mucus collected by fiberoptic bronchoscopy. Adv. Exp. Med. Biol. **144:** 393–395, 1982.
33. CHAKRIN, L. W., BAKER, A. P., CHRISTIAN, P., AND WARDELL, J. R.: Effect of cholinergic stimulation on the release of macromolecules by canine trachea in vitro. Am. Rev. Respir. Dis. **108:** 69–76, 1973.
34. CHAKRIN, L. W., AND SAUNDERS, L. Z.: Experimental chronic bronchitis. Pathology of the dog. Lab. Invest. **30:** 145–154, 1974.
35. CHOW, C. W., LANDAU, L. I., AND TAUSSIG, L. M. Bronchial mucous glands in the newborn with cystic fibrosis. Eur. J. Pediatr. **139:** 240–243, 1982.
36. CLYDE, W. A., JR.: Experimental models for study of common respiratory viruses. Environ. Health Perspect. **35:** 107–112, 1980.
37. COLES, S.: Regulation of the secretory cycles of mucous and serous cells in the human bronchial gland. Adv. Exp. Med. Biol. **89:** 155–168, 1977.
38. COLES, S. J., BHASKAR, K. R., O'SULLIVAN, D. D., NEILL, K. H., AND REID, L. M.: Airway mucus: composition and regulation of its secretion by neuropeptides in vitro. Mucus and mucosa. Ciba Found. Symp. **109:** 40–60, 1984.
39. COLES, S. J., BHASKAR, K. R., O'SULLIVAN, D. D., AND REID, L. M.: Extracellular calcium ion depletion induces release of glycoproteins by canine trachea. Am. J. Physiol. **246:** C494–C501, 1984.
40. COLES, S. J., NEILL, K. H., REID, L. M., AUSTEN, K. F., NII, Y., COREY, E. J., AND LEWIS, R. A.: Effects of leukotrienes C4 and D4 on glycoprotein and lysozyme secretion by human bronchial mucosa. Prostaglandins **25:** 155–170, 1983.
41. COLES, S. J., AND REID, L.: Glycoprotein secretion in vitro by human airway: normal and chronic bronchitis. Exp. Mol. Pathol. **29:** 326–341, 1978.
42. COLLIER, A. M.: Injury of respiratory epithelium. Environ. Health Perspect. **35:** 83–88, 1980.
43. CORRALES, R. J., NADEL, J. A., AND WIDDICOMBE, J. H.: Source of the fluid component of secretions from tracheal submucosal glands in cats. J. Appl. Physiol. **56:** 1076–1082, 1984.
44. CULLUMBINE, H., AND DIRNHUBER, P.: Oral and bronchial fluids in poisoning with anticholinesterases. J. Pharm. Pharmacol. **7:** 580–585, 1955.
45. CUTZ, E., CHAN, W., WONG, V., AND CONEN, P. E.: Endocrine cells in rat fetal lungs. Lab. Invest. **30:** 458–464, 1974.
46. DAVIS, B., MARIN, M., FISCHER, S., GRAF, P., WIDDICOMBE, J., AND NADEL,

J.: New method for study of canine mucous gland secretion in vivo: cholinergic regulation. Am. Rev. Respir. Dis. **113:** 257, 1976.
47. DAVIS, B., MARIN, M. G., YEE, J. W., AND NADEL, J. A.: Effect of terbutaline on Cl- and Na+ fluxes across dog trachea in vitro. Am. Rev. Respir. Dis. **120:** 224–225, 1979.
48. DAVIS, B., AND NADEL, J. A.: New methods used to investigate the control of mucus secretion and ion transport in airways. Environ. Health Perspect. **35:** 121–130, 1980.
49. DAVIS, B., ROBERTS, A. M., COLERIDGE, H. M., AND COLERIDGE, J. C. G.: Reflex tracheal gland secretion evoked by stimulation of bronchial C-fibers in dogs. J. Appl. Physiol. **53:** 985–991, 1982.
50. DE POITIERS, W., LORD, P. W., BILES, B., AND WHIMSTER, W. F.: Bronchial gland histochemistry in lungs removed for cancer. Thorax **35:** 546–551, 1980.
