Ancillary (Nonantimicrobial) Therapy in the Treatment of Bovine Respiratory Disease

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The respiratory system of mammals plays a critical role in external respiration; the exchange of gases between the ambient atmosphere and the blood. In addition to this function, the respiratory system is significantly involved in body temperature control, vocalization, immunity to inhaled antigens, antimicrobial action, detoxication, olfaction and a number of important metalbolic and endocrine functions. In considerations of the pathogenesis of respiratory disease and in the planning of therapeutic regimens, maintaining the primary function of gas exchange is of critical importance, but the effects on other respiratory functions must also be considered. In this review, therapeutic intervention in bovine respiratory disease will be reviewed with the goal of selecting therapetic agents that optimze respiratory and tissue defense mechanisms and minimizing damage produced by infectious agents or tissue reactions to these organisms.

Respiratory Mucus

The mucous coat of the respiratory tract serves an important filtration function. Operating as a gas scrubber, the surface mucus entraps particulate matter in excess of about 2 micron in diameter, preventing its passage from the environment to the alveolar structures involved in gas exchange. The ciliary action of respiratory epithelium results in a continuous movement of the surface mucous coat and its contained materials to the pharynx where these materials may be coughed out or swallowed. This mucous escalator function of the respiratory system is an extremely important defense mechanism against drying of respiratory surfaces and the invasion of respiratory tissue by microorganisms. The maintenance of a normal mucus coat is important in assuring the integrity of this defense against inhaled microorganisms.

Mucus secretions are produced by mixed serous and mucus submucosal glands and several surface epithelial cells, including goblet cells, Clara cells, serous cells and ciliated epithelial cells. These glands and cells produce glycoproteins mixed with other proteins, water and electrolytes, at an optimal pH for the maintenance of mucus integrity. In bovine respiratory disease, alterations in pH, electrolyte and content and in the type of mucin produced results in increased viscosity of respiratory mucus and interferes with the ability of the mucus escalator to effectively move mucus along the epithelial surface.

The respiratory mucous coat is organized into two layers, a surface sol layer, containing a predominance of electrolytes and water, and an overlying gel layer, containing higher concentrations of mucin and lower concentrations of water and electrolytes. The cilia of respiratory epithelia lie within the sol layer in which they move in an organized beat of from 2 to 20 Hz. As cilia beat, they are synchronized so that waves of beats occur across the epithelial surface. The ciliary tips move in such a way that, as they beat toward the pharynx, their tips enter the gel layer. The effectiveness of ciliary beating depends upon normal thickness of the sol layer, neither too thin for cilia to move, nor too thick for their tips to enter the gel layer. In order that cilia move the gel layer, the viscosity of this layer must not be too low, and thus removing friction between the ciliary tips and the layer, nor too high, providing excess friction to mucus movement.

In respiratory disease, the mucus coat is generally dehydrated, (Boat and Cheng, 1980) resulting in thinning of the sol layer, diminishing the ability of cilia to beat, and in increased viscosity of the gel layer and in turn the work of moving mucus. In bovine respiratory disease, there is likely to be large quantities of fibrin in mucus as it is transported from the bronchi. Fibrin adds significantly to the viscosity and elasticity of mucus. Treatments aimed at optimizing the mucous escalator have been directed toward alteration of mucus production and character and enhancing the activity of ciliary action.

Excessive respiratory mucous glycoprotein secretion is a cause of morbidity in a variety of respiratory diseases (Lundgren, et al. 1988). Mechanisms possibly responsible for excess mucous glycoprotein production include the secretory actions of neurohormones (Boat and Kleinerman, 1975; Shelhamer, et al. 1980; Gallagher, et al. 1975), mediators of hypersensitivity (including arachidonic acid metabolites) (Marom, et al. 1984). A variety of lipoxygenase pathway metabolites have been identified in inflamed respiratory epithelia including the peptide leukotrienes (LTC4 and LTD4, or slow reacting substance of anaphylaxis), and monohydroxy derivatives of

eicosatetraenoic acids (5-,9-,11- 12 and 15 HETE), which have powerful stimulatory effects on respiratory glycocongugate secretions (Maron, et al. 1984, 1983, 1982; Hirato, et al. 1980). *Dexamethasone* has been demonstrated to inhibit respiratory glycocojugate secretion in the cat. The mechanism of action appears to be similar to that through which glucocorticoids act as anti-inflammatory agents, involving the synthesis of lipocotins, inhibiting the synthesis of arachidonic acid (Lundgren et al. 1988).

Mucolytic therapy

The use of mucolytic therapy is aimed at decreasing the viscosity of mucous in order to enhance the effectiveness of the mucous escalator. These agents act on mucus glycoproteins, the major determinants of the viscosity of mucus.

Mucus glycoproteins are synthesized within cells of the submucosal glands and by goblet cells, with little contribution from other surface epithelial cells. Under ordinary circumstances there are no mucous secreting cells in small bronchioles, but during chronic inflammation, goblet cell metaplasia occurs, and glycoprotein secretion can occur at near the terminal bronchioles. The glycoproteins produced by the respiratory system are similar to those produced by other mucus producing surfaces. Respiratory glycoproteins resemble a bottle brush in their structure, with a straight peptide backbone, with bristle-like oligosaccharide side chains radiating from the peptide; and with a non glycosylated "handle." The oligosaccharides are attached to the peptide through O-glycosidic linkages between N-acetyl-galactosamine of the saccharides and threonine and serine of the peptide. A single peptide chain constitutes up to 20% of the molecular weight of the molecule (Boat, et al. 1976; Rousel, et al. 1978), but the physical size of the molecule is determined by the saccharide side chains (Rousel et al. 1975). The oligosaccharides resemble those of blood group substances to the degree that mucous inhibition of blood group hemagglutination can be used as an indicator for the presence of tracheobronchial mucous glycoproteins (Boat et al., 1976).

The viscosity and elasticity of tracheobroncial mucus is primarily due to polymerization an aggregation of glycoproteins. Polymerization takes place through the formation of disulfide bonds involving cysteine, a plentiful amino acid of respiratory glycoproteins. Aggregation of mucus glycoproteins by ionic and sugar-sugar interactions, forms a gel matrix (Hill et al., 1977). The ability to polymerize and aggregate is dependent upon glycoprotein concentation, which increases in dehydration (Shih et al., 1977), increased salt concentration and decreased pH (Lutz, et al., 1973).

A large number of mucolytic substancss have been used in the past, most of which has been ineffective in decreasing mucus viscosity. *N-acetylcysteine* (Mucomyst) and *S-carboxymethylcysteine* are effective in depolymerizing mucus and also act on DNA, the component of pus that is responsible for its viscosity. These substances act through free sulfhydryl groups which effectively open the disulfide bonds of mucous glycoproteins. The mucolytic action is most effective at neutral to basic pH's and are ineffective in acid environments. As the pH of respiratory fluids during infections is generally acid, the effectiveness of these products is questionable. Neither agent is effective against fibrin, which may contribute significantly to mucus viscosity in bovine respiratory disease.

Both mucolytic agents must be administered in aerosols, and are therefore not practical for administration to most adult animals. N-acetylcysteine inactivates penicillin and initiates bronchospasm, thus interfering with antibacterial therapy and enhancing histamine induced respiratory distress. There is little evidence that mucolytic agents are effective in enhancing the action of the mucous escalator.

Steam

Steam has often been used as means of adding water to mucus, and thus decreasing its viscosity. As air is inhaled, it is normally heated to body temperature and saturated with water before it reaches the nasopharynx. In open mouth breathing the air may reach the trachea before it acquires these conditions, but in any case the addition of heat and water to inhaled air does little to alter the environment of the respiratory tract.

Enzymes

Enzyme therapy has often been applied, either in aerosols or systemically, to alter the viscosity of respiratory mucus. Deoxyribonuclease, streptokinase, streptodornase and trypsin have been recommended for this purpose. These agents can effectively diminish the viscosity of mucus and may be effective in reducing the viscosity of DNA originating from purulence. The peptide chains of tracheobronchial mucus contains glycosylated and nonglycosylated regions (Roberts, 1976). Nonglycosylated regions are susceptible to digestion by enzymes. These regions contain most of the cysteine residues that are responsible for polymerization (Seawen and Allen, 1977). The effectiveness of enzyme therapy to reduce mucus viscosity has not been tested. The fact that highly viscous mucus often is seen in purulent infections, in which there are large concentrations of proteolytic enzymes suggests limited effectiveness of therapeutic enzymes.

The use of plasminogen as a fibrinolytic agent in respiratory mucus has not been suggested. Plasminogen, however, when activated by plasminogen activating enzyme, which is plentiful in inflammatory tissues, would be effective in breaking down fibrin that is present in the

mucus of animals with infectious respiratory disease.

The thickness of the mucous coat leads one to assume that any agent administered by aerosol will likely have only a surface action. Even if these materials are highly water soluble, they would be markedly diluted in the mucus of the respiratory tract and would have to be administered in high concentrations to be effective.