51. DEY, R. D., SHANNON, W. A., AND SAID, S. I.: Localization of VIP-immunoreactive nerves in airways and pulmonary vessels of dogs, cats, and human subjects. Cell Tissue Res. **220:** 231–238, 1981.
52. DOUGLAS, A. N., LAMB, D., AND RUCKLEY, V. A.: Bronchial gland dimensions in coalminers: influence of smoking and dust exposure. Thorax **37:** 760–764, 1982.
53. EL-BERMANI, A. I., AND GRANT, M.: Acetylcholinesterase-positive nerves of the rhesus monkey bronchial tree. Thorax **30:** 162–170, 1975.
54. ELLEFSEN, P., AND TOS, M.: Goblet cells in the human trachea. Arch. Otolaryngol. **95:** 547–555, 1972.
55. ELMES, P. C., AND BELL, D.: The effects of chlorine gas on the lungs of rats with spontaneous pulmonary disease. J. Pathol. Bacteriol. **86:** 317–326, 1963.
56. ERICSON, L. E., HAKANSON, R., LARSON, B., OWMAN, C., AND SUNDLER, F.: Fluorescence and electron microscopy of amine-storing enterochromaffin like cells in tracheal epithelium of mouse. Z. Zellforsch. Mikrosk. Anat. **124:** 532–545, 1972.
57. ESTEP, J. A., ZORN, J. P., AND MARIN, M. G.: Effect of Cl-, Na^+, and amiloride on the electrical properties of dog tracheal epithelium, in vitro. Am. Rev. Respir. Dis. **126:** 681–685, 1982.
58. FELDHOFF, P. A., BHAVANANDAN, V. P., AND DAVIDSON, E. A.: Purification, properties, and analysis of human asthmatic bronchial mucin. Biochemistry **18:** 2430–2436, 1979.
59. FLOREY, H., CARLETON, H. M., AND WELLS, A. Q.: Mucus secretion in the trachea. Br. J. Exp. Pathol. **13:** 269–284, 1932.
60. FRASCA, J. M., AUERBACH, O., PARKS, V. R., AND JAMIESON, J. D.: Electron microscopic observations of the bronchial epithelium of dogs. Exp. Mol. Pathol. **9:** 363–379, 1968.
61. FREEMAN, G., AND HAYDON, G. B.: Emphysema after low-level exposure to NO_2. Arch. Environ. Health **8:** 125–128, 1964.
62. FREEMAN, G., STEPHENS, R. J., COFFIN, D., AND STARA, J. F.: Changes in dogs' lungs after long-term exposure to ozone. Arch. Environ. Health **26:** 209–216, 1973.
63. FRIZZELL, R. A., WELSH, M. J., AND SMITH, P. L.: Hormonal control of chloride secretion by canine tracheal epithelium: an electrophysiologic analysis. Ann. NY Acad. Sci. **372:** 558–570, 1981.
64. FROHLICH, F.: Die "Helle Zell" der bronchialschleimhaut und ihre beziehungen zum problem der chemoreceptoren. Frankf. Z. Pathol. **60:** 517–559, 1949.
65. GALLAGHER, J. T., KENT, P. W., PASSATORE, M., PHIPPS, R. J., RICHARDSON, P. S., AND LAMB, D.: The composition of tracheal mucus and the nervous control of its secretion in the cat. Proc. R. Soc. Lond. Biol. Sci. **192:** 49–76, 1975.
66. GATTO, L. A., AND AIELLO, E.: Mucus-secreting glands and goblet cells in the trachea of the deer mouse, *Peromyscus leucopus*. Trans. Am. Microsc. Soc. **100:** 355–365, 1981.
67. GERMAN, V. F., CORRALES, R., UEKI, I. F., AND NADEL, J. A.: Reflex stimulation of tracheal mucus gland secretion by gastric irritation in cats. J. Appl. Physiol. **52:** 1153–1155, 1982.
68. GOCO, R. V., KRESS, M. B., AND BRANTIGAN, O. C.: Comparison of mucus glands in the tracheobronchial tree of man and animals. Ann. NY Acad. Sci. **106:** 555–571, 1963.