Autonomic drugs in mucous alteration

The secretion of mucus in airway infections may at least in part be mediated through the autonomic nervous system (Nadel and Davis,1980). Both cholinergic and alpha and beta adrenergic agonists stimulate mucous secretion in normal animals (Gallagher, et al., 1975). Electrical stimulation of the vagus nerves enhance mucin secretion (Ueki, et al. 1980). Electron microscopic studies indicate that both cholinergic and adrenergic axons are located in the vicinity of both serous and mucous cells of submucosal glands (Murlas et al., 1979; Nadel et al., 1985).

The watery content of airway secretions is a critical factor in the effectiveness of mucociliary clearance. The degree of hydration of mucus determines its rheological properties and the depth of the sol layer determines the effectiveness of the cilia. Water movement across the respiratory epithelium involves the active transport mechanisms through which a Cl- rich fluid is secreted (Olver, et al., 1975; Frizzell, et al., 1981; Widdicombe and Welsh, 1980). In ruminants (sheep), this secretion occurs in tracheal epithelium without any apparent stimulus (Boucher, et al., 1981, 1982). Epithelial cells of bronchial passages can be induced to secrete through autonomic stimulation (Boucher and Gatzy, 1981, 1982). Chloride mediated water secretion involves the activity of a Na^+-K^+-ATPase located on the basilateral surface of epithelial cells. In respiratory cells, Na enters the luminal surface and Cl enters the basilateral surface of epithelial cells and the Na is pumped out the basilateral surface of the cell, while the Cl diffuses across the luminal surface. The ATPase, providing energy to drive this secretion is blocked by cardiac glycosides (such as digitalis alkaloids) (Westenfelder et al., 1980; Widdicombe, et al., 1979). Amiloride, a drug that blocks receptors for aldosterone, stimulates Cl induced water secretion in the tracheobronchial tree. Aldosterone, however, apparently does not affect water and ion secretion of the tracheobronchial system (Boucher, et al 1982).

Cholinergic stimulation is quite effective in stimulating fluid secretion involving Na and Cl movement across respiratory epithelium. Atropine effectively blocks this effect. Adrenergic agents that stimulate tracheobronchial secretion have been ranked in order of their potency as: isoproterenol > epinephrine > phenylephrine (Al-Bazzaz and Cheng, 1979). Based on these studies and others, it appears that beta adrenergic receptors dominate over alpha in the stimulation of tracheobronchial electrolytes and water. As beta receptors function through a cAMP mechanisms, it is likely that phosphodiesterase inhibitors, such as *theophylline, theobromine* and *caffeine* should enhance the effects of the sympathetic nervous system in tracheobronchial secretion.

Prostaglandins, PGE_1 and PGE_2 (Al-Bazzaz et al., 1981), leukotrienes (Peatifield et al. 1982), vasoactive intestinal peptide (VIP) (Nathanson, et al. 1983), and histamine (Nadel, et al., 1985), all of which are increased in respiratory disease, have been demonstrated to increase mucus, water and electrolyte secretion by respiratory epithelia. It is not likely that therapeutic intervention to enhance water and electrolyte secretion in bovine respiratory disease will significantly increase the efficiency of the mucus escalator.

Antifoam therapy

In bovine respiratory disease, in which pulmonary edema is present, foam develops from the mixture of surfactant, plasma proteins and mucus components and markedly interferes with the movement of air through airways. Aerosol antifoam agents have been found to be useful in reducing the foaming properties of respiratory fluid. Ethanol, propylene glycol and glycerol serve as surface active agents and reduce foaming. In some cases, assuming that the pulmonary edema is not the limiting lesion in respiratory disease, antifoam therapy may be quite useful, but is difficult to administer in herd situations.

Ciliary augmentation therapy

The respiratory tract is covered with cilia from the nasal passageways to the smallest conducting bronchioles. The action of the cilia is necessary for function of the mucous escalator, moving mucus to the pharynx where it may be coughed out or swallowed. Ciliary activity determines, with the viscosity and elasticity of the mucus, the rate of mucociliary clearance. The rate of ciliary beating is slowest in the terminal bronchioles and demonstrate a gradual increase in rate toward the pharynx. This differential rate is necessary in order that mucus entering the large airways, with a smaller total surface area, from the small airways, with a large surface area, does not accumulate and occlude the air passages. There is no information available concerning physiologic mechanisms for the maintenance of the differential rate of ciliary activity along the respiratory tree. Enhancement of ciliary action, which can be achieved through therapeutic intervention, may not accomplish a differential rate of ciliary beating, and result in accumulation of mucous secretions, thus diminishing air exchange.

A large number of agents have been considered to be ciliary augmentors, including expectorants *(aromatic oils, terpin hydrate, KCl* and *NH_4)*; adrenergic $beta_2$ agonists

(isoproterenol, salbutamol, terbutaline, fenoterol, albuterol and *clenbuterol)*; methylxanthines *(aminophylline* and *theophylline)* and cholinergic agents *(methacholine).* It is difficult to determine whether these agents influence mucociliary clearance through action on the composition and quantity of mucous secretions or through direct actions on the cilia.

Adrenergic $beta_2$ agonists have been demonstrated to increase the frequency of ciliary beating. This action is blocked by propanolol (Verdugo, et al. 1980). Cholinergic agents also stimulate ciliary action (Corssen and Allen, 1959) and result in secretion of both mucous and serous cells, thus acting also on the composition of surface mucus. Atropine impairs mucociliary clearance, but direct action on cilia has not been demonstrated. The use of cholinergic agents is precluded by the significant bronchoconstrictor effect of these drugs.

Ciliary activation by adrenergic $beta_2$ agonists involves the activation of adenylate cyclase in the intracellular production of cAMP. Drugs such as *methylxanthines,* and mediators of inflammatory reactions, such as serotonin and prostaglandins, also increase cAMP in ciliated respiratory epithelial cells. It is not likely, therefore that the use of $beta_2$ agonists will be effective as ciliary augmentators.

There is no direct evidence that expectorants have any action on cilia. These agents may alter secretions of the respiratory mucosa, but generally are inefffective in enhancing mucociliary clearance.

Bronchodilators

A significant contribution to dyspnea of bovine respiratory disease is produced by bronchiolar constriction narrowing, and in many cases, with increased mucus secretion and decreased mucociliary clearance, occlusion of respiratory airways. Hyper-reactivity of airway smooth muscle has long been recognized to be a contributing factor in bronchiolar constriction. A number of influence result in this hyperreactivity, some of which are amenable to therapeutic intervention.

Autonomic nervous system control of bronchiolar smooth muscle.

The autonomic nervous system supplying the airway smooth musculature consists of sympathetic, adrenergic and nonadrenergic and parasympathetic nerve terminals. Alpha adrenergic and muscarinic agonists contract smooth muscle and result in bronchiolar constriction, whereas beta adrenergic agonists and nonadrenergic nerves generally result in muscle relaxation and bronchiolar dilation. Beta receptors are by far the dominant type of adrenergic receptors of the lung (Leff and Munoz, 1981). Stimulation of the sympathetic innervation of the lung normally results in bronchiolar dilatation, which is blocked by beta blocking agents. In bronchiolar smooth muscle, exposed to histamine, serotonin and elevated potassium concentrations, such as occur in the diseased lung, however, an exaggerated alpha adrenergic response has been demonstrated that results in bronchiolar constriction (Barnes, et al. 1983b; Kneussl and Richardson, 1978; Simonsson, et al., 1972). Alpha-adrenergic receptors of bronchiolar smooth muscle are predominantly of the $alpha_2$ type (Barnes, et al, 1983c), but significant numbers of $alpha_1$ type are also present (Barnes, et al 1983a). Sympathetic innervation of pulmonary smooth muscle is sparse, but due to large numbers of $beta_1$ receptors (Barnes et al., 1982; 1981), the overall effect is bronchiolar dilation (Drazen, 1978; Cabezas et al 1971).

Alpha-adrenergic receptors have their effect on bronchiolar smooth muscle through activation of calcium channels in the cell membranes, thus admitting calcium ions into the cytoplasm (Barnes,et al. 1983b) and resulting in muscle contraction. Beta-adrenergic receptors carry out their action through the generation of cytoplasmic cAMP (Rinart, et al. 1980); Sands 1981). The usefulness of *aminophylline* and *theophylline* as bronchodilators results from their ability to inhibit phosphodiesterase, the enzyme that inactivates cAMP. These agents therefore produce a beta-adrenergic effect through indirect means.

Nonadrenergic sympathetic nerve terminals of the lung resulting in relaxation of bronchiolar smooth muscle, contain vasoactive intestinal peptide or VIP (Dey, et al. 1981; Ghatei, et al. 1982; Kitamura, et al. 1980; Partanen, 1982). The means by which VIP produces smooth muscle relaxation is not known. To date, there are no drugs through which the effect of VIP can be enhanced.

Cholinergic, parasympathetic innervation to the respiratory smooth muscle has been amply demonstrated. Acetylcholine produces bronchiolar constriction in both normal and diseased lungs. Parasympatholytic agents, particularly muscarinic blocking drugs, are effective bronchodilators (Cavanaugh and Cooper, 1976; Chamberlain, et al. 1962; Herxheimer, 1959). The role of cholinergic induced bronchoconstriction in pulmonary disease is difficult to evaluate. It is well demonstrated that increased levels of serotonin (Sheller, et al. 1982), prostaglandins (Walters, et al., 1984) and histamine (Loring, et al. 1978; Yanta, et al. 1981), such as occur in pulmonary disease, have the effect of modifying cholinergic bronchodilation. Because muscarinic blocking agents (such as atropine) inhibit the mucociliary escalator function of the lung, these agents are not suitable for use in bovine respiratory disease.

Inflammation mediated bronchiolar constriction

A large number of substances are released in pulmonary inflammation that result in direct action on bronchiolar smooth muscle, inducing broncho-constriction. These chemical mediators are considered to be primary if they

are presynthesized and stored in cell vesicles prior to release by cell activation and secondary if they are synthesized as a result of the cell activation process (Lewis and Austen 1981). Inflammatory mediators of bronchoconstriction include histamine, serotonin and arachidonic acid derivatives. Therapeutic intervention can have the effect of minimizing the effects of these agents in bovine respiratory disease and result in bronchodilation.

Histamine is released in a large number of respiratory disorders, but most prominantly in allergy-mediated disease. Histamine exerts its effects on respiratory smooth muscle through two distinct, H_1 and H_2, receptor types (Ash and Schild, 1966; Black et al. 1972). H_1 receptors mediate bronchiolar constriction and H_2 receptors mediate bronchodilation (Chand and Eyre, 1978). H_1 receptors dominate in respiratory smooth muscle, so that the effect of histamine is bronchoconstriction. H_1 blocking agents, such as *promethazine, pyralamine* and *diphenhydramine* are effective antihistaminics, resulting in bronchodilation in histamine induced bronchoconstriction.

Serotonin, released predominantly from mast cells in allergic responses, is a bronchoconstrictor in the rat, guinea pig and dog, but has minimal potency in humans. Little is known of its effect in other species (Drazen, 1986).

Arachidonic acid is released from reactive cells in tissue inflammation, and is converted to a number of derivatives through the action of an endoperoxide synthetase, which possesses both cyclooxygenase and peroxidase activity (Needleman et al. 1986). The enzyme inserts 2 molecules of oxygen into the molecule to produce prostaglandin G_2 (PGG_1). The peroxidase activity reduces PGG_2 to Prostaglandin H_2 (PGH_2). Depending on the enzymes in the local microenvironment, PGH_2 may be metabolized to the classical prostaglandins PGD_2, PGE_2, PGF_{2alpha}, or PGI_2, or PGG_2 may be converted to an unstable intermediate, thromboxane A_2 (TXA_2), that undergoes transformation to TXB_2, a more stable compound. Thromboxane synthesis is accompanied by the production of 12-hydroxy-heptadeca-trienoic acid (HHTE) (Hamberg and Samuelsson, 1974; Hamberg, et al. 1975).

PGD_2 and PGF_{2alpha} and TXA_2 are bronchoconstrictors, while at least three prostaglandins (PGE_2, PGE_1, and PGI_2) are bronchodilators (Drazen, 1986). Since the synthesis of prostaglandins and thromboxanes are inhibited by nonsteroidal anti-inflammatory drugs (NSAIDS), such as *aspirin, phenylbutazone, oxphenbutazone, dipyrone, isppyrin, piroxicam, ibuprofen, naproxen, meclofenamic acid, acetaminophen,* and *flunixin,* the synthesis of both bronchodilators and bronchoconstrictors is depressed. The net result is uncertain. No controlled experiments with the use of these agents as bronchodilators have been conducted. The use of specific thromboxane inhibitors, such as *imidazoles,* or substituted imidazoles *(dazoxiben, ketoconozole* or *micronozole),* depresses only a bronchoconstrictor and would be the best choice of a NSAIDS agent to aid in bronchodilation (Patrignani, et al. 1984).

Leukotrienes are synthesized from arachidonic acid by a 5-lipoxygenase enzyme found in neutrophils, eosinophils, monocytes, mast cells and lung, spleen, brain and heart cells (Needleman et al., 1986). Slow reactive substance of anaphylaxis (SRA-A), is composed of a mixture of peptidoleukotrienes, LTC_4, LTD_4 and LTE_4. Leukotrienes promote bronchiolar constriction (Hansson et al., 1983; Holyroyd et al., 1981; Barnes, et al., 1984) in addition to stimulating the production of mucus by bronchial epithelial cells (Peatifield et al., 1982) and are inhibitory to ciliary action (Bisgaard and Pedersen, 1983). Although selective and potent leukotriene synthesis inhibitors or receptor antagonists are not available, *diethylcarbamazine* and *propyl gallate* suppress leukotriene synthesis and have demonstrated some protection in experimental cardiovascular shock therapy (Lefer, 1986). These compounds also inhibit the cyclooxygenase of prostaglandin and thromboxane production, and should provide some protection against bronchiolar constriction.

The most effective therapeutic method of interacting with the prostaglandin, thromboxane, leukotriene system in inflammation is the use of *glucocorticoids.* Glucocorticoids exert their antiinflammatory action through the induction of synthesis of lipocortins, peptides generated by cells containing glucocorticoid receptors. Lipocortins, through inhibition of phospholispase A2, prevent the mobilization of arachidonic acid from membrane lipids, and thus block the synthesis of its derivatives. In this way the effects of prostaglandins, thromboxanes and leukotrienes are depressed. The use of *NSAIDS* in bovine respiratory disease is not generally recommended because depression of synthesis of prostaglandins and thromboxanes results in a shift of synthesis toward that of leukotrienes, which, in addition to their role as significant mediators of inflammatory responses are also powerful bronchiolar constrictors. The use of a *glucocorticoid* (dexamethasone), coupled with *amimophylline* and an H_1 receptor blocking *antihistaminic* should provide maximum bronchodilation possible in bovine respiratory disease. It should be recognized, however, that glucocorticoids significantly blunt natural defense mechanisms against bacterial and viral infections. Glucorticoids should not be used in situations in which bacterial infections are not adequately treated with antimicrobials, or in known viral infections.

Pulmonary Macrophages, Neutrophils, Defense and Lung Damage

Pulmonary macrophages are monocyte derived or self replicating cells that reside within the lung (Brain, 1985). These cells are mobile, phagocytic and bactericidal,

maintaining a clean and sterile environment in the respiratory components of the lung. There are at least three types of pulmonary macrophages, *alveolar macrophages* within the lumen of the alveoli, where they lie beneath the surfactant layer; *airway macrophages* within the large and small conducting airways and *interstitial macrophages* within the connective tissues of the lung. Alveolar and airway macrophages are exposed to inhaled air and serve as component of the "air filtration" system of the respiratory tract. It is likely that many airway macrophages are alveolar macrophages that ride the mucous escalator toward the pharynx, but still representing an active phagocytic system. A number of airway macrophages, however, lie beneath the mucous escalator and are adhered to the epithelia lining, and may enter the airways through bronchial epithelium (Brundelet, 1965; Kilburn, 1974; Sorokin and Brain, 1975). Another class of macrophages are found within the vasculature of the lung, particularly in ruminants, where they appear to act in a fashion similar to that of the Kupffer cells of the hepatic sinusoids (Warner and Brain, 1984).

Large numbers of neutrophils are marginated within the pulmonary circulation under normal circumstances, the numbers increasing or decreasing by alterations in physiologic conditions (Ahlborg and Ahlborg, 1970; Foster, et al. 1986; Muir et al. 1984). These neutrophils lie within the lumen of pulmonary vessels, where they adhere and may be released into the systemic circulation producing neutrophilia. The mechanism through which marginated neutrophils adhere to pulmonary endothelial cells appears to be different from that which precedes neutrophil migration through the capillary endothelia in response to infection in the lung. Stimulation of endothelial cells with endotoxin, interleukin 1 (IL-1) or tumor necrosis factor induce an avid binding of neutrophils to capillary endothelia prior to transmural migration (Pohlman et al., 1986). There are few if any neutrophils in the interstitium and air spaces of the normal lung (Weibel, 1984). Chemoattractant agents are derived from bacteria, the complement system and as a consequence of activation of factor XII (Hageman factor) which activates the kinin, coagulation and fibrinolytic systems (Hogg, 1987). In addition, chemotactic substances are released by the neutrophil itself (Cornely, 1966; Wright and Gallin, 1975), lymphocytes (Ward, et al., 1968, 1969) and macrophages (Hunninghake et al., 1980; Kazmierowski, et al., 1977; Merrill, et al., 1980). Preceding neutrophil migration into lung tissue, there is an influx of plasma proteins, including immunoglobulins and complement factors that opsonize bacteria and other antigens in preparation for phagocytosis (Hogg, 1987).