69. GOLDRING, I. R., GREENBURG, L., PARK, S. S., AND RATNER, I. M.: Pulmonary effects of sulfur dioxide exposure in the Syrian hamster. II. Combined with emphysema. Arch. Environ. Health **21:** 32–37, 1970.
70. HAGE, E.: Electron microscopy identification of several types of endocrine cells in the bronchial epithelium of human foetuses. Z. Zellforsch. Mikrosk. Anat. **141:** 401–412, 1973.
71. HAMA, K., AND NAGATA, F.: A stereoscope observation of tracheal epithelium of mouse by means of the high voltage electron microscope. J. Cell Biol. **45:** 654–659, 1970.
72. HANSELL, M. M., AND MORETTI, R. L.: Ultrastructure of the mouse tracheal epithelium. J. Morphol. **128:** 159–163, 1969.
73. HARTMANN, J. F., HUTCHISON, C. F., AND JEWELL, M. E.: Pig bronchial mucous membrane: a model system for assessing respiratory mucus release in vitro. Exp. Lung Res. **6:** 59–70, 1984.
74. HOLTZMAN, M. J., AIZAWA, H., NADEL, J. A., AND GOETZL, E. J.: Selective generation of leukotriene B4 by tracheal epithelial cells from dogs. Biochem. Biophys. Res. Commun. **114:** 1071–1076, 1983.
75. HU, P. C., KIRTZ, J. M., GARDNER, D. E., AND POWELL, D. A.: Experimental infection of the respiratory tract with *Mycoplasma pneumoniae*. Environ. Health Perspect. **35:** 101–106, 1980.
76. HUMPHREYS, M. H.: Inhibition of NaCl absorption from perfused rat ileum by furosemide. Am. J. Physiol. **230:** 1517–1523, 1976.
77. HUNG, K. S., HERTWECK, S., HARDY, J. D., AND LOOSLI, C. G.: Ultrastructure of nerves and associated cells in bronchiolar epithelium of the mouse lung. J. Ultrastruct. Res. **43:** 426–437, 1973.
78. INOUE, S., AND HOGG, J. C.: Intercellular junctions of the tracheal epithelium in guinea pigs. Lab. Invest. **31:** 68–74, 1974.
79. JEFFERY, P. K., AYERS, M., AND ROGERS, D.: The mechanisms and control of bronchial mucous cell hyperplasia. Adv. Exp. Med. Biol. **144:** 399–409, 1982.
80. JEFFERY, P. K., AND REID, L.: New observations of rat airway epithelium: a quantitative and electron microscopic study. J. Anat. **120:** 295–320, 1975.
81. JEFFERY, P. K., AND REID, L. M.: The effect of tobacco smoke, with or without phenylmethyloxadiazole (PMO), on rat bronchial epithelium: a light and electron microscopic study. J. Pathol. **133:** 341–359, 1981.
82. JONES, R., AND REID, L.: Beta-agonists and secretory cell number and intracellular glycoprotein in airway epithelium. Am. J. Pathol. **95:** 407–421, 1979.
83. KARPICK, R. J., PRATT, P. C., AND ASMUNDSSON, T.: Pathological findings in respiratory failure. Ann. Intern. Med. **72:** 189–197, 1970.
84. KILBURN, K. H.: A hypothesis for pulmonary clearance and its implications. Am. Rev. Respir. Dis. **98:** 449–463, 1968.
85. KING, A. S., MCLELLAND, J., COOK, R. D., KING, D. Z., AND WALSH, C.: The ultrastructure of afferent nerve endings in the avian lung. Respir. Physiol. **22:** 21–40, 1974.
86. KNOWLES, M., GATZY, J., AND BOUCHER, R.: Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. N. Engl. J. Med. **305:** 1489–1495, 1981.
87. KNOWLES, M. R., BUNTIN, W. H., BROMBERG, P. A., GATZY, J. T., AND BOUCHER, R. C.: Measurements of transepithelial potential differences in the trachea and bronchi of human subjects in vivo. Am. Rev. Respir. Dis. **126:** 108–112, 1982.