Phagocytosis by macrophages or neutrophils is accompanied by a burst of oxygen utilization by the cells and formation of phagolysosomes by fusion of phagosomes and lysosomes. The burst of oxygen metabolism is associated with a significant increase in activity of the hexose monophosphate pathway of glycolysis, that plays an important role in the metabolism of superoxide anions and H_2O_2. The interaction of the iron salts and H_2O_2 and superoxide (O_2^-) radicals provide an important antimicrobial system, but adds a risk of these radicals injuring surrounding tissue.

Extracellular superoxide radicals have been demonstrated to activate a chemotactic factor of extracellular fluids that enables an activated macrophage or neutrophil to create a signal to surrounding unactivated neutrophils (Petrone et al., 1980). This chemotactic influence causes migration of unactivated neutrophils into an area of an activated neutrophil, but unlike other neutrophil chemotactic factors, such as C5a, does not activate the migrating cells. It appears that the therapeutic use of *superoxide dismutase* and other agents that scavenge O_2^- in extracellular fluid, preventing the activation of the superoxide dependent chemoattractant, represents the basis for anti-inflammatory activity of these agents (McCord et al., 1982; Petrone et al. 1980). The enzymes catalase and glutathione peroxidase of cells are the major intracellular antioxidant defense. Glutathione dependent enzymes also offer protection against lipid peroxidation. The use of antioxidants such as *vitamin C, vitamin E, beta carotene, zinc* (a constituent of superoxide dismutase), *selenium* (a constituent of gluthatione peroxidase), *copper* (a constituent of superoxide dismutase and ceruloplasmin), *iron* (a constituent of catalase) *manganese* (a constituent of mitochondrial superoxide dismutase) (Machlin and Bendich, 1987), and the amino acid *taurine* (Wright, et al. 1986) have demonstrated anti-inflammatory action.

Of these nutrients, only vitamin E, vitamin C, beta carotene and taurine can directly consume (scavenge) free radicals. Vitamin E (alpha-tocopherol) represents the major lipid soluble antioxidant of cell membranes and protects against lipid peroxidation by acting directly on peroxy, hydroxy and superoxide radicals and singlet oxygen (Machlin, 1980; McCay, P.B., 1985; Burton, et al., 1985; Fukuzawa and Gebicki, 1983; Ozawa et al., 1983; Fahrenholtz, et al., 1974; Littarru et al., 1984). Vitamin C reacts directly with superoxide, hydroxyl radicals and singlet oxygen. Vitamin C can also regenerate vitamin E that has been reduced by free radicals. *Beta-carotene,* a precursor of vitamin A, appears to be the most efficient consumer of singlet oxygen of this group and at the same time acts as an antioxidant (Burton and Ingold, 1984). Vitamin A itself, however is quite ineffective in scavenging free radicals (Urbch et al., 1951; Matthews-Roth, 1986).

Macrophages and neutrophils are potent protectors against bacterial invasion of tissues, but contain a number of agents that can produce tissue damage in bovine respiratory disease. Activation and migration of neutrophils as well as activation of macrophages, in addition to

initiating the secretion of oxygen radicals and lysosomal enzymes, result in the release of arachidonic acid and the production of prostaglandins, thromboxanes, leukotrienes and other lipid products that initiate damage to lung tissue (Johnson, et al. 1981; Johnson and Ward, 1981; Martin et al., 1981; Sacks, et al. 1978). These agents damage capillary endothelia, resulting in leakage of plasma proteins and water into the interstitial spaces in the development of lung edema. Plasma proteins in the interstitial spaces lead to extravascular coagulation (Dvorak, et al. 1985; Mantaner, et al. 1984; Hogg, 1987). Endothelial injury, due to infectious agents or, to products of reactive cells leads to intravascular coagulation. Blood coagulation becomes an important part of the pulmonary response to infection. The activation of platelets in the coagulation process also plays an important role in the chemotaxis of neutrophils. Through the release of platelets activating factor, a phospholipid produced by stimulated cells, such as neutrophils (Pinkard, et al 1982; Snyder, 1983; Vargaftig and Benveniste, 1983), activates platelets to begin a coagulation of blood, and serves in neutrophil chemotaxis (Omann, et al. 1987). Inhibitors for platelet activating factor have been demonstrated (Hwang, et al. 1985; Shen, et al. 1985), which should ultimately lead to minimizing this aspect of lung inflammation.

Intervention in the mechanisms of activation of macrophages and neutrophils provides a key focus for therapeutic modulation of pulmonary inflammation. It is clear that in neutrophil activation, a membrane receptor-transducer system involving guanine nucleotide binding peptides (G-peptides) results in the activation of membrane associated enzymes adenylate cyclase and phospholipase C. Adenylate cyclase results in the production of c-AMP from ATP, and phospholipase C generates triphosphoinositol and diacylglycerol from membrane lipids. Triphosphoinositol enters the cytosol and releases large quantities of calcium from endoplasmic reticulum stores. Diacylglycerol acts on membrane calcium channels to increase influx of calcium from the extracellular fluid and with phosphatidylserine and calcium, activates protein kinase C. Arachidonic acid also has the potential of activating protein kinase C. Protein kinase C in turn activates a number of cytoplasmic enzymes to stimulate the production of cytokines that are secreted by the cells and contribute to tissue inflammatory reactions. Protein kinase C inhibition can be accomplished through the use of cationic amphipathic compounds, such as *trifluoperazine* (Ikeda, et al. 1987) and *polymyxin B* (Windmaier, et al. 1987). Inhibitin of arachidonic acid release, through the use of *glucocorticoids,* serves to minimize the activation of this system in inflammation (McPhail et al. 1983). Calcium in the cytoplasm is bound to calmodulin, a cytosilic protein that directs the actions of cellular calcim. Cellular enzymes systems are activated by calcium-calmodulin complexes, resulting in superoxide production and secretion and the release of lysosomal enzymes. Inhibitors of calmodulin with phenothiazine derivatives such a *trifluoroperazine, chlorpromazine,* and *acetylpromazine* effectively limit this action of calmodulin (Shenolikar, 1988; Smolen and Weissmann, 1982; Smolen et al., 1981). The hydrophobic nature of these compounds and the concentrations required for complete inhibition of calmodulin action limit their therapeutic action but therapeutic levels used for tranquilization do effectively depress these systems. Recent development of new compound, such as *calmidazolium* show a greater potency as calmodulin antagonists than do phenothiazine derivatives (Shenolikar, 1988).

Acid-Base Balance Therapy in Bovine Respiratory Disease

The development of intravascular and extravascular coagulation, pulmonary edema and/or emphysema, and obstruction of airways through mucus accumulation produces serious deficiencies in the respiratory function of the lung. Hypoxemia and hypercapnia result from this defect. Hypercapnia produces a respiratory acidosis, and serves as a stimulus for increased respiratory rate and effort. Hypoxia results in an increased dependence of tissues upon anaerobic metabolism and the production of increasing amounts of lactic acid, resulting in a metabolic acidosis. Blood gas and electrolyte analysis are the only means by which the acid-base balance of these animals can be appropriately evaluated.

In respiratory acidosis, the elevation of extracellular and intracellular PCO_2 results in enhanced reabsorption of $NaHCO_3$ from renal tubules, thus conserving this important base. Renal tubule cells and red blood cells (through the chloride shift mechanism) produce increased amounts of $NaHCO_3$ in extracellular fluids. These compensation mechanisms minimize the change in pH produced by hypercapnia. If compensation is complete, no change in pH occurs. In most cases, however, compensation is incomplete, and some acidemia is present.

In metabolic acidosis, increased levels of lactic acid result in the titration of $NaHCO_3$ to Na-lactate, thus lowering the concentation of this important buffer. The degree of metabolic acidosis can be estimated by measuring plasma levels of lactic acid, or by the determination of the anion gap. The anion gap $([Na^+] + [K^+]) - ([Cl^-] + (HCO_3])$, is a measure of the quantity of $NaHCO_3$ that has been titrated by organic acids produced by anaerobic metabolism. The normal anion gap is between 15 and 20 mEq/liter. This represents the quantity of sodium and potassium salts of plasma that are not chloride or bicarbonate. An increase of anion gap above 20 is considered to be due to excess quantities of salts of organic acids, and is a measure of

the intensity of metabolic acidosis even in the face of an overshadowing respiratory acidosis.