88. KNOWLES, M. R., STUTTS, M. J., SPOCK, A., FISCHER, N., GATZY, J. T., AND BOUCHER, R. C.: Abnormal ion permeation through cystic fibrosis respiratory epithelium. Science (Wash. DC) **221:** 1067–1069, 1983.
89. KOKIN, P.: Uber die secretorischen Nerven der Kehlkopf- und Luftrohrenschleim-drusen. Pfluegers Arch. Gesamte Physiol. Menschen Tiere **63:** 622–630, 1896.
90. LAMB, D., AND REID, L.: Mitotic rates, goblet cell increase, and histochemical changes in mucus in rat bronchial epithelium during exposure to sulphur dioxide. J. Pathol. Bacteriol. **96:** 97–111, 1968.
90a. LANGRIDGE-SMITH, J. E., RAO, M. C., AND FIELD, M.: Chloride and sodium transport across bovine tracheal epithelium: effects of secretagogues and indomethacin. Pflugers Arch. Eur. J. Physiol. **402:** 42–47, 1984.
91. LARSELL, O.: The ganglia, plexuses, and nerve-terminations of the mammalian lung and pleura pulmonalis. J. Comp. Neurol. **35:** 97–132, 1923.
92. LAST, J. A., AND KAIZU, T.: Mucus glycoprotein secretion by tracheal explants: effects of pollutants. Environ. Health Perspect. **35:** 131–138, 1980.
93. LAUWERYNS, J. M., AND COKELAERE, M.: Hypoxia-sensitive neuro-epithelial bodies intrapulmonary secretory neuroreceptors, modulated by the CNS. Z. Zellforsch. Mikrosk. Anat. **145:** 521–540, 1973.
94. LAUWERYNS, J. M., COKELAERE, M., AND THEUNYNCK, P.: Neuro-epithelial bodies in the respiratory mucosa of various mammals. Z. Zellforsch. Mikrosk. Anat. **135:** 569–592, 1972.
95. LAUWERYNS, J. M., AND GODDEERIS, P.: Neuroepithelial bodies in the human child and adult lung. Am. Rev. Respir. Dis. **111:** 469–476, 1975.
96. LAUWERYNS, J. M., AND PEUSKENS, J. C.: Argyrophil (kinin and amine producing?) cells in human infant airway epithelium. Life Sci. **8:** 577–585, 1969.
97. LAUWERYNS, J. M., AND PEUSKENS, J. C.: Neuro-epithelial bodies (neuroreceptor or secretory organs?) in human infant bronchial and bronchiolar epithelium. Anat. Rec. **172:** 471–482, 1971.
98. LEV, R., AND SPICER, S. S.: A histochemical comparison of human epithelial mucins in normal and in hypersecretory states including pancreatic cystic fibrosis. Arch. Biochem. Biophys. **177:** 23–47, 1976.
99. LIEDTKE, C. M., BOAT, T. F., AND RUDOLPH, S. A.: Neurohormonal receptors and cyclic AMP-binding proteins in rabbit. Biochim. Biophys. Acta **719:** 169–177, 1982.
100. LIEDTKE, C. M., RUDOLPH, S. A., AND BOAT, T. F.: Beta-adrenergic modulation of mucin secretion in cat trachea. Am. J. Physiol. **244:** C391–C398, 1983.
101. LOPEZ-VIDRIERO, M. T., AND REID, L.: Bronchial mucus in health and disease. Br. Med. Bull. **34:** 63–74, 1978.
102. LUMSDEN, A. B., MCLEAN, A., AND LAMB, D.: Goblet and clara cells of human distal airways: evidence for smoking induced changes in their numbers. Thorax **39:** 844–849, 1984.
103. MANN, S. P.: The innervation of mammalian bronchial smooth muscle: the localization of catecholamines and cholinesterases, Histochem. J. **3:** 319–331, 1971.
104. Marin, M. G., and Culp, D. J.: Isolation and culture of submucosal gland cells. Clin. Chest Med. **7:** 239–245, 1986.
105. MARIN, M. G., DAVIS, B., AND NADEL, J. A.: Effect of acetylcholine on Cl–

and Na+ fluxes across dog tracheal epithelium in vitro. Am. J. Physiol. **231:** 1546–1549, 1976.
106. MARIN, M. G., DAVIS, B., AND NADEL, J. A.: Effect of histamine on electrical and ion transport properties of tracheal epithelium. J. Appl. Physiol **42:** 735–738, 1977.
107. MARIN, M. G., ESTEP, J. A., AND ZORN, J. P.: Effect of calcium on sulfated mucous glycoprotein secretion in dog trachea. J. Appl. Physiol. **52:** 198–205, 1982.
108. MARIN, M. G., AND ZAREMBA, M. M.: Effect of calcium on ion transport and electrical properties of tracheal epithelium. J. Appl. Physiol. **44:** 900–904, 1978.
109. MARIN, M. G., AND ZAREMBA, M. M.: Interdependence of Na^+ and Cl- transport in dog tracheal epithelium. J. Appl. Physiol. **47:** 598–603, 1979.
110. MAROM, Z., SHELHAMER, J. H., AND KALINER, M.: Effects of arachidonic acid, monohydroxyeicosatetraenoic acid, and prostaglandins on the release of mucous glycoproteins from human airways in vitro. J. Clin. Invest. **67:** 1695–1702, 1981.
111. MARSAN, C., CAVA, E., LOUGNON, J., AND ROUJEAU, J.: Cytochemical and histochemical characterization of epithelial mucins in human bronchi. Acta Cytol. (Baltimore) **22:** 562–565, 1978.
112. MCDOWELL, E. M., COMBS, J. W., AND NEWKIRK, C.: Changes in secretory cells of hamster tracheal epithelium in response to acute sublethal injury: a quantitative study. Exp. Lung Res. **4:** 227–243, 1983.
112a. MELON, J.: Activite secretoire de la muqueuse nasale. Acta Oto-rhino-laryngol. Belg. **22:** 11–244, 1968.
113. MEYRICK, B., STURGESS, J. M., AND REID, L.: A reconstruction of the duct system and secretory tubules of the human bronchial submucosal gland. Thorax **24:** 729–736, 1969.
114. MIAN, N., ANDERSON, C. E., POPE, A. J., SMITH, A. R., RICHARDSON, P. S., BALFRE, K., AND KENT, P. W.: Directional Ca^{2+} effect on stimulation of mucin secretion from chicken trachea in vitro. Biochem. J. **208:** 425–433, 1982.
115. MOOSAVI, H., SMITH, P., AND HEATH, D.: The feyrter cell in hypoxia. Thorax **28:** 729–741, 1973.
116. MORIARTY, C. M., GARETT, J. S., AND FONTAINE, M.: Effect of normal and cystic fibrotic serum on ion transport and mucus glycoprotein secretion from the isolated rabbit trachea. J. Med. **13:** 257–273, 1982.
117. MURLAS, C., NADEL, J. A., AND BASBAUM, C. B.: A morphometric analysis of the autonomic innervation of cat tracheal glands. J. Auton. Nerv. Syst. **2:** 23–37, 1980.
118. MURPHY, G. F., BRODY, A. R., AND CRAIGHEAD, J. E.: Exfoliation of respiratory epithelium in hamster tracheal organ cultures infected with *Mycoplasma pneumoniae*. Virchows Arch. A Pathol. Anat. Histol. **389:** 93–102, 1980.
119. NADEL, J. A.: Neural control of airway submucosal gland secretion. Eur. J. Respir. Dis. **64:** 322–326, 1983.
120. NADEL, J. A., AND DAVIS, B.: Autonomic regulation of mucus secretion and ion transport in airways. *In* Asthma, pp. 197–209, Academic Press, New York, 1977.
121. Nadel, J. A., and Davis, B.: Parasympathetic and sympathetic regulation of secretion from submucosal glands in airways. Fed. Proc. **39:** 3075–3079, 1980.