Blood gas analysis, in a condition of mixed respiratory and metabolic acidosis, through the PCO_2 provides a clear measure of the intensity of respiratory acidosis, but does not provide a measure of the underlying metabolic acidosis. The anion gap provides a measure of the $NaHCO_3$ deficit produced by metabolic acidosis, but in a condition with respiratory acidosis does not provide the quantity of $NaHCO_3$ needed for therapy. In many cases of respiratory disease, the plasma [$NaHCO_3$] is above normal levels, even though a severe metabolic acidosis exists and is masked by partial compensation for respiratory acidosis. Unless a clear deficiency of $NaHCO_3$ can be demonstrated, it is unwise to administer this base beyond one half of the base deficit determined by the anion gap. The quantity of $NaHCO_3$ administered in mixed respiratory and metabolic acidosis would be determined by the following equation. Therapeutic $NaHCO_3$ = (0.5 x Body wt in Kg.) x 0.5 (anion gap -20) mEq. $NaHCO_3$ should not be administered as Na lactate, depending on the liver to convert the lactate to HCO_3^-, because lactate, which is already excessive, tends to diminish the energy availability in anaerobic metabolism. Adding more lactate simply amplifies this problem.

If animals with respiratory disease are dehydrated, rehydration fluids should be administered to correct this deficit. This can serve as a ready avenue for the administration of therapeutic $NaHCO_3$. The degree of dehyration is estimated and substantiated to some degree with alterations in packed red cell mass and plasma protein concentrations, generally to be mild (8% body wt. loss), moderate (10% body wt. loss) or severe (12% body wt. loss). To determine the quantity of fluid that is needed to restore body fluid volumes to normal it is necessary to know (or have a fairly accurate estimate of) the dehydrated body weight. If an animal weights 900 lbs. (409 kg.), and is 10% dehydrated, 45.5 liters of fluid will be necessary to restore normal hydration. This value is calculated as follows. dehydrated body weight = orginal body weight - % dehydration x original body weight. Let x = original body weight. In the case presented above, 409 kg = x - .10 x; 409 kg = .9x; x = 409 kg/.9 = 45.5 kg (or liters) of fluid lost. If this animal has an anion gap of 40, 2045 mEq of $NaHCO_3$ will be required to restore the animal's body fluid to normal levels. This value is determined by the equation (dehydrated body weight x .5 x base deficit). 0.5 x the body weight is the bicarbonate space, or the apparent volume of fluid into which administered $NaHCO_3$ is distributed.

Since the 45.5 liters of fluid to be administered does not contain any $NaHCO_3$, it will be necessary to add 25 mEq of $NaHCO_3$ to each liter administered, or a total of 1136 mEq additional $NaHCO_3$. This quantity of $NaHCO_3$ is known as maintenance bicarbonate, and prevents dilution of the therapeutic bicarbonate and the bicarbonate of the animal. Maintenance plus therapeutic bicarbonate provides a total of 3181 mEq of $NaHCO_3$ in 45.5 liters of fluid or 70 mEq of $NaHCO_3$ per liter.

The addition of this amount of $NaHCO_3$ to an isotonic saline (or .45% saline + 2.5% glucose) solution creates a hypertonic solution (osmolality will be 440 mOsm/liter). Hypertonic solutions should be avoided in the correction of fluid and acid-base balances. It is necessary therefore to dilute the final solution to isotonic osmolality (300 mOsm/liter). This can be accomplished (in this case) in the following manner. For each liter of final solution, use 530 ml of the base isotonic solution (isotonic saline or 0.45% saline + 2.5% glucose, containing 300 mOsm per liter), add 70 mEq (5.8 grams) of $NaHCO_3$ powder (1 gm contains 12 mEq.) and 470 ml of water for injection to produce an isotonic solution. These values are determined by the following calculations. One liter of isotonic solution contains 300 mOsmoles of solute. 70 mEq of $NaHCO_3$ represents 140 mOsmoles, therefore only 160 mOsmoles are needed from the isotonic solution, or 53% of that contained in a liter =530 ml. Generally these solutions are prepared in 10 to 15 liter volumes.

As an alternative to the preceding procedure for preparation of isotonic solutions, an isotonic solution of $NaHCO_3$ can be prepared by dissolving the 3181 mEq of $NaHCO_3$ (about 265 grams) in 21.2 liters (calculated by the following formula: (3181 x 2) or 6262 mOsmoles/300). This solution is then administered in a separate vein, or in the side port of an I.V. injection tubing with 24.3 lites (45.5 - 21.2) of the isotonic rehydration solution.

Oxygen therapy

The administration of oxygen may be used in bovine respiratory disease of particularly valuable individuals, or newborn in which it is economically feasible to do so. Oxygen is administered through a nasopharyngeal tube at a concentration of about 40%. This will not result in high enough oxygen concentrations to damage respiratory tract cells, but will result in elevation of alveolar oxygen concentrations to provide increased oxygen diffusion in conditions in which the diffusion barrier is thickened by interstitial edema.

The use of oxygen therapy in the face of increased respiratory effort due to airway constriction may result in reduced alveolar ventilation. With increased respiratory effort, there is a reduced sensitivity of the central nervous system to elevated PCO_2. Hypoxia therefore plays a greater role in the maintenance of respiratory drive. If the respiratory drive of hypoxia is eliminated, hypoventilation results. In animals that have been hypercapnic for a period of time (hours to days), sensitivity to elevated PCO_2 is further decreased, producing further dependence on

hypoxia for respiratory drive (Aubier et al., 1980; Lane and Howell, 1970; Lourenco and Miranda, 1968). This effect can be noted by observation of diminished respiratory effort during the administration of oxygen. If this occurs, oxygen therapy may be a detriment to respiration. Bronchodilators, administered prior to oxygen administration will aid in alleviating this problem.

A second untoward effect of oxygen therapy occurs particularly in lungs that have been hypoxic for a time. Tissue hypoxia results in the inability of cells to maintain a low intracellular concentration of calcium ions. Increased intracellular calcium results in the activation of a calcium binding protease (calpain). The activated enzyme degrades xanthine dehydrogenase (a relatively innocuous enzyme) to produce xanthine oxidase. Xanthine oxidase, rather than producing NADHH (the function of xanthine dehydrogenase), produces superoxide radicals (Roy and McCord, 1983). Tissue injury is ameliorated by cellular dismutase and catalase of the cells. The administration of *allopurinol,* effectively inhibits xanthine oxidase and serves to protect against this cellular damage. *Oxypurinol* exerts greater therapeutic potential than does allopurinol because it is not only an xanthine oxidase inhibitor, but also a significant oxygen radical scavenger (Moorhouse et al., 1987).

Pulmonary edema

In nearly all cases of bovine respiratory disease, there is some element of pulmonary edema. Edema may be limited to perivascular leakage of fluids and plasma proteins, or may be so severe as to fill many alveoli with fluid, rendering them nonfunctional in respiration. It is very unlikely that any therapy is effective in removing this extravasated fluid from the lung, other than correction of the primary disease. Enhanced exchange of oxygen in functional alveoli can take place through the use of oxygen therapy. Decreased airway resistance, and thus more effective ventilation of functional alveoli can be achieved through the use of bronchodilators (H-1 receptor blocking *antihistaminics, beta adrenergic drugs, glucocorticoids* and *aminophyllin).* The use of diuretics such as *furosemide* (Lasix) to enhance fluid loss is not effective in removing edema fluid until it has severely dehydrated the animal. The production of such severe dehydration and the accompanying metabolic acidosis in an animal that is likely already dehydrated and often severely acidotic does not seem to be reasonable therapy.