122. NEWHOUSE, M., SANCHIS, J., AND BIENENSTOCK, J.: Lung defense mechanisms. N. Engl. J. Med. **295:** 990–998, 1976.
123. NYGREN, H., LANGE, S., AND LONNROTH, I.: Development of mucous cells in mouse intrapulmonary airways induced by cholera toxin, dibutyryl cyclic AMP, and prostaglandin E1. Br. J. Exp. Pathol. **65:** 549–556, 1984.
124. OLVER, R. E., DAVIS, B., MARIN, M. G., AND NADEL, J. A.: Active transport of Na^+ and Cl- across the canine tracheal epithelium in vitro. Am. Rev. Respir. Dis. **112:** 811–815, 1975.
125. PACK, R. J., AL-UGAILY, L. H., AND MORRIS, G.: The cells of the tracheobronchial epithelium of the mouse: a quantitative light and electron microscope study. J. Anat. **132:** 71–84, 1981.
126. PACK, R. J., AL-UGAILY, L. H., MORRIS, G., AND WIDDICOMBE, J. G.: The distribution and structure of cells in the tracheal epithelium of the mouse. Cell Tissue Res. **208:** 65–84, 1980.
127. PEATFIELD, A. C., BARNES, P. J., BRATCHER, C., NADEL, J. A., AND DAVIS, B.: Vasoactive intestinal peptide stimulates tracheal submucosal gland secretion in ferret. Am. Rev. Respir. Dis. **128:** 89–93, 1983.
128. PEATFIELD, A. C., PIPER, P. J., AND RICHARDSON, P. S.: The effect of leukotriene C_4 on mucin release into the cat trachea in vivo and in vitro. Br. J. Pharmacol. **77:** 391–393, 1982.
129. PEATFIELD, A. C., AND RICHARDSON, P. S.: the action of dust in the airways on secretion into the trachea of the cat. J. Physiol. **342:** 327–334, 1983.
130. PHIPPS, R. J., DENAS, S. M., AND WANNER, A.: Antigen stimulates glycoprotein secretion and alters ion fluxes in sheep trachea. J. Appl. Physiol. **55:** 1593–1602, 1983.
131. PHIPPS, R. J., NADEL, J. A., AND DAVIS, B.: Effect of alpha-adrenergic stimulation on mucus secretion and on ion transport in cat trachea in vitro. Am. Rev. Respir. Dis. **121:** 359–365, 1980.
132. QUINTON, P. M.: Composition and control of secretions from tracheal bronchial submucosal glands. Nature (Lond.) **279:** 551–552, 1979.
133. QUINTON, P. M.: Possible mechanisms of stimulus-induced vacuolation in serous cells of tracheal secretory glands. Am. J. Physiol. **241:** C25–C32, 1981.
134. REID, L., BHASKAR, K., AND COLES, S. Clinical aspects of respiratory mucus. Adv. Exp. Med. Biol. **144:** 369–391, 1982.
135. REID, L., AND JONES, R.: Bronchial mucosal cells. Fed. Proc. **38:** 191–196, 1979.
136. REID, L. M.: Secretory cells. Fed. Proc. **36:** 2703–2707, 1977.
137. RHODIN, J., AND DALHAMN, T.: Electron microscopy of the tracheal ciliated mucosa in rat. Z. Zellforsch. Mikrosk. Anat. **44:** 345–412, 1956.
138. RHODIN, J. A. G.: The ciliated cell: ultrastructure and function of the human tracheal mucosa. Am. Rev. Respir. Dis. **93:** 1–15, 1966.
139. RICH, B., PEATFIELD, A. C., WILLIAMS, I. P., AND RICHARDSON, P. S.: Effects of prostaglandins E1, E2, and F2alpha on mucin secretion from human bronchi in vitro. Thorax **39:** 420–423, 1984.
140. RICHARDSON, J. B.: Nerve supply to the lungs. Am. Rev. Respir. Dis. **119:** 785–802, 1979.
141. RICHARDSON, P. S., PHIPPS, R. J., BALFRE, K., AND HALL, R. L.: The roles of mediators, irritants, and allergens in causing mucin secretion from the trachea. Control of tracheal mucin secretion. Ciba Found. Symp. **54:** 111–131, 1978.