References

1. Ahlborg, B. and Ahlborg, G. (1970) Exercise leukocytosis with and without beta-adrenergic blockage. Acta Med. Scand. 187:241-246. 2. Al-Bazzaz, F.J., Yadava, V.P., and Westernfelder, C. (1981) Modification of Na and Cl transport in canine tracheal mucosa by prostaglandins. Am. J. Physiol. 140 (Renal Fluid Electrolyte Physiol. 9):F101-F105. 3. Al-Bazzaqz, F.J. and Cheng, E. (1979) Effect of catecholamines on ion transport in dog tracheal epithelium. J. Appl. Physiol. Respirat. Environ. Exercise Physiol, 47:397-403. 4. Ash, A.S.F. and Schild, H.O. (1966) Receptors mediating some actions of histamine. Br. J. Pharmacol. Chemother. 27:427-439. 5. Aubier, M., Murciano, D., Fournier, M., Milic-Emili, J., Pariente, R. and Derenne, J.P. (1980) Central respiratory drive in acute respiratory failure of patients with chronic obstructive pulmonary disease. Am. Rev. Repir. Dis. 122:191-199. 6. Barnes, N., Piper, P.J. and Costello, J.F. (1984) Actions of inhaled leukotrienes and their interactions with other allergic mediators. Prostaglandins. 28:629-631. 7. Barnes, P.J., Basbaum, C.B. Nadel, J.A. and Roberts, J.M. (1982) Localization of beta-adrenoceptors in mammalian lung by light microscopic autoradiography. Nature, London 199:444-447. 8. Barnes, P.J., Brown, M.J., Silverman, M. and Dollery, C.T. (1981) Circulating catecholamines in exercise and hyperventilation induced asthma. Thorax 36:435-440. 9. Barnes, P.J., Basbaum, C.B., Nadel, J.A. and Roberts, J.M,. (1983a) Pulmonary alpha-adrenoceptors: autoradiographic localization using ^{3}H prazosin. Eur. J. Pharmacol. 88:57-62. 10. Barnes, P.J., Nadel, J.A., Skoogh, B.-E. and Roberts, J.M. (1983b) Characterization of beta-adrenoceptors subtypes in canine airway smooth muscle by radioligand binding and physiological responses. J. Pharmacol. Exp. Ther. 225:456-461. 11. Barnes, P.J., Skoogh, B.-E., Brown, J.K. and Nadel, J.A. (1983c) Activation of alpha-adrenergic response in tracheal smooth muscle: a postreceptor mechanism. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 54:570-575. 12. Black, J.W., Duncan, W.A.M., Durant, C.J., Gnellin, C.R. and Parsons, E.M., (1972) Definition and antagonism of histamine H_2-receptors. Nature London 236-385-390. 13. Bisgaard, H., and Pedersen, M. (1982) SRS-A dampens activity of human cilia. (Abstract) Federation Proc. 42:1381. 14. Boat, T.G. and Cheng, P.W. (1980) Biochemistry of airway mucus secretions. Federation Proc. 39:3067-3074. 15. Boat, T.F., Cheng, P.W., Iyer, R.N., Carlson, D.M. and Polony, I. (1976) Human respiratory tract secretions. Mucous glycoproteins of nonpurulent tracheobronchial secretions and sputum of patients with bronchitis and cystic fibrosis. Arch. Biochem. Biophys. 177:95-104. 16. Boat, T.F. and Kleinerman, J.L. (1975) Human respiratory tract secretions. 2. Effect of cholinergic and adrenergic agents on in vitro release of protein and mucus glycoproteins. Chest. 67 (Suppl):32-34. 17. Boucher, R.C. and Gatzy, J.T. (1981) Effect of amiloride (Am) and mucosal sodium removal on canine bronchial ion transport (Abstract). Fed. Proc. 40:447. 18. Boucher, R.C. and Gatzy, J.T. (1982) Characterization of Na^+ absorption across rabbit tracheal epithelium (Abstract) Federation Proc. 41:1510. 19. Boucher, R.C., Navarte, J., Cotton, C., Stutts, J.J., Knowles, M.R., Finn, A.L. and Gatzy, J.T. (1982) Sodium absorption in mammalian airways. In: Quinton, P.M., Martinez, J.R. and Hopfer, U. (eds.) *Fluid and Electrolyte Abnormalities in Exocrine Glands in Cystic Fibrosis.* San Francisco, CA: San Francisco Press. pp. 271-287. 20. Boucher, R.C., Stutts, M.H. and Gatzy, J.T. (1981) Regional differences in bioelectric properties and ion flow in excised canine airways. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 51:706-714. 21. Brain, J.D. (1985) Macrophages in the respiratory tract. In: Fishman, A.P., Fiisher, A.B. and Geiger, S.R. (eds.) *Handbook of Physiology.* Section 3: *The Respiratory System.* Vol. 1. *Circulation and Nonrespiratory Functions.* Bethesda, MD. American Physiological Society. pp. 447-471. 22. Brundelet, J.P. (1965) Experimental study of the dust clearance mechanism of the lung. I. Histological study in rats of the intrapulmonary bronchial route of elimination. Acta Pathol. Microbiol. Scand. Suppl. 175:1-141. 23. Burton, G.W. and Ingold, K.V. (1984) Beta carotene: an unusual type of lipid antioxidant. Science 224:569-573. 24. Burton, G.W., Foste, D.O., Perly, B., Slater, T.F., Smith, I.C.P. and Ingold, K.U. (1985) Biological antioxidants. Philos. Trans. R. Soc. Lond. B Biol. Sci. 311:565-578. 25. Cabeza, G.A., Graf, P.D. and Nadel, J.A. (1971) Sympathetic versus parasympathetic nervous regulation of airways in dogs. J. Appl. Physiol. 31:651-655. 26. Cavanaugh, M.J. and Cooper, (1976) Inhaled atropine sulfate: dose response characteristics. Am. Rev. Respir. Dis.

114:517-524. 27. Chamberlain, D.A., Muir, D.C.F. and Kennedy, K.P. (1962) Atropine methonitrate and isoprenaline in bronchial asthma. Lancet 2:1019-1021. 28. Chand, N. and Eyre, P. (1975) Classification and biological distribution of histamine receptor sub-types. Agents Actions 5:277-295. 29. Cornely, H.P., (1966) Reversal of chemotaxis in vitro and chemotactic activity of leukocyte fractions. Proc. Soc. Exper. Biol. Med. 122:831-835. 30. Courssen, G. and C.R. Allen. (1959) Acetylcholine: its significance in controlling ciliary activity of human respiratory epithelium invitro. J. Appl. Physiol. 14:901-904. 31. Dey, R.D., Shannon, W.A. and Said, S.I. (1981) Localization of VIP-immunoreactive nerves in airways and pulmonary vessels of dogs, cats and human subjects. Cell Tissue Res. 220:231-238. 32. Drazen, J.M. (1986) Chemical mediators of immediate hypersensitivity reactions. In: Fishman, A.P., Macklem, P.T., Mead, J. and Geiger, S.R. (eds.) *Handbook of Physiology;* Section 3, *The Respiratory System;* Vol. III. *Mechanics of Breathing,* Part 2. pp. 711-718. 33. Drazen, J.M. (1978) Adrenergic influences on histamine-mediated bronchoconstriction in the guinea pig. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 44:340-345. 34. Dvorak, H.F., Senger, D.R., Dvorak, A.M., Harvey, V.S. and McDonagh, (1985) Regulation of extravascular coagulation by microvascular permeability. Science Wash. DC 227:1059-1061. 35. Fahrenholtz, S.R., Doleiden, F.H., Trozzolo, A.M. and Lamola. A.A. (1974) On the quenching of singlet oxygen by alpha-tocopherol. Photochem. Photobiol. 20:505-509. 36. Foster, N.K., Martyn, J.B., Rangno, R.E., Hogg, J.C. and Pardy, R.L. (1986). Leukocytosis of exercise: role of cardiac output and catecholamines. J. Appl. Physiol. 61:2218-2223. 37. Frizzell, R.A., Welsh, M.J. and Smith, P.L. (1981) Hormonal control of chloride secretion by canine tracheal epithelium: an electrophysiological analysis. Ann. NY Acad. Sci. 372:558-570. 38. Fukuzawa, K. and Bebicki, J.M. (1983) Oxidation of alpha tocopherol in micelles and liposomes by hydroxyl, perohydroxyl and superoxide free radicals. Arch. Biochem. Biophys. 226:242-251. 39. Gallagher, J.T., Kent, P.W., Passatore, M., Phipps, R.J. and Richardson, P.S. (1975) The composition of tracheal mucus and the nervous control of its secretion in the cat. Proc. R. Soc. Lond. Ser. B 192:49-76. 40. Ghatei, M.A., Sheppard, W.M., O'Shaughnessy, D.J., Adrian, D.J., McGregor, T.E., Polack, J.M. and Bloom, S.R. (1982) Regulatory peptides in the mammalian respiratory tract. Endocrinology 111:1248-1254. 41. Hamberg, M. and Samuelsson, B. 91974) Prostaglandin endoperoxides. Novel transformations of archidonic acid in human platelets. Proc. Natl. Acad. Sci. USA 71:3400-3404. 42. Hamberg, M., Svensson. J. and Samuelsson, B. (1975) Thromboxanes: a new group of biologically active compounds derived from prostaglandin endoperoxides. Proc. Natl. Acad. Sci. USA 72:2944-2998. 43. Hansson, G., Bjorck, T., Dahlen, S.W., Hedqvist, P., Gramstrom, E. and Dahlen, B. (1983) Specific allergen induced contraction of bronchi and formation of leukotrines C_4 D_4 and E_4 in human asthmatic lungs. Adv. Prostaglandin Thromboxane Leukotriene Res. 12:153-159. 44. Herxheimer, H. (1959) Atropine cigarettes in asthma and emphysema. Br. Med. J. 2:167-171. 45. Hill, H.K., Jr., Reynolds, J.A. and Hill, R.L. (1977) Purification, composition, molecular weight, and subunit structure of ovine submaxillary mucin. J. Biol. Chem. 252:3791-3798. 46. Hirato, F., Shiffman, E., Venkatasubramanian, K., Salomon, D. and Axelrod, J. (1980) A phospholipase A2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. Proc. Natl. Acad. Sci. USA 77:2533-2536. 47. Hogg, J.C. (1987) Neutrophil kinetics and lung injury. Physiol. Revs. 67:1249-1295. 48. Holyroyde, M.C., Altounyan, R.E.C., Cole, A.H., Dixon, M. and Elliott, E.V. (1981) Bronchoconstriction produced in man by leukotrienes C and D. Lancet 2:17-18. 49. Hunninghake, G.W., Gadek, J.E., Fales, H.M. and Crystal, R.G. (1980) Human alveolar macrophage-derived chemotactic factor for neutrophils: stimuli and partial characterization. J. Clin. Invest. 66:473-483. 50. Hwang, S.-B., Lam, M.-H., Biftu, T., Beattie, T.R. and Shen, T.-Y. (1985) Trans-2, 5-bis-(3,45-trimethoxy-phenyl) tetrahydrofuran. An orally active specific and competitive receptor antagonist of platelet activating factor. J. Biol. Chem. 260:15639-15645. 51. Iketa, M., Deery, W.J., Ferdows, M.S., Nielsen, T.B. and Field, J.B. (1987) Role of Cellular Ca^{++} in phosphorylation of 21 K and 19 K polypeptides in cultured thyroid cells: effects of phorbol ester, trifluoperazine and 8-diethylaminol-octyl-3,4,5-trimethoxybenzoate hydrochloride. Endocrinology 121:175-181. 52. Johnson, K.J., Fantone, J.C.,Kaplan, J. and Ward, P.A. (1981) In vivo damage of rat lungs by oxygen metabolite, J. Clin. INvest. 67:983-993. 53. Johnson, K.J. and Ward, P.A. (1981) Role of oxygen metabolites in immune complex injury of lung. J. Immunol. 126:2365-2369. 54. Kazmierowski, J.A., Gallin, J.I. and Reynolds, H.Y. (1977) Mechanism for the inflammatory response in primate lungs. Demonstration and partial characterization of an alveolar macrophage-derived chemotactic factor with preferential activity for polymorphonuclear leukocytes. J. Clin. Invest. 59:273-281. 55. Kilburn, K.H. (1974) Functional morphology of the distal lung. Int. Rev. Cytol. 37:153-270. 56. Kitamura, S., Ishihara, Y. and Said, S.I. (1980) Effect of VIP, phenoxybenzamine and prednisoline on cyclic nucleotide content of isolated guinea-pig lung and trachea. Eur. J. Pharmacol. 67:219-223. 57. Kneussl, M.P. and Richardson, J.B. (1978) Alpha-adrenergic receptors in human and canine tracheal and bronchial smooth muscle. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 45:307-311. 58. Lane, D.J. and Howell, J.B.L. (1970) Relationship between sensitivity to carbon dioxide and clinical features in patients with chronic airways obstruction. Thorax 25:150-158. 59. Lefer, A.M. (1986) Leukotrienes as mediators of ischemia and shock. Biochem. Pharmacol. 35:123-127. 60. Leff, A. and Munoz, N.M. (1981) Interrelationship between alpha- and beta-adrenergic agonists and histamine in canine airways. J. Allergy Clin. Immunol. 68:300-309. 61. Lewis, R.A. and Austen, K.F. (1981) Mediation of local homeostasis and inflammation by leukotrienes and other mast cell dependent compounds. Nature, London 293:103-108. 62. Littarru, G.P., Lippa, S., De Sole, P., Oradei, A., Dalla Torre, F. and Macri, M. (1984) Quenching of singlet oxygen by D-alpha-tocopherol in human granulocytes. Biochem. Biophys. Res. Commun. 119:1056-1061. 63. Lourenco, R.V., and Miranda, J.M. (1968) Drive and performance of the ventilatory apparatus in chronic obstructive lung disease. N. Engl. J. Med. 279:53-59. 64. Lundgren, J.D., Hirata, F., Marom, Z., Logun, C., Steel, L., Kaliner, M. and Shelhamer, J. (1988) Dexamethasone inhibits respiratory glycoconjugate secretion from feline airways in vitro by the induction of lipocortin (lipomodulin) synthesis. Am. Rev. Respir. Dis. 137:353-357. 65. Lutz, R., Litt, M. and Chakrin, L.W. (1973) Physical-chemical factors in mucus rheology. In: Gabelnick, H.L. and Litt, N. (eds.) *Rheology of Biological Systems.* Springfield, Il. Charles C. Thomas. 66. McCay, P.B. (1985) Vitamin E: interactions with free radicals and ascorbate. Annu. Rev. Nutr. 5:323-340. 67. Macklin, L.J. and Bendich, A. (1987) Free radical tissue damage: protective role of antioxidant nutrients. FASEB J. 1:441-445. 68. Marom, Z., Shelhamer, J.H. and Kaliner, M. (1984) Human pulmonary macrophage derived mucus secretagogue. J. Exp. Med. 159:844-860. 69. Marom, Z., Shelhamer, J.H., Alling, D. and Kaliner, M. (1984) The effects of corticosteroids on mucous glycoprotein secretin from human airways in vitro. Am. Rev. Respir. Dis. 129:62-65. 70. Marom, Z., Shelhamer, J.H., Sun F. and Kaliner M. (1983) Human airway monohydroxyeicosatetraenoic acid generation and mucus release. J. Clinc. Invest. 72:122-127. 71. Marom, Z., Shelhamer, J.H., Bach, M.K., Morton, D.R. and Kaliner, M. (1982) Slow reacting substances leukotrienes C4 and D4, on glycoprotein and lysozyme secretion by human bronchial mucosa. Am. Rev. Respir. Dis. 126:449-451. 72. Marom, Z., Shelhamer, J.H., kaliner, M. (1981) Effects of arachidonic acid monohydroxyeicosatetraenoic acid and prostaglandins on the release of mucous glycoproteins from human airways in vitrol. J. Clin. Invest. 67:1695-1702. 73. Martin, W.J., Gadek, J.E., Hunninghake, G.W., and Crystal, R.G. (1981) Oxidant injury of lung paranchymal cells. J. Clin. Invest. 68:1277-1288. 74. Matthews-Roth, M.M. (1986) Beta carotene therapy for erythropoietic protoporphyria and other photogensitivity diseases. Biochemie 68:875-884. 75. McCord, J.M., Wong, K., Stokes, S.H., Petrone, W.F. and English, D.K. (1982) A