142. RUDICK, V. L., WOOTEN, M. W., AND RUDICK, M. J.: Secretory activity of hamster tracheal explants and isolated tracheal epithelial cells and the effects of cystic fibrosis serum. J. Cell. Physiol. **118:** 67–78, 1984.
143. SCHULMAN, E. S., NEWBALL, H. H., DEMERS, L. M., FITZPATRICK, F. A., AND ADKINSON, N. F., JR.: Anaphylactic release of thromboxane A2, prostaglandin D2, and prostacyclin from human lung parenchyma. Am. Rev. Respir. Dis. **124:** 402–406, 1981.
144. SHELHAMER, J. H., MAROM, Z., AND KALINER, M.: Immunologic and neuropharmacologic stimulation of mucous glycoprotein release from human airways in vitro. J. Clin. Invest. **66:** 1400–1408, 1980.
145. SHELHAMER, J. H., MAROM, Z., SUN, F., BACH, M. K., AND KALINER, M.: The effects of arachinoids and leukotrienes on the release of mucus from human airways. Chest **81:** 36S–37S, 1982.
146. SHOROFSKY, S. R., FIELD, M., AND FOZZARD, H. A.: Electrophysiology of Cl secretion in canine trachea. J. Membr. Biol. **72:** 105–115, 1983.
146a. SHOROFSKY, S. R., FIELD, M., AND FOZZARD, H. A.: Mechanism of Cl secretion in canine trachea: changes in intracellular chloride activity with secretion. J. Membr. Biol. **81:** 1–8, 1984.
147. SHUL'GA, M. I.: Islet receptors of the lungs. Arkh. Anat. Gistol. Embriol. **47:** 87–89, 1964.
148. SMITH, P. L., WELSH, M. J., AND FRIZZELL, R. A.: Effects of agents which mediate chloride secretion on cAMP content on tracheal epithelium. J. Gen. Physiol. **76:** 26a, 1980.
149. SPENCER, H.: Pulmonary diseases of uncertain aetiology. *In* Pathology of the Lung, p. 566, Macmillan Company, New York, 1962.
150. SPICER, S. S., CHAKRIN, L. W., AND WARDELL, J. R., JR.: Respiratory mucous secretion. *In* Sputum Fundamentals and Clinical Pathology, ed. by M. J. Dulfano, chap. 11, pp. 22–68, C. C. Thomas, Springfield, IL, 1974.
151. STURGESS, J., AND IMRIE, J.: Quantitative evaluation of the development of tracheal submucosal glands in infants with cystic fibrosis and control infants. Am. J. Pathol. **106:** 303–311, 1982.
152. STURGESS, J., AND REID, L.: An organ culture study of the effect of drugs on the secretory activity of the human bronchial submucosal gland. Clin. Sci. **43:** 533–543, 1972.
153. STURGESS, J., AND REID, L.: The effect of isoprenaline and pilocarpine on (*a*) bronchial mucus-secreting tissue and (*b*) pancreas, salivary glands, heart, thymus, liver, and spleen. Br. J. Exp. Pathol. **54:** 388–403, 1973.
154. SWEET, C., MACARTNEY, J. C., BIRD, R. A., CAVANAGH, D., COLLIE, M. H., HUSSEINI, R. H., AND SMITH, H.: Differential distribution of virus and histological damage in the lower respiratory tract of ferrets infected with influenza viruses of differing virulence. J. Gen. Virol. **54:** 103–114, 1981.
155. THURLBECK, W. M., MALAKA, D., AND MURPHY, K.: Goblet cells in the peripheral airways in chronic bronchitis. Am. Rev. Respir. Dis. **112:** 65–69, 1975.
156. TOM-MOY, M., BASBAUM, C. B., AND NADEL, J. A.: Localization and release of lysozyme from ferret trachea: effects of adrenergic and cholinergic drugs. Cell Tissue Res. **228:** 549–562, 1983.
157. TOS, M.: Distribution of mucus producing elements in the respiratory tract. Differences between upper and lower airways. Eur. J. Respir. Dis. **64:** 269–279, 1983.
158. TOS, M., AND MOLLER, K.: Goblet-cell density in human bronchus in chronic bronchitis. Arch. Otolaryngol. **109:** 673–676, 1983.