mechanism for the anti-inflammatory activity of superoxide dismutase in: Autor, A.P. (ed.) *Pathology of Oxygen.* New YorK: Academic press. pp. 75-83. 76. McPhail, L.C., Clayton, C.C. and Snyderman, R. (1984) A potential second messenger role for unsaturated fatty acids: activation of Ca^{++}-dependent protein kinase. Science Wash. DC 224:622-200. 77. Merrill, W.W., Naegel, G.P., Mathay, R.A. and reynolds. H.Y. (1980) Alveolar macrophage-derived chemotactic factor. Kinetics of in vitro production and partial characterization. J. Clin. Invest. 65:268-276. 78. Montaner, J.S.G., Tsang, J., Evans, K.G., Mullen, J.B.M., Burns, A.R., Walker, D.C., Wiggs, B. and Hogg, J.C. (1986) Alveolar epithelial damage: a critical difference between high pressure and oleic acid induced low pressure pulmonary edema. J. Clin. Invest. 77:1786-1796. 79. Moorhouse, C.P., Grootveld, M., Halliwell, B., Quinlan, G.J. and Gutteidge, J.M>C. (1987) Allopurinol and oxypurinol are hydroxyl radical scavengers. FEBS Lett. 213:23-28. 80. Muir, A.L., Cruz, M., Martin, B.A., Thomasen, H.V., Belzberg, A. and Hogg, J.C. (1984) Leukocyte kinetics in the human lung: role of exercise and catecholamine. J. Appl. Physiol. 57:711-719. 81. Murlas, C., Basbaum, C. and Nadel, J. (1979) Morphometric analysis of the adrenergic innervation of cat tracheal glands. (Abstract) Federation Proc. 38:1374. 82. Nadel, J.A., Widdicombe, J.H. and Peatfield, A.C. (1985) Regulation of airway secretions, ion transport and water movement. In: Fishman, A.P., Fisher, A.B. and Geiger, S.R. (eds.) *Handbook of Physiology:* Section 3: *The Respiratory System,* Vol. I. *Circulation and Nonrespiratory Functions.* Bethesda, MD., American Physiological Society. pp. 419-445. 83. Nadel, J.A. and Davis, B. (1980) Parasympathetic and sympathetic regulation of secretion from submucosal glands in airways. Federation Proc. 39:3075-3079. 84. Nathanson, I., Widdicombe, J.H. and Barnes, P.J. (1983) Effects of vasoactive intestinal peptide on ion transport across the dog tracheal epithelium. J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 55:1844-1848. 85. Needleman, P., Turk, J., Jakschik, B.A., Morrison, A.R. and Lefkowith, J.B. (1986) Arachidonic acid metabolism. Ann. Rev. Biochem. 55:69-115. 86. Omann, G.M., Allen, R.A., Bokoch, G.M., Painter, R.G., Traynor, A.E. and Sklar, L.A. (1987) Signal transduction and cytoskeletal activation in the neutrophil. Physiol. Revs. 67:285-322. 87. Olver, R.E., Davis, B., Marin, M.G. and Nadel, J.A. (1975) Active transport of Na^+ and Cl^- across the canine tracheal epithelium in vitro. Am. Rev. Respir. Dis. 112:811-815. 88. Ozawa, T., Hanaki, vA. and Matsuo, M. (1983) Reactions of superoxide ion with tocopherol and model compounds: correlation between the physiological activities of tocopherols and the concentration of chromanoxyl-radicals. Biochem. Int. 6:685-692. 89. Partanen, M., Laitinen, A., Hervonen, A., Topivanen, M. and Laitinen, L.A. (1982) Catecholamine- and acetylcholinesterase-containing nerves in human lower respiratory tract. Histochemistry 76:175-188. 90. Patrignani, P., Filabozzi, P., Catella, F., Pugliese, F. and Patrono, C. (1984) Differential effects of dazoxiben, a selective thromboxane synthase inhibitor, on platelet and renal prostaglandin-endoperoxide metabolism. J. Pharmacol. Exp. Ther. 228:472-477. 91. Peatifield, A.C., Piper, P.J. and Richardson, P.S. (1982) The effect of leukotriene $_4$ on mucin release into the cat trachea in vivo and in vitro. Br. J. Pharmacol. 77:391-393. 92. Petrone, W.G., English, D.K., Wong, K., McCord, J.M. (1980) Free radical and inflammation: superoxide-dependent activation of a neutrophil chemotactic factor in plasma. Proc. Natl. Acad. Sci. USA 77:1159-1163. 93. Pinkard, R.L., McManus, L.M. and Hanahan, D.J. (1982) Chemistry and biology of acetyl glyceryl ether phosphorylcholine (platelet-activating factor). Adv. Inflammation lRes. 4:147-180. 94. Pohlman, T.H., Stanness, K.A., Beatty, P.G., Ochs, H.D. and Harlan, J.M,. (1986) An endothelial cell surface factor(s) induced in vitro by lipopolysaccharide, interleukin 1 and tumor necrosis factor-alpha increases neutrophil adherence by CDw18 dependent mechanism. J. Immunol. 136:4548-4553. 95. Rinart, T.A., Rubinfeld, A.R., Brunton, L.L. and Mayer, S.A. (1980) Depressed cyclic AMP levels in airway smooth muscle from asthmatic dogs. Proc. Natl. Acad. Sci. USA 76:1492-1476. 96. Roberts, G.P. (1976) The role of disulfide bonds in maintaining the gel structure of bronchial mucus. ARch. Biochem. Biophys. 173:528-537. 97. Roussel, P., Degand, P., Lamblin, G., Laine, A. and La Fitte, J.J. (1978) Biochemical definition of human tracheobronchial mucus. Lung 154:241-260. 98. Roussell, P., Lamblin, G., Degand, P., Walker-Nasier, E. and Jeanloz, R.W. (1975) Heterogeneity of the carbohydrate chains of sulfated bronchial glycoproteins isolated from a patient suffering from cystic fibrosis. J. Biol. Chem. 250:2114-2122. 99. Sacks, T., Moldow, C.F., Craddock. R.P., Bowers, T.K. and Jacob, H.S. (1978) Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes: an invitro model of immune vascular damage. J. Clin. Invest. 61:1161-1167. 100. Sands, H. (1981) Effect of drug treatment and aerosoled antigen sensitization on cyclic AMP and beta-adrenergic receptors of guinea pig lung. Exp. Lung Res. 2:1-8. 101. Seawen, M. and Allen, A. (1977) The action of proteolytic enzymes on the glycoprotein of pig gastric mucus. Biochem. J. 163:363-368. 102. Shelhamer, J.H., Marom, Z. and Kaliner, M. (1980) Immunologic and neuropharmacologic stimulation of mucous glycoprotein release from human airways in vitro. J. Clin. Invest. 66:1400-1408. 103. Shen, T.Y. Hwang, S.-B., Chang, M.N., Doebber, T.W., Lam, M.-H.T., Wu, M.S., Wang. X., Han, G.Q. and Li, R.Z. (1985) Characterization of a platelet-activating factor antagonist isolated from haifenteng (*Piper futokadsura*): specific inhibition of in vitro and in vivo platelet-activating factor-induced effects. Proc. Natl. Acad. Sci. USA 82:672-676. 104. Shenolikar, S. (1988) Protein phosphorylation: hormones, drugs and bioregulation. FASEB J. 2:2753-2764. 105. Shih, C.K., Litt, M., Khan, M.A. and Wolf, D.P. (1977) Effect of nondialyzable solids concentration and viscoelasticity on ciliary transport of tracheal mucus. Am. Rev. Respir. Dis. 115:989. 106. Simonsson, B.G., Svedmyr, N., Skoogh, B.-E., Anderson, R. and Bergh, N.P. (1972) In vivo and in vitro studies on alpha-receptors in human airways. Potentiation with bacterial endotoxin. Scand. J. Respir. Dis. 53:227-236, 107. Smolen, J.E. and Weissmann, G. (1982) The effect of various stimuli and calcium antagonists on the fluorescence response of chlorotetracycline-loaded human neutrophils. Biochim. Biophys. Acta 720:172-180. 108. Smolen, J.E., Korchak, A.S. and Weissmann, G. (1981) The role of extracellular and intracellular calcium in lysosomal enzyme release and superoxide anion generation by human neutrophils. Biochim. Biophys. Acta. 677:512-520. 109. Snyder, F. (1983) Platelet activating factor (PAF), a novel type of phospholipid with diverse biological properties. Annu. Rep. Med. Chem. 17:243-252. 110. Sorokin, S.P. ands Brain, J.D. (1975) Pathways of clearance in mouse lungs exposed to iron oxide aerosols. Anat. Rec. 181:581-626. 111. Ueki, I., German, V.F. and Nadel, J.A. (1980) Micropipette measurement of airway submucosal gland secretion: autonomic effects. Am. Rev. Respir. Dis. 121:351-357. 112. Urbach, C., Hickman, K. and Harris, P.L. (1951) Effect of individual vitamins A, C, E and carotene administered at high levels and their concentration in the blood. Exp. Med. Surg. 10:7-20. 113. Vargaftig, B.B. and Benveniste, J. (1983) Platelet-activating factor today. Trends Pharmacol. Sci. 4:341-343. 114. Verdugo, P., Johnson, N.T. and Tam, P.Y. (1980) Beta-adrenergic stimulation of respiratory ciliary activity. J. Appl. Physiol.: Respirat. Envirion. Exercise Physiol. 48:868-871. 115. Ward, P.A., Remold, H.G. and David, J.R. (1969) Leukotactic factor produced by sensitized lymphocytes. Science Wash. DC 163:1079-1081. 116. Ward, P.A., Remold H.G. and David, J.R. (1970) The production of antigen-stimulated lymphocytes of a leukotactic factor distinct from migration inhibitory factor. Cell Immunol. 1:162-174. 117. Warner, A.P. and Brain, J.D. (1984) Intravascular pulmonary macrophages in ruminants actively participate in reticuloendothelial clearance of particles (Abstract). Federation Proc. 43:1001. 118. Weibel, E.R. (1984) Lung cell biology. In: Fishman, A.P., Fisher, A.B. and Geiger, S.R. (eds) *Handbook of Physiology.* Section 3. *The Respiratory System.* Volume 2. *Circulation and Nonrespiratory Functions.* pp. 47-91. 119. Westenfelder, C., Earnest, W.R. and al-Bazzaz, F.J. (1980) Characterization of Na-K-ATPase in dog tracheal epithelium: enzymatic and ion transport measurements. J. Appl. Physiol.; Respirat. Environ. Exercise Physiol. 48:1008-1019. 120.

Widdicombe, J.H. and Welsh, MLJ. (1980) Ion transport by the dog's tracheal epithelium. Federation Proc. 39:3062-3066. 121. Widdicombe, J.H. Basbaum C.B. and Yee, J.Y. (1973) Localization of Na pumps in the tracheal epithelium of the dog. J. Cell Biol. 82:380-390. 122. Windmaier, E.P., Iida, S. and Hall, P.F. (1987) The effect of polymyxin B on steroidogenesis from adrenocortical cells. Endocrinology 121:290-297. 123. Wright, C.E., Tallan, H.H., Lin, YH. Y. and Gaull, G.E. (1986) Taurine: Biological update. Ann. Rev. Biochem. 55:427-453. 124. Wright, D.G. and Gallin, J.E. (1975) Modulation of the inflammatory response by products released from human polymorphonuclear leukocytes during phagocytosis. Generation and inactivation of the chemotactic factor C5a. Inflammation 1:23-39.