159. UDDMAN, R., ALUMETS, J., DENSERT, O., HAKANSON, R., AND SUNDLER, F.: Occurrence and distribution of VIP nerves in the nasal mucosa and tracheobronchial wall. Acta Oto-laryngol. **86:** 443–448, 1978.
160. UEKI, I., GERMAN, V. F., AND NADEL, J. A.: Micropipette measurement of airway submucosal gland secretion. Autonomic effects. Am. Rev. Respir. Dis. **121:** 351–357, 1980.
160a. USSING, H. H., AND ZERAHN, K.: Active transport of sodium as the source of electrical current in the short-circuited isolated frog skin. Acta Physiol. Scand. **23:** 110–127, 1951.
161. VIDIC, B., KAPUR, S. P., AND JENKINS, D. P.: Estrogen and tracheal secretion: the effect of estrogen on the epithelial secretory cells of rat trachea. Cytobiologie **18:** 10–21, 1978.
162. VULLIEMIN, P., DURAND-ARCZYNSKA, W., AND DURAND, J.: Electrical properties and electrolyte transport in bovine tracheal epithelium: effects

of ion substitutions, transport inhibitors, and histamine. Pflugers Arch. Eur. J. Physiol. **396:** 54–59, 1983.
163. WATSON, J. H. L., AND BRINKMAN, G. L.: Electron microscopy of the epithelial cells of normal and bronchitic human bronchus. Am. Rev. Respir. Dis. **90:** 851–866, 1964.
164. WELSH, M. J.: Intracellular potassium activities in canine tracheal epithelium. Am. J. Physiol. **245:** C248–C254, 1983.
165. WELSH, M. J.: Evidence for basolateral membrane potassium conductance in canine tracheal epithelium. Am. J. Physiol. **244:** C377–C384, 1983.
166. WELSH, M. J.: Barium inhibition of basolateral membrane potassium conductance in tracheal epithelium. Am. J. Physiol. **244:** F639–F645, 1983.
167. WELSH, M. J.: Cigarette smoke inhibition of ion transport in canine tracheal epithelium. J. Clin. Invest. **71:** 1614–1623, 1983.
168. WELSH, M. J.: Inhibition of chloride secretion by furosemide in canine tracheal epithelium. J. Membr. Biol. **71:** 219–226, 1983.
169. WELSH, M. J., SMITH, P. L., AND FRIZZELL, R. A.: Chloride secretion by canine tracheal epithelium. III. Membrane resistances and electromotive forces. J. Membr. Biol. **71:** 209–218, 1983.
170. WELSH, M. J., AND WIDDICOMBE, J. H.: Pathways of ion movement in the canine tracheal epithelium. Am. J. Physiol. **239:** F215–F221, 1980.
171. WELSH, M. J., WIDDICOMBE, J. H., AND NADEL, J. A.: Fluid transport across the canine tracheal epithelium. J. Appl. Physiol. **49:** 905–909, 1980.
172. WHIMSTER, W. F., AND REID, L.: The influence of dibutyryl cyclic adenosine monophosphate and other substances on human bronchial mucous gland discharge. Exp. Mol. Pathol. **18:** 234–240, 1973.
173. WIDDICOMBE, J. H., BASBAUM, C. B., AND YEE, J. Y.: Localization of Na pumps in the tracheal epithelium of the dog. J. Cell Biol. **82:** 380–390, 1979.
173a. Widdicombe, J. H., Nathanson, I. T., and Highland, E.: Effects of "loop" diuretics on ion transport by dog tracheal epithelium. Am. J. Physiol. **245:** C388–C396, 1983.
174. WIDDICOMBE, J. H., UEKI, I. F., BRUDERMAN, I., AND NADEL, J. A.: The effects of sodium substitution and ouabain on ion transport by dog tracheal epithelium. Am. Rev. Respir. Dis. **120:** 385–392, 1979.
175. YAMATAKE, Y., AND YANAURA, S.: New method for evaluating bronchomotor and bronchosecretory activities effects of prostaglandins and antigen. Jpn. J. Pharmacol. **28:** 391–402, 1978.